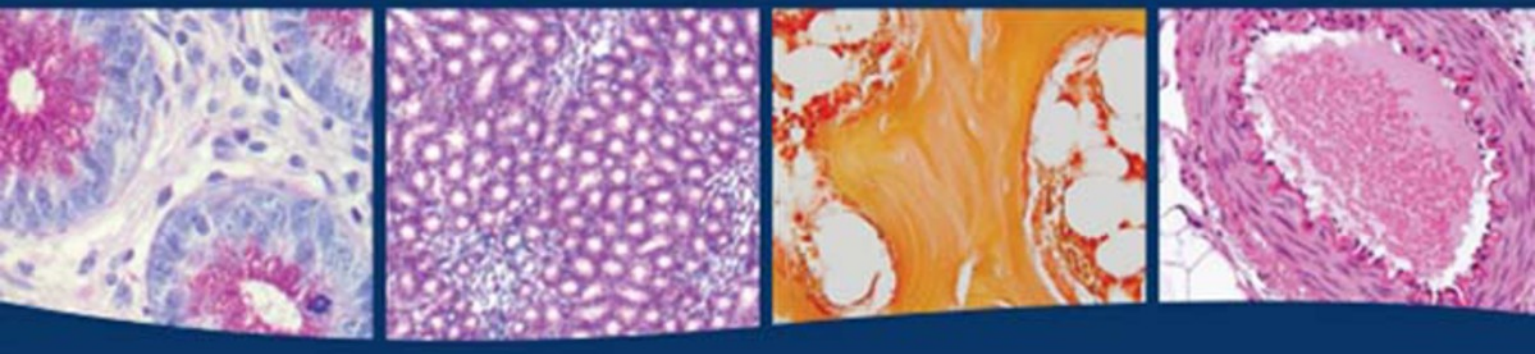
Study smart with Student Consult

TEXTBOOK OF HISTOLOGY

Leslie P. Gartner



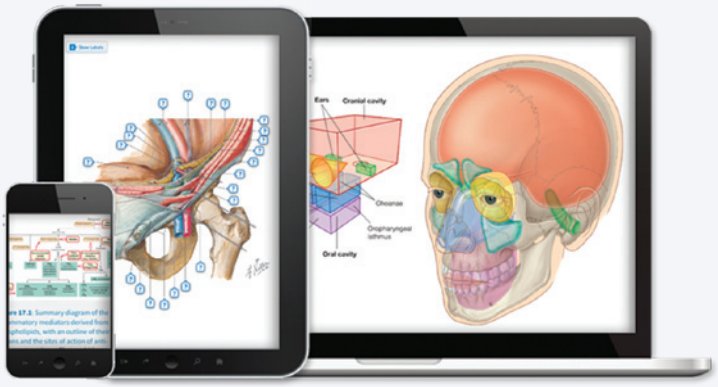
FOURTH EDITION

ELSEVIER

Student CONSULT Built with inkling | E books

Any screen. Any time. Anywhere.

Activate the eBook version of this title at no additional charge.



Student Consult eBooks give you the power to browse and find content, view enhanced images, share notes and highlights—both online and offline.

Unlock your eBook today.

1. Visit **studentconsult.inkling.com/redeem**
2. Scratch off your code
3. Type code into "Enter Code" box
4. Click "Redeem"
5. Log in or Sign up
6. Go to "My Library"

It's that easy!

Scan this QR code to redeem your eBook through your mobile device:

Place Peel Off Sticker Here

For technical assistance:
email studentconsult.help@elsevier.com
call 1-800-401-9962 (inside the US)
call +1-314-447-8200 (outside the US)

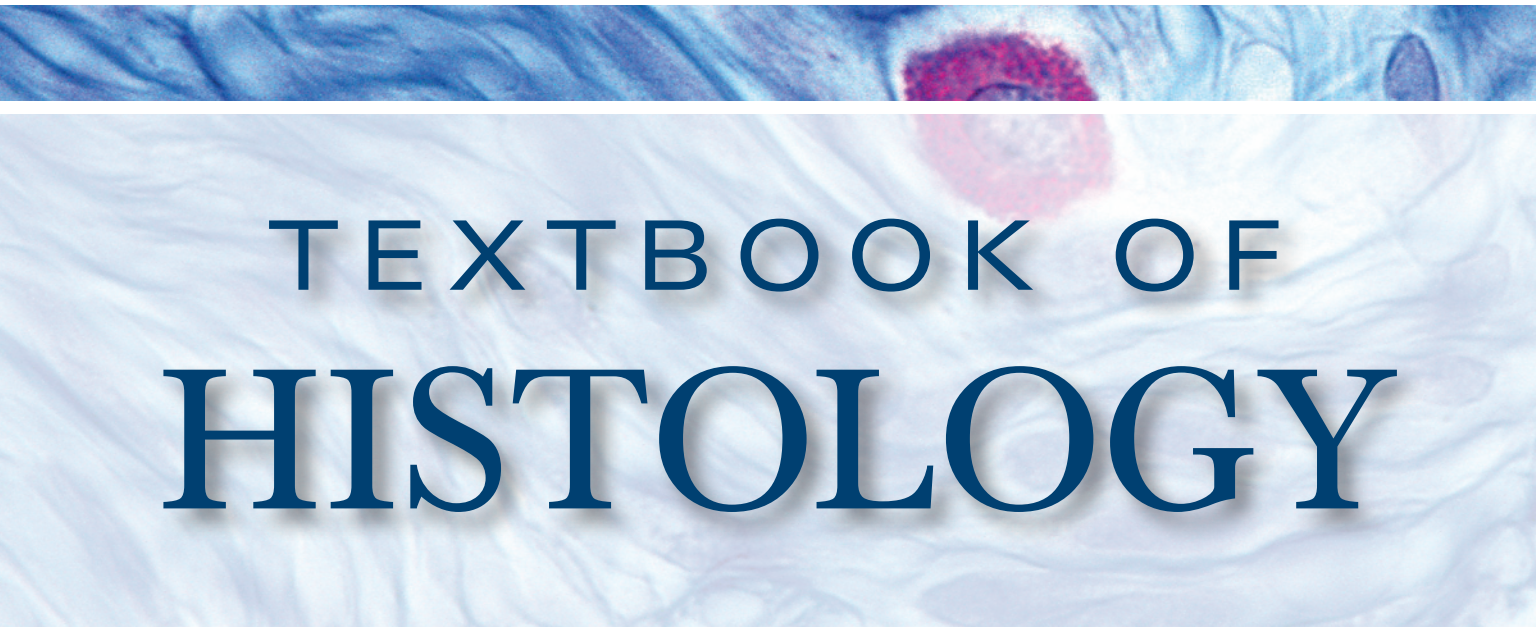
ELSEVIER

Use of the current edition of the electronic version of this book (eBook) is subject to the terms of the nontransferable, limited license granted on studentconsult.inkling.com. Access to the eBook is limited to the first individual who redeems the PIN, located on the inside cover of this book, at studentconsult.inkling.com and may not be transferred to another party by resale, lending, or other means.

2015v1.0

TEXTBOOK OF
HISTOLOGY

This page intentionally left blank



TEXTBOOK OF HISTOLOGY

FOURTH EDITION

Leslie P. Gartner, PhD
Professor of Anatomy (Ret.)
Department of Biomedical Sciences
Baltimore College of Dental Surgery
Dental School
University of Maryland
Baltimore, Maryland

ELSEVIER

ELSEVIER

1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

TEXTBOOK OF HISTOLOGY, FOURTH EDITION ISBN: 978-0-323-35563-6
INTERNATIONAL EDITION ISBN: 978-0-323-39613-4
Copyright © 2017 by Elsevier, Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Previous editions copyrighted 2007, 2001, and 1997.

Library of Congress Cataloging-in-Publication Data

Names: Gartner, Leslie P., 1943- , author.
Title: Textbook of histology / Leslie P. Gartner.
Other titles: Color textbook of histology
Description: Fourth edition.
Philadelphia, PA : Elsevier, [2017].
Preceded by Color textbook of histology / Leslie P. Gartner, James L. Hiatt. 3rd ed. 2007.
Includes bibliographical references.
Identifiers: LCCN 2015037448 ISBN 9780323355636 (pbk. : alk. paper)
ISBN 9780323396134 (international edition)
Subjects: MESH: Histology.
Classification: LCC QM557 NLM QS 504 DDC 611.018—dc23 LC record available at http://lccn.loc.gov/201503744

Associate Content Strategist: Lauren Willis
Senior Content Development Specialist: Jennifer Ehlers
Publishing Services Manager: Patricia Tannian
Project Manager: Amanda Mincher
Book Designer: Margaret Reid

Printed in China

Last digit is the print number: 9 8 7 6 5 4 3 2 1

To my wife Roseann,
my daughter Jennifer,
and my mother Mary.

L.P.G.

This page intentionally left blank

Although it has been stated that writing is a lonely profession,
I have been very fortunate in having the company of my faithful
Airedale Terrier, Skye, who, as is evident in the accompanying photograph,
kept me company as I was sitting at my computer.

This page intentionally left blank

Preface

Once again, I am gratified to release a new edition of a histology textbook that has become well established not only in its original language but also in seven other languages, namely Italian, Portuguese, Indonesian, Korean, Spanish, Greek, and Turkish, into which it has been translated. The place of histology has altered as the biological sciences have progressed in the last half century. It evolved from the purely descriptive science of microscopic anatomy to its current position as the linchpin that connects functional anatomy, cell and molecular biology, physiology, and histopathology.

The current edition, coming out a number of years after the third edition reached the bookshelves, has been revised to be consistent with new information in cell and molecular biology that pertains to the subject matter of histology. While incorporating new material, I was mindful of the time constraints that students face in an ever expanding curriculum and an exponentially increasing information glut; therefore the goal was to maintain the brevity and readability of the textual material.

The most visible change is that my co-author, Dr. James L. Hiatt, was not associated with the writing of this edition. Jim retired from active teaching 18 years ago and decided to make his retirement complete and devote himself to other endeavors. Over the years Jim and I wrote 24 research articles, 47 abstracts, and, counting new editions, 22 textbooks together. It was a very pleasant and fruitful association, and I sorely miss this long-term professional partnership. Needless to say, our personal friendship remains as strong as ever.

As with all of my textbooks, *Textbook of Histology* has been written with the student in mind; thus the material presented is complete but not esoteric. It is not meant to train the reader to be a histologist but to provide the necessary basis for the understanding of the microscopic structure of the human body and to lay a foundation for the student's progress through the biomedical sciences.

Although I have attempted to be accurate and complete, I realize that errors and omissions may have escaped my attention. Therefore I welcome criticisms, suggestions, and comments that would help improve this textbook. Please address them to LPG21136@yahoo.com.

Leslie P. Gartner

Note to instructors:

Contact your Elsevier Sales Representative for teaching resources, including slides, an image bank, and a test bank, for *Textbook of Histology*, 4e, or request these supporting materials at: http://evolve.elsevier.com/Gartner/histology/

This page intentionally left blank

Acknowledgments

I would like to thank the following individuals for the help and support that they provided in the preparation of this book.

Because histology is a visual subject, it is imperative to have excellent graphic illustrations. For that I am indebted to Todd Smith for his careful attention to detail in revising the illustrations from the previous editions and for the creation of new figures. I also thank my many colleagues from around the world and their publishers who generously permitted me to borrow illustrative materials from their publications.

Finally, my thanks go to the project team at Elsevier for all their help: namely Meghan Ziegler, Content Strategist, who was instrumental in initiating and bringing to fruition the possibility of this new edition; Jennifer Ehlers, Senior Content Development Specialist, who worked tirelessly in ensuring that the "i"s were dotted and the "t"s were crossed and was always available to help solve all of the problems that arose doing the revision; Ryan Pettit, Content Coordinator, who assisted with ironing out the contract between Elsevier and the author; Brett MacNaughton, Illustration Buyer; Amanda Mincher, Project Manager, who was so efficient and careful in managing the corrections to the page proofs; Lauren Willis, Associate Content Strategist; and Patricia Tannian, Publishing Services Manager.

This page intentionally left blank

Contents

1 Introduction to Histology and Basic Histological Techniques, *1*

2 Cytoplasm, *13*

3 Nucleus, *55*

4 Extracellular Matrix, *81*

5 Epithelium and Glands, *99*

6 Connective Tissue, *127*

7 Cartilage and Bone, *149*

8 Muscle, *179*

9 Nervous Tissue, *211*

10 Blood and Hemopoiesis, *251*

11 Circulatory System, *287*

12 Lymphoid (Immune) System, *311*

13 Endocrine System, *345*

14 Integument, *373*

15 Respiratory System, *395*

16 Digestive System: Oral Cavity, *419*

17 Digestive System: Alimentary Canal, *435*

18 Digestive System: Glands, *471*

19 Urinary System, *499*

20 Female Reproductive System, *529*

21 Male Reproductive System, *559*

22 Special Senses, *583*

Index, *615*

This page intentionally left blank

1 Introduction to Histology and Basic Histological Techniques

Histology is the branch of the anatomical sciences that studies tissues of animals and plants. This textbook, however, discusses only animal, more specifically, human, tissues. In its broader aspect, the word *histology* is used as if it were a synonym for microscopic anatomy because its subject matter encompasses not only the microscopic structure of tissues but also that of the cell, organs, and organ systems.

It should be understood that the body is composed of cells, intercellular matrix, and a fluid substance—the extracellular fluid (tissue fluid)—which bathes these components. Extracellular fluid, which is derived from plasma of blood, carries nutrients, oxygen, and signaling molecules to cells of the body. Conversely, signaling molecules, waste products, and carbon dioxide released by cells of the body reach blood and lymph vessels by way of the extracellular fluid. Extracellular fluid as well as much of the intercellular matrix is not visible in routine histological preparations, yet their invisible presence must be appreciated by the student of histology.

The subject of histology no longer deals with merely the microscopic structure of the body; it also concerns itself with the body's function. In fact, histology has a direct relationship to other disciplines and is essential for their understanding. This textbook, therefore, intertwines the disciplines of cell biology, biochemistry, physiology, embryology, gross anatomy, and, as appropriate, pathology. Students will recognize the importance of this subject as they refer to the text later in their careers. An excellent example of this relationship should be evident when the reader learns about the histology of the kidney and realizes it is the intricate and almost sublime structure of that organ (down to the molecular level) that is responsible for the kidney's ability to perform its functions. Alterations of the kidney's structure are responsible for a great number of life-threatening conditions. Another example is the microscopic, indeed molecular, structure of muscle cells. The ability to contract is intimately dependent on the microscopic, submicroscopic, and molecular organization of the various components of the muscle cell.

The remainder of this chapter discusses the methods used by histologists to study the microscopic anatomy of the body.

LIGHT MICROSCOPY

Tissue Preparation

Steps required in preparing tissues for light microscopy include (1) fixation, (2) dehydration and clearing, (3) embedding, (4) sectioning, and (5) mounting and staining the sections.

Various techniques have been developed to prepare **tissues** for study so that they closely resemble their natural, living state. The steps involved are **fixation**, **dehydration** and **clearing**, **embedding** in a suitable medium, **sectioning** into thin slices to permit viewing by transillumination, **mounting** onto a surface for ease of handling, and **staining** so that the various tissue and cell components may be differentiated.

Fixation

Fixation refers to treatment of the tissue with chemical agents that not only retard the alterations of the tissue subsequent to death (or after removal from the body) but also maintain its normal architecture. The most common fixative agents used in light microscopy are neutral buffered **formalin** and **Bouin fluid**. Both of these substances cross-link proteins, thus preventing them from altering their position, therefore maintaining a life-like image of the tissue.

Dehydration and Clearing

Because a large fraction of the tissue is composed of water, a graded series of alcohol baths, beginning with 50% alcohol and progressing in graded steps to 100% alcohol, are used to remove the water (**dehydration**). The tissue is then treated with xylene, a chemical that is miscible both with alcohol and melted paraffin. This process is known as **clearing** because the tissue becomes transparent in xylene.

SUMMARY

Microscopic study of the human body requires that small tissue samples be excised so that they may be examined by various types of microscopy. The preparation techniques that were developed ensure that the removed, dead tissues very closely resemble their natural, living state. For examination using **light microscopy**, the excised tissue has to be **fixed**, **dehydrated**, **cleared**, and **embedded** in a suitable medium (such as paraffin) so that they can be **sectioned**, **mounted** on glass slides, rehydrated, and then **stained** with various dyes so that the components of the tissue that have very similar optical densities may be differentiated from each other. After staining, the sections have to be dehydrated so that a thin glass **coverslip** may be affixed over the section. The coverslip not only protects the tissue section but also is necessary for viewing the section with a microscope. Additional types of microscopy in common use include **phase contrast** and **confocal microscopy**, as well as **transmission** and **scanning electron microscopy**.

KEY WORDS

Histology; tissues; light microscopy; fixation; dehydration; clearing; embedding; sectioning; histochemistry; immunocytochemistry; autoradiography; confocal microscopy; transmission electron microscopy; freeze-fracture; scanning electron microscopy

Embedding

In order to distinguish the overlapping cells in a tissue and the extracellular matrix from one another, the histologist must **embed** the tissues in a proper medium and then slice them into thin sections. For light microscopy, the usual embedding medium is paraffin. The tissue is placed in a suitable container of melted paraffin until it is completely **infiltrated** by that waxy substance. Once the tissue is infiltrated, it is placed into a small receptacle, covered with melted paraffin, and allowed to harden, forming a paraffin block containing the tissue.

Sectioning

After the blocks of tissue are trimmed of excess embedding material, they are mounted for **sectioning**. The task of sectioning, in which thin slices are removed from the block, is performed by a microtome, a machine equipped with a blade and an arm that advances the tissue block in specific equal increments. For light microscopy, the thickness of each section is about 5 to 10 µm, and each section or a series of sections is mounted (placed) on glass slides.

Sectioning can also be performed on specimens frozen either in liquid nitrogen or on the rapid-freeze bar of a cryostat. These sections are mounted by the use of a quick-freezing mounting medium and sectioned at subzero temperatures by means of a precooled steel blade. The sections are placed on precooled glass slides, permitted to come to room temperature, and stained with specific dyes (or treated for histochemical or immunocytochemical studies).

Mounting and Staining

Paraffin sections are mounted (placed) on glass slides and then stained by water-soluble stains that permit differentiation of the various cellular components.

The sections for conventional light microscopy, cut by stainless steel blades, are **mounted** on adhesive-coated glass slides. Because many tissue constituents have approximately the same optical densities, they must be stained for light microscopy. **Staining** for light microscopy is performed mostly with water-soluble stains. Therefore, the paraffin must first be removed from the mounted sections, after which the tissue is rehydrated and stained. After staining, the section is again dehydrated so that the coverslip may be permanently affixed by the use of a suitable mounting medium. The coverslip not only protects the tissue from damage but also is necessary for viewing the section with the microscope.

Although various types of stains have been developed for visualization of the many components of cells and tissues, they may be grouped into three classes, as follows:

- Stains that differentiate between acidic and basic components of the cell
- Specialized stains that differentiate the fibrous components of the extracellular matrix
- Metallic salts that precipitate on tissues, forming metal deposits on them

The most commonly used stains in histology are **hematoxylin and eosin**. Hematoxylin is a base that preferentially colors the acidic components of the cell blue. Because the most acidic components are deoxyribonucleic acid (DNA) and ribonucleic acid, the nucleus and regions of the cytoplasm rich in ribosomes stain dark blue; these components are referred to as ***basophilic***. Eosin is an acid that dyes the basic components of the cell a pinkish color. Because many cytoplasmic constituents have a basic pH, regions of the cytoplasm stain pink; these elements are said to be **acidophilic**. Many other stains are also used in preparation of specimens for histological study (Table 1–1).

Molecules of some stains, such as **toluidine blue**, polymerize with each other when exposed to high concentrations of polyanions in tissue. These aggregates are of a color differing from that of their individual molecules. For example, toluidine blue stains tissues blue, except for those that are rich in polyanions (e.g., cartilage matrix, granules of mast cells), which are stained purple. A tissue or cell component that stains purple with this stain is said to be **metachromatic**, and

TABLE 1–1 Common Histological Stains and Reactions

Reagent	Result
Hematoxylin	*Blue:* nucleus, acidic regions of the cytoplasm, cartilage matrix
Eosin	*Pink:* basic regions of the cytoplasm, collagen fibers
Masson trichrome	*Dark blue:* nuclei *Red:* muscle, keratin, cytoplasm *Light blue:* mucinogen, collagen
Weigert elastic stain	*Blue:* elastic fibers
Silver stain	*Black:* reticular fibers
Iron hematoxylin	*Black:* striations of muscle, nuclei, erythrocytes
Periodic acid–Schiff	*Magenta:* glycogen and carbohydrate-rich molecules
Wright and Giemsa stains	Used for differential staining of blood cells *Pink:* erythrocytes, eosinophil granules *Purple:* leukocyte nuclei, basophil granules *Blue:* cytoplasm of monocytes and lymphocytes

toluidine blue is said to exhibit **metachromasia**. Examples of tissues stained with common histological stains are presented at the end of the chapter (see Figs. 1–10 to 1–16).

Light Microscope

Compound microscopes are composed of a specific arrangement of lenses that permit a high magnification and good resolution of the tissues being viewed.

The present-day light microscope uses a specific arrangement of groups of lenses to magnify an image (Fig. 1–1). As a result of the use of more than just a single lens, this instrument is known as a ***compound microscope***. The light source is an electric bulb with a tungsten filament whose light is gathered into a focused beam by the **condenser lens**.

The light beam is located below and is focused on the stained specimen. Light passing through the specimen enters one of the objective lenses; these lenses sit on a movable turret located just above the specimen. Usually, four objective lenses are available on a single turret, providing low, medium, high, and oil magnifications. Generally, in most microscopes, the first three lenses magnify 4, 10, and 40 times, respectively, and are used without oil; the oil lens magnifies the image 100 times.

The image from the objective lens is gathered and further magnified by the ocular lens of the eyepiece. This lens usually magnifies the image by a factor of 10—for total magnifications of 40, 100, 400, and 1000—and focuses the resulting image on the retina of the eye (or on the film of a film camera or on the sensor of a digital camera).

Focusing of the image is performed by the use of knurled knobs that move the objective lens up or down above the specimen. The coarse-focus knob moves the objective lenses in larger increments, and the fine-focus knob moves the objective lens in smaller increments. It is interesting that the image projected on the retina (or film or sensor) is reversed from right to left and is upside down.

The quality of an image depends not only on the capability of a lens to magnify but also on its **resolution**—the ability of the lens to show that two distinct objects are separated by a distance. The quality of a lens depends on how close its resolution approaches the theoretical limit of 0.25 µm, a restriction that is determined by the wavelength of visible light.

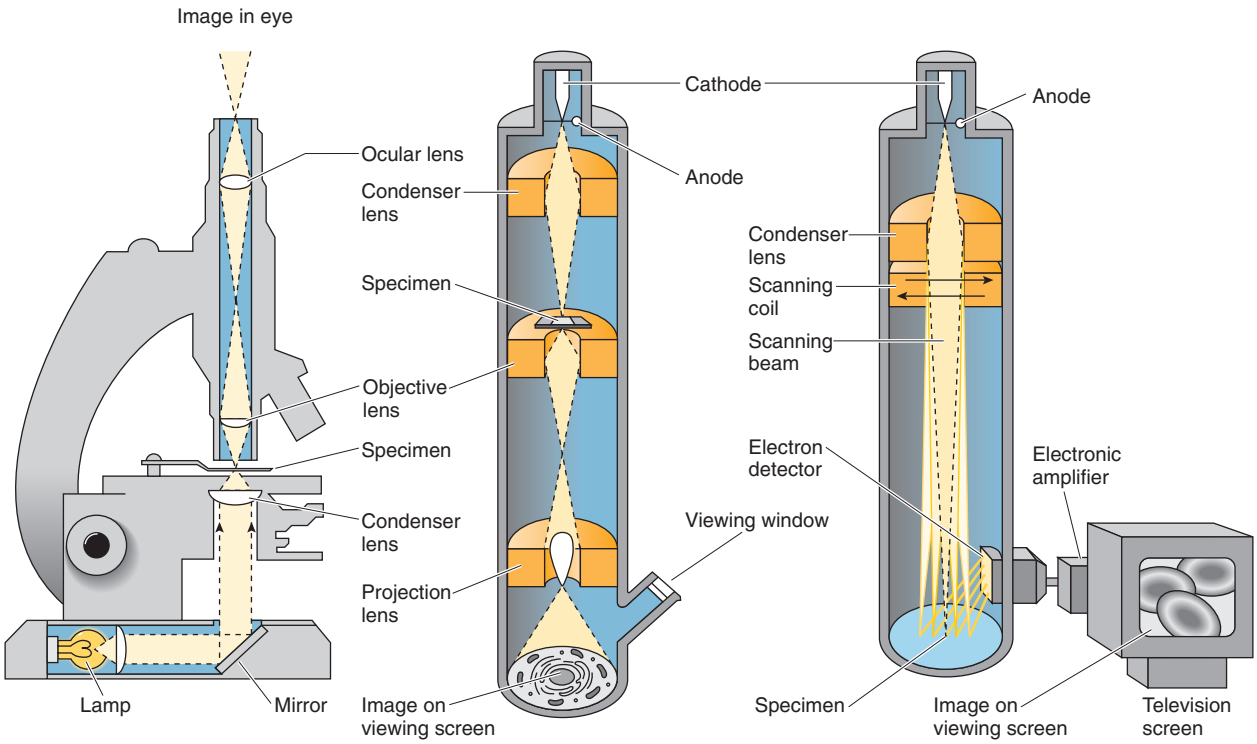


Figure 1–1 Comparison of light, transmission, and scanning electron microscopes.

There are several types of light microscopes, distinguished by the type of light used as a light source and the manner in which they use the light source. However, most students of histology are required to recognize images obtained only from compound light microscopy, transmission electron microscopy, and scanning electron microscopy; therefore, the other types of microscopy are not discussed.

Digital Imaging Techniques

Digital imaging techniques use computer technology to capture and manipulate histologic images.

The advent of computer technology has provided a means of capturing images digitally, without the use of film. Although, in its earlier years, this method of image capturing could not compete with film technology, it is now as good as, if not better than, the film technology. Additionally, digital photomicrography has many advantages that make it a valuable tool, for example:

- Immediate visualization of the acquired image
- Digital modification of the image
- The capability of enhancing the image by the use of commercially available software

In addition, because these images are stored in a digital format, hundreds or thousands of them may be archived on a single CD-ROM/DVD disk and/or removable disk technology, and their retrieval is almost instantaneous. Finally, their digital format permits the electronic transmission of these images by distribution via the Internet.

Interpretation of Microscopic Sections

One of the most difficult, frustrating, and time-consuming skills needed in histology is to learn how to interpret what a two-dimensional section looks like in three dimensions. If one imagines a garden hose coiled as in Figure 1–2 and then takes the indicated thin sections from that hose, it becomes clear that the three-dimensional object is not necessarily discerned from

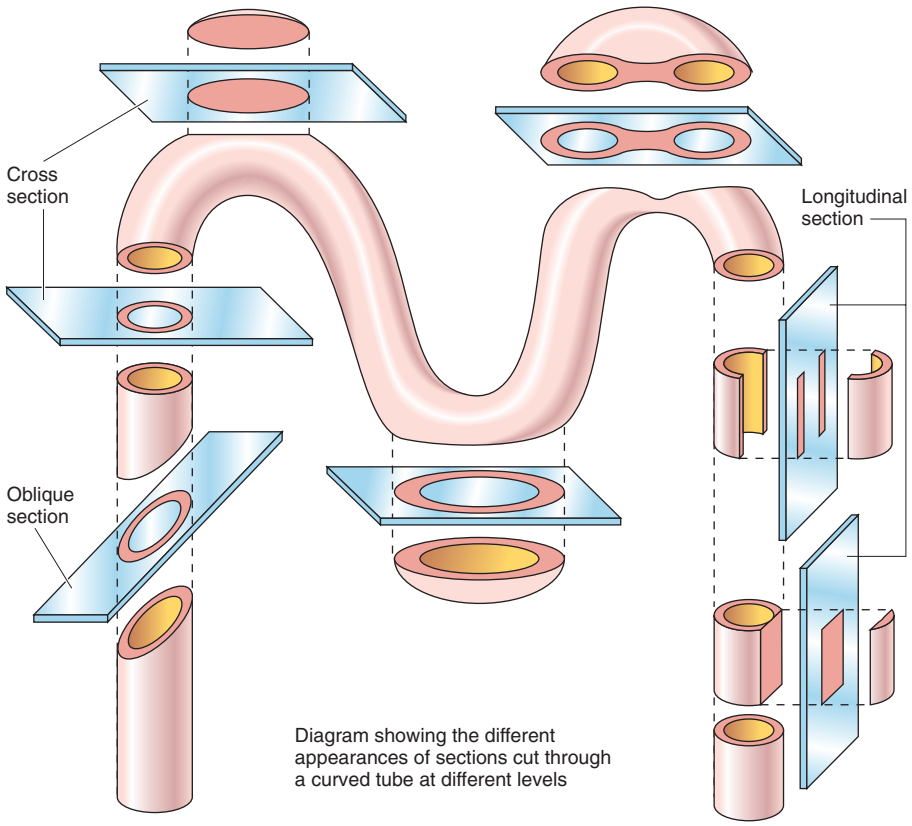


Figure 1–2 Histology requires a mental reconstruction of two-dimensional images into the three-dimensional solid from which they were sectioned. In this diagram, a curved tube is sectioned in various planes to illustrate the relationship between a series of two-dimensional sections and the three-dimensional structure.

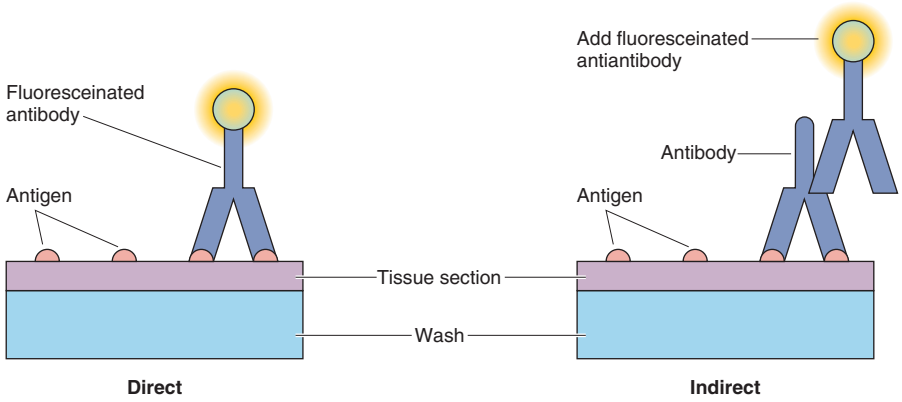


Figure 1–3 Direct and indirect methods of immunocytochemistry. *Left,* An antibody against the antigen was labeled with a fluorescent dye and viewed with a fluorescent microscope. The fluorescence occurs only over the location of the antibody. *Right,* Fluorescent-labeled antibodies are prepared against an antibody that reacts with a particular antigen. When viewed with fluorescent microscopy, the region of fluorescence represents the location of the antibody.

any *one* of the two-dimensional depictions. However, by viewing all of the sections drawn from the coiled tube, one can mentally reconstruct the correct three-dimensional image.

Advanced Visualization Procedures

Histochemistry

Histochemistry is a method of staining tissue that provides information concerning the presence and location of intracellular and extracellular macromolecules.

Specific chemical constituents of tissues and cells can be localized by the method of **histochemistry** and **cytochemistry**. These methods capitalize on the enzyme activity, chemical reactivity, or other physicochemical phenomena associated with the constituent of interest. Reactions of interest are monitored by the formation of an insoluble precipitate that takes on a certain color. Frequently, histochemistry is performed on frozen tissues and can be applied to both light and electron microscopy.

A common histochemical reaction uses the periodic acid–Schiff (PAS) reagent, which forms a magenta precipitate with molecules rich in glycogen and carbohydrate. To ensure that the reaction is specific for glycogen, consecutive sections are treated with amylase. Thus, sections not treated with amylase display a magenta deposit, whereas amylase-treated sections display a lack of staining in the same region.

Although enzymes can be localized by histochemical procedures, the product of enzymatic reaction, rather than the enzyme itself, is visualized. The reagent is designed so that the product precipitates at the site of the reaction and is visible either as a metallic or colored deposit.

Immunocytochemistry

Immunocytochemistry uses fluoresceinated antibodies and antiantibodies to provide more precise intracellular and extracellular localization of macromolecules than is possible with histochemistry.

Although histochemical procedures permit relatively good localization of some enzymes and macromolecules in cells and tissues, more precise localization can be achieved by the use of **immunocytochemistry.** This procedure requires the development of an antibody against the particular macromolecule to be localized and labeling the antibody with a fluorescent dye such as fluorescein or rhodamine.

There are two common methods of antibody labeling: **direct** and **indirect**. In the direct method (Fig. 1–3), the antibody against the macromolecule is labeled with a fluorescent dye. The antibody is then permitted to react with the macromolecule, and the resultant complex may be viewed with a fluorescent microscope (Fig. 1–4).

In the indirect method (see Fig. 1–3), a fluorescent-labeled antibody is prepared against the primary antibody specific for the macromolecule of interest. Once the primary antibody has reacted with the antigen, the preparation is washed to remove unbound primary antibody; the labeled antibody is then added and reacts with the original antigen-antibody complex, forming a secondary complex visible by fluorescent microscopy (Fig. 1–5). The indirect method is more sensitive than the direct method because numerous labeled antiantibodies bind to the primary antibody, making them easier to visualize. In addition, the indirect method does not require labeling of the primary antibody, which often is available in limited quantities only.

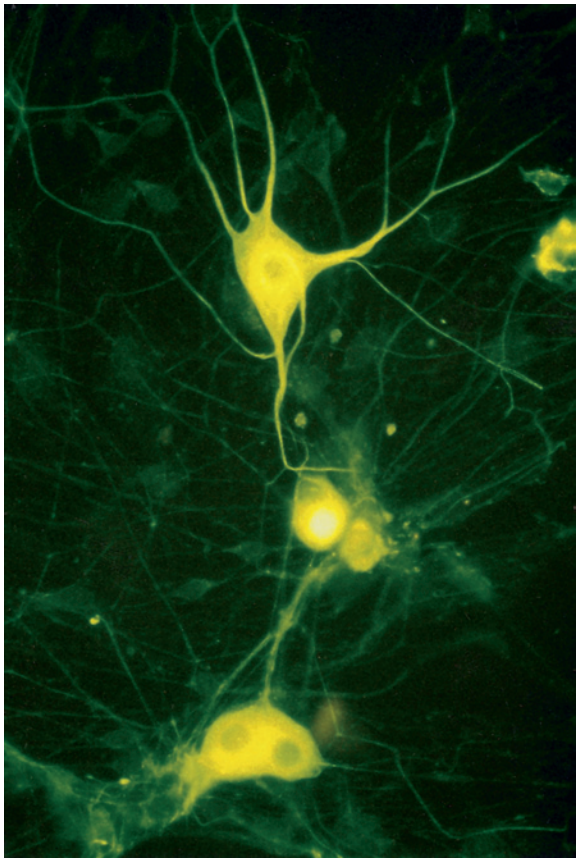


Figure 1–4 Example of direct immunocytochemistry. Cultured neurons from rat superior cervical ganglion were immunostained with fluorescent-labeled antibody specific for the insulin receptor. The bright areas correspond to sites where the antibody has bound to insulin receptors. The staining pattern indicates that receptors are located throughout the cytoplasm of the soma and processes but are missing from the nucleus. (From James S, Patel N, Thomas P, Burnstock G. Immunocytochemical localisation of insulin receptors on rat superior cervical ganglion neurons in dissociated cell culture. *J Anat.* 1993;182:95-100.)

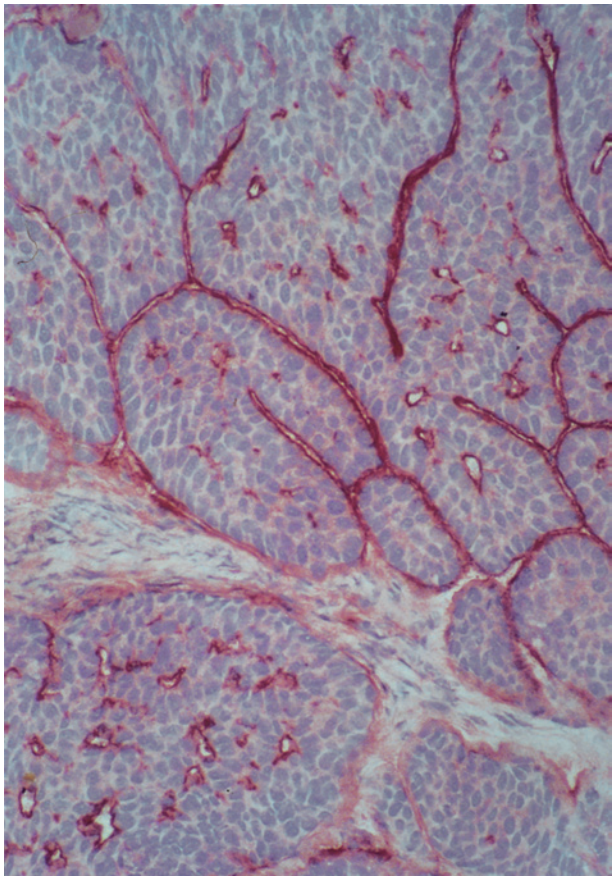


Figure 1–5 Indirect immunocytochemistry. Fluorescent antibodies were prepared against primary antibodies against type IV collagen, to demonstrate the presence of a continuous basal lamina at the interface between malignant clusters of cells and the surrounding connective tissue. (From Kopf-Maier P, Schroter-Kermani C. Distribution of type VII collagen in xenografted human carcinomas. *Cell Tissue Res.* 1993;272:395-405.)

Immunocytochemistry can be used with specimens for electron microscopy by labeling the antibody with ferritin, an electron-dense molecule, instead of with a fluorescent dye. Ferritin labeling can be applied to both the direct and indirect methods.

Autoradiography

Autoradiography is a method that uses the incorporation of radioactive isotopes into macromolecules, which are then visualized by the use of an overlay of film emulsion.

Autoradiography (radioautography) is a particularly useful method for localizing and investigating a specific temporal sequence of events. The method requires the incorporation of a radioactive isotope—most commonly tritium (^{3}H)—into the compound being studied (Fig. 1–6). An example is the use of tritiated amino acids to follow the synthesis and packaging of proteins. After the radiolabeled compound is injected into an animal, tissue specimens are taken at selected time intervals. The tissue is processed as usual and placed on a glass slide; however, instead of the tissue being sealed with a coverslip, a thin layer of photographic emulsion is placed over it. The tissue is placed in a dark box for a few days or weeks, during which time particles emitted from the radioactive isotope expose the emulsion over the cell sites where the isotope is located. The emulsion

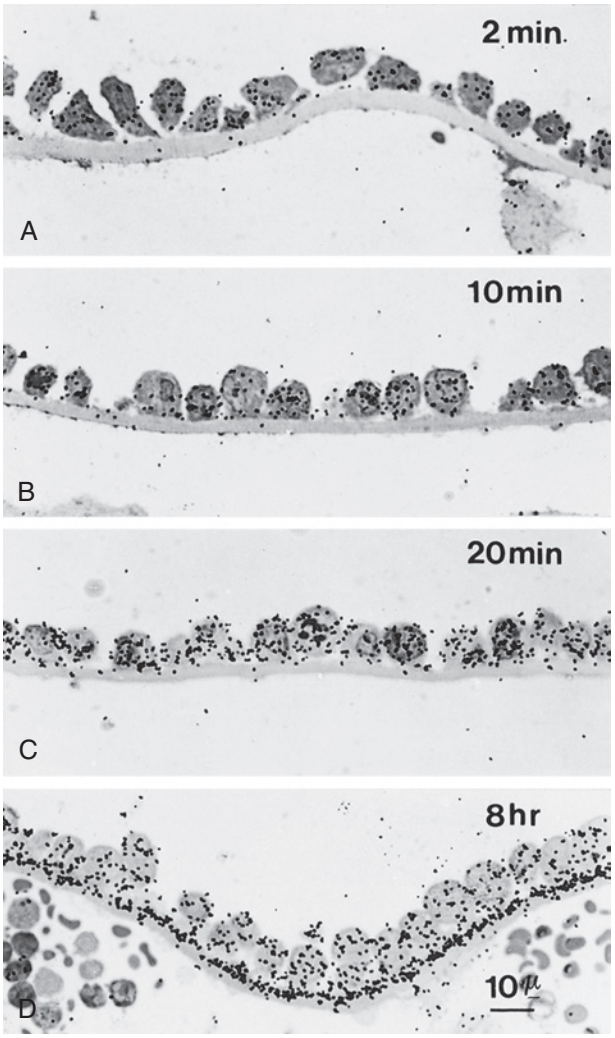


Figure 1–6 Autoradiography. Light microscopic examination of tritiated proline incorporation into the basement membrane as a function of time subsequent to tritiated proline injection. In photomicrographs **A** to **C**, the silver grains *(black dots)* are localized mostly in the endodermal cells; after 8 hours **(D),** however, the silver grains are also localized in the basement membrane. The presence of silver grains indicates the location of tritiated proline. (From Mazariegos MR, Leblond CP, van der Rest M. Radioautographic tracing of ^{3}H-proline in endodermal cells of the parietal yolk sac as an indicator of the biogenesis of basement membrane components. *Am J Anat.* 1987;179:79-93. Reprinted by permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.)

is developed and fixed by means of photographic techniques, and small silver grains are left over the exposed portions of the emulsion. The specimen then is sealed with a coverslip and viewed with a light microscope. The silver grains are positioned over the regions of the specimen that incorporated the radioactive compound.

Autoradiography has been used to follow the time course of incorporation of tritiated proline into the basement membrane underlying endodermal cells of the yolk sac (see Fig. 1–6). An adaptation of the autoradiography method of electron microscopy has been used to show that the tritiated proline first appears in the cytosol of the endodermal cells, then travels to the rough endoplasmic reticulum, then to the Golgi apparatus, then into vesicles, and finally into the extracellular matrix (Fig. 1–7). In this manner, the sequence of events leading to the synthesis of type IV collagen—the main protein in the lamina densa of the basal lamina—was visually demonstrated.

Confocal Microscopy

Confocal microscopy relies on a laser beam for the light source and a pinhole screen to eliminate undesirable reflected light from being observed. Thus, the only light that can be observed is that which is located at the focal point of the objective lens, making the pinhole conjugate of the focal point.

In **confocal microscopy**, a laser beam passes through a dichroic mirror to be focused on the specimen by two motorized mirrors whose movements are computer controlled to scan the beam along the sample. Because the sample is treated by fluorescent dyes, the impinging laser beam causes the emission of light from the dyes. The emitted light follows the same path taken by the laser beam, but in the opposite direction, and the dichroic mirror focuses this emitted light on a pinhole in a plate. A photomultiplier tube collects the emitted light passing through the pinhole while the plate containing the pinhole blocks all of the extraneous light that would create a fuzzy image. It must be remembered that the light emerging from the pinhole at any particular moment in time represents a single point in the sample, and, as the laser beam scans across the sample, additional individual points are collected by the photomultiplier tube. All of these points gathered by the photomultiplier tube are then compiled by a computer, forming a composite image one pixel at a time. Because the depth of field is very small (i.e., only a thin layer of the sample is observed at any one scan), the scanning may be repeated at deeper and deeper levels in the sample, providing a capability of compiling a very good three-dimensional image (Fig. 1–8).

Electron Microscopy

The use of electrons as a light source in electron microscopy permits the achievement of much greater magnification and resolution than that realized by light microscopy.

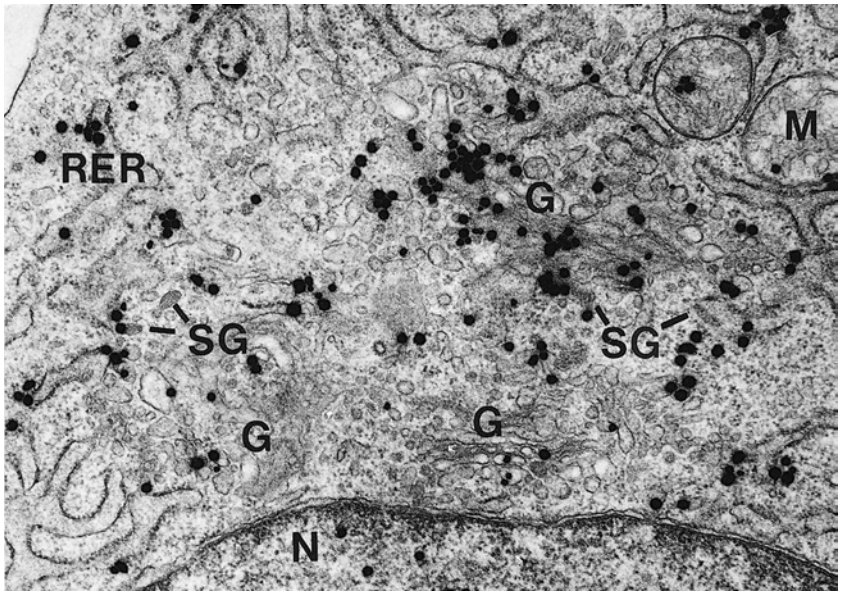


Figure 1–7 Autoradiography. In this electron micrograph of a yolk sac endodermal cell, silver grains (similar to those in Figure 1–6), representing the presence of tritiated proline, are evident overlying the rough endoplasmic reticulum (RER), Golgi apparatus (G), and secretory granules (SG). Type IV collagen, which is rich in proline, is synthesized in endodermal cells and released into the basement membrane. The tritiated proline is most concentrated in organelles involved in protein synthesis. M, Mitochondria; N, nucleus. (From Mazariegos MR, Leblond CP, van der Rest M. Radioautographic tracing of ^{3}H-proline in endodermal cells of the parietal yolk sac as an indicator of the biogenesis of basement membrane components. *Am J Anat.* 1987;179:79-93. Reprinted by permission of Wiley-Liss Inc, a subsidiary of John Wiley & Sons, Inc.)

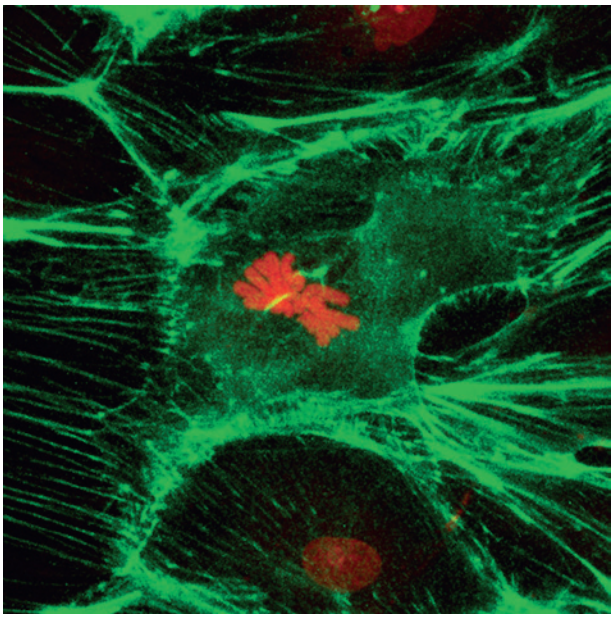


Figure 1–8 Confocal image of a metaphase rat kangaroo cell (PtK2) stained with FITC-phalloidin for F-actin *(green)* and propidium iodide for chromosomes *(red).* (Courtesy Dr. Matthew Schibler, UCLA Brain Research Institute.)

In light microscopes, optical lenses focus visible light (a beam of photons). In electron microscopes, electromagnets serve the function of focusing a beam of electrons. Because the wavelength of an electron beam is much shorter than that of visible light, electron microscopes theoretically are capable of resolving two objects separated by 0.005 nm. In practice, however, the resolution of the **transmission electron microscope** (TEM) is about 0.2 nm, still more than a thousand-fold greater than the resolution of the compound light microscope. The resolution of the **scanning electron microscope** (SEM) is about 10 nm, considerably less than that of TEMs. Moreover, modern electron microscopes can magnify an object as much as 150,000 times; this magnification is powerful enough to see individual macromolecules such as DNA and myosin.

Transmission Electron Microscopy

Transmission electron microscopy uses much thinner sections compared with light microscopy and requires heavy metal precipitation techniques rather than water-soluble stains to stain tissues.

Preparation of tissue specimens for transmission electron microscopy involves the same initial basic steps as in light microscopy. Special fixatives had to be developed for use with transmission light microscopy because the greater resolving power of the electron microscope requires much smaller end products and more specific cross-linking of proteins. These fixatives, which include buffered solutions of **glutaraldehyde, paraformaldehyde, osmium tetroxide**, and **potassium permanganate**, not only preserve fine structural details but also act as electron-dense stains, which permit observation of the tissue with the electron beam.

Because the ability of these fixatives to penetrate fresh tissues is much less than those for light microscopy, relatively small pieces of tissues have to be infiltrated in large volumes of fixatives. Tissue blocks for transmission electron microscopy are usually no larger than 1 mm^3. Suitable embedding media have been developed, such as epoxy resin, so that plastic-embedded tissues may be cut into extremely thin (ultrathin) sections (25 to 100 nm) that absorb only a small fraction of the impinging beam of electrons.

Electron beams are produced in an evacuated chamber by heating a tungsten filament, the **cathode**. The electrons then are attracted to the positively charged **anode**, a donut-shaped metal plate with a central hole. With a charge differential of about 60,000 volts placed between the cathode and the anode, the electrons that pass through the hole in the anode have high kinetic energy.

The electron beam is focused on the specimen by the use of electromagnets, which are analogous to the condenser lens of a light microscope (see Fig. 1–1). Because the tissue is stained with heavy metals that precipitate preferentially on lipid membranes, the electrons lose some of their kinetic energy as they interact with the tissue. The heavier the metal concentration that is encountered by an electron, the less energy the electron will retain.

The electrons leaving the specimen are subjected to the electromagnetic fields of several additional electromagnets, which focus the beam on a fluorescent plate. As the electrons hit the fluorescent plate, their kinetic energy is converted into points of light, whose intensity is a direct function of the electron's kinetic energy. One may make a permanent record of the resultant image by substituting an electron-sensitive film in place of the fluorescent plate and by producing a negative from which a black-and-white photomicrograph can be printed. Recently, digital electron microscopy has been introduced in which the photographic film is replaced by charge-coupled device technology to capture the image produced by the electrons.

Scanning Electron Microscopy

Scanning electron microscopy provides a three-dimensional image of the specimen.

Unlike transmission electron microscopy, scanning electron microscopy is used to view the surface of a solid specimen. Using this technique, one can view a three-dimensional image of the object. Usually, the object to be viewed is prepared in a special manner that permits a thin layer of heavy metal, such as gold or palladium, to be deposited on the specimen's surface.

As a beam of electrons scans the surface of the object, some (backscatter electrons) are reflected and others (secondary electrons) are ejected from the heavy metal coat. The backscatter and secondary electrons are captured by electron detectors. The array of electrons is then interpreted, collated, and displayed on a monitor as a three-dimensional image (see Fig. 1–1). One may make the image permanent either by photographing it or digitizing it for storage in a computer.

Freeze-Fracture Technique

The macromolecular structure of the internal aspects of membranes is revealed by the method of **freeze-fracture** (Fig. 1–9). Quick-frozen specimens that have been treated with cryopreservatives do not develop ice crystals during the freezing process; hence, the tissue does not suffer mechanical damage. As the frozen specimen is hit by a supercooled razor blade, the specimen fractures along cleavage planes, which are regions of least molecular bonding; in cells,

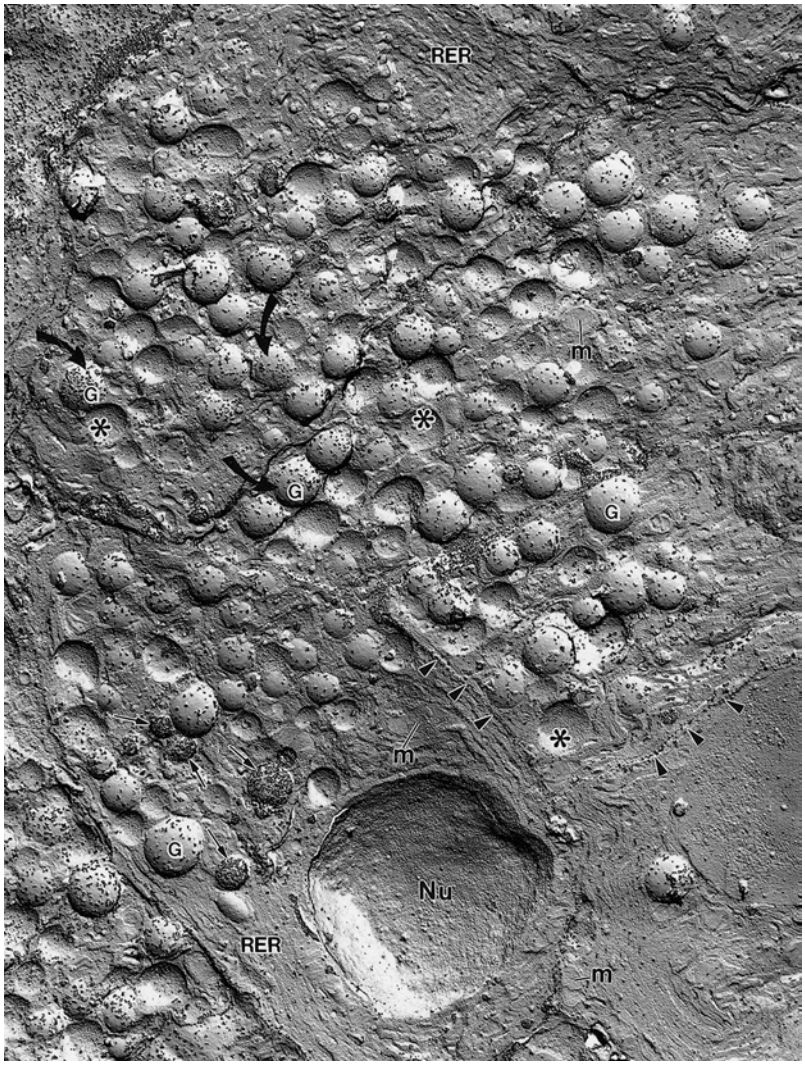


Figure 1–9 Cytochemistry and freeze etching. Fracture-label replica of an acinar cell of the rat pancreas. *N*-acetyl-*d*-galactosamine residues were localized by the use of *Helix pomatia* lectin-gold complex, which appears as black dots in the image. The nucleus (Nu) appears as a depression, the rough endoplasmic reticulum (RER) as parallel lines, and secretory granules (G) as small elevations or depressions. The elevations (lG) represent the E-face half, and the depressions *(asterisk)* represent the P-face of the membrane of the secretory granule. (From Kan FWK, Bendayan M. Topographical and planar distribution of *Helix pomatia* lectin-binding glycoconjugates in secretory granules and plasma membrane of pancreatic acinar cells of the rat: demonstration of membrane heterogeneity. *Am J Anat.* 1989;185:165-176. Reprinted by permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.)

fracture frequently occurs between the inner and outer leaflets of membranes.

The fracture face is coated at an angle by evaporated platinum and carbon, forming accumulations of platinum on one side of a projection and no accumulation on the opposite side next to the projection, thus generating a replica of the surface. The tissue is then digested away, and the replica is examined by transmission electron microscopy. This method enables the transmembrane proteins of cellular membranes to be displayed (Fig. 1–10).

Figures 1–11 to 1–16 show examples of tissues stained with common histological stains.

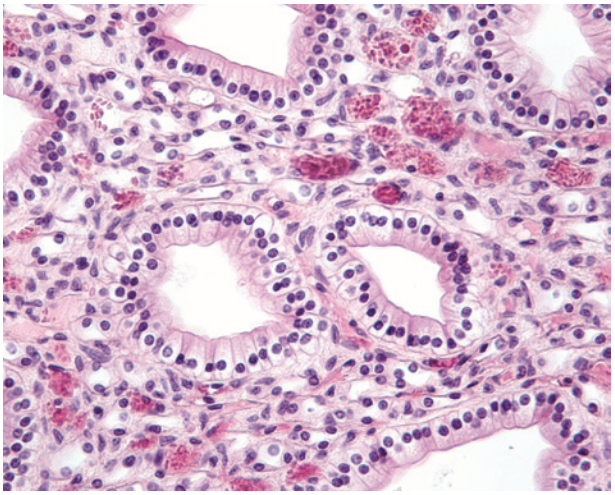


Figure 1–10 Hematoxylin and eosin are the most commonly used stains. Hematoxylin stains acids blue. Nuclei are rich in deoxyribonucleic acids, and, consequently, they stain blue. Basic regions of the cytoplasm stain pinkish-red with eosin.

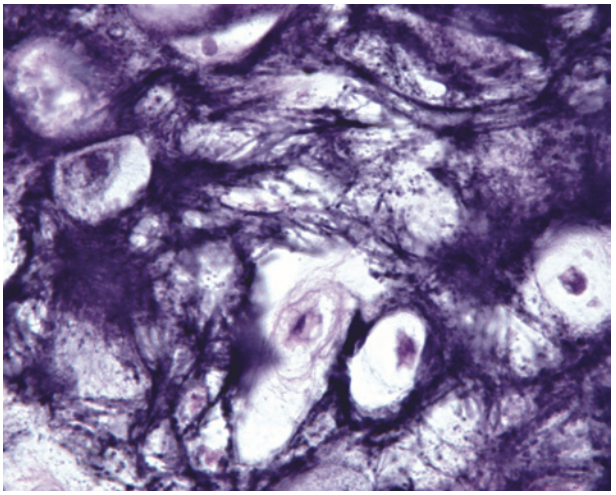


Figure 1–12 Weigert's elastic stain stains elastic fibers blue.

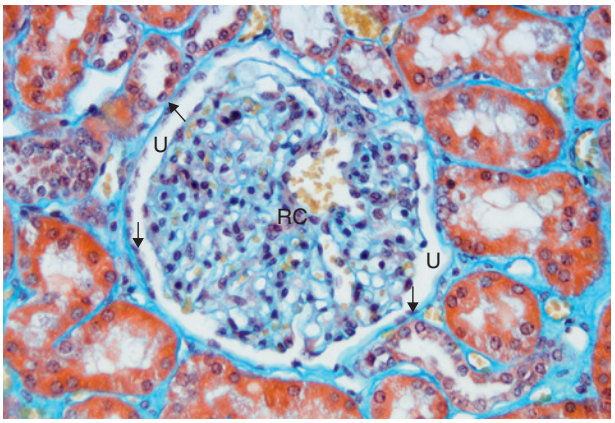


Figure 1–11 Masson-trichrome stains nuclei dark blue, collagen light blue, and cytoplasm pink to red. (From Standring S. *Gray's Anatomy.* 40th ed. Elsevier; 2008.)

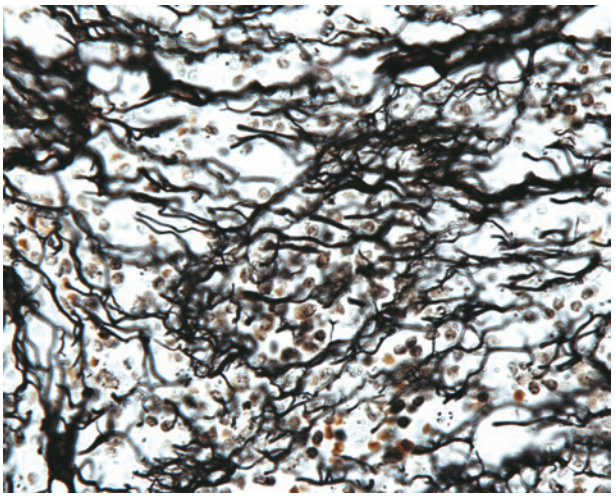


Figure 1–13 Silver stain stains reticular fibers (type III collagen fibers) black.

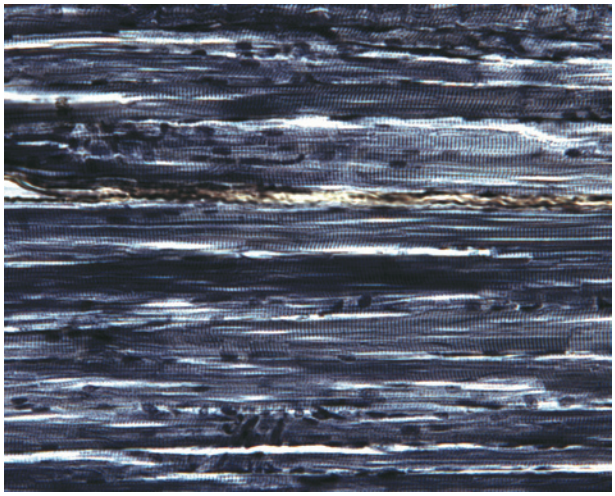


Figure 1–14 Iron hematoxylin stains cross striations and nuclei of striated muscle cells as well as red blood cells black.

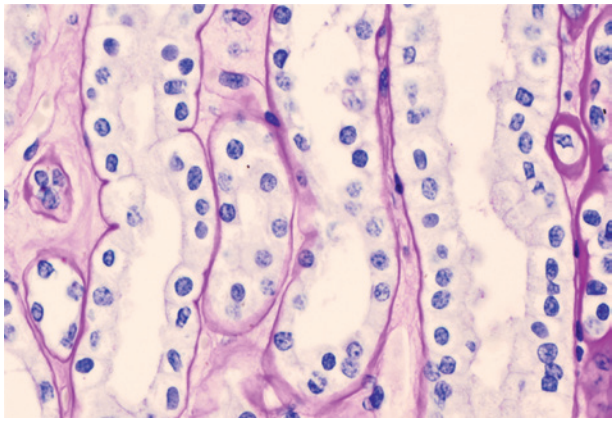


Figure 1–15 Periodic acid–Schiff reagent stains glycogen and carbohydrate-rich molecules a magenta color. (From Standring S. *Gray's Anatomy.* 40th ed. Elsevier; 2008.)

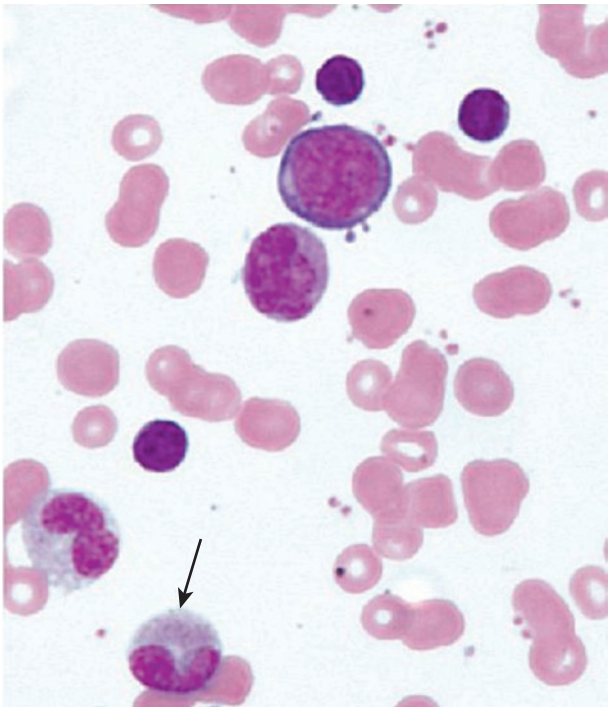


Figure 1–16 Wright and Giemsa stains are used for differential staining of blood cells. Erythrocytes and eosinophilic granules stain pink, white blood cell nuclei *(arrow)* and basophilic granules stain purple, and monocyte and lymphocyte cytoplasm stain blue.

Cytoplasm

2

Cells are the basic functional units of complex organisms. Cells that are related or are similar to each other as well as cells that function in a particular manner or serve a common purpose are grouped together to form tissues. Four basic tissues are recognized in mammals (epithelium, connective tissues, muscle, and nervous tissue), and they are assembled to form organs, which, in turn, are collected into the various organ systems of the body. The task of each organ system is specific, in that it performs a collection of associated functions, such as digestion, reproduction, respiration, and so on.

Although the human body is composed of more than 200 different types of cells, each performing a different function, all cells possess certain unifying characteristics and thus can be described in general terms. Every cell is surrounded by a bilipid plasma membrane, possesses organelles that permit it to discharge its functions, synthesizes macromolecules for its own use or for export, produces energy, and is capable of communicating with other cells (Figs. 2–1 to 2–4). The number and disposition of the organelles vary not only with the cell in question but also with the particular stage in the life cycle of that cell.

Protoplasm, the living substance of the cell, is subdivided into two compartments: the **cytoplasm**, extending from the plasma membrane to the nuclear envelope, and the **karyoplasm**, the material forming the contents of the nucleus. The cytoplasm is detailed in this chapter; the nucleus is discussed in Chapter 3.

The bulk of the cytoplasm is **water**, in which various inorganic and organic chemicals are dissolved and/or suspended. This fluid suspension is called the ***cytosol*** (**intracellular fluid**) and is that portion of the cytoplasm that is left after all organelles and solid materials are removed from the cytoplasm. The cytosol contains **organelles**, metabolically active structures that perform distinctive functions (Figs. 2–5 and 2–6), and solid materials. The shapes of cells, their ability to move, and the intracellular pathways within cells are maintained by a system of tubules and filaments known as the ***cytoskeleton***.

Finally, the cytoplasm contains **inclusions**, which consist of metabolic by-products, storage forms of various nutrients, or inert crystals and pigments. The following topics discuss the structure and functions of the major constituents of organelles, the cytoskeleton, and inclusions.

ORGANELLES

Organelles are metabolically active cellular structures that execute specific functions.

Although some organelles were discovered by light microscopists, their structure and function were not elucidated until the advent of electron microscopy, separation techniques, and sensitive biochemical and histochemical procedures. As a result of the application of these methods, it is now known that the membranes of organelles are composed of a phospholipid bilayer, which not only partitions the cell into compartments but also provides large surface areas for the biochemical reactions essential for the maintenance of life.

Cell Membrane

The cell membrane forms a selectively permeable barrier between the cytoplasm and the external milieu.

Each cell is bounded by a **cell membrane** (also known as the ***plasma membrane*** or ***plasmalemma***) that functions in

- Maintaining the structural integrity of the cell
- Controlling movements of substances in and out of the cell (selective permeability)
- Regulating cell–cell interactions
- Recognizing via receptors, antigens, and foreign cells as well as altered cells
- Acting as an interface between the cytoplasm and the external milieu
- Establishing transport systems for specific molecules
- Sustaining a potential difference between the intracellular and extracellular aspects of the membrane
- Transducing extracellular physical or chemical signals into intracellular events

Cell membranes are not visible with the light microscope. In electron micrographs, the plasmalemma is about 7.5 nm thick and appears as a trilaminar structure of two thin, dense lines with an intervening light area. Each layer is about 2.5 nm in width, and the entire structure is known as the **unit membrane** (Fig. 2–7).

SUMMARY

Protoplasm, the living substance of the cell, is subdivided into two compartments: the **cytoplasm**, extending from the plasma membrane to the nuclear envelope, and the karyoplasm that forms the contents of the nucleus. The bulk of the cytoplasm is **water**, in which various inorganic and organic chemicals are dissolved and/or suspended. This fluid suspension is called the ***cytosol*** (**intracellular fluid**) and is that portion of the cytoplasm that is left after all organelles and solid materials are removed from the cytoplasm. The cytosol contains **organelles**, metabolically active structures that perform distinctive functions and **inclusions,** nonliving metabolic by-products, storage forms of various nutrients, or inert crystals and pigments. The shape of cells, their ability to move, and the intracellular pathways within cells are maintained by a system of tubules and filaments known as the ***cytoskeleton***.

KEY WORDS

Centrioles; cytoplasm; cytoskeleton; cytosol; endoplasmic reticulum; endosomes; Golgi apparatus; inclusions; lysosomes; mitochondria; organelles; plasmalemma; proteasomes; ribosomes

Figure 2–1 Light photomicrograph of typical cells from the renal cortex of a monkey. Note the blue nucleus (N) and the pink cytoplasm. The boundaries (CM) of individual cells may be easily distinguished. The white area in the middle of the field is the lumen (L) of a collecting tubule. (×975)

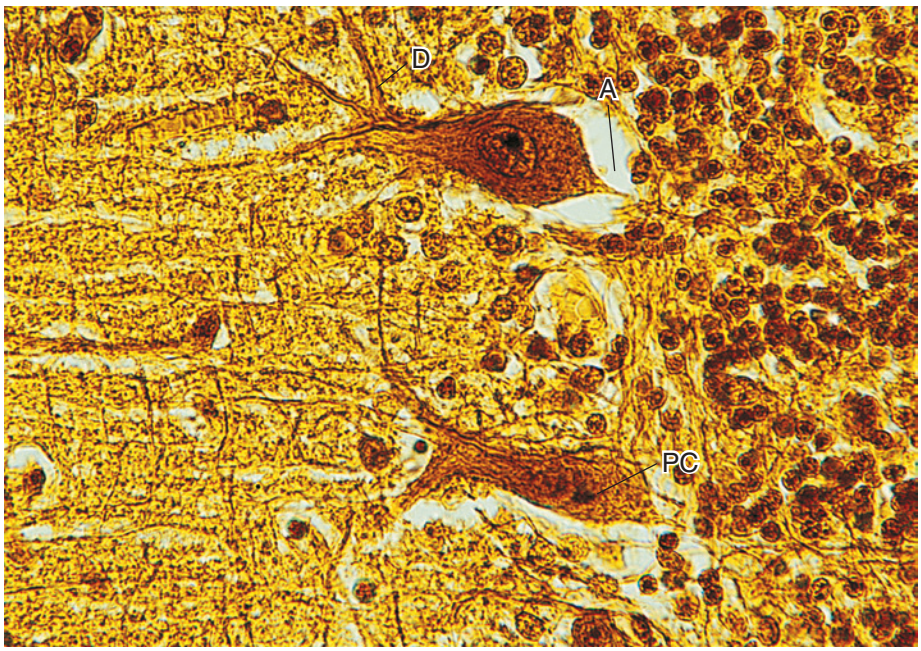


Figure 2–2 Purkinje cells (PC) from the cerebellum of a monkey. Observe the long, branching processes, dendrites (D) and axon (A) of these cells. The nucleus is located in the widest portion of the cell. (×540)

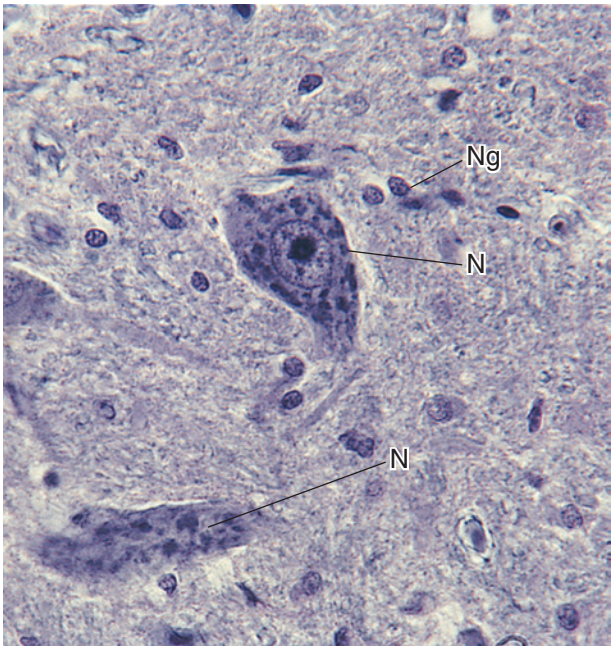


Figure 2–3 Motor neurons from the human spinal cord. These nerve cells have numerous processes (axons and dendrites). The centrally placed nucleus and the single large nucleolus are clearly visible. The Nissl bodies (N) (rough endoplasmic reticulum) are the most conspicuous features of the cytoplasm. Observe also the small nuclei of the neuroglia cells (Ng). (×540)

The inner (cytoplasmic) dense line is its **inner leaflet**; the outer dense line is its **outer leaflet**.

Molecular Composition

The plasmalemma is composed of a phospholipid bilayer and associated integral and peripheral proteins.

Each leaflet is composed of a single layer of **phospholipids** and associated **proteins**, usually in a 1:1 proportion by weight. In certain cases, such as myelin sheaths, however, the lipid component outweighs the protein component by a ratio of 4:1. The two leaflets, composing a **lipid bilayer** in which **proteins** are suspended, constitute the basic structure of all membranes of the cell (Fig. 2–8). Although as viewed by the electron microscope the two leaflets appear indistinguishable from each other, it is known that their phospholipid compositions are different and, therefore, the two leaflets are said to be asymmetrical.

Each **phospholipid molecule** of the lipid bilayer is composed of a **polar head**, located at the surface of the membrane, and two long **nonpolar** fatty acyl tails, usually consisting of chains of 16 to 18 carbon atoms, projecting into the center of the plasmalemma (see Fig. 2–8). The nonpolar fatty acyl tails of the two layers face each other within the membrane and form weak noncovalent bonds with each other, holding the bilayer together. Because the phospholipid molecule is

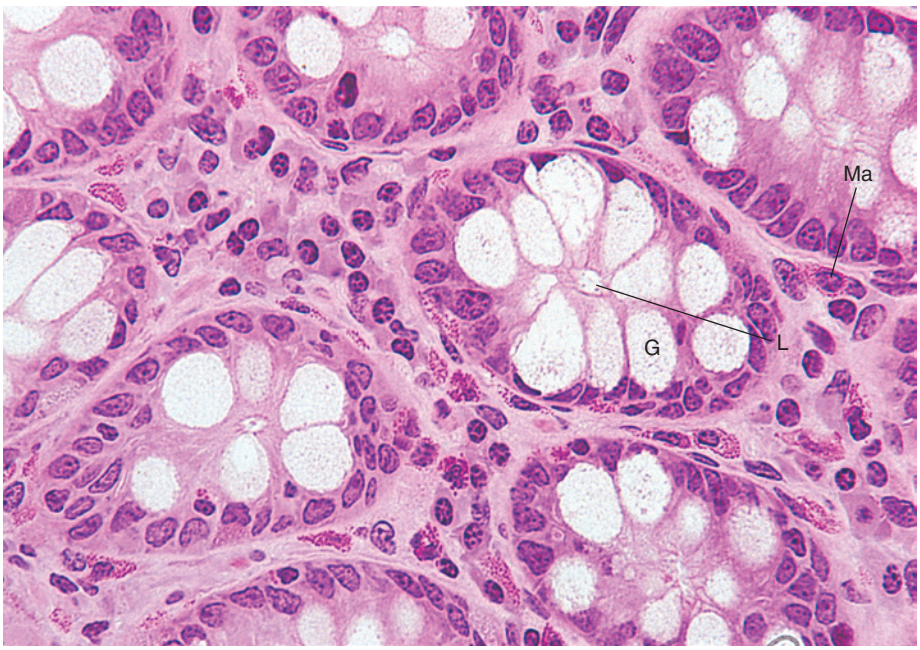


Figure 2–4 Goblet cells (G) from the monkey colon. Some cells, such as goblet cells, specialize in secreting materials. These cells accumulate mucinogen, which occupies much of the cell's volume, and then release it into the lumen (L) of the intestine. During the processing of the tissue, the mucinogen is extracted, leaving behind empty spaces. Observe the presence of a mast cell (Ma). (×540)

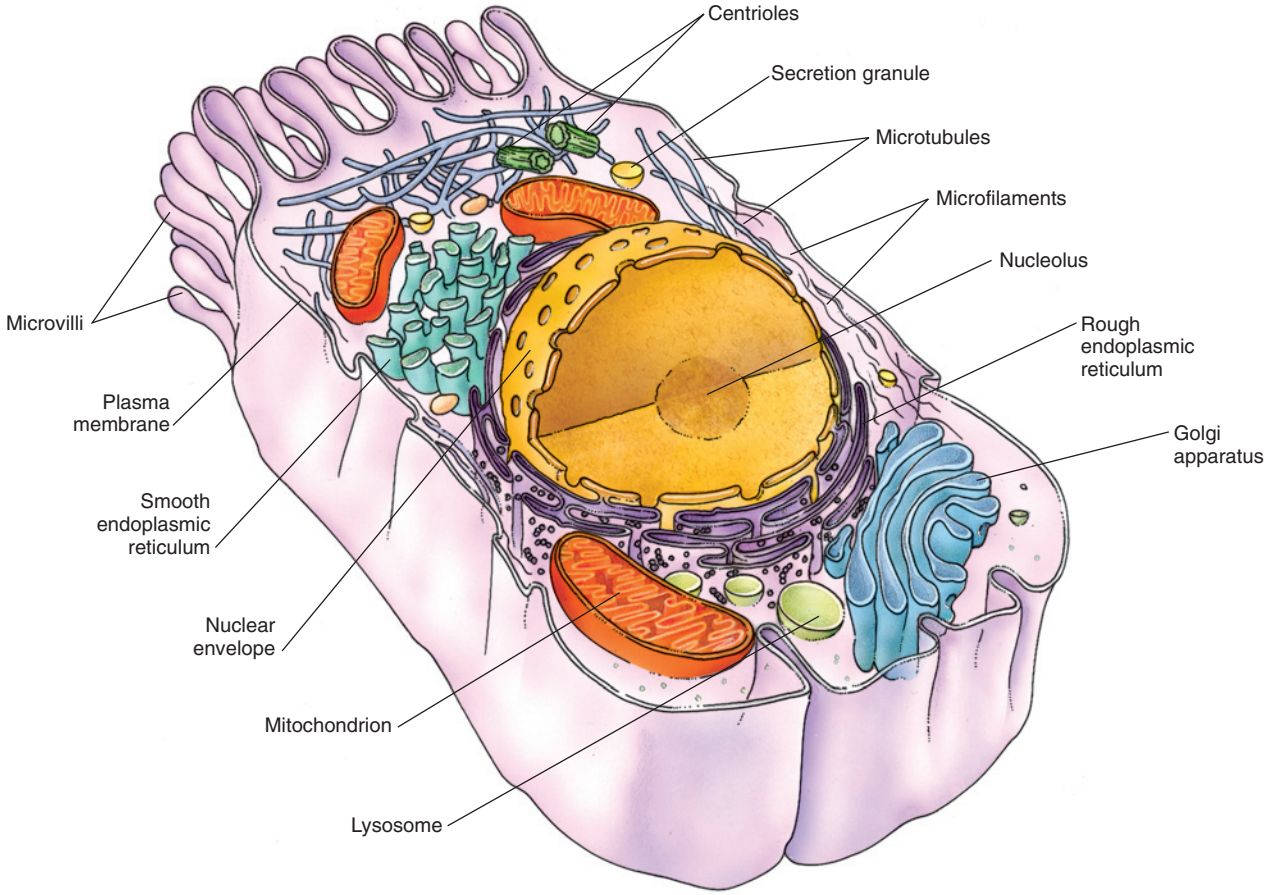


Figure 2–5 Three-dimensional schematic diagram of an idealized cell, as visualized by transmission electron microscopy. Various organelles and cytoskeletal elements are displayed.

composed of a **hydrophilic** head and a **hydrophobic** tail, the molecule is said to be **amphipathic**.

The polar heads are composed of **glycerol**, to which a positively charged nitrogenous group is attached by a negatively charged **phosphate group**. The two fatty acyl tails, only one of which is usually saturated, are covalently bound to glycerol. Other amphipathic molecules, such as **glycolipids, glycosphingolipids,** and **cholesterol**, are also present in the cell membrane. The unsaturated fatty acyl molecules increase membrane fluidity, whereas cholesterol decreases it (although cholesterol concentrations much lower than normal increase membrane fluidity). In fact, certain regions of the plasmalemma are so well endowed with glycosphingolipids and cholesterol that they create a bulge in the cell membrane. These thickened microdomains are known as ***lipid rafts***, and they form a slight bulge into the extracellular space. Frequently, lipid rafts possess protein components that participate in diverse signaling events (discussed in the next paragraph). Therefore, lipid rafts appear to facilitate and enhance the possibilities of communications between and among a variety of cells.

The protein components of the plasmalemma either span the entire lipid bilayer as **integral proteins** or are attached to the cytoplasmic aspect (and, at times, the extracellular aspect) of the lipid bilayer as **peripheral proteins**. Because most integral proteins pass through the entire thickness of the membrane, they are also referred to as ***transmembrane proteins***. Those regions of transmembrane proteins that project into the cytoplasm or the extracellular space are composed of hydrophilic amino acids, whereas the intramembrane region consists of hydrophobic amino acids. Transmembrane proteins frequently form ion channels and carrier proteins that facilitate the passage of specific ions and molecules across the cell membrane.

Many of these transmembrane proteins are quite long and are folded so that they make several passes through the membrane and thus are known as ***multipass proteins*** and are frequently attached to the inner leaflet (and infrequently to the outer leaflet) by prenyl

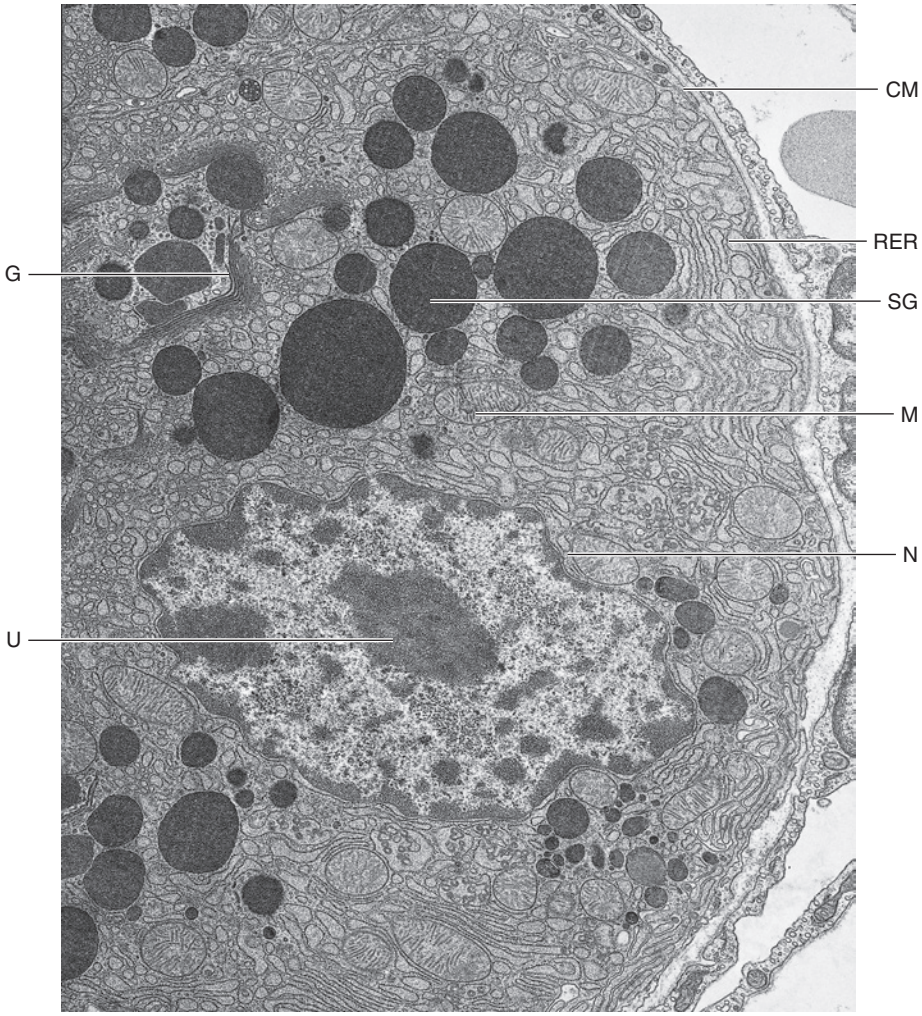


Figure 2–6 Electron micrograph of an acinar cell from the urethral gland of a mouse illustrating the appearance of some organelles (×11327). CM, Cell membrane; G, Golgi apparatus; M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum; SG, secretory granules; U, nucleolus. (From Parr MB, Ren HP, Kepple L, et al. Ultrastructure and morphometry of the urethral glands in normal, castrated, and testosterone-treated castrated mice. *Anat Rec.* 1993;236:449-458. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

groups or fatty acyl groups. The cytoplasmic and extracytoplasmic aspects of these proteins commonly possess receptor sites that are specific for particular **signaling molecules**. Once these molecules are recognized at these receptor sites, the integral proteins can alter their conformation and can perform a specific function.

Because the same integral membrane proteins have the ability to float like icebergs in the sea of phospholipids, this model is referred to as the ***fluid mosaic model*** of membrane structure. However, the integral proteins frequently possess only limited mobility, especially in polarized cells, in which particular regions of the cell serve specialized functions.

Peripheral proteins do not usually form covalent bonds with either the integral proteins or the phospholipid components of the cell membrane. Although they are usually located on the cytoplasmic aspect of the cell membrane, they may also be on the extracellular surface. These proteins may form bonds either with the phospholipid molecules or with the transmembrane proteins. Frequently, they are associated with the secondary messenger system of the cell (see section on signaling molecules) or with the cytoskeletal apparatus.

Using freeze-fracture techniques, one can cleave the plasma membrane into its two leaflets in order to view the hydrophobic surfaces (Figs. 2–9 and 2–10). The outer surface of the inner leaflet is referred to as the ***P-face*** (closer to the ***p****rotoplasm*); the inner surface of the outer leaflet is known as the ***E-face*** (closer to the ***e****xtracellular* space). Electron micrographs

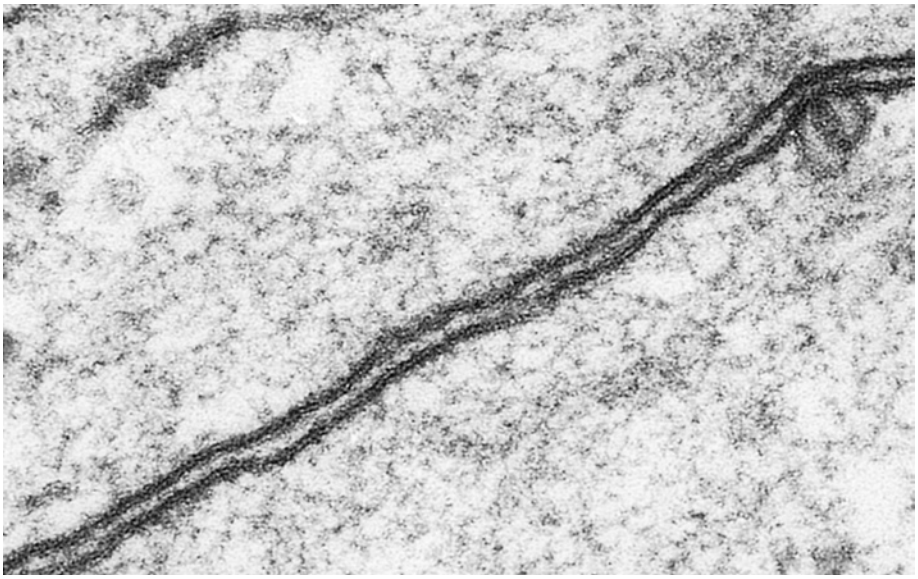


Figure 2–7 A junction between two cells demonstrates the trilaminar structures of the two cell membranes (×240000). (From Leeson TS, Leeson CR, Papparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

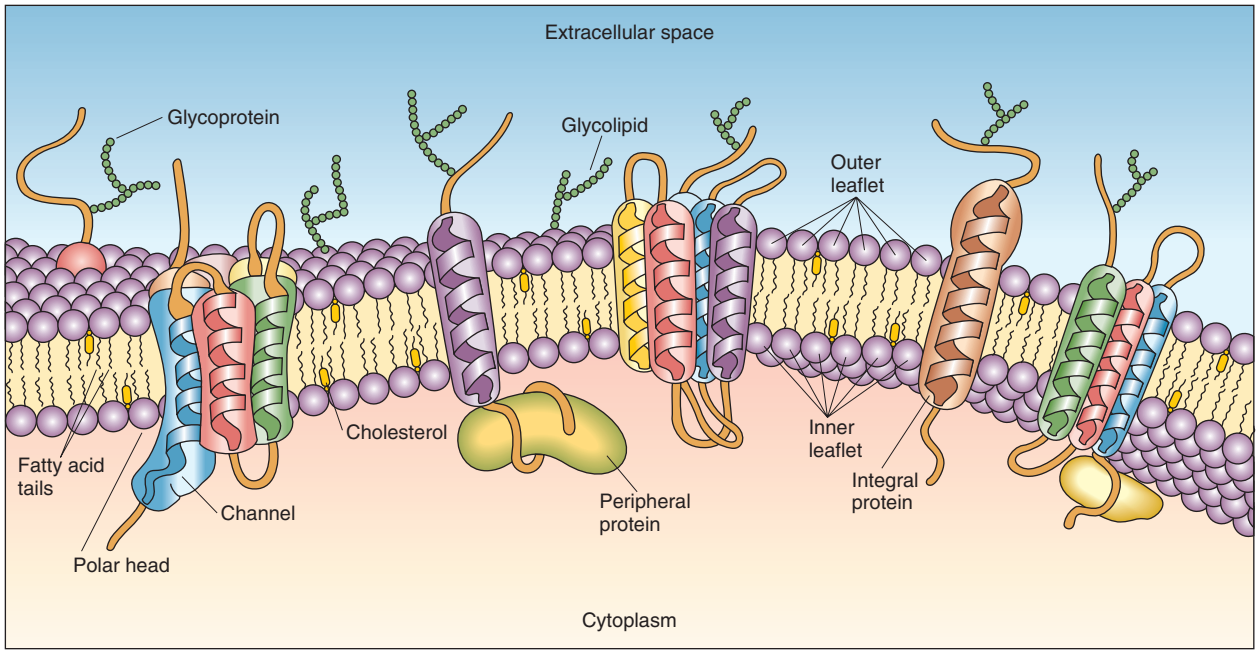


Figure 2–8 Three-dimensional diagrammatic representation of the fluid mosaic model of the cell membrane.

of freeze-fractured plasma membranes show that the integral proteins, visualized by shadowing replica, are more numerous on the P-face than on the E-face (see Fig. 2–10).

Glycocalyx

Glycocalyx, composed usually of carbohydrate chains, coats the cell surface.

A fuzzy coat, referred to as the ***cell coat*** or ***glycocalyx***, is often evident in electron micrographs of the cell membrane. This coat is usually composed of carbohydrate chains that are covalently attached to transmembrane proteins and/or phospholipid molecules of the outer leaflet (see Fig. 2–8). Additionally, some of the extracellular matrix molecules, adsorbed to the cell surface, also contribute to its formation. Its intensity and thickness vary, but it may be as thick as 50 nm on some epithelial sheaths, such as those lining regions of the digestive system.

Because of its numerous negatively charged sulfate and carboxyl groups, the glycocalyx stains intensely with lectins as well as with dyes, such as ruthenium red and Alcian blue, permitting its visualization with light microscopy. The most important function of the glycocalyx is protection of the cell from interaction with inappropriate proteins, from chemical injury, and from physical injury. Other cell coat functions include cell–cell recognition and adhesion, as occurs between endothelial cells and neutrophils as well as T cells and antigen-presenting cells (APCs); facilitation of blood clotting and inflammatory responses; and assistance in reducing friction between blood and the endothelial cells lining blood vessels.

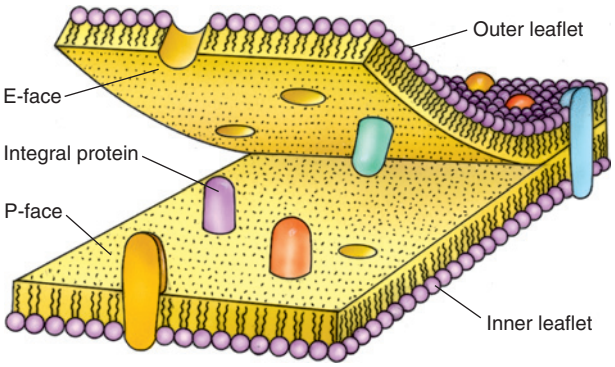


Figure 2–9 Schematic diagram of the E-face and the P-face of the cell membrane.

Membrane Transport Proteins

Membrane transport proteins are of two types, channel proteins and carrier proteins, and they facilitate the movement of aqueous molecules and ions across the plasmalemma.

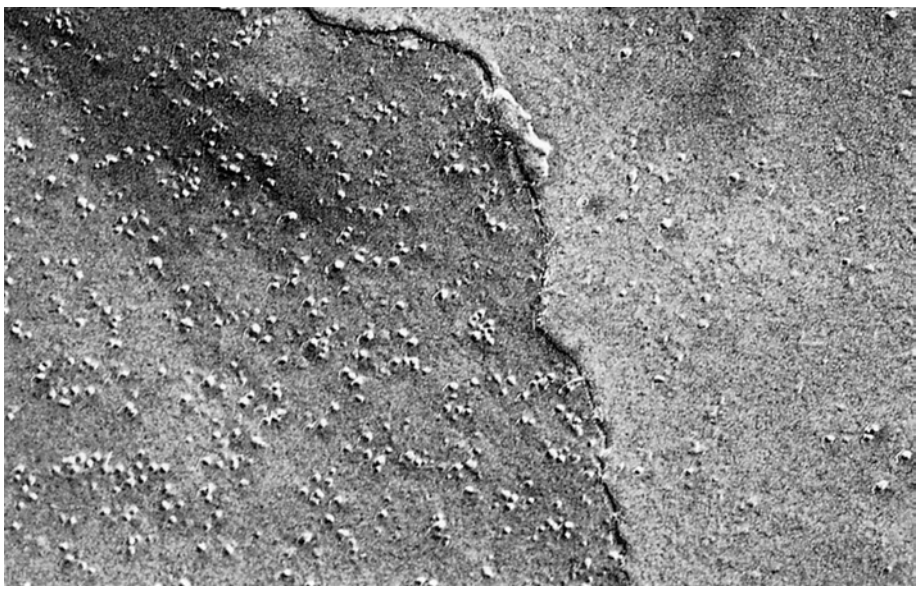


Figure 2–10 Freeze-fracture replica of a cell membrane. The E-face (closer to the *extracellular* space) is on the right, and the P-face (closer to the *protoplasm*) is on the left. Note that the integral proteins are more numerous on the P-face *(left-hand side)* than on the E-face *(right-hand side)*. (×168000) (From Leeson TS, Leeson CR, Papparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

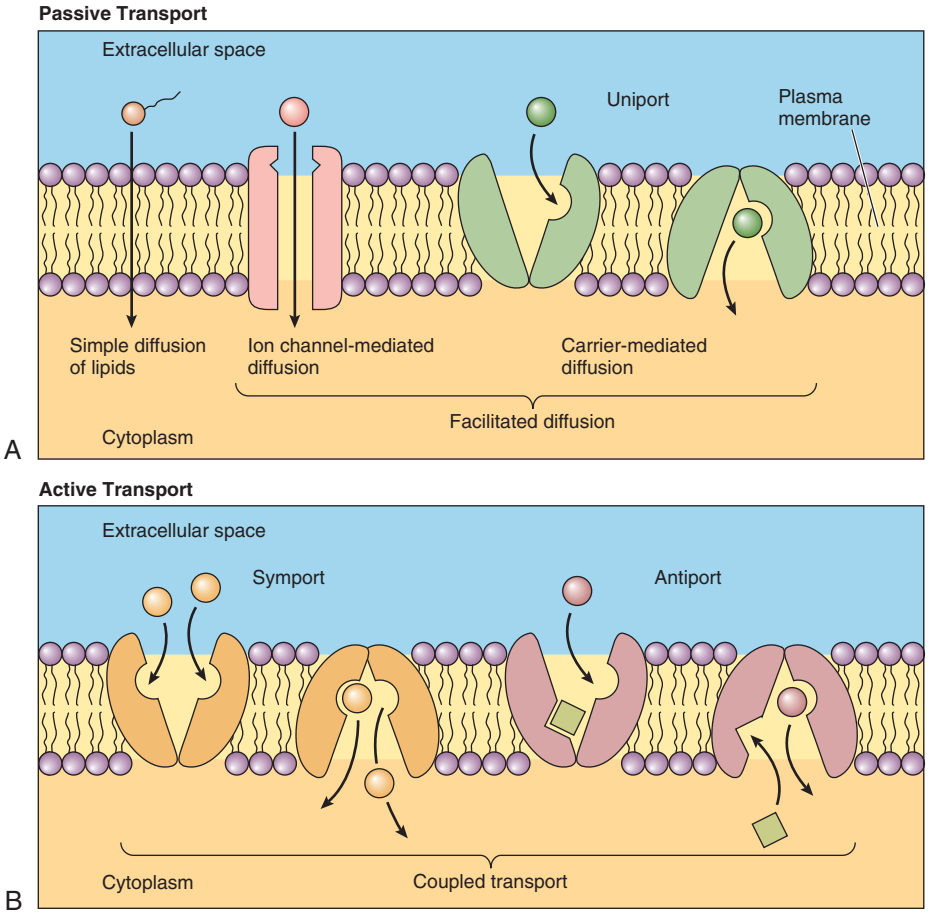


Figure 2–11 Types of transport. **A,** Passive transport: diffusion, ion channel-mediated diffusion, and carrier-mediated diffusion. **B,** Active transport: coupled transport. Symport and antiport.

Although the hydrophobic components of the plasma membrane limit the movement of polar molecules across it, the presence and activities of specialized transmembrane proteins facilitate the transfer of these hydrophilic molecules across this barrier. These transmembrane proteins and protein complexes form **channel proteins** and **carrier proteins**, which are specifically concerned with the transfer of ions and small molecules across the plasma membrane.

A number of small nonpolar molecules (e.g., benzene, oxygen, nitrogen) and uncharged polar molecules (e.g., water, glycerol) can move across the cell membrane by simple diffusion down their concentration gradients. Even when driven by a concentration gradient, however, significant movement of most ions and small molecules across a membrane requires the aid of either channel proteins or carrier proteins. This process is known as ***facilitated diffusion***. Because both types of diffusion occur without any input of energy other than that inherent in the concentration gradient, they represent **passive transport** (Fig. 2–11). By expending energy, cells can transport ions and small molecules against their concentration gradients. Only carrier proteins can mediate such energy-requiring **active transport**. The several channel proteins involved in facilitated diffusion are discussed first, and the more versatile carrier proteins are considered afterward.

Channel Proteins

Channel proteins may be gated or ungated; they are incapable of transporting substances against a concentration gradient.

Channel proteins participate in the formation of hydrophilic pores, called ***ion channels***, across the plasmalemma. In order to form hydrophilic channels, the proteins are folded so that the hydrophobic amino acids are positioned peripherally, interacting with the fatty acyl tails of the phospholipid molecules of the lipid

bilayer; whereas the hydrophilic amino acids face inward, forming a polar inner lining for the channel.

There are more than 100 different types of ion channels; of these, some are specific for one particular ion, but others permit the passage of several different ions and small water-soluble molecules. Although these ions and small molecules follow chemical or electrochemical concentration gradients for the direction of their passage, cells have the capability of preventing these substances from entering these hydrophilic tunnels by means of controllable **gates** that block their opening. Most channels are **gated channels**; only a few are **ungated**. Gated channels are classified according to the control mechanism required to open the gate.

Voltage-Gated Channels

Voltage-gated channels go from the closed to the open position, permitting the passage of ions from one side of the membrane to the other. The most common example is depolarization in the transmission of nerve impulses. In some channels, such as Na^+ channels, the open position is unstable, and the channel goes from an open to an **inactive position**, in which the passage of the ion is blocked, and, for a short time (a few milliseconds), the gate cannot be opened again. This is the **refractory period** (see Chapter 9 on the nervous system). The velocity of response to depolarization may also vary, and some of those channels are referred to as ***velocity-dependent***.

Ligand-Gated Channels

Channels that require the binding of a **ligand** (signaling molecule) to the channel protein to open their gate are known as **ligand-gated channels**. Unlike voltage-gated channels, these channels remain open until the ligand dissociates from the channel protein; they are referred to as ***ion channel–linked receptors***. Some of the ligands controlling these gates are neurotransmitters, whereas others are nucleotides.

Neurotransmitter-gated channels are usually located on the postsynaptic membrane. The neurotransmitter binds to a specific site on the protein, altering its molecular conformation, thus opening the channel or gate and permitting the influx of a specific ion into the cell. Some neurotransmitters are **excitatory**, whereas others are **inhibitory**. Excitatory neurotransmitters (e.g., acetylcholine) facilitate depolarization, whereas inhibitory neurotransmitters facilitate hyperpolarization of the membrane.

In **nucleotide-gated channels,** the signal molecule is a nucleotide (e.g., **cyclic adenosine monophosphate [cAMP]** in olfactory receptors and **cyclic guanosine monophosphate [cGMP]** in rods of the retina) that binds to a site on the protein and, by altering the conformation of the protein complex, permits the flow of a particular ion through the ion channel.

Mechanically Gated Channels

In **mechanically gated channels,** an actual physical manipulation is required to open the gate. An example of this mechanism is found in the hair cells of the inner ear. These cells, located on the basilar membrane, possess **stereocilia** that are embedded in a matrix known as the ***tectorial membrane***. Movement of the basilar membrane causes a shift in the positions of the hair cells, resulting in the bending of the stereocilia. This physical distortion opens the mechanically gated channels of the stereocilia located in the inner ear, permitting the entry of cations into the cell, depolarizing it. This event generates impulses that the brain interprets as sound.

G-Protein–Gated Ion Channels

Certain gated ion channels (e.g., muscarinic acetylcholine receptors of cardiac muscle cells) require the interaction between a receptor molecule and a G-protein complex (see section on G-protein–linked receptors) with the resultant activation of the G protein. The activated G protein then interacts with the channel protein modulating the ability of the channel to open or close.

Ungated Channels

One of the most common forms of an ungated channel is the **potassium (K^+) leak channel,** which permits the movement of K^+ ions across it and is instrumental in the creation of an **electrical potential (voltage) difference** between the two sides of the cell membrane. Because this channel is ungated, the transit of K^+ ions is not under the cell's control; rather, the direction of ion movement reflects its concentration on the two sides of the membrane.

Aquaporins

Currently, there are twelve different types of **aquaporins** that are known; these are a family of multipass proteins that form channels designed for the passage of water from one side of the cell membrane to the other. Some of these channels are pure water transporters (e.g., AqpZ), whereas others transport glycerol (e.g., GlpF). These aquaporins discriminate in the transport of the two molecules by restricting the pore sizes in such a fashion that glycerol is too large to pass through pores of the AqpZ channel. An interesting property of aquaporins is that they are completely impermeable to protons, so that streams of protons cannot traverse the channel even though they readily pass through water molecules via the process of donor-acceptor configurations. Aquaporins interfere with this donor-acceptor model by forcing the water molecules to flip-flop half way along the channel, so that water molecules enter the channel face up (hydrogen side up and oxygen side down, that, is the oxygen enters first followed by the two hydrogens), flip over, and leave the

channel face down (so that the oxygen leaves first, followed by the two hydrogens). Properly functioning aquaporins in the kidney may transport as much as 20 L of water per hour, whereas improperly functioning aquaporins may result in diseases such as diabetes insipidus and congenital cataracts of the eye.

Carrier Proteins

Carrier proteins can use ATP-driven transport mechanisms to ferry specific substances across the plasmalemma against a concentration gradient.

Carrier proteins are **multipass** membrane transport proteins that possess binding sites for specific ions or molecules on both sides of the lipid bilayer. When an ion or molecule specific to the particular carrier protein binds to the binding site, the carrier protein undergoes *reversible* conformational changes; as the ion or molecule is released on the other side of the membrane, the carrier protein returns to its previous conformation.

As stated previously, transport by carrier proteins may be **passive**, along an electrochemical concentration gradient, or **active**, against a gradient, thereby requiring energy expenditure by the cell. Transport may be **uniport**, a single molecule moving in one direction, or **coupled**, two different molecules moving in the same (**symport**) or opposite (**antiport**) directions (see Fig. 2–11). Coupled transporters convey the solutes either simultaneously or sequentially.

Primary Active Transport by Na^+-K^+ Pump

Normally, the concentration of Na^+ is much greater outside of the cell than inside, and the concentration of K^+ is much greater inside of the cell than outside. The cell maintains this concentration differential by expending **adenosine triphosphate** (**ATP**) to drive a coupled antiport carrier protein known as the **Na^+-K^+** pump. This pump transports K^+ ions into and Na^+ ions out of the cell, each against a steep concentration gradient. Because this concentration differential is essential for the survival and normal functioning of practically every animal cell, the plasma membrane of all animal cells possesses a large number of these pumps.

The Na^+-K^+ pump possesses two binding sites for K^+ on its extracellular aspect and three binding sites for Na^+ on its cytoplasmic aspect; thus, for every two K^+ ions conveyed into the cell, three Na^+ ions are transported out of the cell.

Na^+-K^+ ATPase has been shown to be associated with the Na^+-K^+ pump. When three Na^+ ions bind on the cytosolic aspect of the pump, ATP is hydrolyzed to **adenosine diphosphate** (**ADP**), and the released phosphate ion is used to phosphorylate the ATPase, resulting in the alteration of the conformation of the pump, with the consequent transfer of Na^+ ions out of the cell. The binding of two K^+ ions on the external aspect of the pump causes dephosphorylation of the ATPase with an ensuing return of the carrier protein to its previous conformation, resulting in the transfer of the K^+ ions into the cell. Thus, the expenditure of a single ATP molecule provides the energy for the transfer of three Na^+ ions and two K^+ ions across the cell membrane.

The constant operation of this pump reduces the intracellular ion concentration, resulting in decreased intracellular osmotic pressure. If the osmotic pressure within the cell were not reduced by the Na^+-K^+ pump, water would enter the cell in large quantities, causing the cell to swell and eventually to succumb to osmotic lysis (i.e., the cell would burst). Hence, it is through the operation of this pump that the cell is able to regulate its osmolarity and, consequently, its volume. Additionally, this pump supplies minor assistance to the K^+ leak channels in the maintenance of the cell membrane potential.

Because the binding sites on the external aspect of the pump bind not only K^+ but also the glycoside **ouabain,** this glycoside inhibits the Na^+-K^+ pump.

Secondary Active Transport by Coupled Carrier Proteins

The ATP-driven transport of Na^+ out of the cell establishes a low intracellular concentration of that ion. The energy reservoir inherent in the sodium ion gradient can be used by carrier proteins to transport ions or other molecules against a concentration gradient. Frequently, this mode of active transport is referred to as ***secondary active transport***, distinct from the **primary active transport,** which uses the energy released from the hydrolysis of ATP.

The carrier proteins that participate in secondary active transport are either symports or antiports. As a Na^+ ion binds to the extracellular aspect of the carrier protein, another ion or small molecule (e.g., **glucose**) also binds to a region on the same aspect of the carrier protein, inducing in it a conformational alteration. The change in conformation results in the transfer and subsequent release of both molecules on the other side of the membrane.

ATP-Binding Cassette Transporters (ABC-Transporters)

These highly conserved transporters occur in the largest numbers of all carrier proteins. They are present in both prokaryotic organisms, such as bacteria, as well as in all eukaryotic organisms. The major difference is that, in prokaryotic organisms, the ABC-transporters move substances in both directions (into and out of the cell), whereas, in eukaryotic cells, the transport is in a single direction only, namely out of the cell; only the eukaryotic transporters are discussed.

ABC-transporters are transmembrane proteins, thus protruding through the entire cell membrane. The intracellular portion of the transporters possesses binding sites (known as ***ATP-binding cassettes***) for two ATP molecules. When ATP is not present, the intracellular binding sites for specific molecules are exposed,

and the particular ion or molecule adheres to the binding site. When the ATP molecules bind to the ATP-binding cassettes, the transporter's conformation becomes altered, and the ion or molecule is permitted to leave at the transporter's extracellular surface. It should be stated that not all ABC-transporters are located on the plasmalemma; many are present on the membranes of intracellular membranous organelles, such as the trans-Golgi network (TGN), rough endoplasmic reticulum (ER), and mitochondrion. The transfer of epitopes into the TGN or into the rough ER depends on the activities of specialized ABC-transporters (see Chapter 12, Lymphoid [Immune] System).

Many ABC-transporters transport various hydrophobic toxic substances and drugs out of the cell. Many cancer cells possess specific ABC-transporters, known as ***multidrug resistance proteins*** (**MDR proteins**), that drive anticancer drugs out of the cell, thus providing the malignant cells with increased resistance to chemotherapeutic agents.

CLINICAL CORRELATIONS

A member of the ABC-transporters, the **cystic fibrosis transmembrane conductance regulator protein** (CFTR protein, coded for by a mutated form of the CFTR gene), is responsible for the formation of abnormal chloride channel proteins, especially in the respiratory system. These channels formed by these proteins do not permit Cl^- ions to pass through them to leave the cell, thereby the increased negative charges due to the increased concentration of chloride ions in the cytoplasm attract Na^+ ions into the cells. The elevated NaCl content of the cell attracts water from the extracellular milieu into the cell, increasing the viscosity of the mucus lining the respiratory tract. The thickened mucus blocks the smaller bronchioles leading to infection, debilitated lung function, and, eventually, death.

Cell Signaling

Cell signaling is the communication that occurs when signaling cells release signaling molecules that bind to cell surface receptors of target cells.

When cells communicate with each other, the one that sends the signal is called the **signaling *cell***; the cell receiving the signal is called the ***target cell***. Transmission of the information may occur by either the secretion or presentation of **signaling molecules**, which contact **receptors** on the target cell membrane (or intracellularly either in the cytosol or in the nucleus), or by the formation of intercellular pores known as ***gap junctions***, which permit the movement of ions and small molecules (e.g., cAMP) between the two cells. Gap junctions are discussed in Chapter 5.

The signaling molecule, or **ligand**, may be either secreted and released by the signaling cell or may remain bound to its surface and be presented directly by the signaling cell to the target cell. A cell-surface receptor usually is a transmembrane protein, whereas an intracellular receptor is a protein that resides in the cytosol or in the nucleus of the target cell. Ligands that bind to cell-surface receptors usually are **polar** molecules; those that bind to intracellular receptors are **hydrophobic** and thus can diffuse through the cell membrane.

In the most selective signaling process, **synaptic signaling**, the signaling molecule, a neurotransmitter, is released so close to the target cell that only a single cell is affected by the ligand. A more generalized but still local form of signaling, **paracrine signaling**, occurs when the signaling molecule is released into the intercellular environment and affects cells in its immediate vicinity. Occasionally, the signaling cell is also the target cell, resulting in a specialized type of paracrine signaling known as ***autocrine signaling***. The most widespread form of signaling is **endocrine signaling**; in this case, the signaling molecule enters the bloodstream to be ferried to target cells situated at a distance from the signaling cell.

Signaling Molecules

Signaling molecules bind to extracellular or intracellular receptors to elicit a specific cellular response.

Most signaling molecules are hydrophilic (e.g., **acetylcholine**) and cannot penetrate the cell membrane. Therefore, they require receptors on the cell surface. Other signaling molecules are either hydrophobic, such as **steroid hormones**, or are small nonpolar molecules, such as **nitric oxide** (**NO**), both of which have the ability to diffuse through the lipid bilayer. These ligands require the presence of an intracellular receptor. Hydrophilic ligands have a very short life span (a few milliseconds to minutes at most), whereas steroid hormones last for extended time periods (several hours to days).

Signaling molecules often act in concert, so that several different ligands are required before a specific cellular response is elicited. Moreover, the same ligand or combination of ligands may elicit different responses from different cells. For instance, acetylcholine causes skeletal muscle cells to contract, cardiac muscle cells to relax, endothelial cells of blood vessels to release nitric oxide, and parenchymal cells of some glands to release the contents of their secretory granules.

Binding of signaling molecules to their receptors activates an intracellular **second messenger system**, initiating a cascade of reactions that result in the required response. A hormone, for example, binds to its receptors on the cell membrane of its target cell. The receptor alters its conformation, with the resultant activation of **adenylate cyclase,** a transmembrane protein, whose cytoplasmic region catalyzes the transformation of **ATP** to **cAMP**, one of the most common second messengers.

The second messenger, cAMP, activates a cascade of enzymes within the cell, thus multiplying the effects of very few molecules of hormones on the cell surface. The specific intracellular event depends on the enzymes located within the cell; for instance, cAMP activates one set of enzymes within an endothelial cell and another set of enzymes within a follicular cell of the thyroid gland. Therefore, the same molecule can have a different effect in different cells. The system is known as a *second messenger system* because the hormone is the first messenger that activates the formation of cAMP, the second messenger. Other second messengers include calcium (Ca^{2+}), cGMP, inositol triphosphate (IP_3), and diacylglycerol.

Steroid hormones (e.g., cortisol) can diffuse through the cell membrane. Once in the cytosol, they bind to **steroid hormone receptors** (members of the **intracellular receptor family**), and the ligand-receptor complex activates gene expression, or **transcription** (the formation of **messenger ribonucleic acid** [**mRNA**]). Transcription may be induced directly, resulting in a fast **primary response**, or indirectly, bringing about a slower, secondary response. In the **secondary response,** the mRNA codes for the protein that is necessary to activate the expression of additional genes.

Cell-Surface Receptors

Cell-surface receptors are of three types: ion-channel–linked, enzyme-linked, and G-protein–linked.

Most cell-surface receptors are integral **glycoproteins** that function in recognizing signaling molecules and in **transducing** the signal into an intracellular action. The three main classes of receptor molecules are ion channel–linked receptors, enzyme-linked receptors, and G-protein–linked receptors.

Enzyme-Linked Receptors

Enzyme-linked receptors are transmembrane proteins whose extracellular regions act as receptors for specific ligands. When a signaling molecule binds to the receptor site, the receptor's intracellular domain becomes activated so that it now possesses enzymatic capabilities. These enzymes then either induce the formation of second messengers, such as cGMP, or permit the assembly of intracellular signaling molecules that relay the signal intracellularly. This signal then elicits the required response by activating additional enzyme systems or by stimulating gene regulatory proteins to initiate the transcription of specific genes.

G-Protein–Linked Receptors

G-protein–linked receptors are multipass proteins whose extracellular domains act as receptor sites for ligands. Their intracellular regions have two separate sites, one that binds to G proteins and another that becomes phosphorylated during the process of receptor desensitization.

Most cells possess two types of GTPases (monomeric and trimeric), each of which has the capability of binding **guanosine triphosphate** (**GTP**) and **guanosine diphosphate** (**GDP**). Trimeric GTPases, **G proteins**, are composed of a large **α subunit** and two **small subunits**, **β** and **γ**, and can associate with G-protein–linked receptors. There are several types of G proteins, including:

- Stimulatory **(G_s)**
- Inhibitory **(G_i)**
- Pertussis toxin-sensitive **(G_o)**
- G_{olf}
- Pertussis toxin-insensitive **(G_{Bq})**
- Transducin **(G_t)**
- **$G_{12/13}$**

G proteins act by linking receptors with enzymes that modulate the levels of the intracellular signaling molecules (second messengers) cAMP or Ca^{2+}.

Signaling via G_s and G_i Proteins. G_s proteins (Fig. 2–12) are usually present in the **inactive** state, in which a **GDP** molecule is bound to the α subunit. When a ligand binds to the G-protein–linked receptor, it alters the receptor's conformation, permitting it to bind to the α subunit of the G_s protein, which, in turn, exchanges its GDP for a **GTP**. The binding of GTP causes the α subunit to dissociate not only from the receptor, but also from the other two subunits and to bind with **adenylate cyclase**, a transmembrane protein. This binding activates adenylate cyclase to form many molecules of cAMP from ATP molecules. As the activation of adenylate cyclase is occurring, the ligand uncouples from the G-protein–linked receptor, returning the receptor to its original conformation without affecting the activity of the α subunit. Within a few seconds, the α subunit hydrolyzes its GTP to GDP, detaches from adenylate cyclase (thus deactivating it), and reassociates the β and γ subunits.

G_i behaves similarly to G_s, but, instead of activating adenylate cyclase, it inhibits it, so that cAMP is not being produced. The lack of cAMP prevents the phosphorylation—thus activation—of enzymes that would elicit a particular response. Hence, a particular ligand binding to a particular receptor may activate or inactivate the cell, depending on the type of G protein that couples it to adenylate cyclase.

Cyclic AMP and Its Role as a Second Messenger. Cyclic AMP (cAMP) is an intracellular signaling molecule that activates cAMP-dependent protein kinase **(PKA, A-kinase)** by binding to it. The activated A-kinase dissociates into its **regulatory component** and two **active catalytic subunits**. The active catalytic subunits phosphorylate other enzymes in the cytosol, thus initiating a cascade of phosphorylations and resulting in a specific response. Elevated levels of cAMP in some cells result

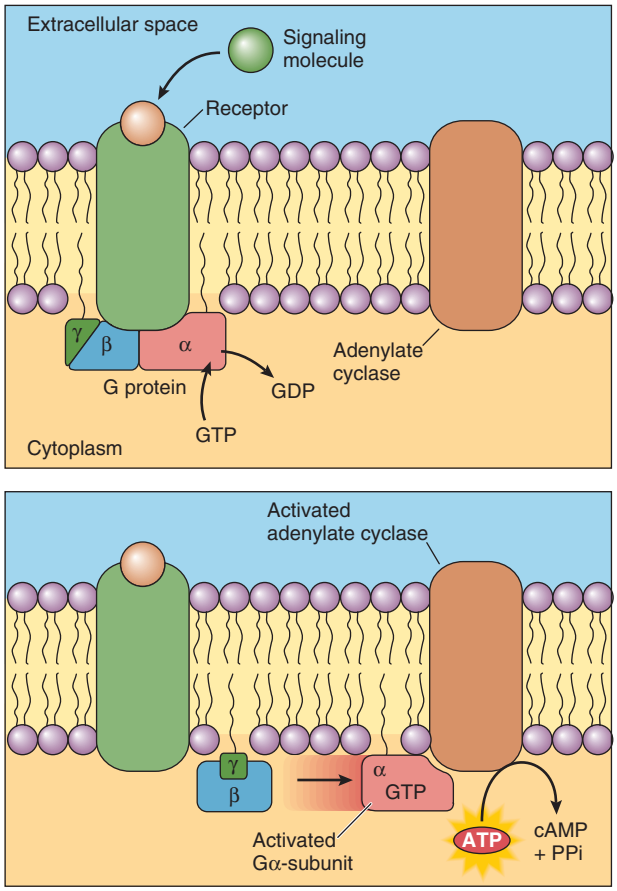


Figure 2–12 G-protein–linked receptor. When the signaling molecule contacts its receptor, the α subunit dissociates from the G protein and contacts and activates adenylate cyclase, which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). GTP, Guanosine triphosphate; PPi, pyrophosphate.

in the transcription of those genes whose regulatory regions possess **cAMP response elements** (**CREs**). A-kinase phosphorylates, and thus activates, a gene regulatory protein known as ***CRE-binding protein*** **(CREB)** whose binding to the CRE stimulates the transcription of those genes.

As long as cAMP is present at a high enough concentration, a particular response is elicited from the target cell. In order to prevent responses of unduly long duration, cAMP is quickly degraded by **cAMP phosphodiesterases** to 5′-AMP, which is unable to activate A-kinase. Moreover, the enzymes phosphorylated during the cascade of phosphorylations become deactivated by becoming dephosphorylated by another series of enzymes **(serine/threonine phosphoprotein phosphatases)**.

Signaling via G_o Protein. When a ligand becomes bound to **G_o-protein–linked receptor,** the receptor alters its conformation and binds with **G_o.** This trimeric protein dissociates, and its subunit activates **phospholipase C**, the enzyme responsible for cleaving the membrane phospholipid phosphatidylinositol bisphosphate (PIP_2) into IP_3 and **diacylglycerol**. **IP_3** leaves the membrane and diffuses to the ER, where it causes the release of Ca^{2+}—another second messenger—into the cytosol. Diacylglycerol remains attached to the inner leaflet of the plasma membrane and, with the assistance of Ca^{2+}, activates the enzyme protein kinase C **(C-kinase)**. C-kinase, in turn, initiates a phosphorylation cascade, whose end result is the activation of gene regulatory proteins that initiate transcription of specific genes.

IP_3 is rapidly inactivated by being dephosphorylated, and diacylglycerol is catabolized within a few seconds after its formation. These actions ensure that responses to a ligand are of limited duration.

Ca^{2+} and Calmodulin. Because cytosolic Ca^{2+} acts as an important second messenger, its cytosolic concentration must be carefully controlled by the cell. These control mechanisms include the sequestering of Ca^{2+} by the ER, by specific Ca^{2+}-binding molecules in the cytosol and by mitochondria, and by the active transport of this ion out of the cell.

When IP_3 causes elevated cytosolic Ca^{2+} levels, the excess ions bind to **calmodulin,** a protein found in high concentration in most animal cells. The Ca^{2+}-calmodulin complex activates a group of enzymes known as ***Ca^{2+}-calmodulin–dependent protein kinases*** **(CaM-kinases)**. CaM-kinases have numerous regulatory functions in the cell, such as initiation of glycogenolysis, synthesis of catecholamines, and contraction of smooth muscle.

Signaling via Other G Proteins. **G_{olf}** is an olfactory specific protein that reacts to recognize specific odorants; **$G_{12/13}$** prompts actin formation in the cytosol, thus remodeling the cytoskeleton and, in that manner, facilitates cell motility. Pertussis toxin-insensitive **(G_{Bq}) G protein** activates substance P which, in the brain, regulates the opening of potassium channels.

Protein Synthetic and Packaging Machinery of the Cell

The primary components of the protein synthetic machinery of the cell are ribosomes (and polyribosomes), RER, and the Golgi apparatus.

Ribosomes

Ribosomes are small particles, approximately 12 nm wide and 25 nm long, composed of proteins and **ribosomal RNA (rRNA)**. They function as a surface for the synthesis of proteins. Each ribosome is composed of a **large subunit** and a **small subunit**, both of which are manufactured or assembled in the nucleolus and released as separate entities into the cytosol. The small

subunit has a sedimentation value of 40S and is composed of 33 proteins and an 18S rRNA. The sedimentation value of the large subunit is 60S, and it consists of 49 proteins and 3 rRNAs. The sedimentation values of the RNAs are 5S, 5.8S, and 28S.

The small subunit has a site for binding mRNA, a **P site** for binding *peptidyl* **transfer ribonucleic acid (tRNA)**, an **A site** for binding *aminoacyl* tRNAs, and an **E site** where the tRNA that gave up its amino acid *exits* the ribosome. Some of the rRNAs of the large subunit are referred to as **ribozymes** because they have enzymatic activity and catalyze peptide bond formation. The small and large subunits are present in the cytosol individually and do not form a ribosome until protein synthesis begins.

Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** is the largest membranous system of the cell, comprising approximately half of the total membrane volume. It is a system of interconnected tubules and vesicles whose lumen is referred to as the ***cistern***. The ER has two components: **smooth endoplasmic reticulum (SER)** and **rough endoplasmic reticulum (RER)**. Although only the RER participates in protein synthesis, the SER is also discussed at this point but only as an aside, and the reader should keep in mind that distinction.

Smooth Endoplasmic Reticulum

A system of anastomosing tubules and occasional flattened membrane-bound vesicles constitute the SER (Fig. 2–13). The lumen of the SER is assumed to be continuous with that of the RER. Except for cells active in synthesis of steroids, cholesterol, and triglycerides, and cells that function in detoxification of toxic materials (e.g., alcohol and barbiturates), most cells do not possess an abundance of SER. The SER has become specialized in some cells (e.g., skeletal muscle cells), where it is known as the ***sarcoplasmic reticulum*** (see Chapter 8, Muscle), where it functions in sequestering calcium ions from the cytosol, assisting in the control of muscle contraction.

Rough Endoplasmic Reticulum

Cells that function in the synthesis of proteins that are to be exported are richly endowed with RER (see Fig. 2–6). The membranes of this organelle are somewhat different from those of its smooth counterpart because it possesses integral proteins that function in recognizing and binding ribosomes to its cytosolic surface and also maintains the flattened morphology of the RER. For the purposes of this textbook, the integral proteins of interest are (1) **signal recognition particle receptor (docking protein),** (2) **ribosome receptor protein** (ribophorin I and ribophorin II), and (3) **pore protein**. Their functions are discussed in the section on synthesis of proteins on the RER.

RER participates in the synthesis of all proteins that are to be packaged or delivered to the plasma membrane as well as proteins that are destined to remain in the RER. It also performs posttranslational modifications of these proteins, including sulfation, folding, glycosylation, and, when necessary, their degradation. Additionally, lipids and integral proteins of all membranes of the cell are manufactured by the RER. The cisterna of the RER is also continuous with the perinuclear cistern, the space between the inner and outer nuclear membranes.

Polyribosomes

Proteins to be packaged are synthesized on the RER surface, whereas proteins destined for the cytosol are manufactured within the cytosol. The information for the primary structure of a protein (sequence of amino acids) is housed in the **deoxyribonucleic acid (DNA)** of the nucleus. This information is **transcribed** into a strand of mRNA, which leaves the nucleus and enters the cytoplasm. The sequence of **codons** of the mRNA thus represents the chain of amino acids, in which each codon is composed of three consecutive nucleotides. Because any three consecutive nucleotides constitute a codon, it is essential that the protein synthetic machinery recognize precisely the beginning and the end of the message; otherwise, an incorrect protein will be manufactured.

The three types of RNA play distinctive roles in protein synthesis:

- The **mRNA** carries the coded instructions specifying the sequence of amino acids.
- The **tRNAs** form covalent bonds with amino acids, forming **aminoacyl tRNAs**. These enzyme-catalyzed reactions are specific; that is, each tRNA reacts with its own corresponding amino acid. Each tRNA also contains the **anticodon** that recognizes the codon in mRNA corresponding to the amino acid it carries.
- Several **rRNAs** associate with a large number of proteins to form the small and large ribosomal subunits.

Protein Synthesis (Translation)

Protein synthesis (translation) occurs on ribosomes in the cytosol or on the surface of the RER.

The requirements for protein synthesis are:

- An **mRNA** strand
- The **tRNAs**, each of which carries an amino acid and possesses the anticodon that recognizes the codon of the mRNA coding for that particular amino acid
- Small and large **ribosomal subunits**

It is interesting that the approximate time of synthesis of a protein composed of 400 amino acids is about 20 seconds. Because a single strand of mRNA may have as many as 15 ribosomes translating it simultaneously, a large number of protein molecules may be synthesized

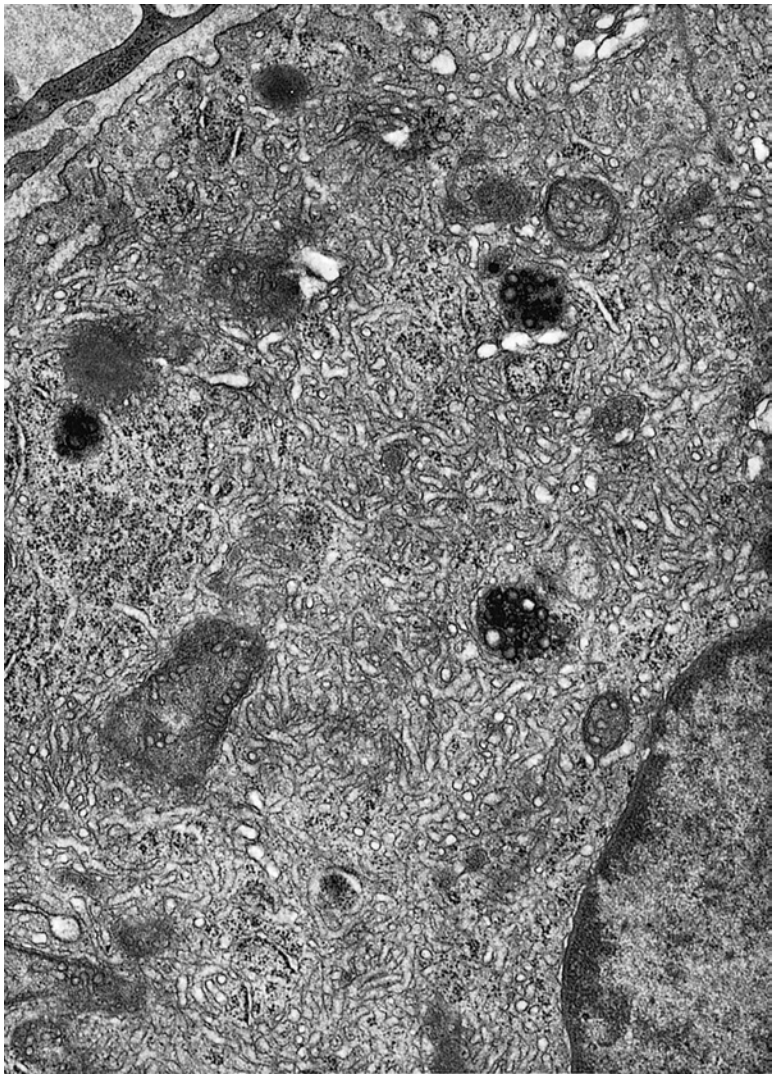


Figure 2–13 Electron micrograph of the smooth endoplasmic reticulum of the human suprarenal cortex. (From Leeson TS, Leeson CR, Papparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

in a short period of time. This conglomeration of mRNA–ribosome complex, which usually has a spiral or long, hairpin form, is referred to as a ***polyribosome***, or ***polysome*** (Fig. 2–14).

Synthesis of Cytosolic Proteins

The general process of protein synthesis in the cytosol is outlined in Figure 2–15.

STEP 1

- The process begins when the P site of the small ribosomal subunit is occupied by an **initiator tRNA**, whose anticodon recognizes the triplet **codon AUG**, coding for the amino acid **methionine**.
- An **mRNA** binds to the small subunit.
- The small subunit assists the anticodon of the tRNA molecule to recognize the **start codon AUG** on the mRNA molecule. This step acts as a registration step so that the next three nucleotides of the mRNA molecule may be recognized as the next codon.

STEP 2

- The large ribosomal subunit binds to the small subunit, and the ribosome moves along the mRNA chain, in a 5′ to 3′ direction, until the next codon lines up with the A site of the small subunit.

STEP 3

- An acylated tRNA (a tRNA bearing an amino acid) compares its anticodon with the codon of the mRNA; if they match, the tRNA binds to the A site.

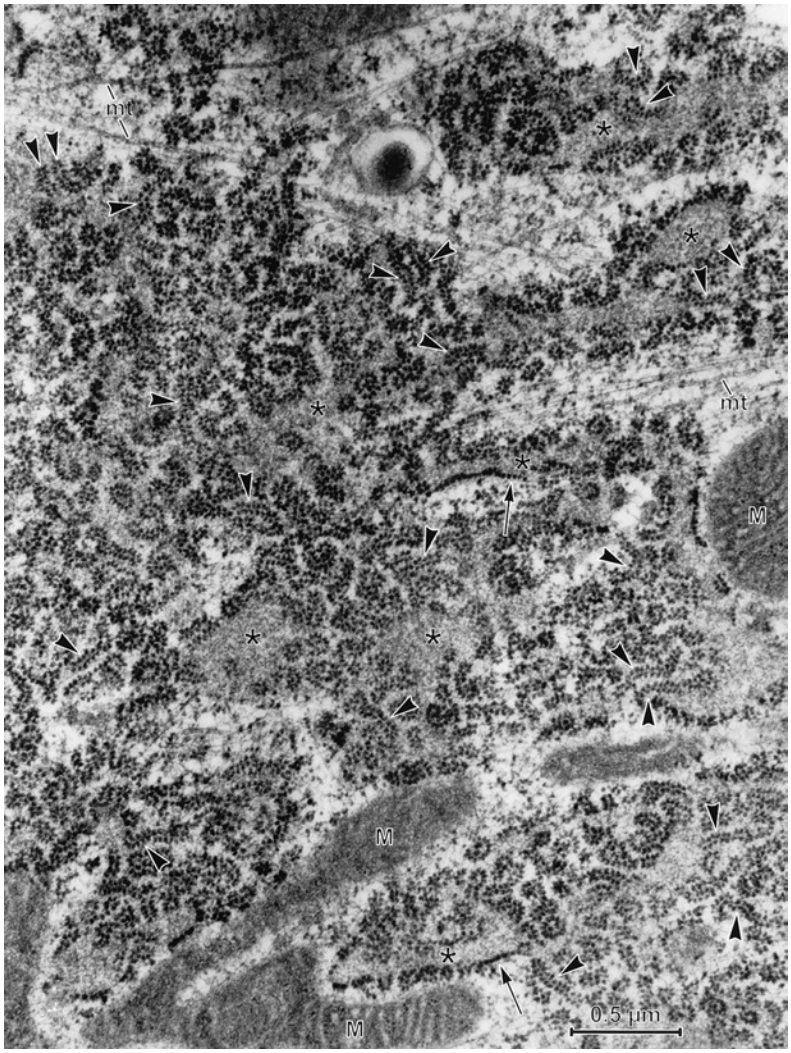


Figure 2–14 Electron micrograph of bound polysome. (From Christensen AK, Bourne CM. Shape of large bound polysomes in cultured fibroblasts and thyroid epithelial cells. *Anat Rec.* 1999;255:116-129. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

STEP 4
- The amino acids at the A site and the P site form a peptide bond.
- The tRNA on the P site yields its amino acid to the tRNA at the A site, which now has two amino acids attached to it. These reactions are catalyzed by peptidyl transferase, the rRNA-based enzyme of the large ribosomal subunit.

STEP 5
- The deaminated tRNA leaves the P site and binds to the E site; the tRNA with its two amino acids attached moves from the A site to the P site. Concurrently, the ribosome moves along the mRNA chain until the next codon lines up with the A site of the small ribosomal subunit and the tRNA from the E site is ejected. The energy required by this step is derived from the hydrolysis of GTP.

STEP 6
- Steps 3 through 5 are repeated, elongating the polypeptide chain until the stop codon is reached.
- There are three stop codons (**UAG, UAA,** and **UGA**), each one of which may halt translation.

STEP 7
- When the A site of the small ribosomal subunit reaches a stop codon, the **release factors eRF1** and **eRF3** bind to the A site. The eRF1 can bind to each of the three stop codons UAG, UAA, and UGA.

STEP 8
- The tRNA moves from the P site to the E site, and eRF3, a GTPase, assists eRF1 in releasing the

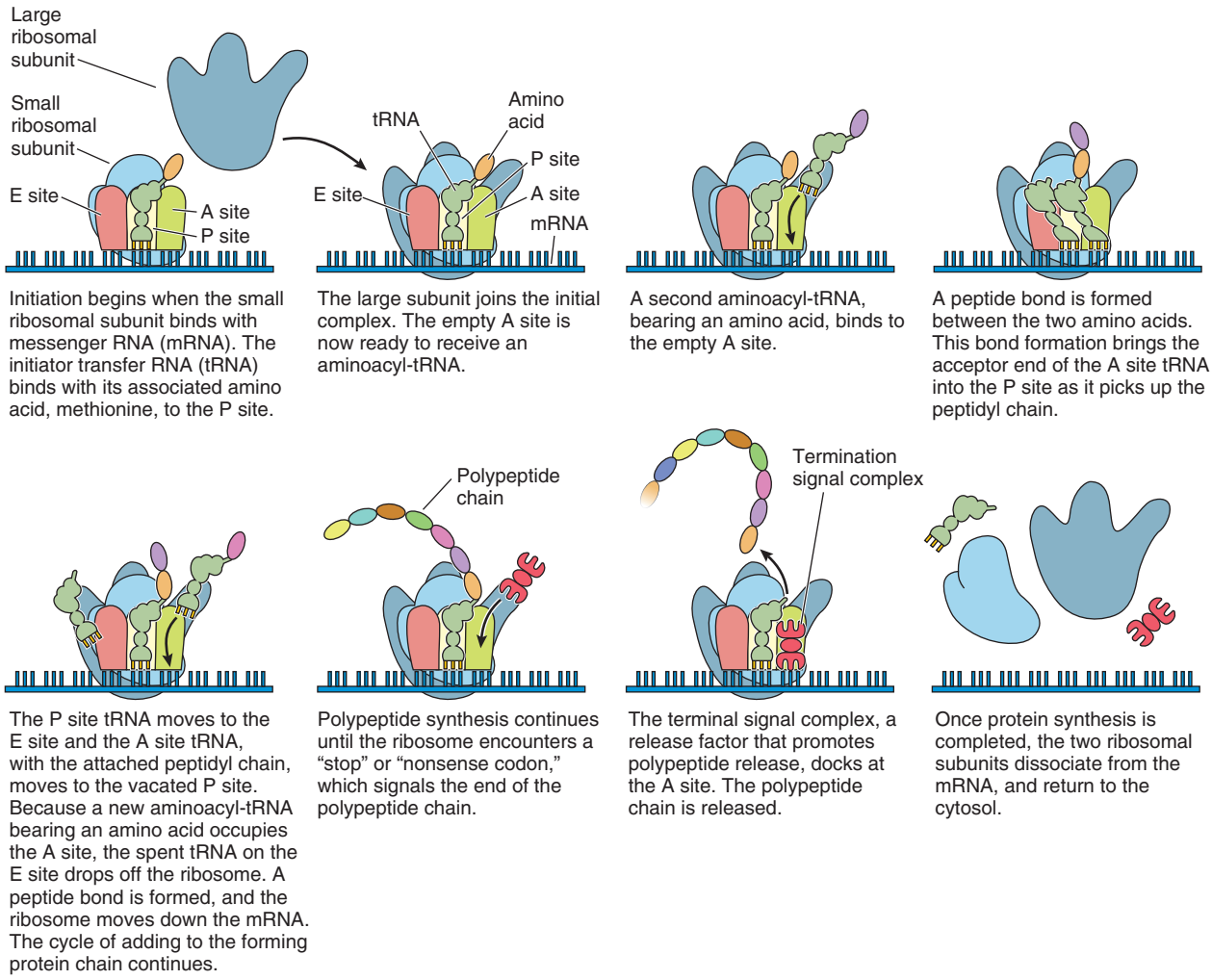


Figure 2–15 Diagram of protein synthesis in the cytosol.

polypeptide from the ribosome, and the ribosome leaves the mRNA and dissociates into a small and a large subunit.

Synthesis of Proteins on the Rough Endoplasmic Reticulum

Proteins that need to be packaged for delivery outside of the cell, inserted into the cell membrane, sent to an cytoplasmic organelle, retained in the RER, or merely isolated from the cytosol must be identified and be delivered **cotranslationally** (during the process of synthesis) into the RER cistern. The mode of identification resides in a small segment of the mRNA, located immediately following the start codon, which codes for a sequence of amino acids, known as the ***signal peptide***.

Using the sequence just outlined for the synthesis of protein in the cytosol, the mRNA begins to be translated, forming the signal peptide (Fig. 2–16). This peptide is recognized by the **signal-recognition particle** (**SRP**), a ribonucleated protein (protein–RNA complex) located in the cytosol. The SRP becomes attached to the signal peptide and, by occupying the P site on the small subunit of the ribosome, halts translation; it then directs the polysome to migrate to the RER.

The **SRP receptor protein** (**docking protein**) in the RER membrane contacts the SRP, and the ribosome receptor protein contacts the large subunit of the ribosome, attaching the polysome to the cytosolic surface of the RER. The following events then occur almost simultaneously:

1. A group of proteins, the **protein translocators**, assemble, forming a **pore** through the lipid bilayer of the RER.
2. The signal peptide contacts the pore protein and begins to be translocated (amino terminus first) into the cistern of the RER.

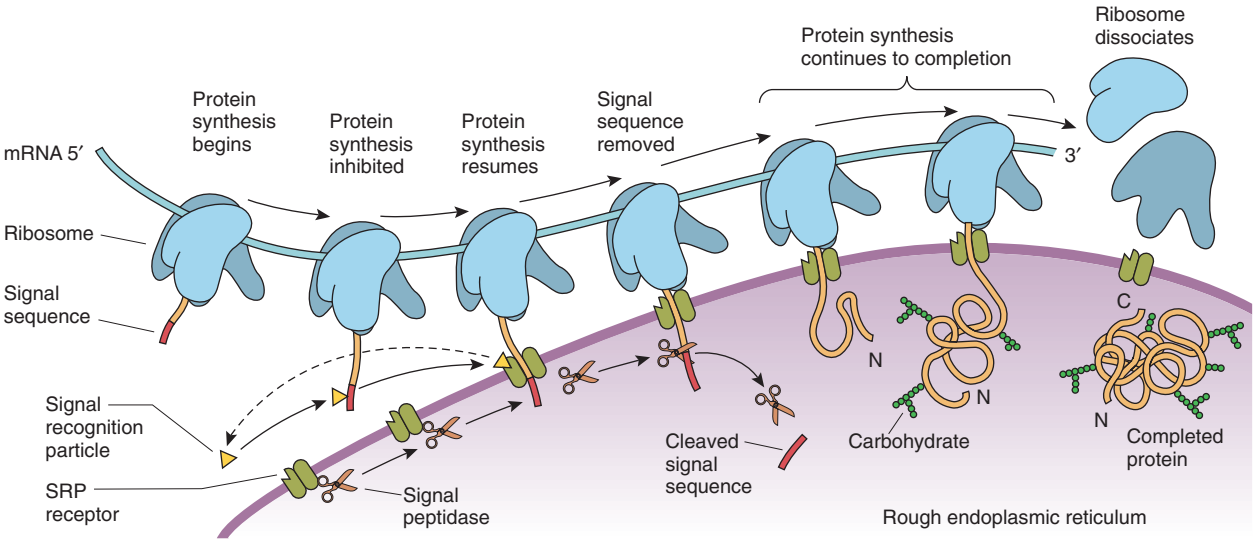


Figure 2–16 Schematic diagram of protein synthesis on the rough endoplasmic reticulum. C, Carboxyl terminus; mRNA, messenger ribonucleic acid; N, amino terminus; SRP, signal recognition particle.

3. The SRP is dislodged, reenters the cytosol, and frees the P site on the small ribosomal subunit. The ribosome remains on the RER surface.
4. As translation resumes, the nascent protein continues to be channeled into the cistern of the RER.
5. An enzyme attached to the cisternal aspect of the RER membrane, known as ***signal peptidase***, cleaves the signal peptide from the forming protein. The signal peptide becomes degraded into its amino acid components.
6. As detailed in the previous section in steps 6 through 8 under the synthesis of cytosolic proteins, when the stop codon is reached, protein synthesis is completed, and the small and large ribosomal subunits dissociate and reenter the cytosol to join the pool of ribosomal subunits.
7. The newly formed proteins are sulfated, folded so that the proteins are no longer linear in shape, glycosylated, and undergo additional posttranslational modifications within the RER cisternae.
8. The modified proteins leave the cistern via small **COP II** coated (coat protein complex II, coatomer II) **transfer vesicles** at regions of the RER known as the ***transitional endoplasmic reticulum*** **(TER)**, and these are elements of the RER devoid of ribosomes.

Golgi Apparatus

The Golgi apparatus functions in the synthesis of carbohydrates and in the modification and sorting of proteins manufactured on the RER.

Proteins manufactured, modified, and packaged in the RER follow a **default pathway** to the Golgi apparatus for posttranslational modification and packaging. Proteins destined to remain in the RER or to go to a compartment other than the Golgi apparatus possess a signal that will divert them from the default pathway.

The Golgi apparatus is composed of one or more series of flattened, slightly curved membrane-bounded **cisternae** (known as faces), the **Golgi stack**, which resemble a stack of pita breads that do not quite contact each other (Figs. 2–17 to 2–19). The periphery of each cisterna is dilated and is rimmed with vesicles that are in the process of either fusing with or budding off that particular compartment. It is now believed that the flattened shape of the faces of the Golgi apparatus and the budding of vesicles from the periphery of the Golgi faces are due to several proteins that are associated with the Golgi. The membranes of the cisternae possess transmembrane proteins known as **phosphatidylinositol-4-phosphate (PtdIns4P)** which bind the **Golgi protein H3 (GOLPH3)**, which, in turn, forms bonds with a special type of myosin, **MYO18A**. This special form of myosin interacts with actin filaments of the cytoskeleton. Therefore, the Golgi faces are connected to F-actin, and, in this fashion, the Golgi faces are stretched along the entire periphery of the cisternae, forming the flattened configuration of the faces of the Golgi apparatus. As vesicles are formed at the periphery of the cisternae, the vesicles migrate along the GOLPH3 to the MYO18A and to the actin filaments, which lead the vesicles to the microtubule "highways" of the cell.

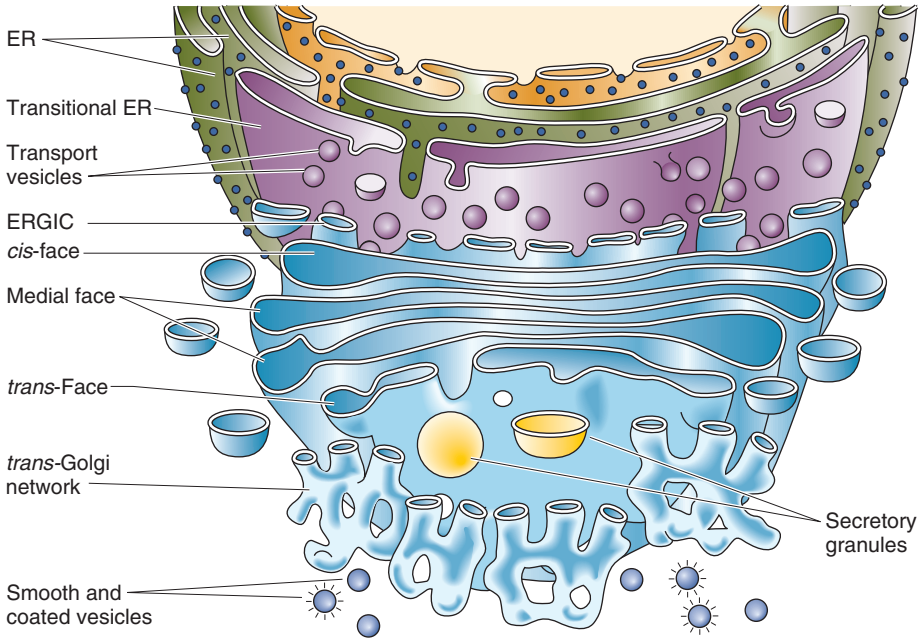


Figure 2–17 Schematic diagram illustrating the rough endoplasmic reticulum (ER) and the Golgi apparatus. Transfer vesicles contain newly synthesized protein and are ferried to the vesicular-tubular clusters, previously known as the endoplasmic reticulum/Golgi intermediate compartment (ERGIC) and from there to the Golgi apparatus. The protein is modified in the various faces of the Golgi complex and enters the *trans*-Golgi network for packaging.

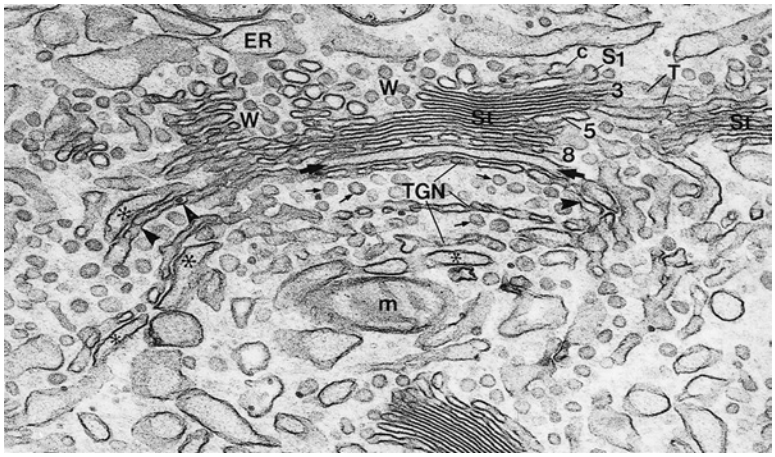


Figure 2–18 Electron micrograph of the Golgi apparatus of the rat epididymis. ER, Endoplasmic reticulum; m, mitochondrion; TGN, *trans*-Golgi network; numbers represent the saccules of the Golgi apparatus. (From Hermo L, Green H, Clermont Y. Golgi apparatus of epithelial principal cells of the ependymal initial segment of the rat: structure, relationship with endoplasmic reticulum, and role in the formation of secretory vesicles. *Anat Rec.* 1991;229:159-176. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

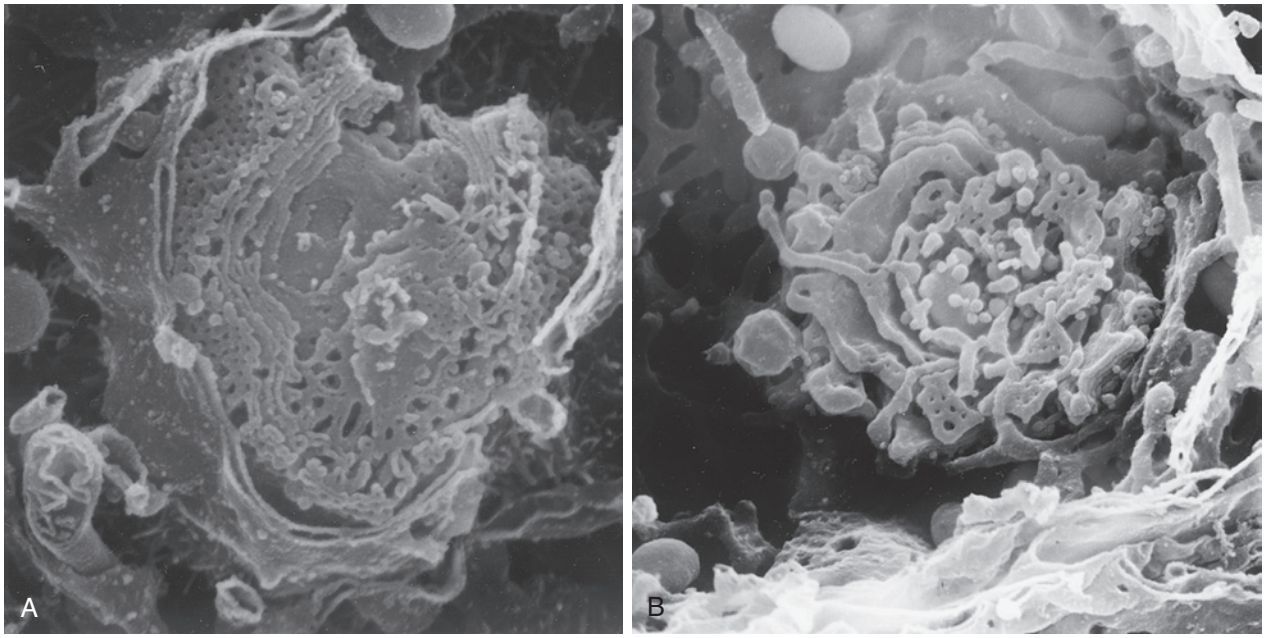


Figure 2–19 **A,** Face view of the *cis* Golgi network in a step 6 spermatid. The *cis*-most saccule is a regular network of anastomotic membranous tubules, capped by the endoplasmic reticulum. Some of the medial saccules with fewer but larger and more irregular pores are visible under the *cis* Golgi saccule. **B,** Face view of another *cis* Golgi network in a step 6 spermatid. Note the fenestration at the edges of the irregular *trans*-Golgi saccules. (From Ho HC, Tang CY, Suarez SS. Three-dimensional structure of the Golgi apparatus in mouse spermatids: a scanning electron microscopic study. *Anat Rec.* 1999;256:189-194. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Each Golgi stack has three levels of cisternae:

- The *cis*-face (and the *cis* Golgi network)
- The medial face (intermediate face)
- The *trans*-face (and the *trans* Golgi network)

The *cis*-face is closest to the RER. It is convex in shape and is considered to be the entry face because newly formed proteins from the RER enter the *cis*-face before they are permitted to enter the other cisternae of the Golgi apparatus. The *trans*-face is concave in shape and is considered to be the exit face because the modified protein is ready to be packaged and to be sent to its destination from here.

There are two additional compartments of interest, one associated with the *cis*-face and the other with the *trans*-face. Located between the RER and the *cis*-face of the Golgi apparatus is an intermediate compartment of vesicles, known as the ***vesicular-tubular clusters*** **(VTC)**, previously known as the ***endoplasmic reticulum/Golgi intermediate compartment*** **(ERGIC)**. The second compartment, known as the ***trans*-Golgi *network*** **(TGN)**, is located at the distal side of the Golgi apparatus. The VTC is a collection of vesicles and tubules formed from the fusion of **transfer vesicles** derived from the **TER**. These transfer vesicles bud off of the TER and contain nascent proteins synthesized on the surface and modified within the cisternae of the RER.

Vesicles derived from the VTC enter the cis Golgi network from which additional vesicles make their way to and fuse with the periphery of the *cis*-face of the Golgi apparatus, thus delivering the protein to this compartment for further modification. The modified proteins are transferred from the *cis* to the medial and finally to the *trans* cisternae (see the next section) via vesicles that bud off and fuse with the rims of the particular compartment (Fig. 2–20). As the proteins pass through the Golgi apparatus, they are modified within the Golgi stacks. Proteins that form the cores of glycoprotein molecules become heavily glycosylated, whereas other proteins acquire or lose sugar moieties.

Mannose phosphorylation occurs within the *cis*-face cisterna, whereas the removal of mannose from certain proteins takes place within the *cis* and medial compartments of the Golgi stack. *N*-acetylglucosamine is added to the protein within the medial cisternae. The addition of sialic acid (*N*-acetylneuraminic acid) and galactose as well as phosphorylation and sulfation of amino acids occurs in the *trans*-face and in the *trans* Golgi network.

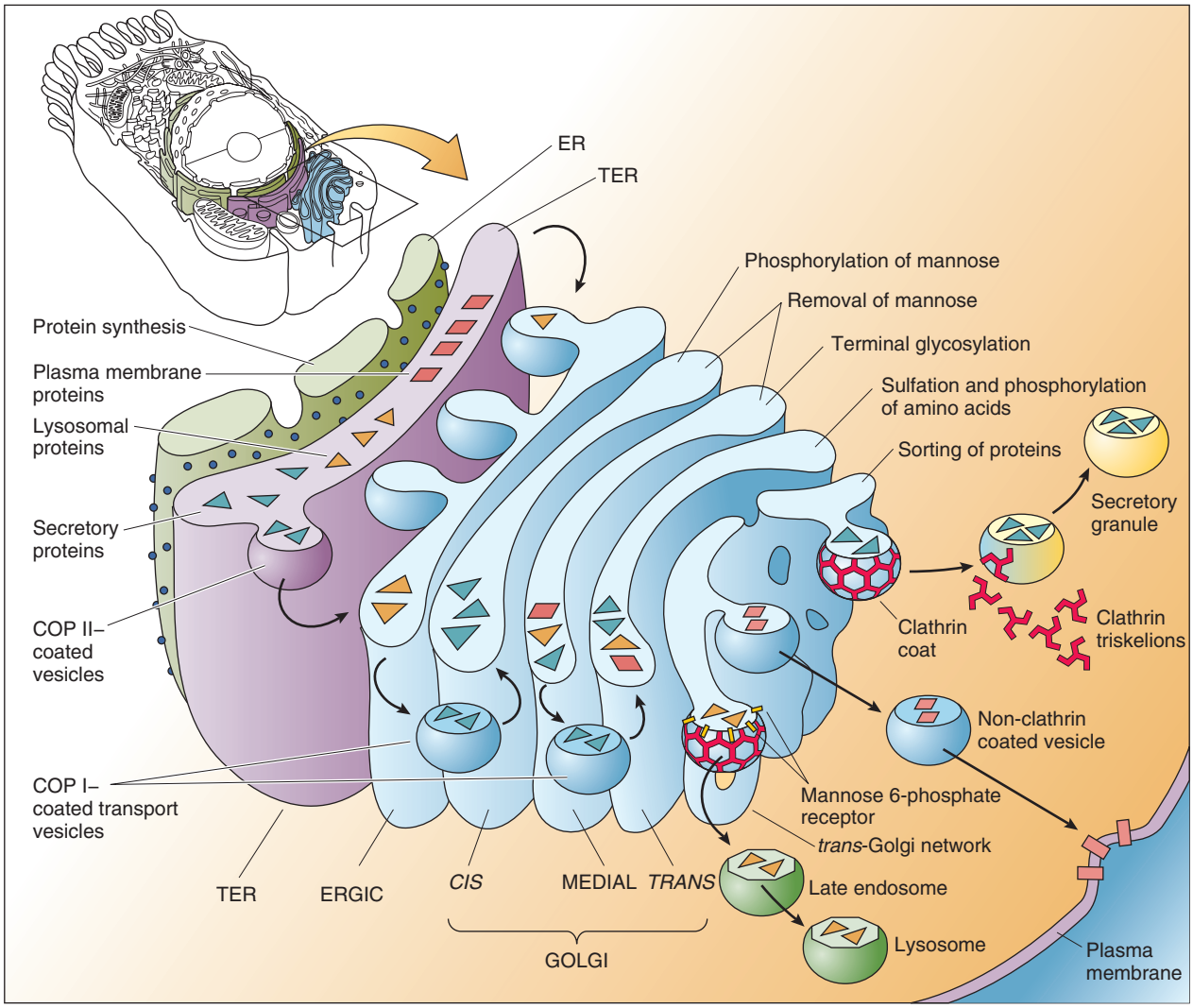


Figure 2–20 Schematic diagram of the Golgi apparatus and packaging in the *trans*-Golgi network. COP, Coatomer; ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum/Golgi intermediate compartment (now known as the VTC); TER, transitional endoplasmic reticulum. (Note that the *cis* Golgi network is not shown; it is between the VTC and the *cis* face.)

Coated Vesicles

Most vesicles possess a protein coat that assist them in reaching their destination; different protein coats are used for different destinations in the cell.

Vesicles that transport proteins **(cargo)** between organelles and regions of organelles must have a way of budding off of the organelle and must be labeled as to their destination. The process of budding is facilitated by the assembly of a proteinaceous coat on the cytosolic aspect of the organelle. Five types of proteins are known to elicit the formation of cargo-bearing vesicles: **coatomer I (COP I)**, **COP II**, **clathrin, retromer,** and **caveolin**. Thus, there are COP I–coated, COP II–coated, clathrin-coated, retromer-coated, and caveolin-coated vesicles. At the site of future vesicle formation, these proteins coalesce, attach to the membrane, draw out the vesicle, and coat its cytosolic surface.

Vesicles that arise from endosomes to be returned to the TGN are coated by retromer. Caveolin-coated vesicles are present in smooth muscle cells and endothelial cells. In the former, they are associated with calcium transfer (see Chapter 8, Muscle), and, in the latter, their functions appear to be those of endocytosis, transcytosis (see Chapter 11, Circulatory System), and cell signaling.

COP I–, COP II–, and clathrin-coated vesicles are discussed in the following sections.

Vesicles Associated with the Golgi Apparatus

Nascent protein-bearing vesicles reach the Golgi apparatus from the TER and deposit the newly formed protein into the Golgi complex for modification within the Golgi cisternae, packaging, and distribution throughout the cell.

Transfer vesicles leaving the transitional ER are always COP II–coated until they reach the VTC, where they shed their COP II coat, which is recycled. It is believed by most investigators that vesicles that arise from the VTC to carry recently delivered cargo to the *cis* Golgi network are coated by COP I, as are all other vesicles that proceed through the *cis*-, medial, and to the *trans*-face and to the TGN. Most of the vesicles that arise from the TGN, however, are coated by **clathrin** for their formation. The transport mechanism has a quality control aspect, in that if RER (or transitional ER) resident proteins are packaged in vesicles, and these "stowaway" molecules reach the VTC, they are returned to the RER in **COP I–coated** vesicles. This is referred to as ***retrograde transport*** in contrast to **anterograde transport** of cargo, that was just described.

Proteins that are destined to be transported out of the RER do not have the additional four amino acids, known as the ***KDEL sequence*** (lysine, asparagine, glutamine, and leucine), attached to their C terminus. However, if proteins bearing the KDEL sequence escape from the RER, they are returned via the retrograde pathway in COP I–coated vesicles.

Because these vesicles are formed at a particular site in the cell and must reach their destination, an additional set of information should be considered, namely, how the vesicles are transported to their destination. Although these are interesting concepts to contemplate, the complexity of the mechanism precludes a complete discussion here; instead, a cursory overview is presented. (For more information, consult a textbook on cell biology.)

As the cargo-containing vesicles form, they possess not only a coatomer or clathrin coat but also other surface markers and receptors. Some of these receptors interact with microtubules and the motor protein complexes that are responsible for vesicle movement. As discussed in the following section on the cytoskeleton, microtubules are long, straight, rigid, tubule-like structures that originate in the **microtubule organizing center (MTOC)** and extend to the cell periphery. The major MTOC of the cell is known as the *centrosome,* and it houses a pair of centrioles embedded in a matrix of proteins rich in γ-tubulin ring complexes.

The MTOC is located in the vicinity of the Golgi complex, and these ends of the microtubules, each emanating from a γ-tubulin ring complex, are referred to as the ***minus end***; the other end of each microtubule, near the periphery of the cell, is the **plus end**. The molecular motor that drives vesicles to the minus end (toward the MTOC) is dynein and its accessory protein complex. The molecular motor that drives vesicles toward the positive end (away from the MTOC) is kinesin and its associated protein complex. Thus, vesicles derived from the ER as well as from the VTC are driven toward the MTOC and are driven by dynein, whereas vesicles that leave the Golgi complex in a retrograde direction to the VTC or to the RER are driven by kinesin.

Sorting in the Trans*-Golgi Network*

The TGN is responsible for the sorting of proteins to their respective pathways so that they reach the plasma membrane, secretory granules, or lysosomes.

Cargo that leaves the TGN is enclosed in vesicles that may do one of the following (see Fig. 2–20):

- Insert into the cell membrane as membrane proteins and lipids
- Fuse with the cell membrane such that the protein they carry is *immediately* released into the extracellular space
- Congregate in the cytoplasm near the apical cell membrane as **secretory granules (vesicles)**, and, upon a given signal, fuse with the cell membrane for *eventual* release of the protein outside of the cell
- Fuse with **late endosomes** (see later), releasing their content into that organelle, which then becomes a lysosome

The first three processes are known as ***exocytosis*** because material leaves the cytoplasm proper. Neither immediate release into the extracellular space nor insertion into the cell membrane requires a particular regulatory process; thus, both processes are said to follow the **constitutive secretory pathway (default pathway)**. In contrast, the pathways to lysosomes and to secretory vesicles are known as the ***regulated secretory pathway***.

Transport of Lysosomal Proteins

The sorting process begins with the phosphorylation of mannose residues of the lysosomal proteins (lysosomal hydrolases) in the *cis* cisterna of the Golgi stack. When these proteins reach the TGN, their **mannose-6-phosphate (M6P)** is recognized as a signal, and they become bound to **mannose-6-phosphate receptors**, transmembrane proteins of the TGN membrane.

A small pit is formed with the assistance of **clathrin triskelions**, protein complexes composed of three heavy and three light chains forming a structure with three arms that radiate from a central point (Fig. 2–21; see Fig. 2–20). The triskelions self-assemble, coating the cytoplasmic aspect of the TGN rich in M6P receptors to which M6P is bound. As the pit deepens, it pinches off the TGN and forms a **clathrin-coated**

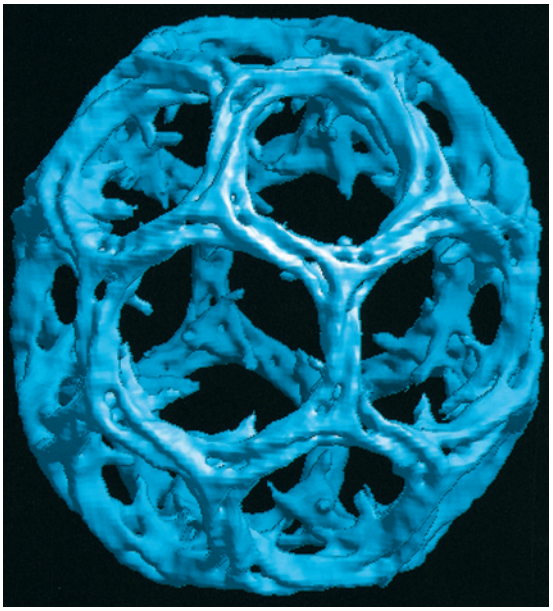


Figure 2–21 A map of clathrin coat at 21 Å resolution. To allow a clear view of the path of the triskelion legs, the amino-terminal domain and most of the linker have been removed from this map. (From Smith CJ, Grigorieff N, Pearse BM. Clathrin coats at 21 Å resolution: a cellular assembly designed to recycle multiple membrane receptors. *Embo J.* 1998;17:4943-4953. Reprinted with permission of Oxford University Press.)

vesicle. The clathrin coat, also referred to as the ***clathrin basket***, is composed of 36 triskelion molecules that completely envelop the vesicle.

The clathrin-coated vesicle quickly loses its clathrin coat, which, unlike the formation of the clathrin basket, is an energy-requiring process. The uncoated vesicle reaches, fuses with, and releases its contents into the late endosome (see later section on endosomes).

Because clathrin coats are used for many other types of vesicles, an intermediary protein complex, known as an ***adaptor* (adaptin complex)**, composed of four of the six types of the protein **adaptin**, is interposed between the cytoplasmic aspect of the receptor molecule and the clathrin. Many different types of adaptors exist, and each has a binding site for a particular receptor as well as a binding site for clathrin.

Transport of Regulated Secretory Proteins

Proteins that are to be released into the extracellular space in a discontinuous manner also require the formation of clathrin-coated vesicles. The signal for their formation is not known; however, the mechanism is believed to be similar to that for lysosomal proteins.

Unlike vesicles that ferry lysosomal enzymes, secretory granules are quite large and carry many more proteins than there are receptors on the vesicle surface. Additionally, the contents of the secretory granules become condensed with time as a result of the loss of fluid from the secretory granules (see Figs. 2–6 and 2–20). During this process of increasing concentration, these vesicles are frequently referred to as ***condensing vesicles***. Moreover, secretory granules of polarized cells remain localized in a particular region of the cell. They remain as clusters of secretory granules that, in reaction to a particular signal (e.g., neurotransmitter or hormone), fuse with the cell membrane to release their contents into the extracellular space.

Transport Along the Constitutive Pathway

All vesicles that participate in nonselective transport, such as those passing between the RER and the *cis* Golgi network or among the cisternae of the Golgi stack or using the constitutive pathway between the TGN and the plasma membrane, also require a coated vesicle (see Fig. 2–20). However, as indicated previously, the coating is composed of coatomer instead of clathrin.

Vesicles derived from the TGN are driven along microtubule tracts by the use of kinesin and its associated protein complex. However, these vesicles also use an alternative, and perhaps their primary pathway, of actin filaments. The motor that drives these vesicles is myosin II; it is believed that myosin II is brought to the TGN subsequent to, or in conjunction with, the recruitment of the clathrin triskelions to the site of vesicle formation.

Alternative Concept of the Golgi Apparatus

An alternative concept of the Golgi apparatus suggests the process of cisternal maturation instead of anterograde vesicle transport.

The two predominant theories of **anterograde vesicle transport** and **cisternal maturation** are mutually incompatible, and ample evidence exists to support both theories. The theory of cisternal maturation suggests that instead of the cargo being ferried through the various regions of the Golgi apparatus, it remains stationary, and the various enzyme systems of the Golgi are transported in a retrograde fashion in the correct sequence and at the designated time, so that a given sedentary cisterna matures into the subsequent cisternae.

At first glance, the cisternal maturation theory may appear to be dubious; however, it may be illustrated with a commonly observed phenomenon. If one is sitting in a stationary train and watches another stationary train on the neighboring railroad track when one of the trains begins to move, it is difficult initially to determine which train is moving, and without external visual aids we cannot make a reasonable determination. The current state of research cannot determine which of the two theories is correct, but most histology and cell biology textbooks favor the anterograde vesicle transport theory.

Endocytosis, Endosomes, and Lysosomes

Endocytosis, endosomes, and lysosomes are involved in the ingestion, sequestering, and degradation of substances internalized from the extracellular space.

The process whereby a cell ingests macromolecules, particulate matter, and other substances from the extracellular space is referred to as ***endocytosis***. The endocytosed material is engulfed in a vesicle appropriate for its volume. If the vesicle is large (>250 nm in diameter), the method is called ***phagocytosis*** (cell eating), and the vesicle is a **phagosome**. If the vesicle is small (<150 nm in diameter), the type of endocytosis is called ***pinocytosis*** (cell drinking), and the vesicle is a **pinocytotic vesicle**.

Endocytotic Mechanisms

Endocytosis is divided into two categories: phagocytosis and pinocytosis.

Phagocytosis

The process of engulfing larger particulate matter, such as microorganisms, cell fragments, and cells (e.g., defunct red blood cells), is usually performed by specialized cells known as ***phagocytes***. The most common phagocytes are the white blood cells, **neutrophils,** and **monocytes**. When monocytes leave the bloodstream and enter the connective tissue domain to perform their task of phagocytosis, they become known as ***macrophages***.

Phagocytes can internalize particulate matter because they possess receptors that recognize certain surface features of the material to be engulfed. Two of the better understood of these surface features come from the study of immunology and are the **constant regions (Fc regions)** of antibodies and a blood-borne series of proteins known as ***complement***. Because the variable region of the antibody binds to the surface of a microorganism, the Fc region projects away from its surface.

Macrophages and neutrophils possess Fc receptors that bind the Fc regions of the antibody upon contact. This relationship acts as a signal for the cell to extend pseudopods, surround the microorganism, and internalize the microorganism by forming a **phagosome**. Complement on the surface of the microorganism probably assists phagocytosis in a similar manner because macrophages also possess complement receptors on their surface. Interaction between complement and its receptor presumably activates the cell to form pseudopods and engulf the invading microorganism.

Pinocytosis

Because most cells export substances into the extracellular space, they continually add the membranes of vesicles that transport those substances from the TGN to the plasma membrane. These cells, in order to maintain their shape and size, must continually remove the excess membrane and return it for recycling. This cycle of membrane shuffling during exocytosis and endocytosis is known as ***membrane trafficking***, the movement of membranes to and from various compartments of the cell. In most cells, pinocytosis is the most active transporting process and contributes most to the recapturing of membranes (Fig. 2–22).

Receptor-Mediated Endocytosis

Many cells specialize in the pinocytosis of several types of macromolecules. The most efficient form of capturing these substances depends on the presence of receptor proteins **(cargo receptors)** in the cell membrane. Cargo receptors are transmembrane proteins that become associated with the particular macromolecule **(ligand)** extracellularly and with a **clathrin coat** intracellularly (see Fig. 2–20).

The assembly of clathrin triskelions beneath the cargo receptors pulls on the plasma membrane, forming a clathrin-coated pit (Figs. 2–23 and 2–24), which eventually becomes a **pinocytotic vesicle**, enclosing the ligand as a droplet of fluid about to drip from a surface. To release this pinocytotic vesicle, several molecules of **dynamin**, a GTPase, surrounds the constricted neck of the vesicle, pinches its neck closed, and the pinocytotic vesicle is released from its membrane origin into the cytoplasm. This method of endocytosis, known as ***receptor-mediated endocytosis***, permits the cell to increase the concentration of the ligand (e.g., low-density lipoprotein) within the pinocytotic vesicle.

A typical pinocytotic vesicle may have as many as 1000 cargo receptors of several types because they may bind different macromolecules. Each cargo receptor is linked to its own **adaptin**, the protein with a binding site for the cytoplasmic aspect of the receptor as well as a binding site for the clathrin triskelion.

Endosomes

Endosomes are divided into two compartments: early endosomes, near the periphery of the cell, and late endosomes, situated deeper within the cytoplasm.

Shortly after their formation, pinocytotic vesicles lose their clathrin coats (which return to the pool of clathrin triskelions in the cytosol) and fuse with **early endosomes** (Fig. 2–25; see Fig. 2–22), a system of vesicles and tubules located near the plasma membrane. If the entire content of the pinocytotic vesicle requires degradation, the material from the early endosome is transferred to a **late endosome**. This similar set of tubules and vesicles, located deeper in the cytoplasm near the Golgi apparatus, helps prepare its contents for eventual destruction by lysosomes.

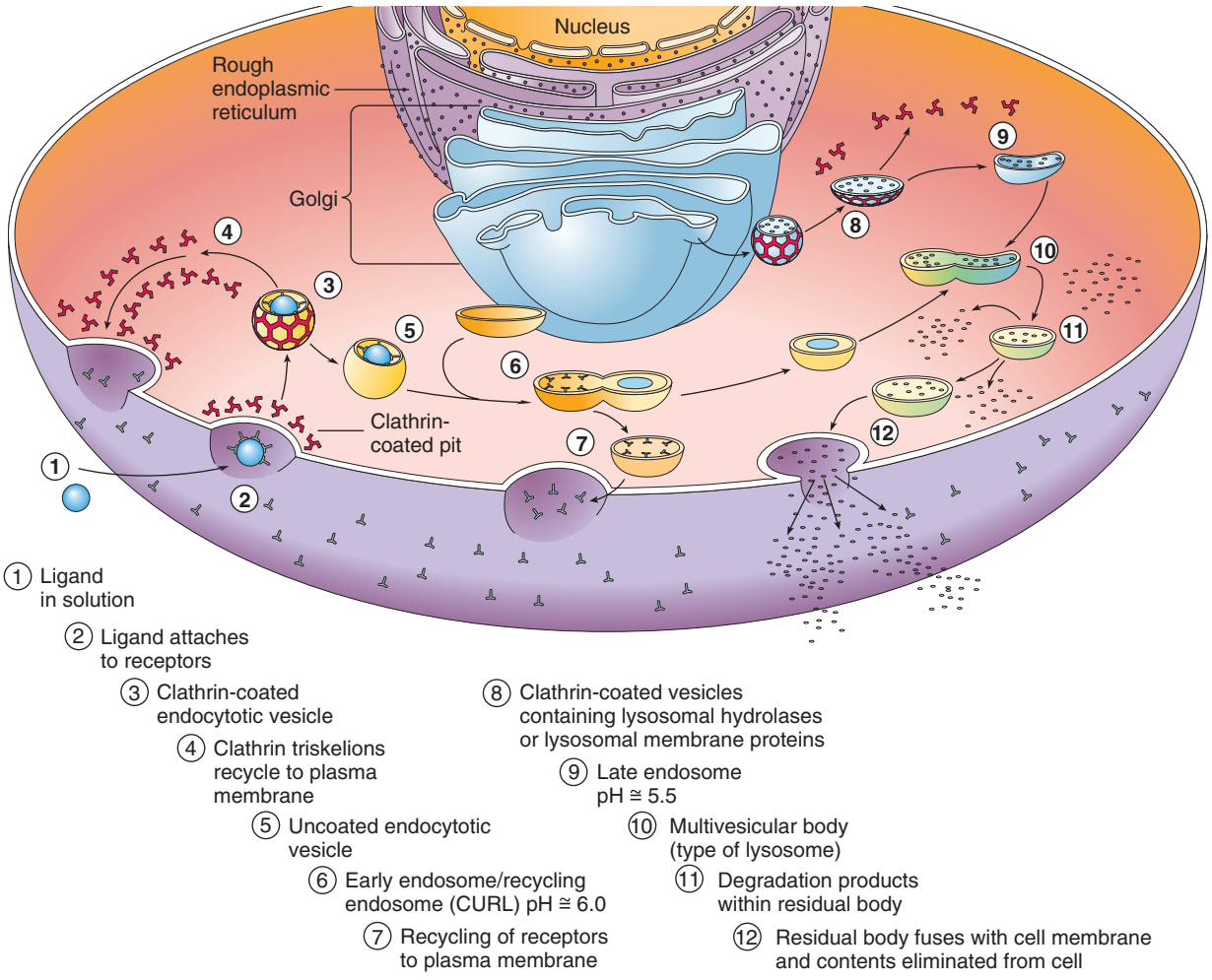


Figure 2–22 Schematic diagram illustrating the endosomal pathways. CURL, Compartment for uncoupling of receptor and ligand.

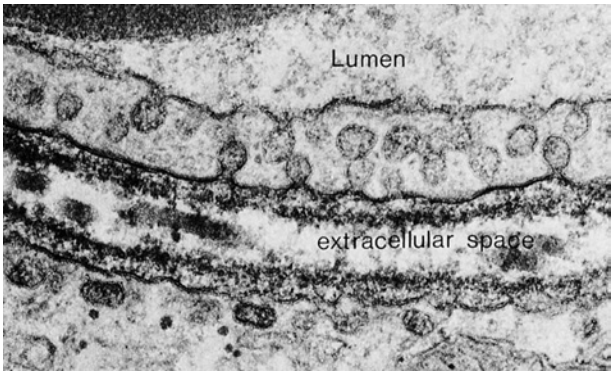


Figure 2–23 Electron micrograph of endocytosis in a capillary. (From Hopkins CR. *Structure and Function of Cells.* Philadelphia: WB Saunders; 1978.)

Early and late endosomes collectively constitute the **endosomal compartment**. The membranes of all endosomes contain ATP-linked H^+ pumps that acidify the interior of the endosomes by actively pumping H^+ ions into the interior of the endosome so that the early endosome has a pH of 6.0, and the late endosome has a pH of 5.5.

Material entering the early endosome may be retrieved from that compartment and returned to its earlier location, as occurs with cargo receptors that need to be recycled. When a pinocytotic vesicle fuses with the early endosome, the acidic environment causes an uncoupling of the ligand from its receptor molecule. The ligand remains within the lumen of the early endosome, whereas the receptor molecules (e.g., low-density

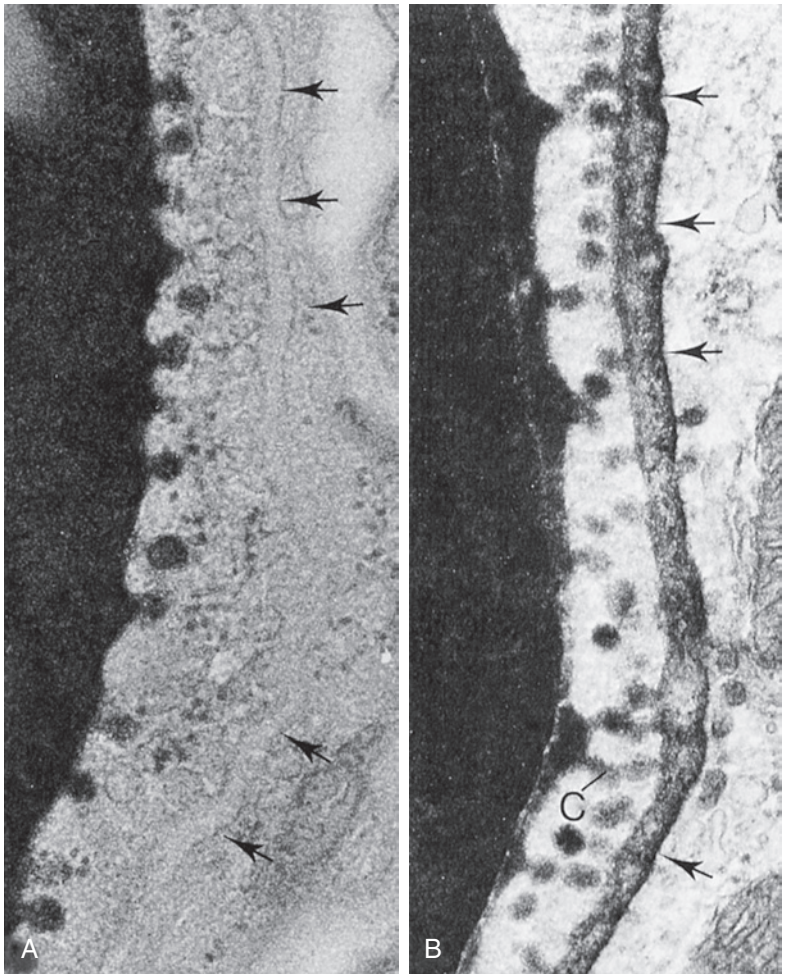


Figure 2–24 Electron microscopy of transport of microperoxidase, a trace molecule, across the endothelial cell of a capillary (×35840). **A,** The lumen of the capillary is filled with the tracer; note its uptake of pinocytotic vesicles on the luminal aspect and the absence of tracer in the extracellular space (demarcated by *arrows*). **B,** One minute later, the tracer has been conveyed across the endothelial cell and exocytosed on the connective tissue side into the extracellular space (demarcated by *arrows*). The letter C indicates a region of fused vesicles, forming a temporary channel between the lumen of the capillary and the extracellular space. (From Hopkins CR. *Structure and Function of Cells.* Philadelphia: WB Saunders; 1978.)

lipoprotein receptors) are returned to the plasma membrane where they originated, or to the plasma membrane of another region of the cell, a process known as *transcytosis*. Some authors refer to this type of early endosome as a compartment for uncoupling of receptor and ligand or, more recently, as a **recycling endosome** (see Figs. 2–22 and 2–25).

Within 10 to 15 minutes of entering the early endosome, the ligand is either transferred to a late endosome (as in the case of low-density lipoprotein) or packaged to be returned to the cell membrane, where it is released (e.g., transferrin) into the extracellular space. Occasionally, both the receptor and the ligand (e.g., epidermal growth factor and its receptor) are transferred to the late endosome, and then to a lysosome, for eventual degradation.

The transport between early and late endosomes has not been elucidated. Some authors suggest that early endosomes migrate along microtubule pathways into a deeper location within the cell and become late endosomes. Others postulate that early and late endosomes are two separate compartments and that specific **endosomal carrier vesicles** ferry material from early to late endosomes. These are believed to be large vesicles containing numerous small vesicles that have been noted as **multivesicular bodies** in electron micrographs.

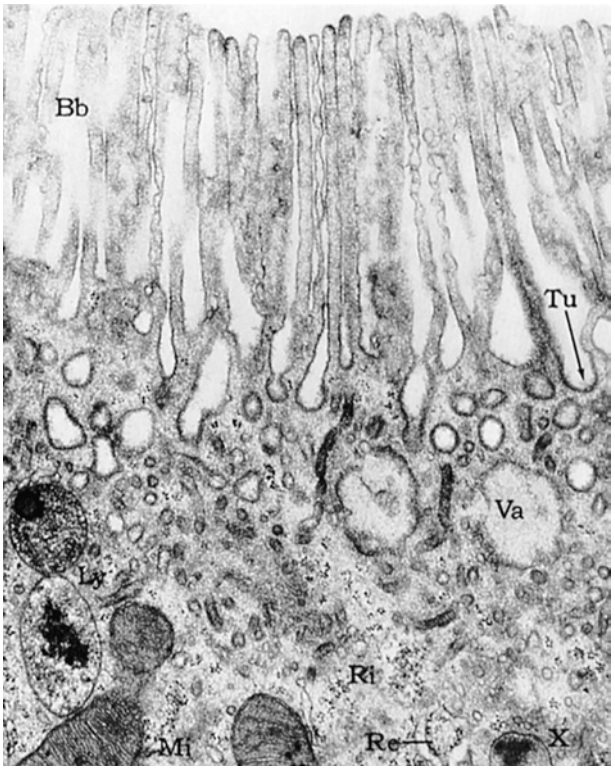


Figure 2–25 Endocytotic vesicles (Tu) of the proximal tubule cell of the kidney cortex. Note the presence of microvilli (Bb), lysosome (Ly), mitochondria (Mi), rough endoplasmic reticulum (Re), free ribosomes (Ri), and, possibly, early endosomes (Va) (×25000). (From Rhodin JAG. *An Atlas of Ultrastructure.* Philadelphia: WB Saunders; 1963.)

Both theories recognize the presence of a system of microtubules along which either the early endosome or the endosomal carrier vesicle negotiates its way to the late endosome.

Lysosomes

Lysosomes have an acidic pH and contain hydrolytic enzymes.

The contents of late endosomes are delivered for enzymatic digestion into the lumina of specialized organelles known as **lysosomes** (Fig. 2–26; see Fig. 2–25). Each lysosome is round to polymorphous in shape. Its average diameter is 0.3 to 0.8 µm, and it contains at least 40 different types of **acid hydrolases**, such as sulfatases, proteases, nucleases, lipases, and glycosidases, among others. Because all of these enzymes require an acid environment for optimal function, lysosomal membranes possess proton pumps that actively transport H^+ ions into the lysosome, maintaining its lumen at a pH of about 5.0 (see Fig. 2–22).

Lysosomes aid in digesting not only macromolecules, phagocytosed microorganisms, cellular debris, and cells but also excess or senescent organelles, such as mitochondria and RER. The various enzymes digest the engulfed material into small, soluble end products that are transported by carrier proteins in the lysosomal membrane from the lysosomes into the cytosol and are either reused by the cell or exported from the cell into the extracellular space.

Formation of Lysosomes

Lysosomes receive their hydrolytic enzymes as well as their membranes from the TGN; however, they arrive in different vesicles. Although both types of vesicles possess a clathrin coat as they pinch off the TGN, the clathrin coat is lost shortly after formation. The uncoated vesicles then fuse with late endosomes.

Vesicles ferrying lysosomal enzymes possess **mannose-6-phosphate receptors**, to which these enzymes are bound. In the acidic environment of the late endosome, the lysosomal enzymes dissociate from their receptors, their mannose residue becomes dephosphorylated, and the receptors are recycled by being returned to the TGN. It should be understood that the dephosphorylated lysosomal hydrolases can no longer bind to the mannose-6-phosphate receptors and therefore stay in the late endosome (see Figs. 2–20 and 2–22). Thus, proenzymes as well as lysosomal membrane proteins are present in late endosomes.

When late endosomes possess both enzymatic and membrane components, some authors hypothesize that the late endosome fuses with a lysosome. However, others suggest that it matures to become a lysosome.

Transport of Substances Into Lysosomes

Substances destined for degradation within lysosomes reach these organelles in one of three ways: through phagosomes, pinocytotic vesicles, or autophagosomes (see Fig. 2–22).

Phagocytosed material, contained within **phagosomes**, moves toward the interior of the cell. The phagosome either joins a lysosome or a late endosome. The hydrolytic enzymes digest most of the contents of the phagosome, especially the protein and carbohydrate components. Lipids, however, are more resistant to complete digestion, and they remain enclosed within the spent lysosome, now referred to as a ***residual body***.

Senescent organelles, such as mitochondria and organelles no longer required by the cell, or the RER of a quiescent fibroblast, need to be degraded. The organelles in question become surrounded by elements of the ER and are enclosed in vesicles called ***autophagosomes***. These structures fuse either with late endosomes or with lysosomes and share the same subsequent fate as the phagosome.

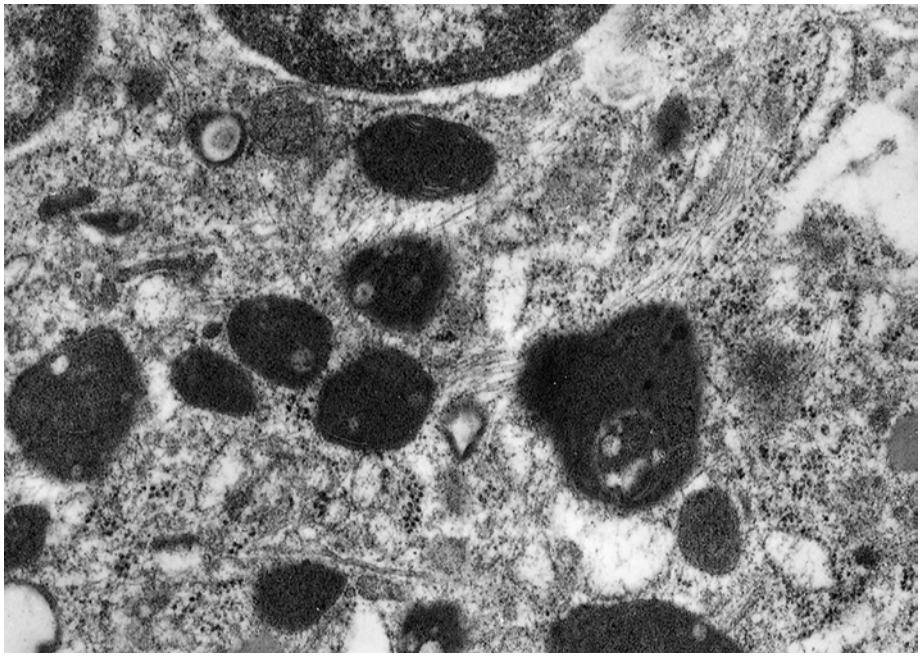


Figure 2–26 Lysosomes of rat cultured alveolar macrophages (×45000). (From Sakai M, Araki N, Ogawa K. Lysosomal movements during heterophagy and autophagy: with special reference to nematolysosome and wrapping lysosome. *J Electron Microsc Tech*. 1989;12:101-131. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

CLINICAL CORRELATIONS

Lysosomal Storage Disorders

Certain individuals with hereditary enzyme deficiencies are incapable of completely degrading various macromolecules into soluble by-products. As the insoluble intermediaries of these substances become amassed within the lysosomes of their cells, the size of these lysosomes increases sufficiently to interfere with the abilities of these cells to perform their function (Table 2–1).

Probably the most commonly known of these conditions is **Tay-Sachs disease**, occurring mostly in children of Northeast European Jewish ancestry and in certain individuals of Cajun ancestry in Louisiana. These children display a deficiency in the enzyme hexosaminidase and cannot catabolize GM_2 gangliosides. Although most cells in these children accumulate GM_2 ganglioside in the lysosomes, it is the neurons in their central and peripheral nervous systems that are the most problematic. Lysosomes of these cells become so engorged that they interfere with neuronal function, causing the children to become vegetative within the first year or two and to die by the third year of life.

Peroxisomes

Peroxisomes are self-replicating organelles that contain oxidative enzymes.

Peroxisomes (microbodies) are small (0.2 to 1.0 µm in diameter), spherical to ovoid membrane-bound organelles that contain more than 40 oxidative enzymes, especially **urate oxidase, catalase,** and **D-amino acid oxidase** (Fig. 2–27). They are present in almost all animal cells and function in the catabolism of long-chained fatty acids **(beta oxidation)**, forming **acetyl coenzyme A (CoA)**, begin the manufacture of plasmalogen (the primary myelin phospholipid), and form **hydrogen peroxide (H_2O_2)** by combining hydrogen from the fatty acid with molecular oxygen. Acetyl CoA is used by the cell for its own metabolic needs or is exported into the extracellular space to be used by neighboring cells. Hydrogen peroxide detoxifies various noxious agents (e.g., ethanol) and kills microorganisms. Excess hydrogen peroxide is degraded into water and molecular oxygen by the enzyme **catalase**.

Proteins destined for peroxisomes are not manufactured on the RER but in the cytosol and are transported into the peroxisomes by two specific peroxisome targeting signals that direct the protein from the cytosol to

TABLE 2–1 Major Lysosomal Storage Diseases

Disease Type	Disease Name	Enzyme Deficiency	Metabolite Buildup
Glycogenosis	Pompe's disease (type II)	Lysosomal glucosidase	Glycogen
Sphingolipidosis	GM_1-gangliosidoses	GM_1-ganglioside beta-galactosidase	GM_1 ganglioside; oligosaccharides containing galactose
Sphingolipidosis	GM_2-gangliosidoses (Tay-Sachs disease)	Hexosaminidase A	GM_2-ganglioside
Sphingolipidosis	GM_2-gangliosidoses (Gaucher's disease)	Glucocerebrosidase	Glucocerebroside
Sphingolipidosis	GM_2-gangliosidoses (Neimann-Pick disease)	Sphingomyelinase	Sphingomyelin
Mucopolysaccharidosis	MPS I H (Hurler)	α-L-iduronidase	Heparan and dermatan sulfate
Mucopolysaccharidosis	MPS II (Hunter)	L-iduronosulfate sulfatase	Heparan and dermatan sulfate
Glycoproteinosis		Enzymes that degrade polysaccharide side chains of glycoproteins	Several, depending on enzyme

Modified from Kumar V, Cotran RS, Robbins SL. *Basic Pathology.* 5th ed. Philadelphia: WB Saunders; 1992.

the peroxisome where they recognize membrane-bound import receptors unique to the targeting signal. However, some peroxisomal membrane proteins may be manufactured on and targeted to the peroxisomes via the RER. Similar to mitochondria, peroxisomes increase in size and undergo fission to form new peroxisomes; however, they possess no genetic material of their own.

Proteasomes

Proteasomes are small organelles composed of protein complexes that are responsible for proteolysis of malformed and ubiquitin-tagged proteins.

The protein population of a cell is in a constant flux as a result of the continuous synthesis, export, and degradation of these macromolecules. Frequently proteins, such as those that act in metabolic regulation, have to be degraded to ensure that the metabolic response to a single stimulus is not prolonged. Additionally, proteins that have been denatured, damaged, or malformed have to be eliminated; moreover, antigenic proteins that have been endocytosed by APCs have to be cleaved into small polypeptide fragments **(epitopes)** so that they can be presented to T lymphocytes for recognition and the mounting of an immune response.

The process of cytosolic proteolysis is carefully controlled by the cell, and it requires that the protein be recognized as a potential candidate for degradation. This recognition involves **ubiquitination,** a process whereby several ubiquitin molecules (76-amino acid long-polypeptide chains) are attached to a lysine residue of the candidate protein to form a **polyubiquitinated protein.** Once a protein has been thus tagged, it is degraded by **proteasomes,** multisubunit protein complexes that have a molecular weight in excess of 2 million Da. An average cell may have as many as 30,000 protasomes. In order to be permitted entry into the proteasome (**translocated** into the proteasome), the ubiquitin molecules must be released from the protein, and the protein must be unfolded. The released ubiquitin molecules reenter the cytosolic pool. The mechanism of ubiquitination requires

- The cooperation of a series of enzymes, including **ubiquitin-activating enzyme**
- A family of **ubiquitin-conjugating enzymes**
- A number of **ubiquitin ligases**, each of which recognizes one or more substrate proteins

Ubiquitination, the release of ubiquitin from the candidate protein, and the mechanism of protein degradation by the proteasome are all energy-requiring processes. Each proteasome resembles a 15-nm tall barrel, by 12 nm in diameter with a central lumen between 1.3 and 5.3 nm in diameter. Both the top and bottom of the barrel possess a **regulatory particle** that limit entrance into and exit out of the proteasome, whereas the bulk of the proteasome is known as the ***core particle***. Two pairs of subunits constitute the core particle, the α units that form the top and the bottom of the core particle, each of which binds the regulatory particles, and the β units that form the bulk of the core particle. It is the β units whose internal regions digest the proteins delivered to the proteasome, into small polypeptides, seven to eight amino acids in length.

It should be noted that ubiquitination can be skipped under exceptional circumstances when the cell is placed into conditions of major stress; during high stress levels,

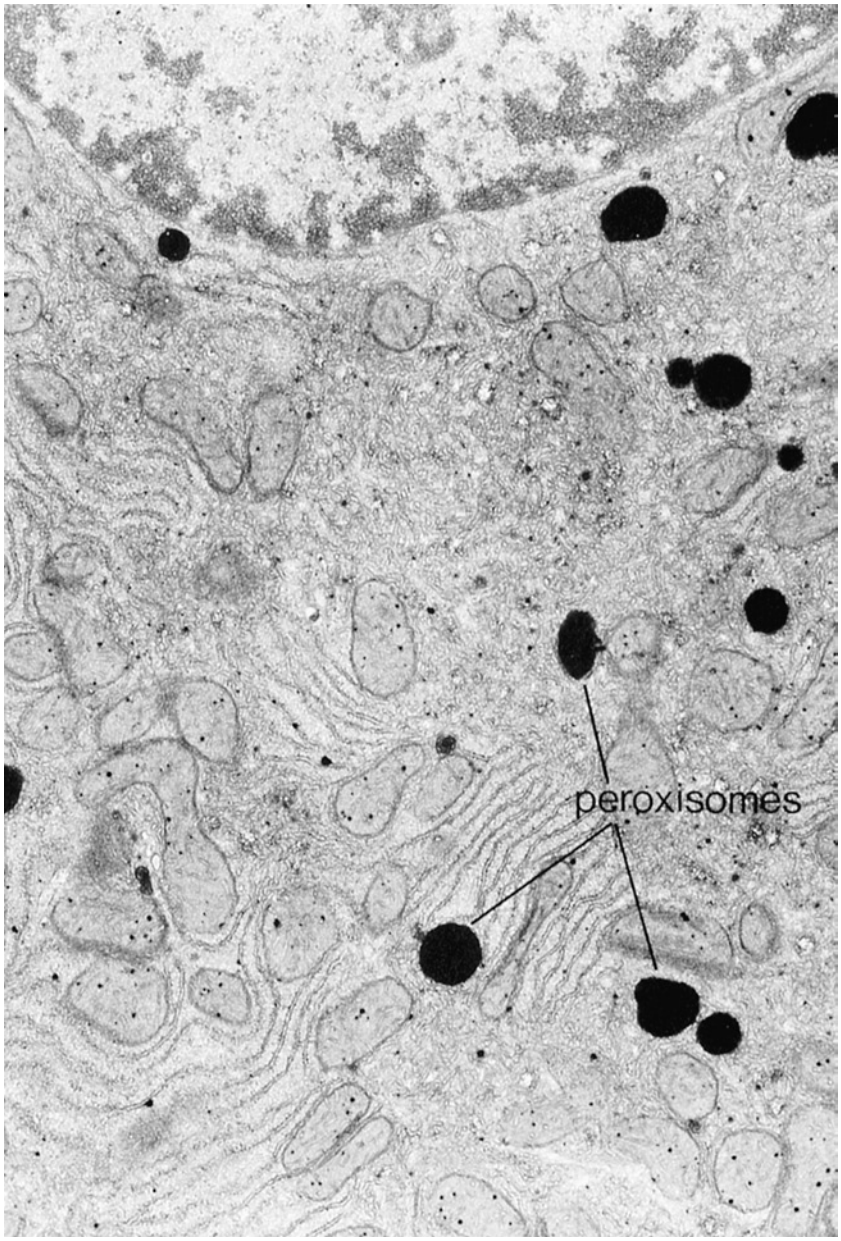


Figure 2–27 Peroxisomes in hepatocytes (×10700). The cells were treated with 3′,3′-diaminobenzidine and osmium tetroxide, yielding a black reaction product caused by the enzyme catalase located within peroxisomes. (From Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

proteasomes will degrade certain proteins in the absence of ubiquitination.

Mitochondria

Mitochondria possess their own DNA and perform oxidative phosphorylation and lipid synthesis.

Mitochondria are flexible, rod-shaped organelles, about 0.5 to 1 μm in girth and sometimes as much as 7 μm in length. Most animal cells possess a large number of mitochondria (as many as 2000 in each liver cell) because, via **oxidative phosphorylation**, they produce **ATP**, a stable storage form of energy that can be used by the cell for its various energy-requiring activities.

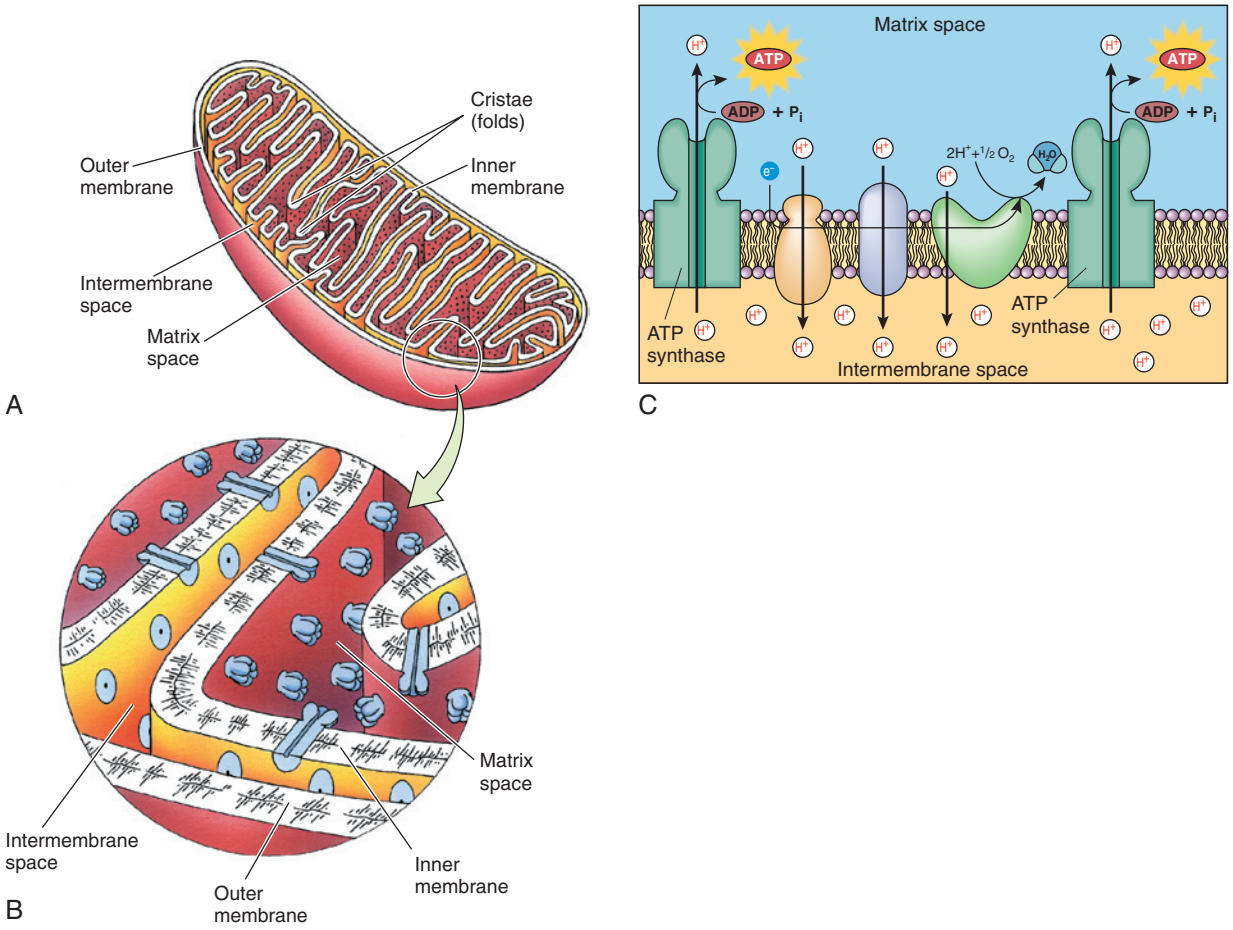


Figure 2–28 Diagrams illustrating the structure and function of mitochondria. **A,** Mitochondrion sectioned longitudinally to demonstrate its outer and folded inner membranes. **B,** Diagram of a negatively stained preparation at higher magnification of the region circled in *A*, displaying the inner membrane subunits, adenosine triphosphate (ATP) synthase. **C,** Diagram displaying two ATP synthase complexes and three of the five members of the electron transport chain that also function to pump hydrogen (H^+) from the matrix into the intermembrane space.

Each mitochondrion possesses a smooth **outer membrane** and a folded **inner membrane** (Fig. 2–28; see Fig. 2–6). The folds of the inner membrane, known as ***cristae***, greatly increase the surface area of that membrane. The number of cristae possessed by a mitochondrion is related directly to the energy requirement of the cell; thus, a cardiac muscle cell mitochondrion has more cristae than an osteocyte mitochondrion has. The narrow space (10 to 20 nm in width) between the inner and outer membranes is called the ***intermembrane space***, whereas the large space enclosed by the inner membrane is termed the ***matrix space*** **(intercristal space)**. The contents of the two spaces differ somewhat and are discussed in the following paragraphs.

Outer Mitochondrial Membrane and Intermembrane Space

The **outer mitochondrial membrane** possesses a large number of **porins**, multipass transmembrane proteins. Each porin forms a large aqueous channel through which water-soluble molecules, as large as 10 kD, may pass. Because this membrane is relatively permeable to small molecules, including proteins, the contents of the **intermembrane space** resemble those of the cytosol. Additional proteins located in the outer

membrane are responsible for the formation of mitochondrial lipids.

Inner Mitochondrial Membrane

The inner mitochondrial membrane is folded into cristae to provide a larger surface area for ATP synthase and the respiratory chain.

The inner mitochondrial membrane, which encloses the matrix space, is folded to form cristae. This membrane is richly endowed with **cardiolipin**, a phospholipid that possesses four, rather than the usual two, fatty acyl chains. The presence of this phospholipid in high concentration makes the inner membrane nearly impermeable to ions, electrons, and protons.

In certain regions, the outer and inner mitochondrial membranes contact each other; these **contact sites** act as pathways for some proteins and small molecules to enter and leave the matrix space. The contact sites are composed of carrier proteins for the transport and regulatory proteins for the recognition of markers denoting the transportability of the specific macromolecules. These same contact sites are also used for the transport of proteins into the intermembrane space, provided that the proteins bear markers specific for entry into that space.

Additional sites are also available for the transport of macromolecules that are destined for the outer or inner mitochondrial membrane or for the matrix. At these sites, the two membranes do not contact one another, but both inner and outer membranes possess receptor molecules that recognize not only the macromolecule that is being transported but also cytosolic carrier molecules (and chaperones) responsible for the delivery of that particular macromolecule. In order for most proteins destined for the mitochondrion to enter that organelle, they must have two signals: a **positively charged amino acid sequence** at their initial terminus as well as an associated protein known as ***heat shock protein 70***. A carrier protein, **translocase of the outer mitochondrial membrane**, recognizes these two signals and transports the protein into the intermembrane compartment. Another carrier protein, located in the inner membrane, known as the ***translocase of the inner mitochondrial membrane complex***, translocates the protein from the intermembrane compartment into the matrix where the heat shock protein 70 dissociates from the translocated protein and removes the amino acid presequence from its initial terminus.

Viewed in negatively stained preparations, the inner membrane displays the presence of a large number of lollipop-like inner membrane subunits, protein complexes known as ***ATP synthase***, which are responsible for the generation of ATP from ADP and inorganic phosphate. The globular head of the subunit, about 10 nm in diameter, is attached to a narrow flattened cylinder-like stalk, 4 nm wide and 5 nm long, projecting from the inner membrane into the matrix space (see Fig. 2–28).

Additionally, a large number of protein complexes, the **respiratory chains**, are present in the inner membrane. Each respiratory chain is composed of three respiratory enzyme complexes: (1) **NADH dehydrogenase complex,** (2) **cytochrome b-c_1 complex**, and (3) **cytochrome oxidase complex**. These complexes form the **electron transport chain** that is responsible for the passage of electrons along this chain and, more important, that function as proton pumps that transport energy-rich H^+ from the matrix into the intermembrane space, establishing an **electrochemical gradient** that provides energy for the ATP-generating action of ATP synthase. Because ADP is required for ATP synthesis, and the newly formed ATP has to leave the matrix space of the mitochondrion to enter the cytosol, both inner and outer mitochondrial membranes house the antiport carrier proteins, **ADP/ATP exchange proteins**, to import ADP into the mitochondrion and export ATP out of the mitochondrion.

Matrix

The **matrix space** is filled with a dense fluid composed of at least 50% protein, which accounts for its viscosity. Much of the protein component of the matrix is enzymes responsible for the stepwise degradation of fatty acids and pyruvate to the metabolic intermediate **acetyl CoA** and the subsequent oxidation of this intermediate in the **tricarboxylic acid (Krebs) cycle**. Mitochondrial ribosomes, tRNA, mRNA, and dense spherical **matrix granules** (30 to 50 nm in diameter) are also present in the matrix.

The function of matrix granules is not understood. They are composed of phospholipoprotein, although, in some cells, especially of bone and cartilage, they may also bind magnesium and calcium. Moreover, in injured cells whose cytosolic Ca^{2+} levels are dangerously high, matrix granules may sequester calcium to protect the cell from calcium toxicity.

The matrix also contains the double-stranded mitochondrial **circular deoxyribonucleic acid (mDNA)** and the enzymes necessary for the expression of the mitochondrial genome. The mDNA contains information for the formation of only 13 mitochondrial proteins, 16S and 12S rRNA, and genes for 22 tRNAs. Therefore, most of the codes necessary for the formation and functioning of mitochondria are located in the genome of the nucleus.

Oxidative Phosphorylation

Oxidative phosphorylation is the process responsible for the formation of ATP.

Acetyl CoA, formed through the β-oxidation of fatty acids and the degradation of glucose, is oxidized in the

citric acid cycle to produce, in addition to carbon dioxide (CO_2), large quantities of the reduced cofactors nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). Each of these cofactors releases a hydride ion (H^-), which is stripped of its two high-energy electrons and becomes a proton (H^+). The electrons are transferred to the electron transport chain and during mitochondrial respiration reduce oxygen (O_2) to form water (H_2O).

According to the **chemiosmotic theory**, the energy released by the sequential transfer of the electrons is used to transport H^+ from the matrix into the intermembrane space, establishing a high proton concentration in that space exerting a **proton motive force** (see Fig. 2–28). Only through ATP synthase may these protons leave the intermembrane space and reenter the matrix. As the protons pass down this electrochemical gradient, the energy differential in the proton motive force is transformed into the stable high-energy bond of ATP by the globular head of the inner membrane subunit, which catalyzes the formation of ATP from ADP + P_i, where P_i is inorganic phosphate. The newly formed ATP is either used by the mitochondrion or is transported, through the ADP/ATP antiport system, into the cytosol. During the entire process of glycolysis, tricarboxylic acid cycle, and electron transport, each glucose molecule yields 36 molecules of ATP.

In some cells, such as the brown fat of hibernating animals, oxidation is uncoupled from phosphorylation, resulting in the formation of heat instead of ATP. This uncoupling is dependent on the presence of proton shunts, known as ***thermogenins*,** that resemble ATP synthase but that cannot generate ATP. As the protons pass through thermogenins to reenter the matrix, the energy of the proton motive force is transformed into heat. It is this heat that awakens the animal from its state of hibernation. Mitochondria that uncouple oxidation from phosphorylation have a somewhat different morphology and are known as ***condensed mitochondria*** rather than the classic mitochondria **(orthodox mitochondria)**, in that they appear swollen, have a denser matrix, and present with a larger intermembrane compartment.

Origin and Replication of Mitochondria

Because of the presence of the mitochondrial genetic apparatus, it is believed that mitochondria were free-living organisms that either invaded or were phagocytosed by anaerobic eukaryotic cells, developing a **symbiotic relationship**. The mitochondrion-like organism received protection and nutrients from its host and provided its host with the capability of reducing its O_2 content and simultaneously supplying it with a stable form of chemical energy.

Mitochondria are self-replicating, in that they are generated from preexisting mitochondria. These organelles enlarge in size, replicate their DNA, and undergo fission. The division usually occurs through the intracristal space of one of the centrally located cristae. The outer mitochondrial membrane of the opposing halves extends through that intracristal space; the halves meet and fuse with each other, thus dividing the mitochondrion into two nearly equal halves. The process of fission usually takes place where mitochondria become surrounded by and contact the ER. The ER at the contact sites houses membrane-bound **inverted formin 2 (INF2)**, which induces actin polymerization and the accumulation of actin filaments between the ER and the mitochondria. It has been suggested that the presence of these actin filaments begins the constriction process **(primary constriction)** of the mitochondria and then **secondary constriction** follows, a process directed by the **dynamin-related protein 1**, resulting in mitochondrial fission and formation of two mitochondria. The two new mitochondria move away from each other. The average life span of a mitochondrion is about 10 days.

Annulate Lamella

Annulate lamellae are parallel aggregates of membranes that enclose cistern-like spaces, thus resembling multiple copies, usually 6 to 10, of nuclear envelopes. They possess nuclear pore complex–like regions **(annuli)** that are in register with those of neighboring membranes. The cisternae of these organelles are relatively evenly spaced, separated by about 80 to 100 nm, and are continuous with the cisternae of the RER.

These organelles are normally present only in cells that have high mitotic indices, such as oocytes, tumor cells, and embryonic cells. Because of their resemblance to the nuclear envelope, some authors suggest that they act as reserves for the nuclear envelope in these rapidly dividing cells. However, immunocytochemical studies of annulate lamellae do not lend support to that supposition, and neither their function nor their significance is understood.

INCLUSIONS

Inclusions are considered to be nonliving components of the cell that do not possess metabolic activity and are not bounded by membranes. The most common inclusions are glycogen, lipid droplets, pigments, and crystals.

Glycogen

Glycogen is the storage form of glucose.

Glycogen is the most common storage form of glucose in animals and is especially abundant in cells of muscle and liver. It appears in electron micrographs as clusters,

or **rosettes**, of β particles (and larger α particles in the liver) that resemble ribosomes, located in the vicinity of the SER. On demand, enzymes responsible for glycogenolysis degrade glycogen into individual molecules of glucose.

CLINICAL CORRELATIONS

Glycogen Storage Disorders

Some individuals have glycogen storage disorders as a result of their inability to degrade glycogen, resulting in excess accumulation of this substance in the cells. There are three classifications of this disease: (1) hepatic, (2) myopathic, and (3) miscellaneous. The lack or malfunction of one of the enzymes responsible for the degradation is responsible for these disorders (Table 2–2).

Lipids

Lipids are storage forms of triglycerides.

Lipids, triglycerides in storage form, not only are stored in specialized cells **(adipocytes)** but also are located as individual droplets in various cell types, especially **hepatocytes**. Most solvents used in histological preparations extract triglycerides from cells, leaving empty spaces indicative of the locations of lipids. However, with the use of osmium and glutaraldehyde, the lipids (and cholesterol) may be fixed in position as gray-to-black intracellular droplets. Lipids are very efficient forms of energy reserves; twice as many ATPs are derived from 1 g of fat as from 1 g of glycogen.

Pigments

The most common pigment in the body, besides **hemoglobin** of red blood cells, is **melanin,** manufactured by melanocytes of skin and hair, pigment cells of the retina, and specialized nerve cells in the substantia nigra of the brain. These pigments have protective functions in skin and aid in the sense of sight in the retina, but their role in hair and neurons is not understood. Additionally, in long-lived cells, such as neurons of the central nervous system and cardiac muscle cells, a yellow-to-brown pigment, **lipofuscin**, has been demonstrated. Unlike other inclusions, lipofuscin pigments are membrane-bound and are believed to represent the indigestible remnants of lysosomal activity. They are formed from fusion of several **residual bodies**.

Crystals

Crystals are not commonly found in cells, with the exception of Sertoli cells **(crystals of Charcot-Böttcher)**, interstitial cells **(crystals of Reinke)** of the testes, and, occasionally, in macrophages (Fig. 2–29). It is believed that these structures are crystalline forms of certain proteins.

CYTOSKELETON

The cytoskeleton has three major components: thin filaments, intermediate filaments, and microtubules.

The cytoplasm of animal cells contains a **cytoskeleton**, an intricate three-dimensional meshwork of protein filaments that are responsible for the maintenance of cellular morphology. Additionally, the cytoskeleton is an active participant in cellular motion, whether of organelles or vesicles within the cytoplasm, regions of the cell, or the entire cell. The cytoskeleton has three components: thin filaments (microfilaments), intermediate filaments, and microtubules.

Thin Filaments

Thin filaments are actin filaments that interact with myosin to bring about intracellular or cellular movement.

Thin filaments (microfilaments) are composed of two chains of globular subunits, **G-actin**, coiled around each other to form a filamentous protein, **F-actin**

TABLE 2–2 Major Subgroups of Glycogen Storage Disorders

Type	Deficient Enzyme	Tissue Changes	Clinical Signs
Hepatic Hepatorenal (von Gierke disease)	Glucose-6-phosphatase	Intracellular accumulation of glycogen in hepatocytes and cortical tubules of kidneys	Enlarged liver and kidneys; hypoglycemia with subsequent convulsions; gout; bleeding; 50% mortality
Myopathic McArdle syndrome	Muscle phosphorylase	Glycogen accumulation in skeletal muscle cells	Cramps following vigorous exercise; adult onset
Miscellaneous Pompe disease	Lysosomal acid maltase	Glycogen accumulation Enlarged lysosomes in hepatocytes	Massively enlarged heart; cardiac and respiratory failure within 2 years of onset; adults have milder form involving only skeletal muscle

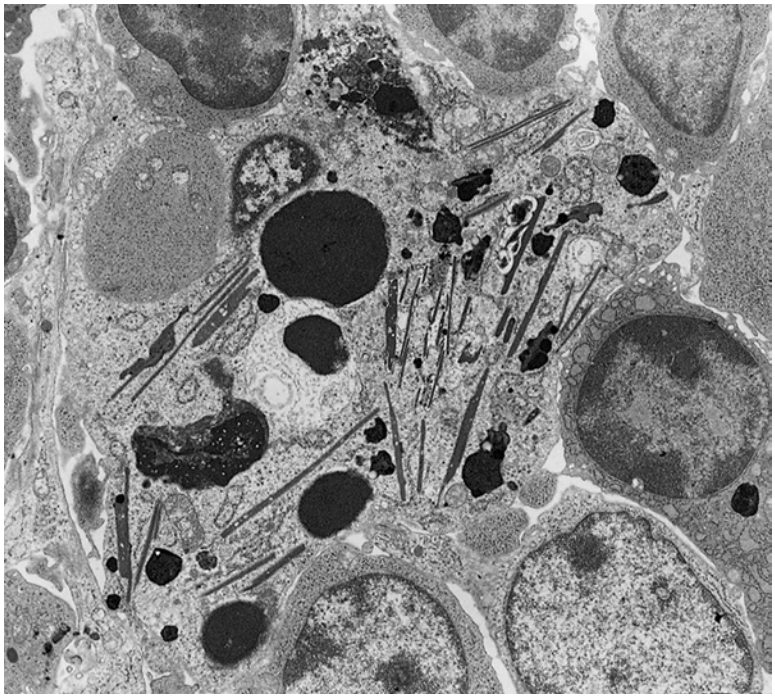


Figure 2–29 Electron micrograph of crystalloid inclusions in a macrophage (×5100). (From Yamazaki K. Isolated cilia and crystalloid inclusions in murine bone marrow stromal cells. *Blood Cells.* 1988;13:407-416.)

(Figs. 2–30 and 2–31). Actin constitutes about 15% of the total protein content of non-muscle cells. Only about half of their total actin is in the filamentous form because the monomeric G-actin form is bound by small proteins, such as **profilin** and **thymosin**, which prevent their polymerization. Actin molecules, present in the cells of many different vertebrate and invertebrate species, are very similar to each other in their amino acid sequence, attesting to their highly conserved nature.

Thin filaments are 7 nm thick and possess a faster-growing **plus end (barbed end)** and a slower-growing **minus end (pointed end)**. When the actin filament reaches its desired length, members of a family of small proteins, **capping proteins**, attach to the plus end, terminating the lengthening of the filament. The process of shortening of actin filaments is regulated in the presence of ATP, ADP, and Ca^{2+} by capping proteins, such as gelsolin, which prevent polymerization of the filament. The cell membrane phospholipid **polyphosphoinositide** has the opposite effect; it removes the gelsolin cap, permitting elongation of the actin filament.

Depending on their isoelectric point, there are three classes of actin: **α-actin** of muscle, and **β-actin**, and **γ-actin** of non-muscle cells. Although actin participates in the formation of various cellular extensions as well as in assembling structures responsible for motility, its basic composition is unaltered. It is capable of fulfilling its many roles via its association with different actin-binding proteins. The most commonly known of these proteins is **myosin**, but numerous other proteins, such as α-actinin, spectrin, fimbrin, filamin, gelsolin, and talin, also bind to actin to perform essential cellular functions (Table 2–3).

Actin filaments form bundles of varied lengths, depending on the function that they perform in non-muscle cells. These bundles form three types of associations:

- Contractile bundles
- Gel-like networks
- Parallel bundles

Contractile bundles, such as those responsible for the formation of cleavage furrows (contractile rings) during mitotic division, are usually associated with myosin. Their actin filaments are arranged loosely, parallel to each other, with the plus and minus ends alternating in direction. These assemblies are responsible for movement not only of organelles and vesicles within the cell but also for cellular activities, such as exocytosis and endocytosis, as well as the extension of filopodia and cell migration.

The myosin associated with these contractile bundles may be one of several types: **myosin-I** through **myosin**

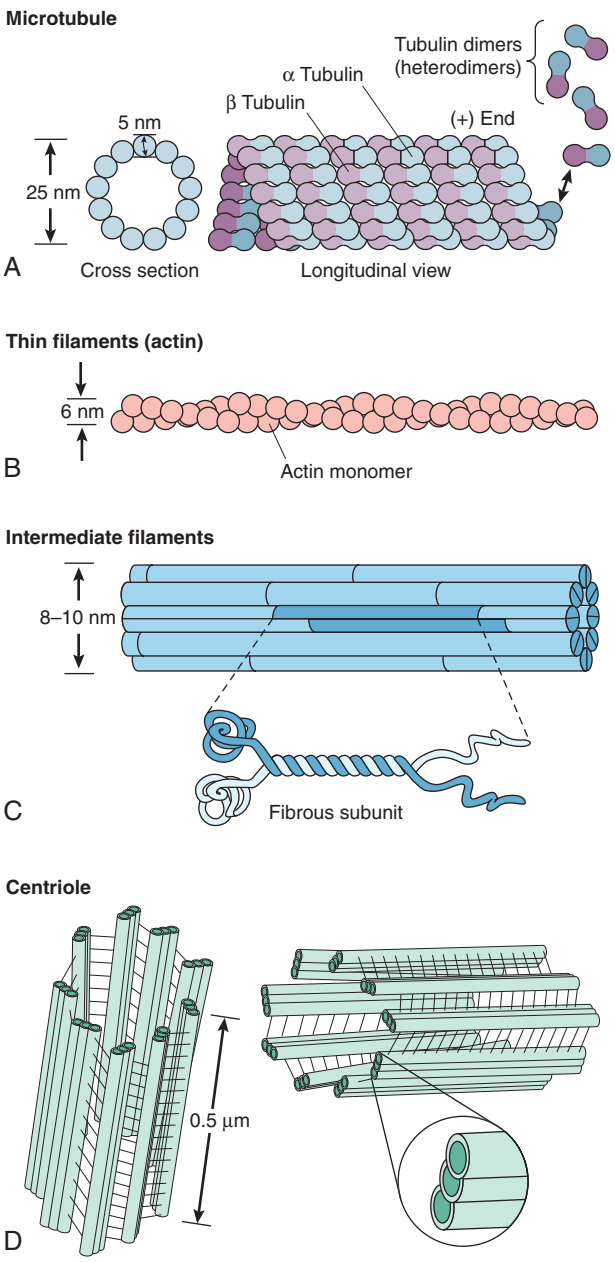


Figure 2–30 **A–D,** Diagram of the elements of the cytoskeleton and centriole.

IX. Myosin II forms **thick filaments** (15 nm in diameter) and moves actin filaments, especially in muscle cells. Myosin V can bind not only to actin filaments but also to other cytoplasmic components, such as vesicles, moving them along an actin filament from one position in the cell to another, whereas myosin I has been implicated in the formation and retraction of actin directed protrusions of the cell cortex, such as in the formation of pseudopods.

Gel-like networks provide the structural foundation of much of the cell cortex. Their stiffness is due to the protein filamin, which assists in the establishment of a loosely organized network of actin filaments resulting in localized high viscosity. During the formation of filopodia, the gel is liquefied by proteins such as **gelsolin**, which, in the presence of ATP and high concentration of Ca^{2+}, cleaves the actin filaments and, by forming a cap over their plus end, prevents them from lengthening.

The proteins **fimbrin** and **villin** are responsible for forming actin filaments into closely packed **parallel bundles** that form the core of microspikes and microvilli, respectively. These bundles of actin filaments are anchored in the **terminal web**, a region of the cell cortex composed of a network of intermediate filaments and the protein **spectrin**. Spectrin molecules are flexible, rod-like tetramers that assist the cell in maintaining the structural integrity of the cortex.

Actin is also important in the establishment and maintenance of **focal contacts** of the cell with extracellular matrix (Fig. 2–32). At focal contacts, the **integrin** (a transmembrane protein) of the cell membrane binds to structural glycoproteins, such as **fibronectin**, of the extracellular matrix, permitting the cell to maintain its attachment. Simultaneously, the intracellular region of the integrin contacts the cytoskeleton via intermediary proteins that attach it to actin filaments. The mode of attachment involves integrin binding to **talin**, which contacts both **vinculin** and the actin filament. Vinculin binds to α-actinin, the actin-binding protein that assembles actin into contractile bundles. These contractile bundles, referred to as ***stress fibers*** in fibroblasts maintained in tissue culture, resemble myofibrils of striated muscle. Stress fibers may extend between two focal points or a focal point and intermediate filaments and assist the cell in exerting a tensile force on the extracellular matrix (as in the wound contraction function of fibroblasts).

Intermediate Filaments

Intermediate filaments and their associated proteins assist in the establishment and maintenance of the three-dimensional framework of the cell.

Electron micrographs display a category of filaments in the cytoskeleton whose diameter of 8 to 10 nm places them between thick and thin filaments and are consequently named ***intermediate filaments*** (see Fig. 2–30). These filaments and their associated proteins accomplish the following:

- Provide structural support for the cell
- Form a deformable three-dimensional structural framework for the cell
- Anchor the nucleus in place

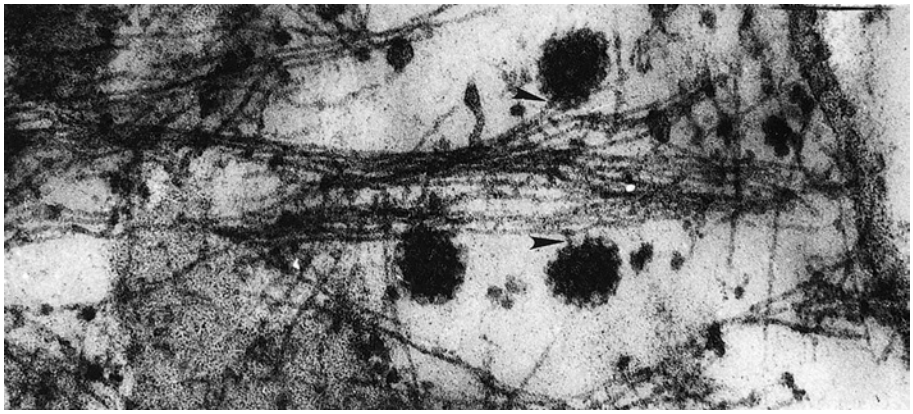


Figure 2–31 Electron micrograph of clathrin-coated vesicles contacting filaments *(arrowheads)* in granulosa cells of a rat ovary (×35000). (From Batten BE, Anderson E. The distribution of actin in cultured ovarian granulosa cells. *Am J Anat.* 1983;167:395-404. Reprinted with permission of John Wiley & Sons, Inc.)

TABLE 2–3 Actin-Binding Proteins

Protein	Molecular Mass of Each Subunit (D)	Function
α-Actinin	100,000	Bundling actin filaments for contractile bundles
Capping protein	74,000	Attaches to the growing (barbed) end of the actin filament, stabilizing its length
Cofilin	19,000	Active in depolymerization of the actin filament, especially during filopodia formation
Fimbrin	68,000	Bundling actin filaments for parallel bundles
Filamin	270,000	Cross-link actin filaments into gel-like network
Formin	22,000	Attaches to growing (barbed) end and promotes the filament polymerization
Myosin-II	260,000	Contraction by sliding actin filaments
Myosin-V	150,000	Movement of vesicles and organelles along actin filaments
Prolifin	15,000	Restructuring of the cytoskeleton by increasing actin polymerization
Spectrin α β	 265,000 260,000	Forms supporting network for plasma membrane of red blood cell
Gelsolin	90,000	Cleaves and caps actin filaments
Thymosin	5,000	Binds to G-actin subunits, maintaining them in monomeric form

- Provide an adaptable connection between the cell membrane and the cytoskeleton
- Furnish a structural framework for the maintenance of the nuclear envelope as well as its reorganization subsequent to mitosis

When microbeads bound to integrin molecules of the cell membrane are micromanipulated, as when one pulls on them, the tensile forces produce distortion of the cytoskeleton, with resultant deformation of the nucleus and rearrangement of the nucleoli. Thus, it appears that the cytoskeleton, and specifically the intermediate filaments, reacts to forces generated in the extracellular matrix, and, by forcing modulations in the shape and location of cellular constituents, they protect the structural and functional integrity of the cell from external stresses and strains.

Biochemical investigations have determined that there are several categories of intermediate filaments that share the same morphological and structural characteristics. These rope-like intermediate filaments are constructed of eight tetramers of rod-like proteins that are tightly bundled into long helical arrays. The individual subunit, the **monomer**, of each tetramer differs considerably for each type of intermediate filament, but their morphology is similar in that each monomer has an N-terminus **(head)** and C-terminus **(tail)** that are

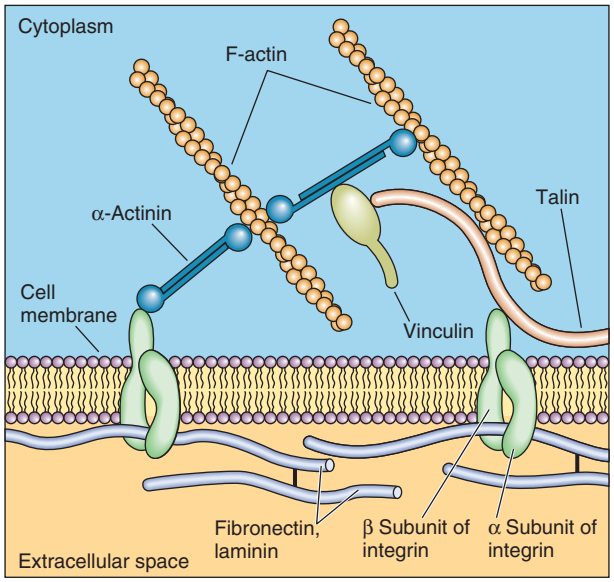


Figure 2–32 Schematic diagram of the cytoskeleton. Fibronectin or laminin receptor regions of integrin molecules bind to fibronectin or laminin, respectively, in the extracellular space. Intracellular talin or α-actinin binding regions of integrin molecules bind to talin or α-actinin, respectively. Thus, integrin molecules bridge the cytoskeleton to an extracellular support framework.

both folded into globular domains, whereas its central region, the **central domain**, is composed of an elongated alpha helix. The categories of intermediate filaments include keratins, desmin, vimentin, glial fibrillary acidic protein, neurofilaments, and nuclear lamins (Table 2–4). Several intermediate filament-binding proteins have been discovered. As they bind to intermediate filaments, they link them into a three-dimensional network that facilitates the formation of the cytoskeleton. The best known of these proteins are as follows:

- **Filaggrin** binds keratin filaments into bundles.
- **Synemin** and **plectin** bind desmin and vimentin, respectively, into three-dimensional intracellular meshworks.
- **Plakins** assist the maintenance of contact between the keratin intermediate filaments and hemidesmosomes of epithelial cells as well as actin filaments with neurofilaments of sensory neurons.

CLINICAL CORRELATIONS

Immunocytochemical methods, using specific immunofluorescent antibodies, are used to distinguish intermediate filament types in tumors of unknown origin. Knowledge of the source of these tumors assists not only in their diagnosis but also in devising effective treatment plans.

Microtubules

Microtubules are long, straight, rigid tubular-appearing structures that act as intracellular pathways.

The **centrosome** is the region of the cell in the vicinity of the nucleus that houses the centrioles, as well as several hundred ring-shaped **γ-tubulin ring complex** molecules. These γ-tubulin molecules act as nucleation sites for **microtubules**, long, straight, rigid, hollow-appearing cylindrical structures 25 nm in outer diameter, with a luminal diameter of 15 nm (Fig. 2–33; see Fig. 2–30). Therefore, the centrosome is considered to be the MTOC (microtubule organizing center) of the cell.

Microtubules are polarized, having a **plus end** (β-tubulin) as well as a **minus end** (α-tubulin), which must be stabilized or it will depolymerize, thus shortening the microtubule. The minus end is stabilized by being embedded in a γ-tubulin molecule. They are dynamic structures that frequently change their length by undergoing growth spurts and then become shorter; both processes occur at the plus ends, so that the average half-life of a microtubule is only about 10 minutes. The main functions of microtubules are to

- Provide rigidity and maintain cell shape
- Regulate intracellular movement of organelles and vesicles
- Establish intracellular compartments
- Provide the capability of ciliary (and flagellar) motion

Each microtubule consists of 13 parallel **protofilaments** composed of heterodimers of the globular polypeptide α- and β-tubulin subunits, each consisting of about 450 amino acids and each having a molecular mass of about 50,000 Da (see Fig. 2–30). Polymerization of the heterodimers requires the presence of magnesium (Mg^{2+}) and GTP. During cell division, rapid polymerization of existing as well as new microtubules is responsible for the formation of the spindle apparatus.

CLINICAL CORRELATIONS

Disruption of the polymerization process by antimitotic drugs, such as colchicine, blocks the mitotic event by binding to the tubulin molecules, preventing their assembly into the protofilament.

Microtubule-Associated Proteins

Microtubule-associated proteins are motor proteins that assist in the translocation of organelles and vesicles inside the cell.

In addition to tubulin heterodimers, microtubules also possess **microtubule-associated proteins (MAPs)** bound to their periphery at 32-nm intervals. There are various types of MAPs (MAP1, MAP2, MAP3, MAP4,

TABLE 2–4 Predominant Types of Intermediate Filaments

Type	Filament	Polypeptide Component Size (Da)	Cell Type	Function
I	Acidic keratins	40,000–70,000	Epithelial cells	Support cell assemblies and provide tensile strength to cytoskeleton
II	Basic keratins	40,000–70,000	Cells of hair and nails	
III	Desmin	53,000	All types of muscle cells	Links myofibrils in striated muscle (around Z disks); attaches to cytoplasmic densities in smooth muscle
III	Vimentin	54,000	Cells of embryo as well as cells of mesenchymal origin: fibroblasts, leukocytes, endothelial cells	Surrounds nuclear envelope; is associated with cytoplasmic aspect of nuclear pore complex
III	Glial fibrillary acidic protein (GFAP)	50,000	Astrocytes, Schwann cells, oligodendroglia	Supports glial cell structure
IV	Neurofilaments NF-L: low-molecular-weight NF-M: medium-molecular-weight NF-H: high-molecular-weight	 68,000 160,000 210,000	Neurons	Form cytoskeleton of axons and dendrites; assist in the formation of the gel state of the cytoplasm; cross-linking responsible for great tensile strength
IV	Syncoilin	64,000	Skeletal muscle cells	Forms bonds with dystrobrevin in skeletal muscle
V	Nuclear lamins A B C	65,000–75,000	Lining nuclear envelopes of all cells	Control and assembly of the nuclear envelope; organization of perinuclear chromatin
VI	Phakinin and filensin	49,000 94,000	Lens fiber cells of the eyeball	Sustain the transparency of the lens

MAP tau, and Lis 1), ranging in molecular weight from about 50,000 to more than 300,000 Da. Their primary functions are to prevent depolymerization of microtubules and to assist in the intracellular movement of organelles and vesicles, whereas Lis 1 functions during brain development and is responsible for the formation of sulci and gyri of the cerebral hemispheres.

Movement along a microtubule occurs in both directions and is toward both the **plus end** and the **minus end**. The two major families of microtubule motor proteins, the MAPs **dynein** and **kinesin**, both bind to the microtubule as well as to vesicles (and organelles). It is believed that different members of each motor protein family transport their cargo at disparate, meticulously controlled rates and that different organelles have their own particular motor protein. In the presence of ATP, dynein moves the vesicle toward the minus end of the microtubule (toward the MTOC). Kinesin effects vesicular (and organelle) transport in the opposite direction, toward the plus end (away from the MTOC), but the mechanism of ATP utilization by these MAPs is not understood. Additionally, dynein and kinesin participate in the organization of the minus and plus ends, respectively.

Centrioles and Centrosome

Centrioles are small, cylindrical structures composed of nine microtubule triplets; two centrioles, embedded in a matrix of pericentriolar material, constitute the core of the microtubule organizing center, or the centrosome.

Centrioles are small, cylindrical structures, 0.2 µm in diameter and 0.5 µm in length (see Fig. 2–30). Usually centrioles are paired structures, arranged perpendicular to each other and are embedded in a matrix of **pericentriolar material**. The entire complex is known as the ***centrosome*** or *MTOC* and is located in the vicinity of the Golgi apparatus. The pericentriolar material is composed of the proteins **γ-tubulin** and **pericentrin**, both of which interact with the minus ends of the microtubules and anchor them into the centrosome. The centrosome assists in the formation and organization of microtubules as well as in its self-duplication before cell division.

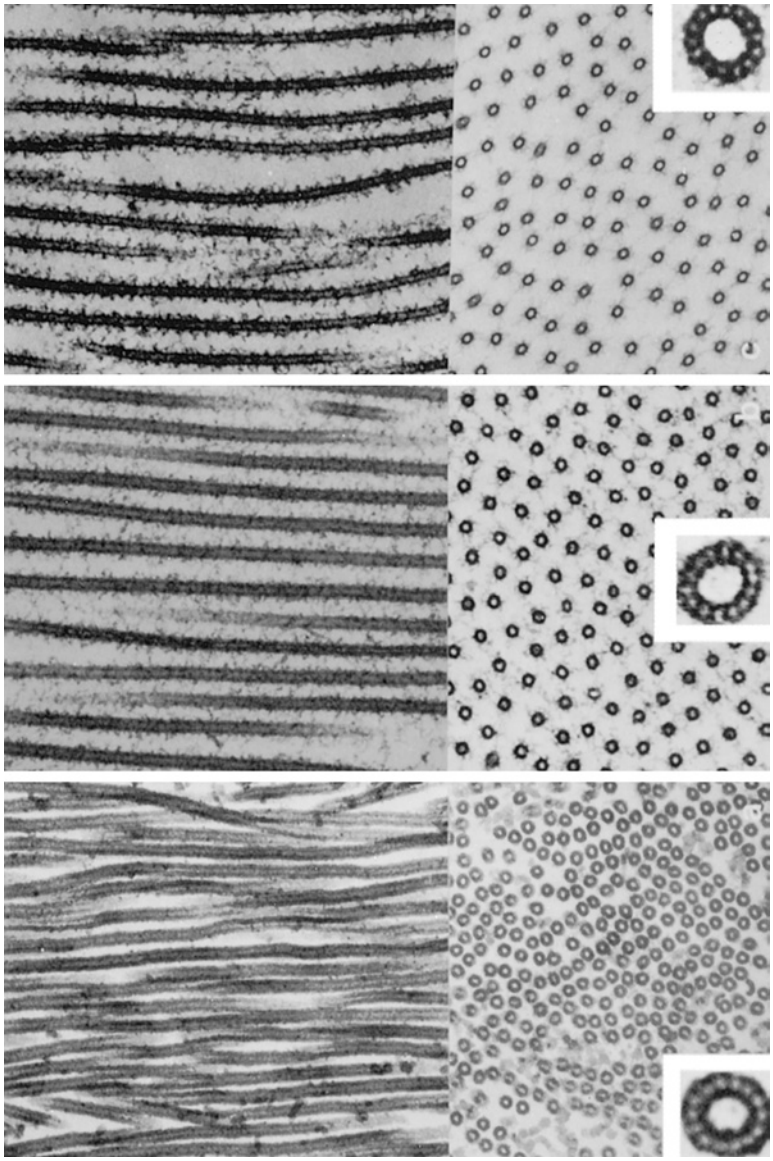


Figure 2–33 Electron micrograph of microtubules assembled with and without microtubule-associated proteins (MAPs) (×65790). *Top,* Microtubules assembled from unfractionated MAPs. *Center,* Microtubules assembled in the presence of MAP_2 subfraction only. *Bottom,* Microtubules assembled without MAPs. (From Leeson TS, Leeson CR, Papparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

Centrioles are composed of a specific arrangement of nine triplets of microtubules arranged around a central axis (9 + 0 axoneme pattern). Each microtubule triplet consists of one complete and two incomplete microtubules fused to each other, so that the incomplete ones share three protofilaments. The complete microtubule "A" is positioned closest to the center of the cylinder; "C" is the farthest away. Adjacent triplets are connected to each other by a fibrous substance of unknown composition, extending from microtubule A to microtubule C. Each triplet is arranged so that it forms an oblique angle with the adjacent and a straight angle with the fifth triplet. Centrioles are also associated with the calcium binding proteins **centrin (caltractin)** which function in centriole duplication.

During the S phase of the cell cycle, each centriole of the pair replicates, forming a procentriole in some unknown manner, at 90 degrees to itself. This

procentriole initially possesses no microtubules, but tubulin molecules begin to polymerize closest to the parent centriole, with the plus end growing away from the parent. The actual replication of the centriole requires the presence of γ-tubulin rings, structures that do not become part of but serve to direct the elongation of the forming microtubules by occupying the forming plus and minus ends. It is believed that the γ-tubulin rings and pericentrin serve as beams that support the developing centriole. Each γ-tubulin initiates the formation of a single microtubule. Additionally, δ-tubulins, related to the α- and β-tubulin superfamily, are also required to form the triplet structure of the microtubule arrays.

Centrioles function in the formation of the centrosome, and, during mitotic activity, they are responsible for the formation of the spindle apparatus. Additionally, centrioles are the basal bodies that guide the formation of cilia and flagella.

PATHOLOGICAL CONSIDERATIONS

See Figures 2–34 and 2–35.

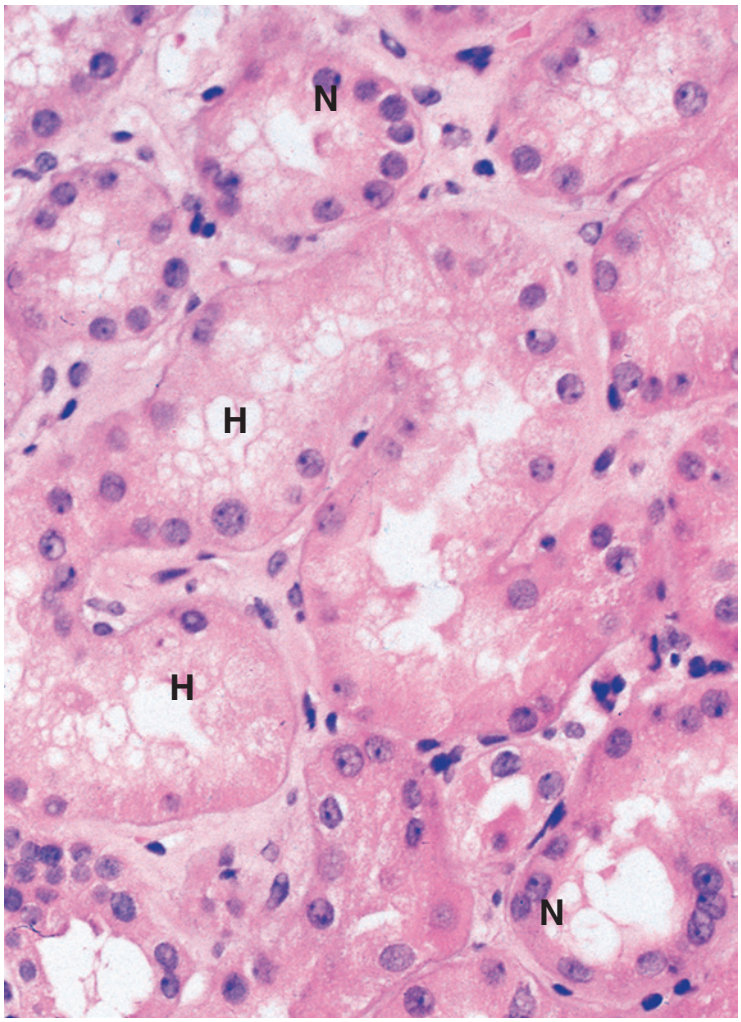


Figure 2–34 Hydropic degeneration. Blood flow to the cortex of this kidney was diminished due to severe hypotension. Note that the cells have a pale, vacuolated appearance with enlarged nuclei. If the hypotension is not corrected, the patient could succumb to acute renal failure. (From Young B, et al. *Wheater's Basic Pathology.* 5th ed. London: Churchill Livingstone; 2010.)

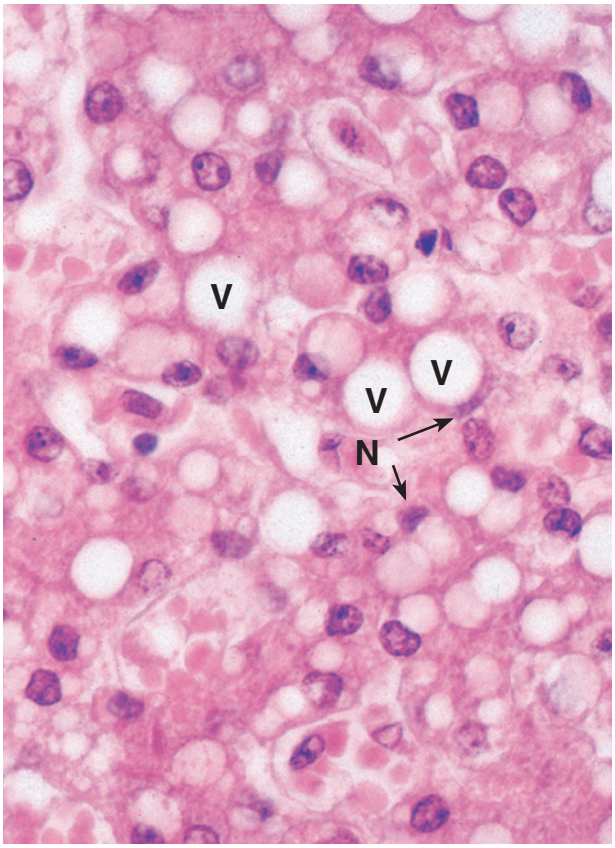


Figure 2–35 Steatosis. Fatty alteration, steatosis, is most common in the liver, frequently due to exposure to toxins, especially alcohol abuse. Other causes are obesity, diabetes mellitus, and chronic hypoxia. This particular specimen is from an individual with long-term alcohol addiction. In the living individual, the vacuoles (V) were filled with lipid, and, frequently, the enlarged vacuoles displaced the nuclei (N), which normally occupies the center of the cell in a healthy hepatocyte. (From Young B, et al. *Wheater's Basic Pathology.* 5th ed. London: Churchill Livingstone; 2010.)

REVIEW QUESTIONS

1. The fluidity of the plasmalemma has well-established clinical significance. Which of the following molecules decreases the cell membrane fluidity?
   a. glycerol
   b. phosphate group
   c. cholesterol
   d. integral proteins
   e. peripheral proteins
2. A patient with asthma has difficulty breathing and is treated with albuterol, a drug that relaxes bronchiolar smooth muscles. Albuterol acts as a(n)
   a. cholinergic receptor antagonist
   b. cholinergic receptor agonist
   c. adrenergic receptor antagonist
   d. adrenergic receptor agonist
   e. inhibitor of kinase phosphorylases
3. A patient who had surgery for ovarian cancer is placed on a combination of cisplatin and taxol therapy. Taxol is an antimitotic agent that
   a. affects microfilaments
   b. affects intermediate filaments
   c. has defective dynein
   d. prevents the polymerization of microtubules
   e. stabilizes microtubules
4. A 9-month-old female Jewish baby, whose parents were born in Poland, is seen by her pediatrician because of neurological problems, including generalized paralysis and blindness. The pediatrician should suspect that the child has
   a. spina bifida
   b. cranial nerve X (vagus nerve) disorder
   c. Tay-Sachs disease
   d. Fabry disease
   e. Refsum disease
5. Acid hydrolases are synthesized on the rough endoplasmic reticulum and are delivered to the Golgi complex to be packaged and distributed to their final destination. Patients whose protooncogenes have mutated to form ras protein related products known as ADP-ribosylation factor (ARF) may interfere with
   a. coatomer II (COP II) synthesis
   b. coatomer II (COP II) assembly
   c. coatomer II (COP II) dissociation from vesicles
   d. clathrin synthesis
   e. clathrin assembly
6. Patients with sickle cell anemia possess hemoglobin S, a mutated form of hemoglobin. Hemoglobin S is
   a. synthesized on free ribosomes
   b. synthesized on the rough endoplasmic reticulum
   c. modified in the Golgi apparatus
   d. packaged in the *trans*-Golgi network
   e. stored in condensing vesicles
7. A neonatal baby boy is observed to have malformed hands, feet, and skull. Additional examination displays jaundice, hepatomegaly, and hypotonic muscles with psychomotor retardation. The pediatrician diagnoses the baby with cerebrohepatorenal syndrome (Zellweger syndrome). This condition is due to problems with which of the following organelles?
   a. mitochondria
   b. lysosomes
   c. early endosomes
   d. late endosomes
   e. peroxisomes
8. A 20-year-old man presenting with deteriorating vision in both eyes, especially his central vision, seeks help from his ophthalmologist. The patient also evidences cardiac dysrhythmia and an apparent demyelination of the optic nerve. The ophthalmologist suspects Leber hereditary optic neuropathy, a condition caused by a point mutation that is not X-linked but is transmitted only by females. This condition is due to problems with which of the following?
   a. lysosomes
   b. early endosomes
   c. late endosomes
   d. mitochondria
   e. peroxisomes
9. Which description refers to cadherins?
   a. They are peripheral proteins.
   b. They are associated with cell–cell adhesion.
   c. They are associated with adherence to the basal lamina.
   d. They have binding sites for heparin sulfate.
   e. They are associated with hemidesmosomes.
10. Dynein arms are always attached to one of the following. Which one is that structure?
    a. central sheet
    b. singlets
    c. subunit A
    d. subunit B
    e. radial spoke

ANSWERS

1. C. Cholesterol decreases membrane fluidity. The polar heads of phospholipids molecules are composed of glycerol, to which a positively charged nitrogenous group is attached by a negatively charged phosphate group. Unsaturated fatty acyl molecules increase membrane fluidity. Integral and peripheral proteins do not have an effect on membrane fluidity.
2. D. Albuterol is an agonist that acts on $beta_2$-adrenergic receptors, which are located mainly on the cell membranes of bronchial tissues and, therefore, do not have many side effects. If it were an antagonist, then it would cause smooth muscle constriction.
3. E. Unlike most antimitotic agents that interfere with polymerization or depolymerization of microtubules, taxol stabilizes microtubules so that they cannot shorten or lengthen. In this fashion, the mitotic spindle apparatus cannot change its length, thus preventing the movement of the chromosomes during the mitotic event. Dynein, microfilaments, and intermediate filaments are unaffected by taxol.
4. C. Tay-Sachs disease affects mostly children of Eastern European Jewish ancestry as well as in certain families in Louisiana of Cajun ancestry. Children with this lysosomal storage disease cannot catabolize GM_2 gangliosides that accumulate in their lysosomes, and the enlarged lysosomes interfere with cell function. The interference is most problematic in the neurons of the central nervous system and causes death by the third year of life. Neither spina bifida nor vagus nerve disorders would cause generalized paralysis or blindness. Fabry disease, although a lysosomal-storage, is due to an X chromosomal defect and affects males only. Refsum disease is due to the accumulation of phytanic acid, a by-product of chlorophyll metabolism and affects older children who already eat fresh fruits and vegetables.
5. B. Delivery of proteins manufactured on the rough endoplasmic reticulum to the Golgi complex is accomplished in COP II–coated vesicles. ARF catalyzes the assembly of COP II molecules to form coatomer on the cytoplasmic aspect of these transfer vesicles. Synthesis of COP II proteins as well as the disassembly of the coatomer is not influenced by ARF. Clathrin coats mediate the endocytotic pathway as well as the pathway that leads away from the *trans*-Golgi network.
6. A. Hemoglobin, whether normal or mutated, is present freely in the cytosol; therefore, it is synthesized on free ribosomes. Only proteins that are to be packaged are synthesized on the rough endoplasmic reticulum. Because hemoglobin is not packaged, it does not enter the Golgi apparatus.
7. E. Children with cerebrohepatorenal syndrome die at an early age due to mutations in their peroxin genes that code for defective receptors that are unable to transfer enzymes destined for peroxisomes into these organelles. Mitochondria, lysosomes, and endosomes are not affected by this mutation.
8. D. Leber hereditary optic neuropathy is due to a point mutation on the mitochondrial DNA. Because the spermatozoon does not contribute mitochondria to the zygote, only the female can transmit this condition to her offspring. Lysosomes, peroxisomes, early and late endosomes do not possess DNA.
9. B. Cadherins are integral proteins that are designed to assist cells to maintain contact with one another. Hemidesmosomes, heparin sulfate, and basal lamina are all associated with cell to extracellular matrix contact.
10. C. Dynein arms are microtubule-associated proteins that function in the achievement bending the cilium. These molecular motors are attached to the subunit A of the axoneme. The central sheet and the radial spokes are both composed of an elastic protein that become stretched during the "cocking" of the axoneme and, as they return to their normal length, they effect ciliary motion.

Nucleus

3

The **nucleus** is the largest organelle of the cell (Fig. 3–1). It contains nearly all of the **deoxyribonucleic acid (DNA)** possessed by the cell as well as the mechanisms for **ribonucleic acid (RNA)** synthesis, and its resident nucleolus is the location for the assembly of ribosomal subunits. The nucleus, bounded by the nuclear envelope composed of two concentric lipid membranes, houses three major components:

- **Chromatin,** the genetic material of the cell
- **Nucleolus,** the center for ribosomal RNA (rRNA) synthesis
- **Nucleoplasm,** containing macromolecules and nuclear particles involved in the maintenance of the cell

The nucleus is usually spherical and is centrally located in the cell; however, in some cells, it may be spindle shaped to oblong shaped, twisted, lobulated, or even disk shaped. Although, usually, each cell has a single nucleus, some cells (such as osteoclasts) possess several nuclei, whereas mature red blood cells have extruded their nuclei. The size, shape, and form of the nucleus are generally constant for a particular cell type, a fact useful in clinical diagnoses of the degree of malignancy of certain cancerous cells.

NUCLEAR ENVELOPE

The nuclear envelope is composed of two parallel unit membranes that fuse with each other at certain regions to form perforations known as nuclear pores.

The nucleus is surrounded by the **nuclear envelope,** composed of two concentric parallel unit membranes: the **inner** and **outer nuclear membranes,** separated from each other by a 20- to 40-nm space called the ***perinuclear cisterna*** (Figs. 3–2 and 3–3). The nuclear envelope is perforated at various intervals by **nuclear pores** that permit communication between the cytoplasm and the nucleus. At these pores, the inner and outer nuclear membranes are continuous with one another. The nuclear envelope helps control movement of macromolecules between the nucleus and the cytoplasm and assists in organizing the chromatin.

Inner Nuclear Membrane

The **inner nuclear membrane** is about 6 nm thick and faces the nuclear contents. It is in close contact with the **nuclear lamins,** an interwoven meshwork of **intermediate filaments**, 80 to 300 nm thick, composed of **lamins A**, $\mathbf{B_1}$, $\mathbf{B_2}$, and **C** and located at the periphery of the nucleoplasm. The nuclear lamins maintain their anatomical position of lining the inner nuclear membrane by binding to integral membrane proteins, such as **lamina-associated polypeptides** and **emerin** of the inner nuclear membrane. The nuclear lamins help in organizing and providing support to the lipid bilayer membrane and the perinuclear chromatin as well as playing a role in the formation of nuclear pore complexes and assembly of vesicles to reform the nuclear envelope subsequent to cell division. Certain integral proteins of the inner nuclear membrane act either directly or via other nuclear matrix proteins as contact sites for nuclear RNAs and chromosomes.

Outer Nuclear Membrane

The **outer nuclear membrane** is also about 6 nm thick, faces the cytoplasm, and is continuous with the rough endoplasmic reticulum (RER) and is considered by some authors as a specialized region of the RER whose lumen is continuous with the perinuclear cistern (see Figs. 3–2 and 3–3). Its cytoplasmic surface is surrounded by a thin, loose meshwork of the intermediate filaments, **vimentin**. Its cytoplasmic surface usually possesses ribosomes actively synthesizing transmembrane proteins that are destined for the outer or inner nuclear membranes.

Nuclear Pores

Nuclear pores are interruptions in the nuclear envelope, where the inner and outer nuclear membranes fuse with each other, establishing sites where communication may occur between the nucleus and the cytoplasm.

At certain locations on the surface of the nuclear envelope, the outer and inner nuclear membranes are continuous with each other, creating openings about 80 nm in diameter known as ***nuclear pores,*** which permit communication between the nuclear compartment and the cytoplasm (Figs. 3–4 and 3–5). The number of nuclear pores in a nucleus ranges between a few dozen to several thousand, correlated directly with the metabolic activity of the cell.

High-resolution electron microscopy has revealed that the nuclear pore is surrounded by nonmembranous structures embedded in its rim. These structures and the pore are called the ***nuclear pore complex,*** which selectively guards passage through the pore (Fig. 3–6). The evidence suggests that each of the nuclear pore complexes is in communication with the others via the nuclear lamina and certain pore-connecting fibers.

SUMMARY

The **nucleus**, the largest organelle of the cell, houses the **nucleoplasm**, **nucleolus,** and the **chromatin**. The nucleus is separated from the cytoplasm by a double membrane, known as the ***nuclear envelope***, which is perforated by numerous openings, known as ***nuclear pores*** and their associated structures that, together with the pore, form the **nuclear pore complex** that functions in a bidirectional nucleocytoplasmic transport. Chromatin is a complex of **DNA** and associated proteins that, during cell division, are folded to form **chromosomes**. It is the DNA that comprises the genetic material of the cell and acts as a template for **RNA transcription**. The **nucleolus** is the deeply staining region within the nucleus where ribosomal RNA synthesis and **ribosome** assembly occur. The cell cycle is a series of exquisitely controlled events that prepare the somatic cell for **mitosis** and the gametes for a unique nuclear division known as ***meiosis***.

KEY WORDS

Apoptosis; cell cycle; chromatin; chromosomes; DNA; genes; interphase; meiosis; mitosis; nuclear envelope; nuclear pore complex; nucleoplasm; nucleus; ploidy; RNA

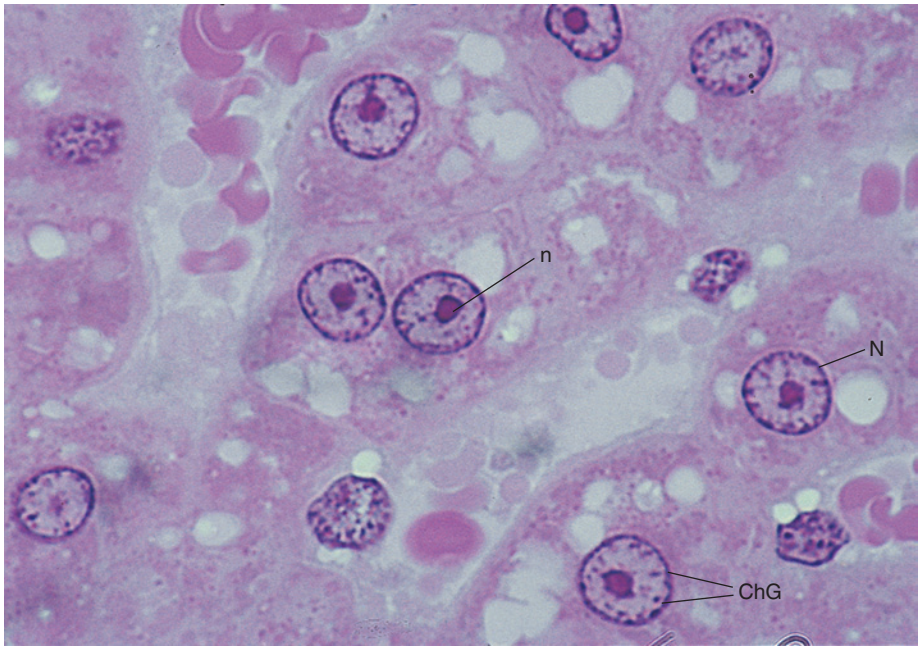


Figure 3–1 Cell nuclei. Light micrograph (×1323). Typical cells, each containing a spherical nucleus (N). Observe the chromatin granules (ChG) and the nucleolus (n).

Nuclear Pore Complex

The nuclear pore complex is composed of the nuclear pore and its associated glycoproteins.

The **nuclear pore complex** is about 100 to 125 nm in diameter and spans the two nuclear membranes. It is composed of about a hundred different proteins, collectively known as ***nucleoporins***, arranged in three ring-like arrays of proteins stacked on top of the other, each ring displaying eightfold symmetry and interconnected by a series of spokes arranged in a vertical fashion. In addition, the nuclear pore complex has cytoplasmic fibers, a central plug, and a nuclear basket (Fig. 3–6).

The **cytoplasmic ring**, composed of eight subunits, is located on the rim of the cytoplasmic aspect of the nuclear pore. Each subunit possesses a filamentous fiber **(cytoplasmic filament)**, believed to be a Ran-binding protein (a family of guanosine triphosphate [GTP]-binding proteins), that extends into the cytoplasm. It has been suggested that these fibers may mediate import into the nucleus through the nuclear pore complex by moving substrates along their length toward the center of the pore.

The **luminal spoke ring (middle ring)** is composed of a set of eight transmembrane proteins that project into the lumen of the nuclear pore as well as into the perinuclear cistern. These spoke-like proteins appear to anchor the glycoprotein components of the nuclear pore complex into the rim of the nuclear pore.

The center of the middle ring is occupied by an oblong-shaped structure known as the ***central plug***, previously known as the ***transporter*****,** which is coupled to the spoke-like proteins of the middle ring. It is now believed to be the material being transported into or out of the nucleus, thus it is not presented within the model (see Fig. 3–7). The central lumen of the middle ring is believed to be a gated channel that restricts passive diffusion between the cytoplasm and the nucleoplasm. It is associated with additional protein complexes that facilitate the regulated transport of materials across the nuclear pore complex.

A **nuclear ring (nucleoplasmic ring)**, analogous to the cytoplasmic ring, is located on the rim of the nucleoplasmic aspect of the nuclear pore and assists in the export of several types of RNA. A filamentous, flexible, basket-like structure, the **nuclear basket**, appears to be suspended from the nucleoplasmic ring and protruding into the nucleoplasm. The nuclear basket becomes deformed during the process of nuclear export. Attached to the distal aspect of the nuclear basket is the **distal ring**.

Function of the Nuclear Pore Complex

The nuclear pore complex functions in bidirectional nucleocytoplasmic transport.

Although the nuclear pore is relatively large, it is nearly filled with the structures constituting the nuclear pore

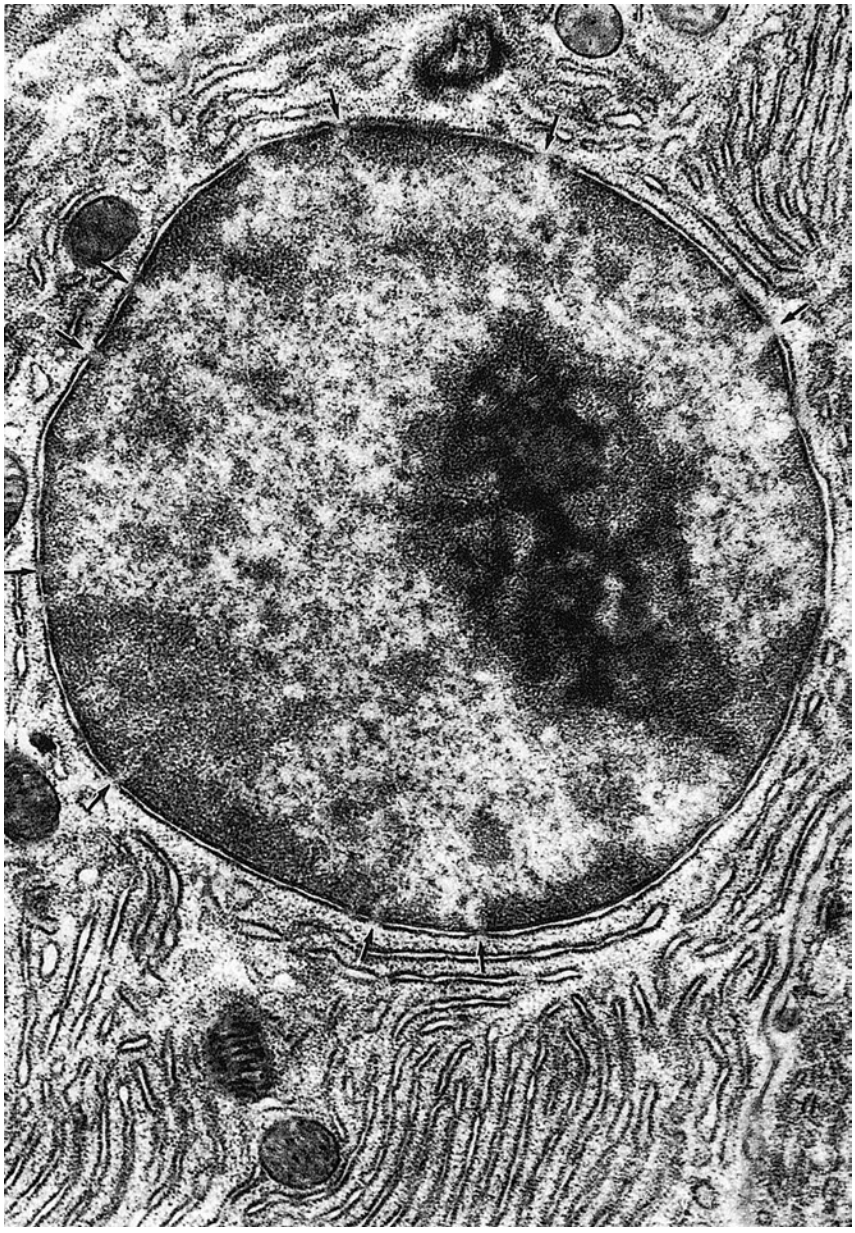


Figure 3–2 Cell nucleus. Electron micrograph (×16762). Observe the electron-dense nucleolus, the peripherally located dense heterochromatin, and the light euchromatin. The nuclear envelope surrounding the nucleus is composed of an inner nuclear membrane and an outer nuclear membrane that is interrupted by the nuclear pores *(arrows)*. (From Fawcett DW. *The Cell.* Philadelphia: WB Saunders; 1981.)

complex. Because of the structural conformation of those subunits, several 9- to 11-nm wide channels are available for simple diffusion of ions and small molecules. However, macromolecules and particles larger than 11 nm cannot reach or leave the nuclear compartment via simple diffusion; instead, they are selectively transported via a **receptor-mediated transport** process. Signal sequences of molecules to be transported through the nuclear pores must be recognized by one of the many receptor sites of the nuclear pore complex. Transport across the nuclear pore complex is frequently an energy-requiring process.

The bidirectional traffic between the nucleus and the cytoplasm is mediated by a group of target proteins

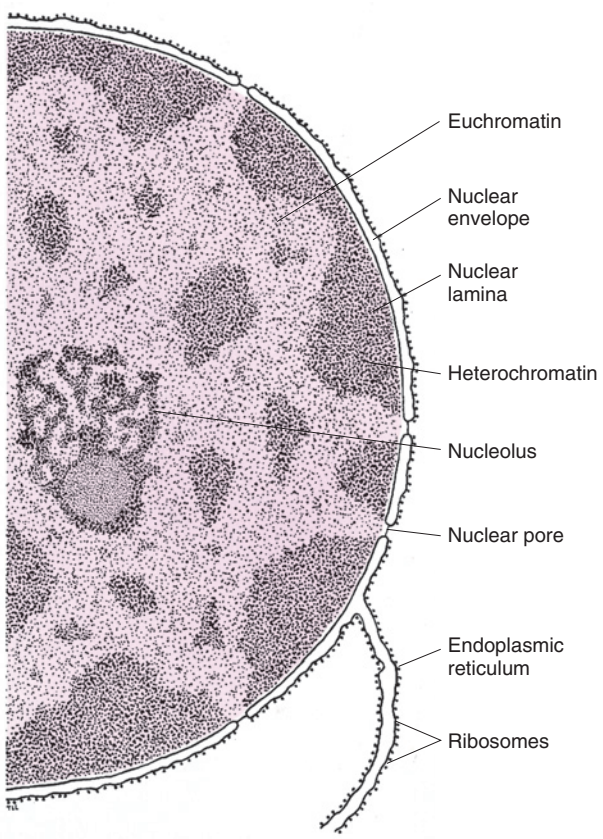


Figure 3–3 Nucleus. The outer nuclear membrane is studded with ribosomes on its cytoplasmic surface, and it is continuous with the rough endoplasmic reticulum. The space between the inner and outer nuclear membranes is the perinuclear cistern. Observe that the two membranes are united at the nuclear pores.

containing **nuclear localization signals (NLSs)** known as ***importins*** and **nuclear export signals** known as ***exportins*** (also known as *karyopherins*, *PTACs*, *transportins*, and *Ran-binding proteins*). **Exportins** transport macromolecules (e.g., RNA) from the nucleus into the cytoplasm, whereas **importins** transport cargo (e.g., protein subunits of ribosomes) from the cytoplasm into the nucleus. Exportin and importin transport is regulated by a family of GTP-binding proteins known as **Ran** (see Fig. 3–7). These specialized proteins along with other **nucleoporins** located along receptor sites in the nuclear pore complex facilitate the signal-mediated import and export processes. Some protein trafficking is more like shuttling because some proteins pass back and forth between the cytoplasm and the nucleus in a continuous fashion. Recently, it has been reported that certain other transport mechanisms literally shuttle in both directions. These transport signals are called ***nucleocytoplasmic shuttling*** signals. Proteins that carry these signals interact with mRNA.

CHROMATIN

Chromatin is a complex of DNA and proteins and represents the relaxed, uncoiled chromosomes of the interphase nucleus.

DNA, the cell's genetic material, resides in the nucleus in the form of **chromosomes**, which are clearly visible during cell division. In the interval between cell divisions, the chromosomes are unwound in the form of **chromatin** (see Figs. 3–2 and 3–3). Depending on its transcriptional activity, chromatin may be condensed as heterochromatin or extended as euchromatin.

Heterochromatin, a condensed inactive form of chromatin, stains deeply with Feulgen stains, which make it visible with the light microscope. It is located mostly at the periphery of the nucleus and comprises almost 90% of the total chromatin of the nucleus. The remainder of the chromatin scattered throughout the nucleus and not visible with the light microscope is **euchromatin**. This represents the active form of chromatin where the genetic material of the DNA molecules is being transcribed into RNA. Therefore, heterochromatin is the folded, non-euchromatin that is not being transcribed.

When euchromatin is examined with electron microscopy, it is seen to be composed of a thread-like material 30 nm thick. More careful evaluation indicates that these threads may be unwound, resulting in a 10- to 11-nm wide structure resembling beads on a string. The beads are termed ***nucleosomes,*** and the string, which is the **DNA molecule**, appears as a thin filament 2 nm in diameter (Fig. 3–8).

Each nucleosome is composed of an octomer of proteins, duplicates of each of four types of **histones** (**H_2 A, H_2 B, H_3,** and **H_4**). The nucleosome is also wrapped with two complete turns (approximately 150 nucleotide pairs) of the DNA molecule that continues as **linker DNA** extending to the next bead. The spacing between each nucleosome is about 200 base pairs. This configuration of the nucleosome with its coils of DNA represents the simplest arrangement of chromatin packaging in the nucleus. Because only a small amount of the chromatin in the cell is in this configuration, it is thought to represent regions where the DNA is being transcribed.

During the cell cycle, **chromatin assembly factor 1** expedites the rapid assembly of the nucleosomes of the newly synthesized DNA into chromatin so that it cannot become a template. Therefore, the nucleosome/histone assembly not only provides structural framework for the chromatin but also imparts control mechanisms important in DNA repair, replication, and transcription.

Figure 3–4 Nuclear pores. Electron micrograph (×47778). Many nuclear pores may be observed in this freeze-fractured preparation of a nucleus. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

Electron microscopic studies of the nuclear contents following more careful manipulation have revealed chromatin fibers exhibiting diameters of 30 nm. Packaging of chromatin into 30-nm threads is believed to occur by helical coiling of consecutive nucleosomes at six nucleosomes per turn of the coil and cooperatively bound there with **histone H_1** (see Fig. 3–8). Nonhistone proteins are also associated with the chromatin, but their function is not clear.

Chromosomes

Chromosomes are chromatin fibers that become so condensed and tightly coiled during mitosis and meiosis that they are visible with the light microscope.

As the cell leaves the interphase stage and prepares to undergo mitotic or meiotic activity, the chromatin fibers are extensively condensed to form **chromosomes,** visible with light microscopy. Tighter condensing of the chromatin material is accomplished by looping the coiled 30-nm fibers into 300-nm loops, held together by specific protein/DNA-bound complexes located at their bases. Further coiling of the 300-nm loops into tightly woven 700-nm helical loops forms the maximally condensed chromosomes observed in the metaphase stage of mitosis or meiosis (Fig. 3–9).

The number of chromosomes in somatic cells is specific for the species and is called the ***genome*,** the total genetic makeup. In humans, the genome consists of 46 chromosomes, representing 23 homologous pairs of chromosomes. One member of each of the chromosome pairs is derived from the maternal parent; the other comes from the paternal parent. Of the 23 pairs, 22 are called **autosomes**; the remaining pair that

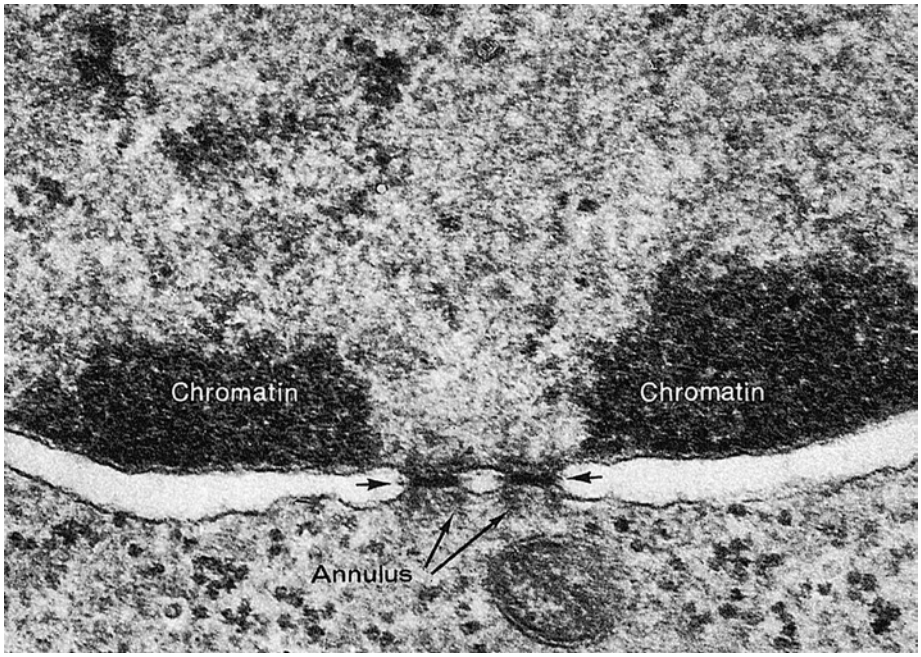


Figure 3–5 Nuclear pore. Electron micrograph (×24828). Note the heterochromatin adjacent to the inner nuclear membrane and that the inner and outer nuclear membranes are continuous at the nuclear pore. (From Fawcett DW. *The Cell*. Philadelphia: WB Saunders; 1981.)

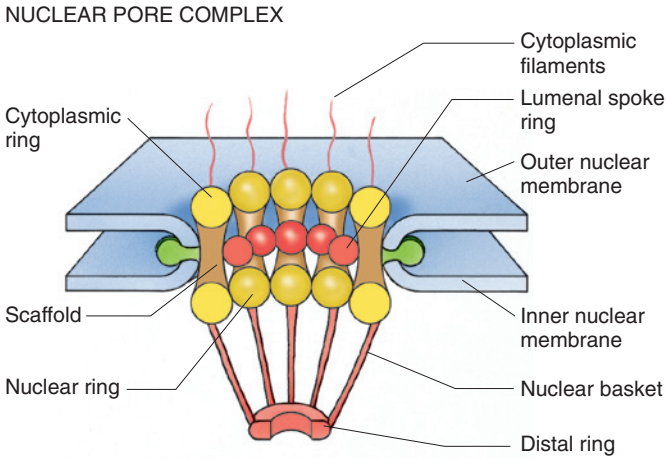


Figure 3–6 Nuclear pore complex. This schematic representation of the current understanding of the structure of the nuclear pore complex demonstrates that it is made up of several combinations of eight units each. Note the model does not include a transporter (see text). (Modified from Alberts B, Bray D, Lewis J, et al. *Molecular Biology of the Cell*. 3rd ed. New York: Garland Publishing; 1994.)

determines gender are the **sex chromosomes**. The sex chromosomes of the female are two X chromosomes **(XX)**; those of a male are the X and Y chromosomes **(XY)** (see Fig. 3–9).

The three-dimensional arrangement of chromosomes in the nuclear milieu varies with cell types, in that the same cell types, in the same functional state, have their chromosomes arranged in a similar fashion, and it differs in cells of different types. It appears that specific chromosomes occupy their own, well-defined space in the nucleus and that chromosomes do not violate the spaces that belong to other chromosomes. It

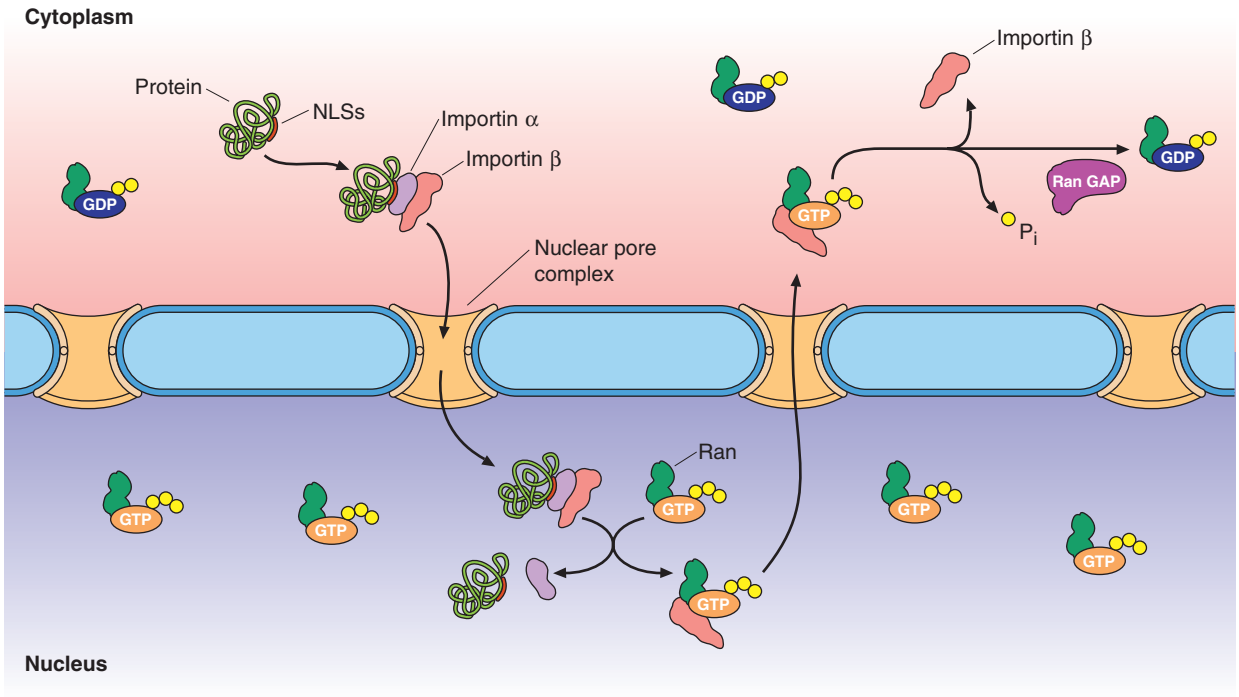


Figure 3–7 Role of Ran in nuclear import. Ran/GDP is present in high concentration in the cytoplasm, whereas Ran/GTP is present in high concentration in the nucleus. Proteins to be imported into the nucleus form complexes with nuclear localization signals (NLS) importin α and importin β. Upon import through the nuclear pore complex, Ran/GTP binds to importin β, thus releasing importin α and the imported protein. To complete the cycle, the Ran/GTP/importin β complex exits the nucleus to enter the cytoplasm via the nuclear pore complex. Here, the Ran/GTPase activating protein (RanGAP) hydrolyzes the GTP forming Ran GDP, thus releasing importin β back into the cytoplasm.

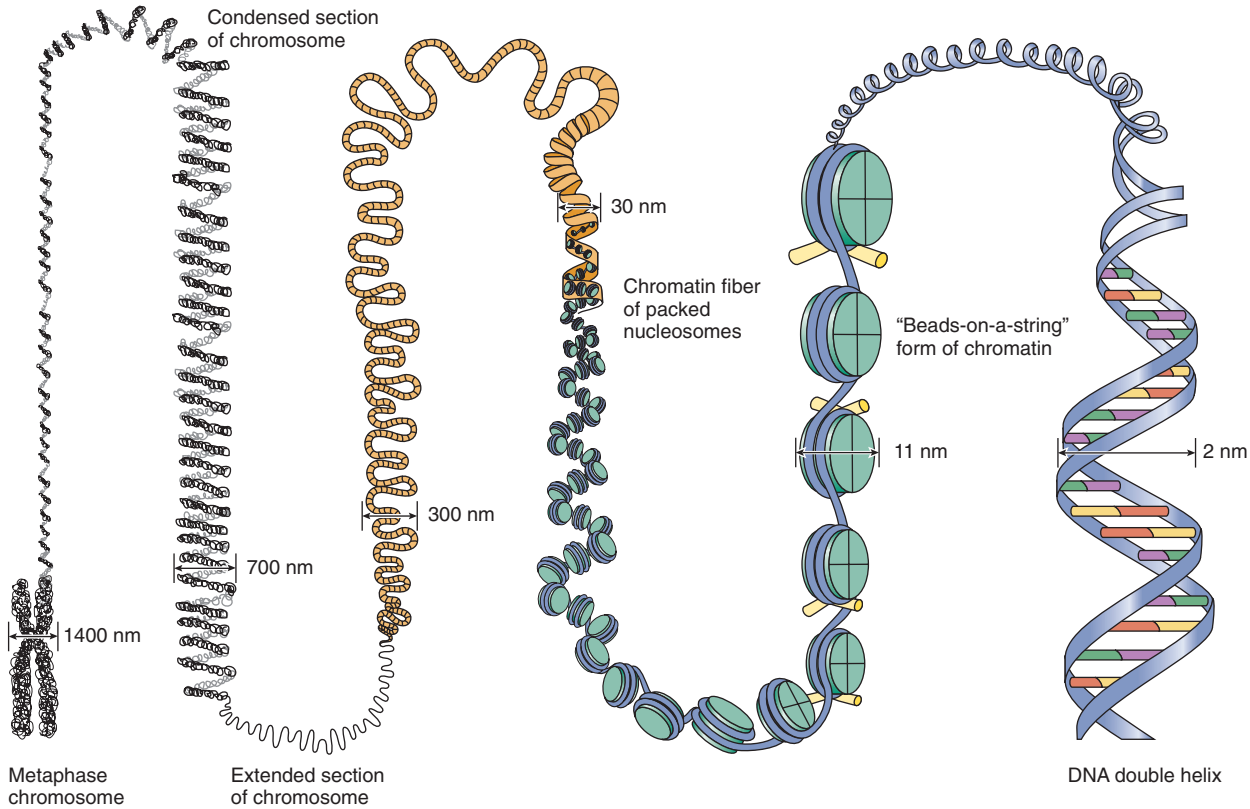


Figure 3–8 Chromatin packaging. Schematic diagram displaying the complex packaging of chromatin to form a chromosome.

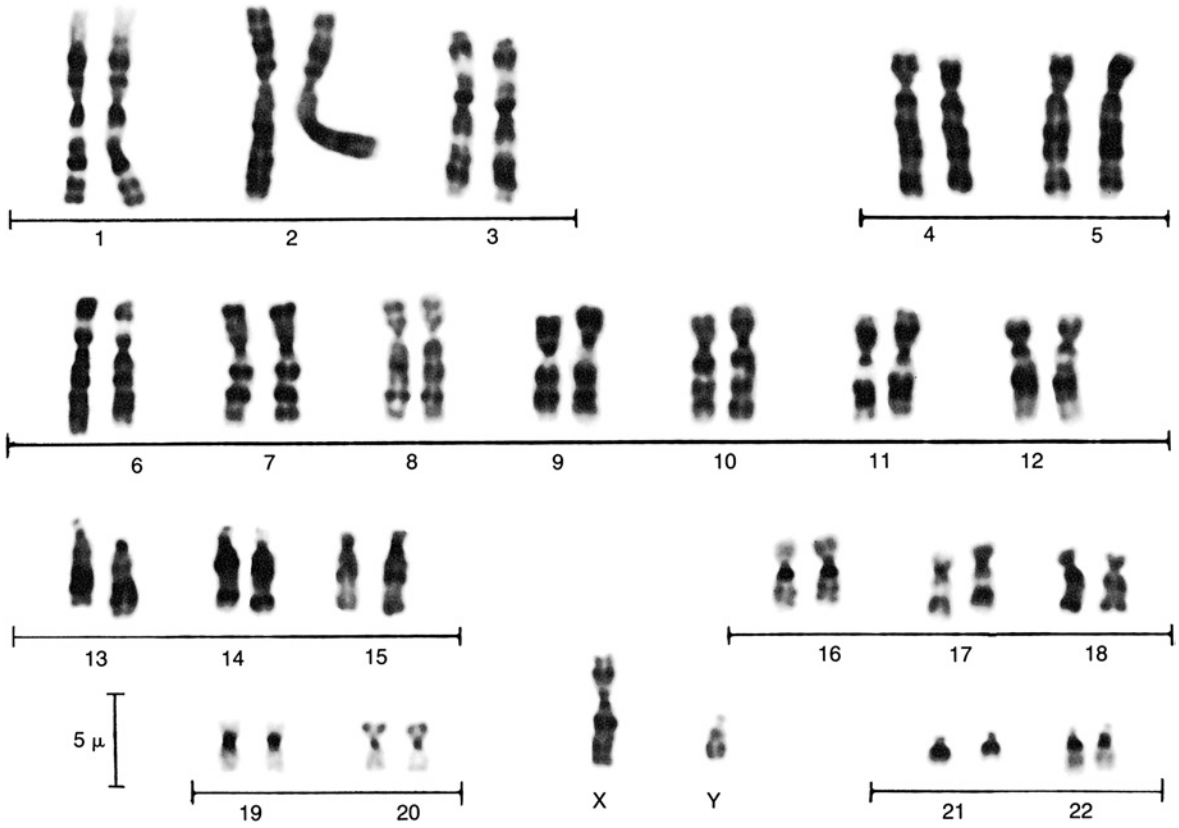


Figure 3–9 Human karyotype. A normal human karyotype illustrating banding. (From Bibbo M. *Comprehensive Cytopathology*. Philadelphia: WB Saunders; 1991.)

has been demonstrated that, in human leukocytes, some chromosomes, such as chromosome 19, are always near the center of the nucleus; others, such as chromosome 7, are more peripherally positioned; and still others, such as chromosome 18, are near the inner nuclear membrane. Although this situation is consistent, changes in chromosomal location do occur, especially when the cell alters its metabolic activity. It has been noted that chromosomes at the periphery of the nucleus are not being transcribed, whereas those in the center of the nucleus are undergoing active transcription; therefore, when a particular gene is to be transcribed, the region of the chromosome that is occupied by that gene moves from a peripheral nuclear location to a central nuclear location, known as a ***transcription factory***, a space rich in transcription factors and RNA polymerases.

Telomeres are short, repeated, DNA sequences at the ends of chromosomes. They appear to protect the ends of the chromosomes from degradation, and in oocytes and spermatogonia as well as in stem cells an enzyme–RNA complex, known as ***telomerase***, maintains the telomere length. Interestingly, the RNA portion of the enzyme is used as a template to synthesize the additional DNA necessary to maintain telomere length. Somatic cells do not possess telomerase, and, with each successive cell division, the telomeres become shorter, and eventually they become short enough that they can no longer protect the chromosome, and the cell becomes unable to replicate itself. This built in senescence is absent in cancer cells because many malignant cells are able to express the gene that codes for telomerase.

Sex Chromatin

Only one of the two X chromosomes in female somatic cells is transcriptionally active. The inactive X chromosome, randomly determined early in development, remains inactive throughout the life of that individual (see section on RNA).

CLINICAL CORRELATIONS

It has been reported that individuals who exercise and people who have higher education appear to possess longer **telomeres,** whereas people who are long-term smokers, individuals who drink alcohol excessively, and people who are under very high stress levels have shorter telomeres. It has been suggested that people with shorter telomeres tend to have a shorter life span.

In some individuals, mostly males, the X chromosome displays a narrowed region near its terminus. About 80% of the males possessing such X chromosomes are mentally retarded and display a decreased ability to accomplish certain tasks; they are also hyperactive and display anxious behavior. This condition is known as the ***fragile X syndrome***, due to the morphology of the X chromosome whose tip appears to be almost broken. The altered region of the X chromosome is the locus for the ***FMR1* gene** (fragile X mental retardation 1 gene) that codes for the **FMR protein**. This protein represses translation of certain mRNAs and thereby inhibits the formation of cytoskeletal elements at synapses; in that fashion, it interferes with neuronal plasticity.

Microscopic study of interphase nuclei of cells from females displays a very tightly coiled clump of chromatin, the **sex chromatin (Barr body)**, the inactive counterpart of the two X chromosomes. Epithelial cells obtained from the lining of the cheek and neutrophils from blood smears are especially useful for studying sex chromatin. The sex chromatin is observed at the edge of the nuclear envelope in smears of the oral epithelial cells and as a small drumstick-like evagination of the nuclei of the neutrophils. A number of cells must be examined to observe sex chromatin because the X chromosome must be in the proper orientation to be displayed for observation.

Ploidy

Cells containing the full complement of chromosomes (46) are said to be **diploid (2n)**. Germ cells (mature ova or spermatozoa) are said to be **haploid (1n)**; that is, only one member of each of the homologous pairs of chromosomes is present. Upon fertilization, the chromosomal number is restored to the 2n amount as the nuclei of the two germ cells unite.

Certain alkaloids, such as colchicine, a plant derivative, arrest a dividing cell in the metaphase stage of mitosis when the chromosomes are maximally condensed, thus permitting the pairing and numbering of the chromosomes via a conventional system of **karyotyping,** an analysis of chromosome number (see Fig. 3–9).

CLINICAL CORRELATIONS

One item that may be observed from the karyotype is **aneuploidy,** an abnormal chromosome number. People with **Down syndrome,** for example, have an extra chromosome 21 **(trisomy 21)**; they exhibit mental retardation, stubby hands, and many congenital malformations, especially of the heart, among other manifestations.

Certain syndromes are associated with abnormalities in the number of sex chromosomes. **Klinefelter syndrome** results when an individual possesses three sex chromosomes **(XXY)**. These persons exhibit the male phenotype, but they do not develop secondary sexual characteristics and are usually sterile. **Turner syndrome** is another example of aneuploidy called ***monosomy*** of the sex chromosomes. The karyotype exhibits only one sex chromosome **(XO)**. These individuals are females whose ovaries never develop and who have undeveloped breasts, a small uterus, and exhibit mental retardation.

Giemsa reagent stains the adenine-thymine–rich regions of chromosomes, producing a pattern of **G bands** that is unique for each chromosome pair and is characteristic for each species. Careful analysis of the G bands can help reveal deletions of certain portions of the chromosome, nondisjunctions, translocations, and so on, that may assist in the diagnosis of certain genetic disorders or diseases resulting from chromosomal anomalies.

Deoxyribonucleic Acid

Deoxyribonucleic acid (DNA), the genetic material of the cell, is located in the nucleus, where it acts as a template for RNA transcription.

Nearly the entire DNA**,** a 2-m long, double-stranded polynucleotide chain wound into a double helix, is housed in the nucleus of the cell. Each nucleotide is composed of a nitrogenous base, a deoxyribose sugar, and a phosphate molecule. Further, the nucleotides are linked to one another by phosphodiester bonds formed between the sugar molecules.

There are two types of bases: **purines** (adenine and guanine) and **pyrimidines** (cytosine and thymine). A double helix is established by the formation of hydrogen bonds between complementary bases on each strand of the DNA molecule. These bonds are formed between **adenine (A)** and **thymine (T)** and between **guanine (G)** and **cytosine (C)**.

Genes

The biological information that is passed from one cell generation to the next—the units of heredity—are

located at specific regions on the DNA molecule called ***genes***. Each gene represents a specific segment of the DNA molecule that codes for the synthesis of a particular protein or set of closely related proteins and is transcribed as a single RMA molecule. The sequential arrangement of bases constituting the gene represents the sequence of amino acids of the protein. The genetic code is designed in such a manner that a triplet of consecutive bases, a **codon,** denotes a particular amino acid. Each amino acid is represented by a different codon. The complete set of genes that belongs to an individual, that is both the **coding** and **noncoding segments** of the DNA, is known as the ***genome***. Coding segments possess the codons that determine the sequence of amino acids ion a protein (or polypeptide), and noncoding segments possess regulatory or other functions.

Prior to beginning the Human Genome Project, it was believed that the 3 billion nucleotide bases in the human genome represented about 100,000 genes. Preliminary analysis at the conclusion of the project indicated that the number of genes was far less than expected. Currently, the data indicate that the human genome contains about 25,000 protein coding genes, all of which were sequenced and mapped, which represents only 2% of the genome; 98% of the genome has regulatory or other functions. Findings from these studies have already increased our understanding of some genetic disorders as well as indicated better treatment modalities for several diseases with the promise of many other discoveries and applications in future years.

Epigenetics

Epigenetics is a relatively new field of study that explores chemically induced heritable changes that occur to the genome without altering the sequence of nucleotides of the DNA molecule. These changes are caused by the addition of small molecules, such as **methyl groups** or **acetyl groups,** to the histones that compose the core of the chromatid, and these small molecules act as markers that either silence the genes or cause the expression of the genes that are wrapped around the methylated or acetylated histones. The addition of these small molecules can transpire not only during embryogenesis but also in the adult individual and may be caused by various environmental insults, such as toxic agents, or environmental stimuli, such as stress. It is important to realize that these are inheritable alterations in the same fashion that mutations in the sequence of DNA nucleotides are inheritable. Methyl groups have been shown to increase the tightness of the winding of the DNA thread around the histones, making that stretch less accessible to transcription; acetyl groups cause a looser wrapping of the DNA thread around the histones, making that stretch of the DNA more available for transcription. Thus, generally speaking, methylation tends to silence genes, whereas acetylation facilitates gene expression.

CLINICAL CORRELATIONS

Recent evidence has demonstrated that individuals who committed suicide had a much greater degree of **methylation of their chromosomes** of their **hippocampus** (the part of the brain responsible for memory formation) than did members of the control group who died suddenly but not due to suicide.

In another related study, it was shown that children who grew up in an orphanage exhibited a greater degree of **chromosome methylation** than did the control group consisting of children who grew up in the home of their biological parents. Most of the methylated genes were related to **brain development** and **neural function**. Although the inheritance of these traits have not been proven in humans, mouse studies have shown that stress-related epigenetic alterations are transmitted from parents to pups.

Ribonucleic Acid

RNA is similar to DNA, except that it is single-stranded, one of its bases is uracil instead of thymine, and its sugar is ribose instead of deoxyribose.

RNA (Table 3-1) is similar to DNA, with both composed of a linear sequence of nucleotides, but RNA is single stranded (see Fig. 3–9), and the sugar in RNA is ribose not deoxyribose. One of the bases, thymine, is replaced by uracil, which, similar to thymine, is complementary to adenine. There are two major types of RNA, **coding RNA** and **noncoding RNAs**. Coding RNA, namely messenger RNA (mRNA), carries the code for protein synthesis, and noncoding RNAs assist in protein synthesis (transfer RNA [tRNA] and ribosomal RNA [rRNA]) as well as in regulatory functions.

The DNA in the nucleus serves as a template for synthesis of a complementary strand of RNA, a process called ***transcription***. Synthesis of three of the various types of RNA is catalyzed by three different **RNA polymerases**:

- **mRNA** by RNA polymerase II
- **tRNA** by RNA polymerase III
- **rRNA** by RNA polymerase I

The mechanism of transcription is generally the same for all three types of RNA. It should be noted that only mRNA is transcribed from the **coding segments** of the DNA. The rRNA, tRNA, and regulatory RNAs are transcribed from the **noncoding segments** of the DNA.

TABLE 3–1 Types of RNA

Abbreviation	Name	Function
mRNA	Messenger RNA	A coding RNA that functions as the template for protein synthesis
rRNA	Ribosomal RNA	Combines with proteins to form the two ribosomal subunits to function in protein synthesis
tRNA	Transfer RNA	Binds to amino acids to carry them to the correct sites on the mRNA in protein synthesis
miRNA	Micro RNA	Short RNA segments (19 to 25 nucleotides) that have regulatory functions by blocking protein synthesis; they also promote cancerogenesis by blocking apoptotic pathways
siRNA	Small interfering RNA Silencing RNA	Short double-stranded RNA segments (20 to 22 nucleotides) that interfere with protein synthesis. They are released by viruses or are transposons that enter the cell. There are synthetic siRNAs that are manufactured for therapeutic purposes.
lincRNA, also known as lncRNA	Long intergenic noncoding RNA Long noncoding RNA	Long chain RNA (200 or more nucleotides) that regulate cancerogenesis and embryogenesis and inactivate the second X chromosomes in females
piRNA	Piwi-interacting RNA	Interfere with the expression of transposons; facilitates the placement of epigenetic markers on chromosomes

Messenger RNA

The mRNA carries the genetic code from the nucleus to the cytoplasm to act as a template for protein synthesis.

The **mRNA** serves as an intermediary for carrying the genetic information encoded in DNA that specifies the primary sequence of proteins from the nucleus to the protein-synthesizing machinery in the cytoplasm (see Fig. 3–9). Each mRNA is a complementary copy of the region of the DNA molecule that codes for a single protein or a combination of proteins. An mRNA molecule thus consists of a series of codons corresponding to particular amino acids. It also contains a **start codon** (AUG), which is necessary for initiating protein synthesis, and one or more **stop codons** (UAA, UAG, or UGA), which act to terminate protein synthesis. Once formed in the nucleus, mRNA is transported to the cytoplasm, where it is translated into protein (see Chapter 2).

Transcription

Transcription of DNA into mRNA begins with the attachment of RNA polymerase II to a **core promoter**, a specific DNA sequence located adjacent to a gene. In the presence of a series of cofactors, RNA polymerase II initiates transcription by unwinding the double helix of the DNA two turns, thus exposing the nucleotides and, therefore, codons on the DNA strand. The enzyme uses one of the exposed DNA strands as a template on which to assemble and polymerize complementary bases of the RNA molecule.

The process repeats as a new region of the DNA double helix is unwound, and more nucleotides are polymerized into the growing mRNA chain. As the enzyme moves along the DNA molecule, the polymerized mRNA chain is separated from the template DNA strand, permitting the two DNA strands to re-form into the double-helix configuration (Fig. 3–10).

Transcription begins at a DNA triplet corresponding to the start codon AUG and is concluded when the RNA polymerase II recognizes a **chain-terminator** site complementary to the stop codons UAA, UAG, or UGA. When the enzyme reaches the chain terminator, it is released from the DNA molecule, permitting it to repeat the process of transcription. Simultaneously, the newly formed RNA strand (known as the ***primary transcript***) is released from the DNA molecule, leaving it free in the nucleoplasm.

The primary transcript is a long, single-stranded RNA molecule, called ***precursor messenger RNA*** (**pre-mRNA**). It contains both coding segments (**exons**) and noncoding segments **(introns)**. The introns must be removed, and the exons have to be spliced together. For that to occur, pre-mRNA and nuclear processing proteins form complexes of **heterogeneous nuclear ribonucleoprotein particles (hnRNPs)** that begin **RNA splicing,** thus reducing the length of the

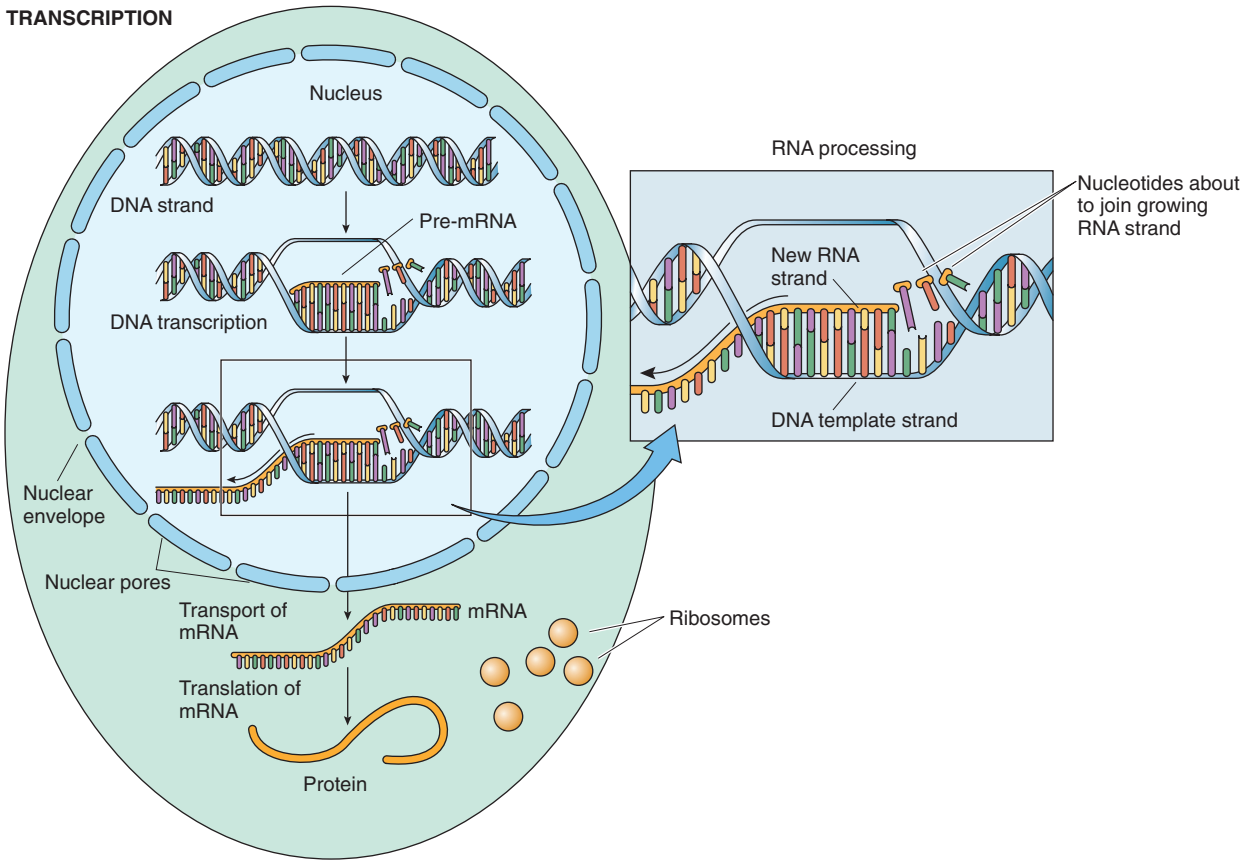


Figure 3–10 DNA transcription. A schematic illustrating transcription of DNA into messenger RNA (mRNA). (Modified from Alberts B, Bray D, Lewis J, et al. *Molecular Biology of the Cell*. 3rd ed. New York: Garland Publishing; 1994.)

pre-mRNA molecule. Additional processing involves **spliceosomes**, complexes of five **small nuclear ribonucleoprotein particles (snRNPs)** and a large number of **non-snRNP splicing factors** that assist in the splicing mechanism to produce **messenger ribonucleoprotein**. Finally, the nuclear processing proteins are removed from the complex, leaving mRNA ready to be transported out of the nucleus via the nuclear pore complexes (see Fig. 3–10).

This description of mRNA synthesis is only a brief overview and omits many details. Readers desiring more information should consult textbooks of molecular and cellular biology.

Because there is an abundance of DNA within the eukaryotic genome, much of it was believed to represent evolutionary remnants that do not ultimately code for a protein from the RNA transcript. During transcription, DNA unwinds and codes for strands of RNA forming exons (coding segments) and introns (noncoding segments). Later in this process, the exons are spliced together forming continual sequences of mRNA for translation to a protein in the cytoplasm. On the other hand, the introns that were removed from the primary RNA transcript were thought to have no function, even though they represent about 95% or more of the RNA than the protein-coding genes.

Recent evidence suggests that, although these intronic RNA segments do not encode protein, they perform regulatory functions that are in parallel with regulatory proteins. Their role may relate to differentiation, development, gene expression, and evolution. If proven correct, this could have a profound impact on understanding certain disease processes and their treatment. For example, it is known that noncoding RNAs are linked to several cancers, autism, and schizophrenia.

Transfer RNA

Transfer RNA ferries activated amino acids to the ribosome/mRNA complex, resulting in the formation of the protein.

Transfer RNA **(tRNA)** is a small RNA molecule produced from DNA by RNA polymerase III. It is about 80 nucleotides in length and is folded upon itself to resemble a cloverleaf with base pairing between some of its nucleotides.

Two regions of the tRNA are of special significance. One of these, the **anticodon,** recognizes the codon of the mRNA; the other is the amino acid-bearing region, which resides at the 3′ end of the molecule. The tRNA is aminoacylated not only in the cytoplasm but also in the nucleus. This is believed to be a "proofreading" step that facilitates functional readiness in the cytoplasm. The tRNA then transfers activated amino acids to the ribosome–mRNA complex, where they are incorporated into the polypeptide chain forming the protein (see Chapter 2).

Ribosomal RNA

Ribosomal RNA forms associations with proteins and enzymes in the nucleus to form ribosomes.

Ribosomal RNA (rRNA) is synthesized in the fibrillar (pars fibrosa) region of the nucleolus by RNA polymerase I (Fig. 3–11). The primary transcript is called ***45S rRNA*** **(pre-rRNA)**, a huge molecule of about 13,000 nucleotides. Ribosomal proteins synthesized in the cytoplasm are transported through the nuclear pore complexes, into the nucleus, and from there into the nucleolus to join a 5S rRNA molecule. Once there, they associate with the 45S rRNA molecules, forming a very large **ribonucleoprotein particle (RNP)**. This RNP is processed by several resident molecules into precursors of the large and small ribosomal subunits in the pars granulosa region of the nucleolus. Thereafter, assembled small ribosomal subunits, made up of 18S rRNAs and other ribosomal proteins, make their way from the nucleolus to the cytoplasm by transport via the nuclear pore complexes. The remaining 28S, 5.8S, and 5S rRNAs are assembled into large ribosomal subunits and are transported out of the nucleus to the cytoplasm by way of the nuclear pore complexes.

Regulatory RNAs

There are several types of regulatory RNAs, such as micro RNA (miRNA), small interfering RNA (siRNA), large intergenic noncoding RNA ([lincRNA], also known as *long noncoding RNA* [lncRNA]), and piwi-interacting RNA (piRNA). All of these are noncoding, meaning that none of these RNAs are translated into proteins.

Micro RNAs **(miRNAs)**, as their name implies, are usually very short RNA segments, composed of only 19

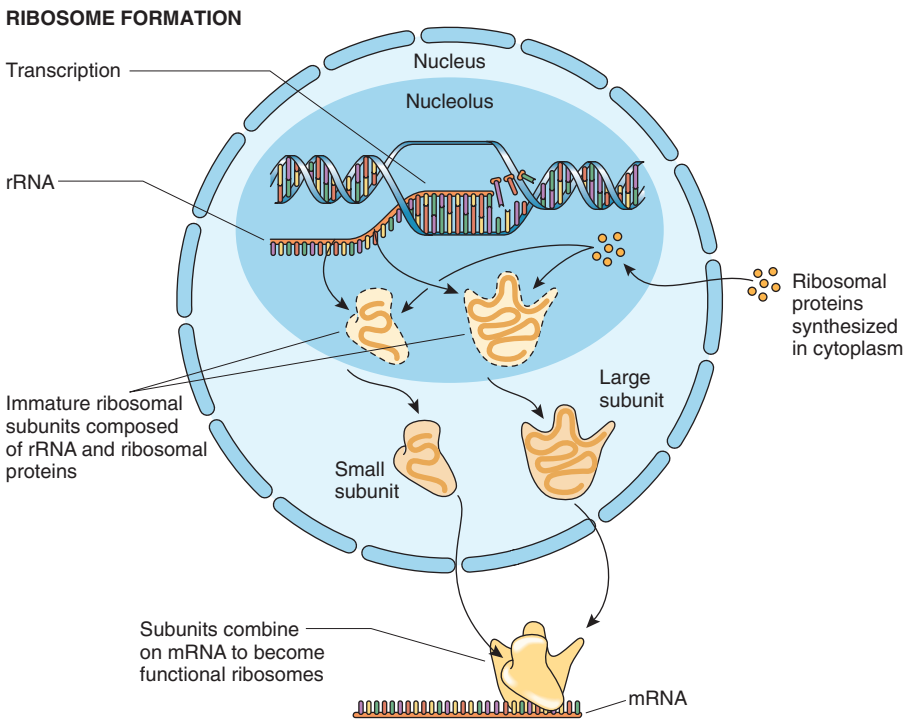


Figure 3–11 Ribosome formation. A schematic presentation of the nuclear events in ribosome formation. (Modified from Alberts B, Bray D, Lewis J, et al. *Molecular Biology of the Cell*. 3rd ed. New York: Garland Publishing; 1994.)

to 25 nucleotides. They compose a very large group of regulatory RNAs, consisting of more than 1000 members each with a specific function. The miRNAs are either transcribed from DNA or are formed from the introns that are spliced from the pre-mRNA. The newly formed long nucleotide sequences (that can be composed of hundreds of nucleotides) are known as ***primary miRNA* (pri-miRNA)** parts of which are folded in several double stranded "hairpin loop" configurations, where each double-stranded hairpin loop is a future miRNA precursor. The pre-miRNA is processed in the nucleus by a group of proteins, known as the ***microprocessor complex***, that liberates individual hairpin loop groups composed of approximately 70 nucleotides, known as **pre-miRNA**. These liberated pre-miRNAs are exported from the nucleus into the cytoplasm through nuclear pore complexes, an energy required step that uses the shuttle protein **exportin-5**. Once in the cytoplasm, the pre-miRNA is modified by the **RNAse III enzyme** known as **dicer**, which cleaves the loop portion of the hairpin, thus forming two strands of nucleotides, each with two free ends. The two strands become separated, and one of the strands becomes destroyed, whereas dicer facilitates the other strand, now known as ***miRNA***, to form an miRNA–protein complex, known as ***RNA-induced silencing complex-miRNA* (RISC-miRNA)**. It is the RISC–miRNA that accomplishes the attachment of the miRNA to the mRNA by way of the formation of complementary nucleotide bonds. The nucleotides of the miRNA do not have to match perfectly with the nucleotide sequence of the mRNA; in fact, in mammals, the match is usually imperfect. The presence of the RISC–miRNA complex on the mRNA not only blocks protein synthesis but also facilitates the rapid degradation of the mRNA and, in that fashion, inhibits gene expression. In addition to blocking protein synthesis, miRNAs interfere with the ability of cells to enter the apoptotic pathway, making those cells "immortal" and thereby promoting cancerogenesis, whereas other miRNAs have been shown to suppress metastasis in some human breast cancers. The miRNAs also function in facilitating some and interfering with other cellular signaling activities.

The **siRNAs**, also known as ***silencing RNAs***, are similar to miRNAs in that they are initially double stranded and are about 20 to 22 nucleotides in length. They are usually released by viruses or are transposons that enter the cell and are attached to RISC, forming an RISC–siRNA complex. These complexes bind, in a complementary fashion, to mRNAs and interfere with protein synthesis; additionally, just as RISC–miRNA complexes, they expedite the degradation of the mRNAs to which they are attached and inhibit gene expression.

The **lincRNA**, also known as ***lncRNA***, are long chains composed of 200 or more nucleotides. Although some investigators believe these lincRNAs to have no function and are destined to be degraded, others have demonstrated that there are at least 35,000 known lincRNAs. Some are present in the nucleus, and others are located in the cytoplasm, suggesting that many of them must have metabolic roles; otherwise, they would not have been conserved during evolution. It is known that at least two lincRNAs, H19 and XIST (X-inactive specific transcript), have important functions, the former in cancerogenesis and embryogenesis and the latter in inactivating the second X chromosomes of females.

- **H19**, although present on both maternally and paternally derived chromosomes, is expressed only on the maternally derived chromosome. It is believed to act as regulators of gene expression at the chromosomal level by specifically enlisting groups of regulatory proteins to those sites of the genes that control transcription, thereby enhancing or inhibiting mRNA formation. The RNA gene product of H19 is especially present in high concentration in malignant cells derived from cancers of the esophagus, colon, bladder, liver, ovary, lung, testis, breast, and uterus.
- The lincRNA, **XIST,** is especially active in females as a product of one of the two X chromosomes, specifically the one that will become silenced. Although the process of inactivating the second X chromosome in females is not understood completely, it is known that XIST binds with a protein complex, known as ***polycomb repressive complex 2***, that inactivates the chromosome in question by forming a microscopically visible enclosure around the entire chromosome that is attached to specific sites on the chromosome. Interestingly, some of the genes are permitted to be expressed, and these chromosomal segments are noted to be protruding outside of the physical enclosure.

The piRNA are about 25 to 30 nucleotides in length and compose a very large group of RNA in mammals, consisting of more than 50,000 members. In mammals, they are located primarily in the testes and in the ovaries; they are housed in both the cytosol and the nucleus. The precise functions of piRNA are not known, but they are believed to repress gene expression and are believed to function in the formation of spermatozoa.

- Recently, it was noted that pi-RNA recruits **piwi protein** and binds to it, and the piRNA–piwi protein complex migrates to specific sites on the DNA and recruits a host of proteins that place **epigenetic marks** on the histones at that position. These epigenetic marks become permanent marks on the chromosomes and cause that particular gene to be silenced or expressed.
- It has also been demonstrated that, during embryogenesis, piRNA interferes with the expression of transposons.
- There are two categories of transposons: (1) segments of DNA that are excised from one chromosome to be inserted into another chromosome,

and (2) DNA sequences that were synthesized from RNA templates via the enzyme reverse transcriptase.

- Transposons are responsible for mutations as well as an increase in the DNA content of the cell that may cause cell death.

CLINICAL CORRELATIONS

Not all miRNAs are synthesized by cells of the individual organism; in fact, some enter the body as part of the digestive process. It has been demonstrated that certain plant miRNAs that were ingested by patients were present in their native state in the blood of these individuals. An example of an miRNA, known as ***MIR168a***, present in rice has been noted to be abundant in the blood of individuals whose diet contains rice. This miRNA binds with **low-density lipoprotein (LDL) receptor adapter protein 1 mRNA** in the liver, whose protein product is responsible for LDL clearing from the blood stream.

Recently, investigators have created **siRNAs** that act as naturally introduced siRNAs in that they form **RISC-siRNA** that complement regions of the mRNA that the investigators want silenced. It is believed that these synthetic siRNAs will have therapeutic benefits in fighting certain diseases.

A relatively recent development is a form of genetic engineering that involves the synthesis of an RNA molecule whose nucleotides complement the region of interest of the human genome. This is performed by proteins known as **Cas proteins** (**CRSPR-associated proteins**, where the acronym stands for clustered regularly interspaced short palindromic repeats). The Cas proteins associated with the synthetic RNA molecule seek out the complimentary regions of the DNA molecule and the complex excises the targeted DNA nucleotide sequence. Future therapeutic actions plan to replace the excised DNA region with the corrected patch of DNA sequence, thus not only eliminating the disease-causing sequence but also supplying the corrective action to repair the mutated gene.

Nucleoplasm

The nucleoplasm consists of interchromatin and perichromatin granules, RNPs, and the nuclear matrix.

Nucleoplasm, separated from the cytoplasm by the nuclear envelope, is a somewhat viscous substance that surrounds the chromosomes and the nucleoli and is composed of interchromatin and perichromatin granules, water, ribonucleoproteins (RNPs), and the nuclear matrix.

Interchromatin granules, which are 20 to 25 nm in diameter, contain RNPs and several enzymes, including ATPase, GTPase, β-glycerophosphatase, and NAD-pyrophosphatase. They are located in clusters scattered throughout the nucleus among the chromatin material and appear to be connected to each other by thin fibrils. Their function is unclear.

Perichromatin granules are 30 to 50 nm in diameter and are located at the margins of the heterochromatin. These electron-dense particles are surrounded by a 25-nm wide halo of a less dense region. They are composed of densely packed fibrils of 4.7S low-molecular-weight RNA complexed to two peptides, resembling **hnRNPs (heterogenous nuclear ribonucleoprotein particles)**.

The **snRNPs (small nuclear ribonucleoprotein particles)** participate in splicing, cleaving, and transporting hnRNPs. Although most snRNPs are located in the nucleus, some are limited to nucleoli. Several minor subgroups of these particles have been discovered recently, but their function has yet to be elucidated.

Nuclear Matrix

The **nuclear matrix** is defined in both structural and biochemical terms. It appears that differences reported in its components may be due to the extraction methods employed in studying its contents. Biochemically, the matrix contains about 10% of the total protein, 30% of the RNA, 1% to 3% of the total DNA, and 2% to 5% of the total nuclear phosphate. The structural components include the nuclear pore–nuclear lamina complex, residual nucleoli, residual RNP networks, and fibrillar elements. Recent studies have revealed that the nucleus possesses a nucleoplasmic reticulum that is continuous with the ER of the cytoplasm and the nuclear envelope. This reticulum houses nuclear calcium that functions within the nucleus. Further, this reticulum possesses receptors for inositol 1,4,5-trisphosphate that ultimately regulate calcium signals within certain compartments of the nucleus, namely, regions dedicated to protein transport, transcription of certain genes, and possibly others.

Functionally, the nuclear matrix is associated with DNA replication sites, rRNA and mRNA transcription and processing, steroid receptor binding, heat shock proteins, carcinogen binding, DNA viruses, and viral proteins. This list is not inclusive and does not address the functional natures of each of these associations because they are still unclear. It has been suggested, however, that the nucleus may contain many interactive subcompartments that function spatially and temporally in a tightly coordinated fashion to facilitate gene expression.

Nucleolus

The nucleolus is the deeply staining non–membrane-bounded structure within the nucleus that is involved in rRNA synthesis and in the assembly of small and large ribosomal subunits.

The **nucleolus,** a dense nonmembranous structure located in the nucleus, is observed only during

interphase because it dissipates during cell division. It stains basophilic with hematoxylin and eosin, being rich in rRNA and protein. The nucleolus contains only small amounts of DNA, which is also inactive, and thus does not stain with Feulgen stains. Usually, there are no more than two or three nucleoli per cell; however, their number, size, and shape are generally species specific and relate to the synthetic activity of the cell. In cells that are actively synthesizing protein, the nucleolus may occupy up to 25% of the nuclear volume. Densely staining regions are the **nucleolus-associated chromatin,** which is being transcribed into rRNA (see Figs. 3–2 and 3–3).

Four distinct areas of the nucleolus have been described:

- **A pale-staining fibrillar center,** containing inactive DNA (not being transcribed)
- **Pars fibrosa,** containing nucleolar DNAs being transcribed into rRNA as well as the protein **nucleolin**, small nucleolar ribonuclear proteins, and ribonucleoprotein enzyme **fibrillarin**, necessary for the converting of the pre-rRNA into mature rRNA
- **Pars granulosa,** in which maturing ribosomal subunits are assembled into the small and large ribosomal subunits
- **Nucleolar matrix,** a network of fibers active in nucleolar organization

Also located in the pale-staining regions are the tips of chromosomes 13, 14, 15, 21, and 22 (in humans), containing the **nucleolar-organizing regions (NORs)**, where gene loci that encode rRNA are located. It should be noted that the regional description of the nucleolus indicated here is more of a historical than functional significance.

The cell's ribosomal subunits are organized and assembled within the nucleolus, except those located in the mitochondria. However, recent evidence shows that the nucleolus performs additional functions. These include the synthesis of some of the regulatory RNAs, regulating some of the events in the cell cycle such as cytokinesis; the inactivation of mitotic cyclin-dependent kinases by sequestering cell cycle regulatory proteins, thus inactivating them; modification of small RNAs that moderate and modify pre-rRNA; assembly of RNP; engagement in nuclear export; and role-play in aging.

CLINICAL CORRELATION

Some suggest that the region of the DNA that codes for ribosomal RNA in the nucleolus may become unstable, thereby accelerating the aging process. In malignant cells, the nucleolus may become hypertrophic. Further, it is known that, in tumor cells, the NORs become larger and more numerous, thus indicating a poorer clinical prognosis.

THE CELL CYCLE

The cell cycle is a series of events within the cell that prepare the cell for dividing into two daughter cells.

The **cell cycle** is divided into two major events: **mitosis,** the short period of time during which the cell divides its nucleus and cytoplasm, giving rise to two **daughter cells**, and **interphase,** a longer period of time during which the cell increases its size and content and replicates its genetic material (Fig. 3–12). The cell cycle may be thought of as beginning at the conclusion of the telophase stage in **mitosis,** after which the cell enters interphase. Interphase is subdivided into three phases:

- **G_1 (gap) phase,** when the synthesis of macromolecules essential for DNA duplication begins
- **S (synthetic) phase,** when the DNA is duplicated
- **G_2 phase,** when the cell undergoes preparations for mitosis

Cells that become highly differentiated after the last mitotic event may cease to undergo mitosis either

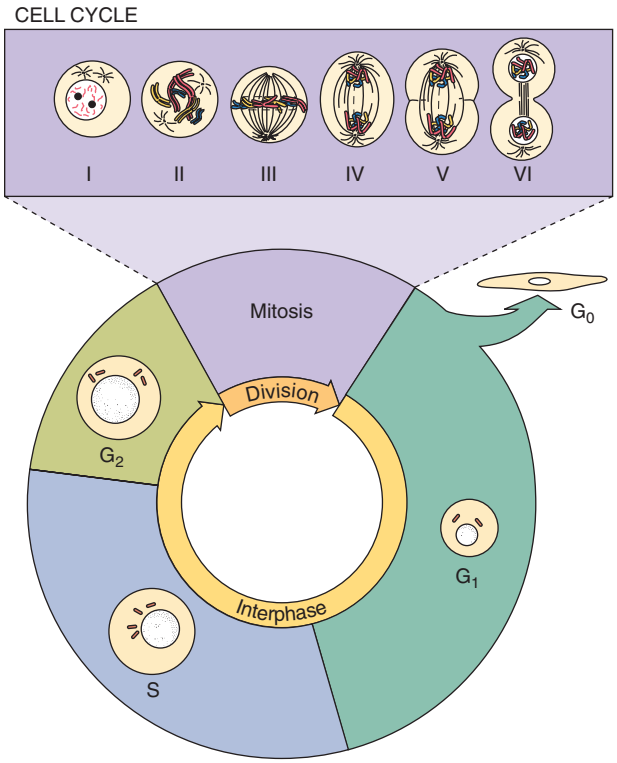


Figure 3–12 Cell cycle. A diagram illustrating the cell cycle in actively dividing cells. Nondividing cells, such as neurons, leave the cycle to enter the G_0 phase (resting stage). Other cells, such as lymphocytes, may return to the cell cycle.

permanently (e.g., neurons, muscle cells) or temporarily (e.g., peripheral lymphocytes) and return to the cell cycle at a later time. Cells that have left the cell cycle are said to be in a resting stage, the **G_0 (outside) phase**, or the **stable phase**.

Interphase

Interphase, the time between mitotic events, is subdivided into three phases: gap 1, synthesis, and gap 2.

Gap 1

G_1 phase (gap 1) is a period of cell growth, RNA synthesis, and other events in preparation for the next mitosis.

Daughter cells formed during mitosis enter the first phase of interphase, the **G_1 phase**. During this phase, the cells synthesize RNA, regulatory proteins essential to DNA replication, and enzymes necessary to carry out these synthetic activities. Thus, the cell volume, reduced by dividing the cell in half during mitosis, is restored to normal. Additionally, the nucleoli are reestablished during the G_1 phase. It is during this time that the centrioles begin to duplicate themselves, a process that is completed by the **G_2 phase**.

The triggers inducing the cell to enter the cell cycle may be (1) a mechanical force (e.g., stretching of smooth muscle), (2) injury to the tissue (e.g., ischemia), and (3) cell death. All of these incidents cause the release of ligands by signaling cells in the involved tissue. Frequently, these ligands are growth factors that indirectly induce the expression of **proto-oncogenes**, genes that are responsible for controlling the proliferative pathways of the cell.

It should be obvious that the expression of proto-oncogenes must be very strictly regulated to prevent unwanted and uncontrolled cell proliferation; in fact, mutations in the proto-oncogenes that enable the cell to escape control and divide in an unrestrained fashion are responsible for many cancers. Such mutated proto-oncogenes are known as ***oncogenes***.

Ligands designed to induce proliferation bind to cell surface receptor proteins of the target cell and activate one of the **signal transduction pathways** described in Chapter 2. Hence, extracellular signals that are perceived at the cell surface are transduced into intracellular events, most of which involve the sequential activation of a cascade of cytoplasmic **protein kinases**. These kinases activate a series of intranuclear **transcription factors** that regulate the expression of proto-oncogenes, resulting in cell division.

The capability of the cell to begin and advance through the cell cycle is governed by the presence and interactions of a group of related proteins known as ***cyclins*** with specific **cyclin-dependent kinases (CDKs)**. Thus,

- Cyclin D, synthesized during **early G_1 phase**, binds to CDK4 as well as to CDK6. Additionally, in the **late G_1 phase,** cyclin E is synthesized and binds to CDK2. These three complexes, through other intermediaries, permit the cell to enter and progress through the **S phase**.
- Cyclin A binds to CDK2 and CDK1, and these complexes permit the cell to leave the **S phase** and enter the **G_2 phase** and induce the formation of cyclin B.
- Cyclin B binds to CDK1, and this complex allows the cell to leave the **G_2 phase** and enter the **M phase**.

After the cyclins have performed their specific functions, they enter the ubiquitin–proteasome pathway, where they are degraded into their component molecules. The cell also employs quality control mechanisms, known as ***checkpoints***, to safeguard against early transition between the phases. These checkpoints ensure the meticulous completion of essential events, such as adequate cell growth, correct DNA synthesis, and proper chromosome segregation, before permitting the cell to leave its current phase of the cell cycle. The cell accomplishes such delays in the progression through the cell cycle by activating inhibitory pathways and/or by suppressing activating pathways.

The actual control mechanisms are considerably more involved and complicated than the steps just described. For example, it appears that the nucleolus plays a regulatory role in the cell cycle by sequestering certain proteins, thus inhibiting their function. The complete sequence of steps is beyond the scope of this textbook. (For more details, see relevant textbooks of cell biology as well as the current literature of the cell cycle.)

S Phase

DNA synthesis occurs during the S phase.

During the **S phase**, the synthetic phase of the cell cycle, the genome is duplicated. All of the requisite nucleoproteins, including the histones, are imported and incorporated into the DNA molecule, forming the chromatin material. The cell now contains twice the normal complement of its DNA. The amount of DNA present in autosomal and germ cells also varies. Autosomal cells contain the 2n amount of DNA before the synthetic (S) phase of the cell cycle when the 2n amount of DNA is doubled (4n) in preparation for cell division. In contrast, germ cells produced by meiosis possess the 1n number of chromosomes and also the 1n amount of DNA.

G_2 Phase

The gap 2 phase (G_2 phase) is the period between the end of DNA synthesis and the beginning of mitosis.

During the **G_2 phase**, the RNA and proteins essential to cell division are synthesized, the energy for mitosis is stored, tubulin is synthesized for assembly into microtubules required for mitosis, DNA replication is analyzed for possible errors, and any of these errors is corrected.

Mitosis

Mitosis is the process of cell division that results in the formation of two identical daughter cells.

Mitosis occurs at the conclusion of the **G_2 phase** and thus completes the cell cycle. Mitosis is the process whereby the cytoplasm and the nucleus of the cell are divided equally into two identical daughter cells (Figs. 3–13 to 3–15). First, the nuclear material is divided in a process called ***karyokinesis***, followed by division of the cytoplasm called **cytokinesis**. The process of mitosis is divided into five distinct stages: **prophase, prometaphase, metaphase, anaphase,** and **telophase** (Fig. 3–16).

Prophase

During prophase, the chromosomes condense, and the nucleolus disappears.

At the beginning of prophase, the chromosomes are condensing to become visible microscopically. Each chromosome consists of two parallel **sister chromatids**, joined together at one point along their length, the **centromere**. As chromosomes condense, the nucleolus disappears. The **centrosome** also divides into two regions, each half containing a pair of **centrioles** in a sea of **γ-tubulin rings**. It is the **microtubule-organizing center (MTOC)** of the cell. The centrosomes migrate away from each other to

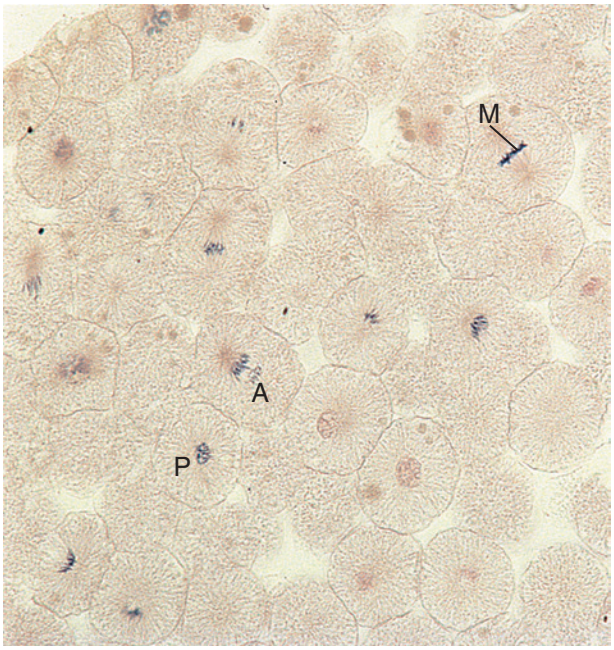


Figure 3–13 Stages of mitosis. Light micrograph (×270). Note the various stages: A, Anaphase; M, metaphase; P, prophase.

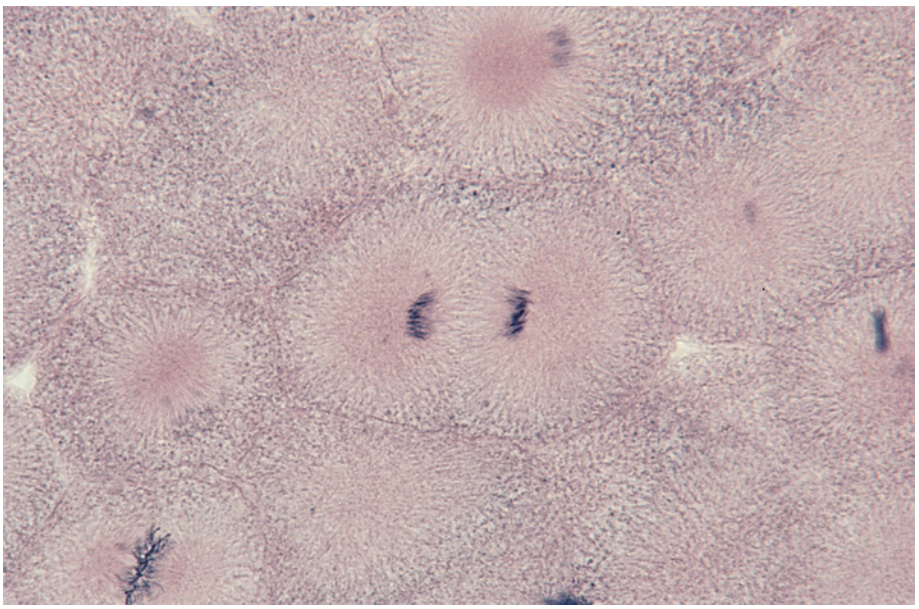


Figure 3–14 Anaphase stage of mitosis (×540). Sister chromatids have separated from the metaphase plate and are now migrating away from each other to opposite poles.

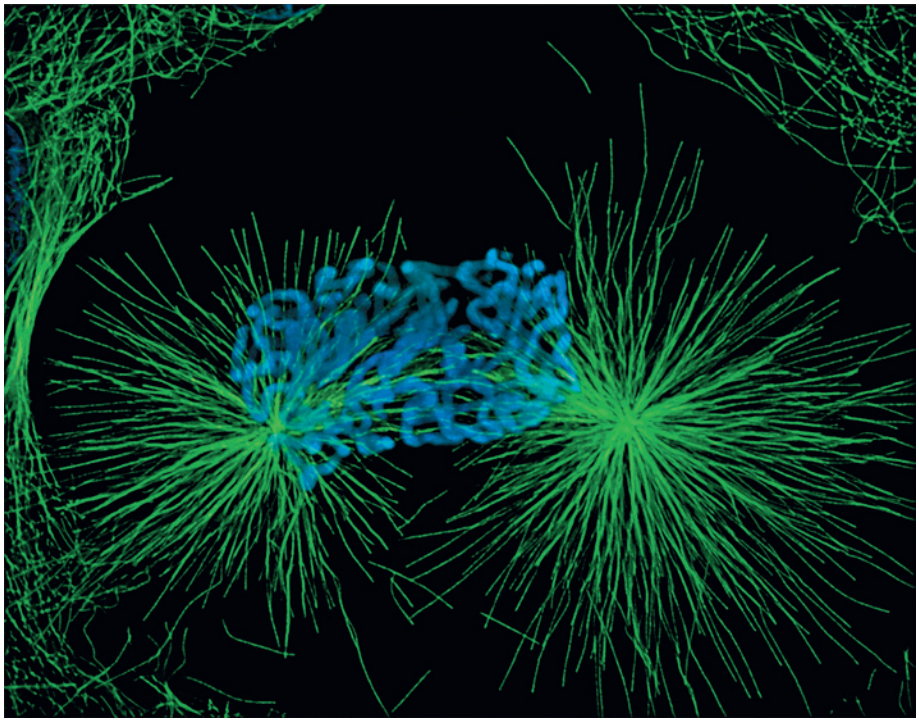


Figure 3–15 Immunofluorescence image of a cell in the prometaphase stage of mitosis. Note the spindle microtubules *(green)* and the chromosomes *(blue)*. (Courtesy Dr. Alexey Khodjakov, 1999.)

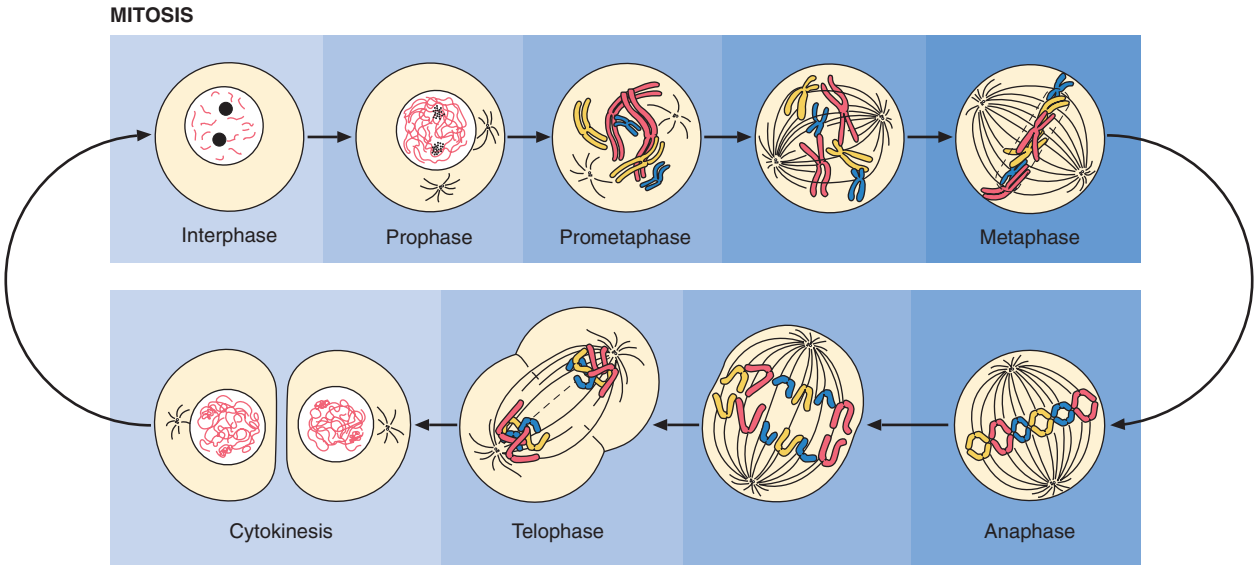


Figure 3–16 Stages of mitosis. Schematic representation of mitosis in a cell containing a diploid (2n) number of six chromosomes.

opposite poles of the cell where they each form a new MTOC.

From each MTOC, astral microtubules, kinetochore microtubules, and polar microtubules develop, giving rise to the mitotic spindle apparatus.

- It is thought that the **astral microtubules** that radiate out from the pole of the spindle may assist in retaining each MTOC at its pole of the cell and ensure that the spindle apparatus is oriented in the correct fashion.
- The microtubules that attach to the kinetochores of sister chromatids are **kinetochore microtubules**. They assist in separating the sister chromatids from each other during anaphase and pulling them to opposite poles of the cell. In the absence of centrioles, the microtubule-nucleating material is dispersed within the cytoplasm, astral microtubules and kinetochore microtubules do not form properly, and mitosis does not proceed in the appropriate manner.
- **Polar microtubules** originate from the two microtubule-organizing centers located at opposite poles of the cell, and, as these polar microtubules elongate, they meet at the center of the cell. In this manner, they ensure that the two MTOCs maintain their respective locations at opposite poles of the cell and do not migrate toward each other.

Prometaphase

Prometaphase begins when the nuclear envelope disappears.

Prometaphase begins as the nuclear lamins are phosphorylated, resulting in the breakdown and disappearance of the nuclear envelope. During this phase, the chromosomes are arranged randomly throughout the cytoplasm; each chromosome is composed of two sister chromatids held to each other by a complex of proteins, known as ***cohesions*** and ***condensins***. Microtubules that become attached to the kinetochores are known as ***kinetochore microtubules*,** whereas microtubules that do not become incorporated into the spindle apparatus are called ***polar microtubules***, which are responsible for maintaining the correct locations of the two microtubule-organizing centers during the mitotic event. As indicated previously, the kinetochore microtubules assist in the migration of the chromosomes so that they become oriented into an alignment with the mitotic spindle.

Metaphase

Metaphase begins as the newly duplicated chromosomes align themselves on the equator of the mitotic spindle.

During **metaphase**, the chromosomes become maximally condensed and are lined up at the equator of the mitotic spindle (**metaphase plate** configuration). Each chromatid parallels the equator, and kinetochore microtubules are attached to its kinetochore, radiating to the spindle pole. Sister chromatids must be maintained in close proximity as the chromosome condenses and aligns on the metaphase mitotic spindle. During **anaphase**, cohesion proteins located between the sister chromatids disappear as the sister chromatids separate and begin their migration to opposite poles.

Anaphase

During anaphase, the sister chromatids separate and begin to migrate to opposite poles of the cell, and a cleavage furrow begins to develop.

Anaphase begins when sister chromatids, located at the equator of the metaphase plate, pull apart and begin their migration toward the opposite poles of the mitotic spindle. The spindle/kinetochore attachment site leads the way, with the arms of the chromatids simply trailing, apparently contributing nothing to the migration or its pathway.

It has been postulated that the observed movement of the chromatids toward the pole in anaphase may be the result of shortening of the microtubules via depolymerization at the kinetochore end. This, coupled with the recent discovery of dynein associated with the kinetochore, may be analogous to vesicle transport along microtubules. In **late anaphase**, a cleavage furrow begins to form at the plasmalemma, indicating the region where the cell will be divided during cytokinesis.

Telophase

Telophase, the terminal phase of mitosis, is characterized by cytokinesis, reconstitution of the nucleus and nuclear envelope, disappearance of the mitotic spindle apparatus, and unwinding of the chromosomes into chromatin.

At **telophase**, each set of chromosomes has reached its respective pole, the nuclear lamins are dephosphorylated, and the nuclear envelope is reconstituted. The chromosomes uncoil and become organized into heterochromatin and euchromatin of the interphase cell. The nucleolus is developed from the **nucleolar organizing regions** on each of five pairs of chromosomes.

Cytokinesis

Cytokinesis is the division of the cytoplasm into two equal parts during mitosis.

The cleavage furrow continues to deepen until only the **midbody,** a small bridge of cytoplasm, and remaining

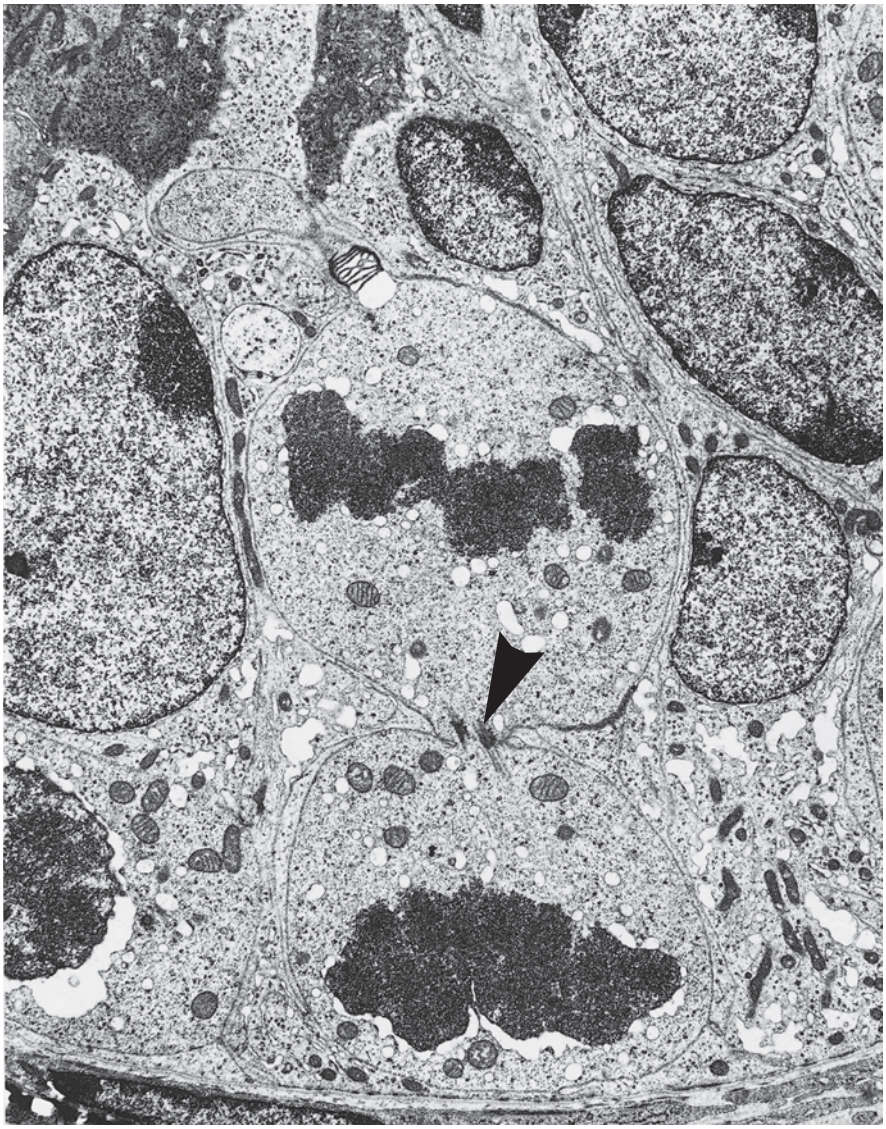


Figure 3–17 Cytokinesis. Electron micrograph (×8092). A spermatogonium in late telophase demonstrating the forming midbody *(arrowhead)*. The chromosomes in the daughter nuclei are beginning to uncoil. (From Miething A. Intercellular bridges between germ cells in the immature golden hamster testis: evidence for clonal and nonclonal mode of proliferation. *Cell Tissue Res.* 1990;262:559-567.)

polar microtubules connect the two daughter cells (Fig. 3–17). The polar microtubules are surrounded by a **contractile ring**, which lies just inside the plasma membrane. The contractile ring is composed of **actin** and **myosin filaments** attached to the plasma membrane. Constriction of the ring is followed by depolymerization of the remaining polar microtubules separating the two daughter cells. During separation of the daughter cells and shortly thereafter, the elements of the contractile ring and the remaining microtubules of the mitotic apparatus are disassembled, concluding cytokinesis.

Each daughter cell resulting from mitosis is identical in every respect, including the entire genome, and each daughter cell possesses a 2n amount of chromosomes.

CLINICAL CORRELATIONS

A more complete understanding of mitosis and the cell cycle has greatly aided cancer chemotherapy, making it possible to use drugs at a time when the cells are in a particular stage of the cell cycle. For example, **vincristine** and similar drugs disrupt the mitotic spindle, arresting the cell in mitosis. **Colchicine,** another plant alkaloid that produces the same effect, has been used extensively in studies of individual chromosomes and karyotyping. **Methotrexate,** which inhibits purine synthesis, and **5-fluorouracil,** which inhibits pyrimidine synthesis, both halt the cell cycle in the S phase, preventing cell division; both are common chemotherapy agents.

Oncogenes are mutated forms of normal genes called *proto-oncogenes*, which code for proteins that control cell division. Oncogenes may result from a viral infection or random genetic accidents. When present in a cell, oncogenes dominate genes over the normal proto-oncogene alleles, causing unregulated cell division and proliferation. Examples of cancer cells arising from oncogenes include **bladder cancer** and **acute myelogenous leukemia**.

Meiosis

Meiosis is a special type of cell division resulting in the formation of gametes, cells whose chromosome number is reduced from 2n to 1n.

Meiosis is a specialized type of cell division that produces the germ cells—the ova and the spermatozoa. This process has two crucial results:

1. Reduction in the number of chromosomes from the **diploid (2n)** to the **haploid (1n)** number, ensuring that each gamete carries the haploid amount of DNA and the haploid number of chromosomes
2. Recombination of genes, ensuring genetic variability and diversity of the gene pool

Meiosis is divided into two separate events:

Meiosis I, or **reductional division** (first event). Homologous pairs of chromosomes line up, members of each pair separate and go to opposite poles and the cell divides; thus, each daughter cell receives half the number of chromosomes (haploid number).

Meiosis II, or **equatorial division** (second event). The two **sister chromatids** of each chromosome are separated, as in mitosis, followed by migration of the chromatids to opposite poles and the formation of two daughter cells. These two events produce four cells (gametes), each with the haploid number of chromosomes and haploid DNA content.

Meiosis I

Meiosis I (reductional division) separates the homologous pairs of chromosomes, thus reducing the number from 2n to 1n.

Meiosis begins at the conclusion of interphase in the cell cycle. In gametogenesis, when the germ cells are in the **S phase** of the cell cycle preceding meiosis, the amount of DNA is doubled to **4n**, whereas the chromosome number remains at **2n** (46 chromosomes). Meiosis I proceeds as outlined in Figure 3–18.

Prophase I

Prophase I, the commencement of meiosis, begins after the DNA has been doubled to 4n in the S phase.

Prophase of meiosis I lasts a long time and is subdivided into the following five phases:

1. *Leptotene.* Individual chromosomes, composed of two chromatids joined at the centromere, begin to condense, forming long strands in the nucleus.

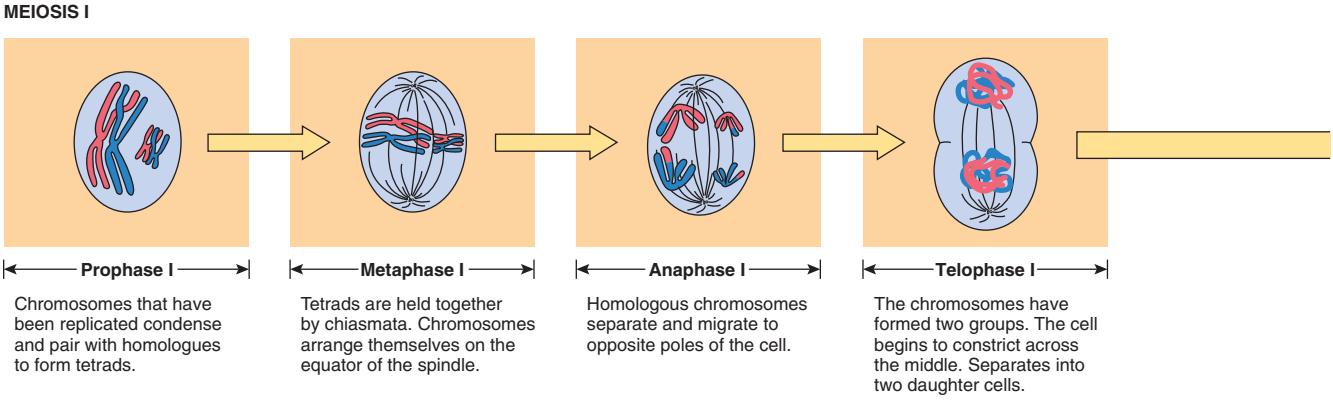


Figure 3–18 Stages of meiosis. Schematic presentation of the events in meiosis in an idealized cell containing a diploid (2n) number of four chromosomes.

2. *Zygotene.* Homologous pairs of chromosomes approximate each other, lining up in register (gene locus to gene locus), and make synapses via the **synaptonemal complex,** forming a tetrad.
3. *Pachytene.* Chromosomes continue to condense, becoming thicker and shorter; **chiasmata** (crossing over sites) are formed as random exchange of genetic material occurs between homologous chromosomes.
4. *Diplotene.* Chromosomes continue to condense and then begin to separate, revealing chiasmata.
5. *Diakinesis.* Chromosomes condense maximally and the nucleolus disappears, as does the nuclear envelope, freeing the chromosomes into the cytoplasm.

Metaphase I

Metaphase I is characterized by homologous pairs of chromosomes, each composed of two chromatids, lining up on the equatorial plate of the meiotic spindle.

During **metaphase I**, homologous chromosomes align as pairs on the equatorial plate of the spindle apparatus in random order, ensuring a subsequent reshuffling of the maternal and paternal chromosomes. Kinetochore microtubules become attached to the kinetochores of the chromosomes, but the four sister chromatids are attached to each other.

Anaphase I

Anaphase I is evident when the homologous pairs of chromosomes begin to pull apart, commencing their migrations to opposite poles of the cell.

In **anaphase I**, homologous chromosomes migrate away from each other, going to opposite poles. Unlike in mitotic anaphase where the sister chromatids are separated from each other, in meiosis, the four sister chromatids are separated to form two pairs of sister chromatids. Each pair of sister chromatids is pulled to opposing poles of the cell.

Telophase I

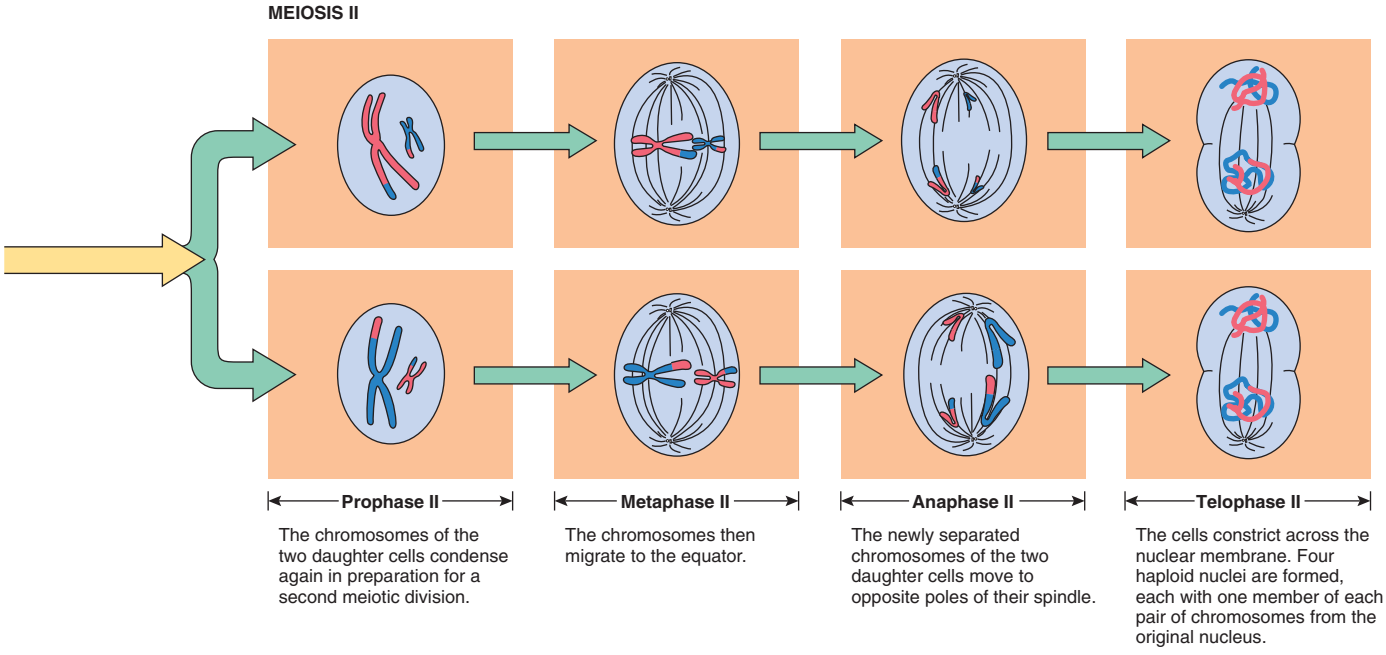
During telophase I, the migrating chromosomes, each consisting of two chromatids, reach opposite poles.

Telophase I is similar to telophase of mitosis. The chromosomes reach the opposing poles, nuclei are re-formed and cytokinesis occurs, giving rise to two daughter cells. Each cell possesses 23 chromosomes, the **1n** number, but, because each chromosome is composed of two sister chromatids, the DNA content is still diploid. Each of the two newly formed daughter cells enters meiosis II.

Meiosis II

Meiosis II (equatorial division) occurs without DNA synthesis and proceeds rapidly through four phases and cytokinesis to form four daughter cells, each with the haploid chromosome number.

The **equatorial division** is not preceded by an **S** phase. It is very similar to mitosis and is subdivided into **prophase II, metaphase II, anaphase II, telophase II,** and **cytokinesis** (see Fig. 3–18). The chromosomes line up on the equator, the kinetochores attach to



kinetochore microtubules, followed by the now separated sister chromatids migrating to opposite poles, and cytokinesis divides each of the two cells, giving a total of four daughter cells from the original diploid germ cell. Each of the four cells contains a haploid amount of DNA content and a haploid chromosome number.

Unlike the daughter cells resulting from mitosis, each of which contains the diploid number of chromosomes and is an identical copy of the other, the four cells resulting from meiosis contain the haploid number of chromosomes and are genetically distinct because of reshuffling of the chromosomes and crossing over. Thus, every gamete contains its own unique genetic complement.

CLINICAL CORRELATIONS

Abnormalities in chromosome numbers may occur during meiosis. During meiosis I, when homologous pairs normally separate, **nondisjunction** may occur; thus, one daughter cell will have both rather than one chromosome of the homologous pair, resulting in 24 chromosomes; the other daughter cell will have 22 chromosomes only. At fertilization, with a normal gamete (containing 23 chromosomes), the resultant zygote will have either 47 chromosomes **(trisomy)** or 45 chromosomes **(monosomy)**. Nondisjunction occurs more frequently with certain chromosomes (i.e., trisomy of chromosomes 8, 9, 13, 18, 21) that produce unique characteristics (e.g., the characteristics of Down syndrome [trisomy 21]).

APOPTOSIS AND NECROSIS

Cells die as a result of various factors, including (1) acute injury, (2) accidents, (3) lack of a vascular supply, (4) destruction by pathogens or by the immune system, and (5) genetic programming. During embryogenesis, many cells, such as those that would give rise to a tail in the human embryo, are driven into the genetically determined process of dying. This process continues on throughout adult life to establish a balance between cell proliferation and cell death. For example, in an adult human, billions of cells die each hour within the bone marrow and digestive tract to balance cell proliferation in these tissues. Cell death by this means is called ***apoptosis* (programmed cell death)**. In contrast to apoptosis, during **necrosis,** the cell dies in an unregulated fashion because of attack or traumatic injury causing the cell to rupture, thereby exposing its contents to neighboring cells, thus initiating an inflammatory response. Unlike apoptosis, which has beneficial effects for the organism, necrosis almost always harms the organism and may even cause death (Table 3-2). Because apoptosis has formidable consequences for the cell involved as well as for the organism, it must be carefully regulated, controlled, and monitored.

TABLE 3–2 Histological Signs of Apoptosis Versus Necrosis

Apoptosis	Necrosis
Caspase-induced destruction of cytoskeletal elements results in rounded cells.	Cells become swollen, and bleb formation ensues.
Pyknotic nuclei due to chromatin condensation is followed by nuclear fragmentation as a result of DNA degradation (karyorrhexis).	Cellular contents are freely present in the extracellular space.
Blebs form at the cell periphery.	Nuclei become pyknotic and become lysed, a process known as *karyolysis*.
Cytoplasm becomes condensed and consequently stains darker.	Necrotic cells and their remnants are not phagocytosed.
Cell becomes fragmented into apoptotic bodies that are phagocytosed by macrophages.	The immune system does not react to necrotic tissues.

The process of apoptosis is regulated by a number of highly conserved genes that code for a family of enzymes known as ***caspases* (cysteine proteases),** which degrade regulatory and structural proteins in the nucleus and in the cytoplasm, whereas **necrosis is caspase-independent**.

Activation of caspases is induced in at least two ways, the **extrinsic** and **intrinsic pathways**.

- The **extrinsic pathway** is activated when extracellular conditions cause the release of certain cytokines, such as **tumor necrosis factor (TNF)**, by signaling cells, which then binds to the TNF receptor of the target cell. These TNF receptors are transmembrane proteins whose cytoplasmic aspect binds to adapter molecules to which caspases are bound. After TNF binds to the extracellular moiety of its receptor, the signal is transduced, and caspase becomes activated. The activated caspase is released and, in turn, triggers a cascade of caspases that results in the degradation of chromosomes, nuclear lamins, and cytoskeletal proteins. Finally, the entire cell becomes fragmented. The cell fragments, known as ***apoptotic bodies***, are then phagocytosed by macrophages. However, these macrophages do not release cytokines that would initiate an inflammatory response.

- The **intrinsic pathway** can be activated when injury occurs intracellularly, and it is essential that the immune system does not become involved and that the inflammatory reaction does not occur. The best understood type of the intrinsic pathway is **mitochondrial apoptosis**, which occurs as a result of damage to the DNA, damage to the endoplasmic reticulum, or other intracellular stresses. Numerous intracellular proteins interact with each other to form a complex, known as ***death-inducing signaling complex***, and activate a pathway that results in the formation of a factor that is transported into mitochondria where they cause the leakage of cytochrome C into the cytosol. When in the cytosol, cytochrome C activates the apoptotic pathway, and the cell enters apoptosis.

PATHOLOGICAL CONSIDERATIONS

See Figures 3–19 and 3–20.

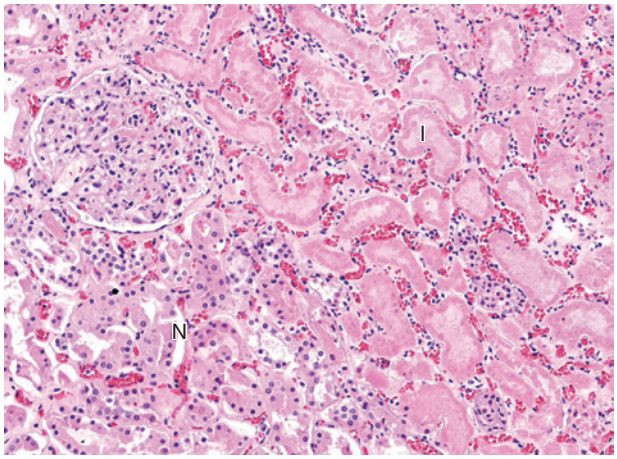


Figure 3–19 Microscopic image of the boundary of normal kidney cortex (N) on the left side and kidney infarct (I) on the right side. Observe that the necrotic side displays cellular blebs and the absence of nuclei in the cells of the tubules. Note the presence of inflammatory infiltrate in the connective tissue between the tubules. (From Kumar V, et al. *Robbins and Cotran: Pathologic Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015.)

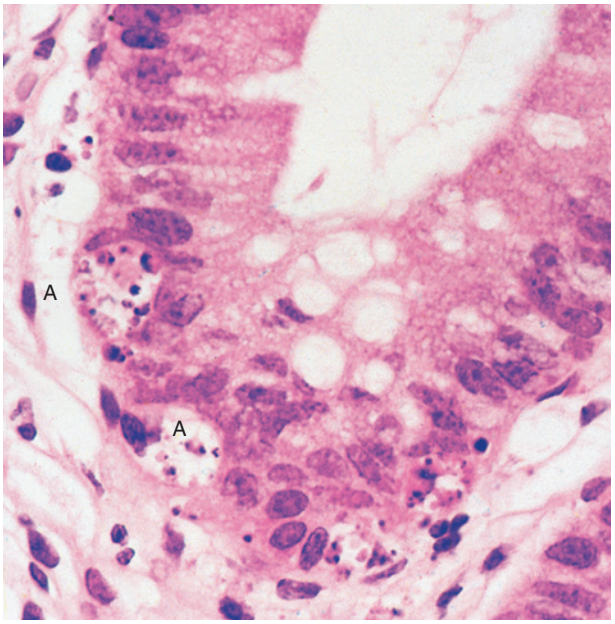


Figure 3–20 Photomicrograph displaying the presence of apoptotic bodies (A) in the colon of a patient who is suffering from graft-versus-host disease. (From Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010.)

REVIEW QUESTIONS

1. A 2-month-old baby is brought to be examined, and the physician notices that he has flaccid muscles, a smaller head than usual, a large tongue, a short nose, and a broad face. The parents say that the baby is very quiet and hardly ever cries. The pediatrician suspects aneuploidy. The child probably has which of the following chromosomal configurations?
   a. Monosomy
   b. Trisomy
   c. Normal diploid complement
   d. Haploid complement
   e. Tetraploid complement
2. A patient with a chromosomal complement of XXY has
   a. trisomy of the autosomes
   b. Turner syndrome
   c. monosomy of the sex chromosomes
   d. Klinefelter syndrome
   e. a female phenotype
3. A patient with a chromosomal complement of XO has
   a. trisomy of the sex chromosomes
   b. Turner syndrome
   c. monosomy of the autosomes
   d. Klinefelter syndrome
   e. a male phenotype
4. A patient with a chromosomal complement of XXX has
   a. male phenotype
   b. female phenotype
   c. a very short life span
   d. a higher IQ than his/her siblings
   e. trisomy of autosomes
5. Which RNA synthesis is catalyzed by RNA polymerase II?
   a. tRNA
   b. rRNA that codes for the large ribosomal subunit
   c. rRNA that codes for the small ribosomal subunit
   d. mRNA
   e. tRNA that carries the start codon

Questions 6 and 7 refer to the following clinical scenario:

During a routine examination of a 3-year-old child, the pediatrician notes that the color of the pupil is white and that the child is cross-eyed. She asks the father if there are any cases of retinoblastoma in the family, and, because the answer is in the affirmative, she calls in a pediatric ophthalmologist. The ophthalmologist knows that retinoblastoma is due to the mutation of the Rb gene (retinoblastoma gene) and that it is a recessive trait.

6. Retinoblastoma can metastasize to the brain via the
   a. optic nerve
   b. superior division of the oculomotor nerve
   c. trochlear nerve
   d. abducent nerve
   e. inferior division of the oculomotor nerve
7. Not all cases of retinoblastoma have a familial component. There is the sporadic form of retinoblastoma in which the child's normal genetic complement does not predispose the child to have retinoblastoma. If this child had the sporadic form of retinoblastoma, then
   a. one copy of the Rb genes mutated
   b. both copies of the Rb genes mutated
   c. the patient's future children will most probably have one mutated Rb gene
   d. the patient's future children will most probably have two mutated Rb genes
   e. the mutated Rb gene is known as a proto-oncogene
8. Chiasmata formation
   a. occurs during metaphase II
   b. results in the exchange of genetic material between non-homologous chromosomes
   c. occurs during the pachytene phase
   d. results in nondisjunction
   e. results in the formation of the synaptonemal complex
9. During meiosis, some chromosomes do not separate from each other. This is called
   a. diplotene
   b. nondisjunction
   c. diakinesis
   d. zygotene
   e. leptotene
10. The binding of cyclin B to CDK1 (cyclin-dependent kinase 1) permits the cell to progress from
    a. S phase into G_2 phase
    b. G_2 phase into M phase
    c. G_1 phase into G_2 phase
    d. G_1 phase into S phase
    e. prometaphase into metaphase

ANSWERS

1. B. The baby most probably has trisomy 21, Down syndrome, in which chromosome 21 is present instead of two chromosomes, one from the mother and the other from the father. During meiosis, nondisjunction occurs, so that one cell has the extra chromosome 21 and the other cell is monosomic; that is, it is missing chromosome 21.
2. D. The patient has Klinefelter syndrome and exhibits a male phenotype. Because there are three sex chromosomes, this is not a monosomy or a trisomy of the autosomes. Turner syndrome is exhibited by females who lack the second X chromosome.
3. B. Patients with Turner syndrome are females who lack the second X chromosomes; therefore, they are monosomal for the sex chromosomes not for the autosomes. Turner syndrome is a male phenotype with an extra X chromosome.
4. B. The patient is a female who has the triple X syndrome, thus she has an extra sex chromosome rather than trisomy of the autosomes. These patients usually have a lower IQ than their siblings but have a normal life span and can even give birth to children with a normal chromosomal complement.
5. D. RNA polymerase II catalyzes the synthesis of messenger RNA (mRNA). The synthesis of all tRNA is catalyzed by RNA polymerase III, whereas all rRNA synthesis is catalyzed by RNA polymerase I.
6. A. The retina is attached to the brain via the optic nerve; therefore, malignant cells can use the optic nerve as a passageway to the brain. The superior and inferior divisions of the oculomotor nerve, the trochlear nerve, and the abducent nerve all innervate the external muscles of the eye and do not contact the retina.
7. B. Because retinoblastoma is a recessive trait, both copies of the Rb gene would have to have mutated. The normal gene, which codes for the Rb protein is known as a *proto-oncogene*, whereas its mutated form is known as an *oncogene*. Because the mutations did not occur in the gametes, the patient's future children will not have any mutated Rb genes.
8. C. Chiasmata formation occurs during the pachytene phase of prophase I of the first meiotic event. Homologous pairs of chromosomes line up during the zygotene phase of prophase I, forming the synaptonemal complex, thus permitting crossing over to occur during the pachytene phase so that there could be a random exchange of genetic material that contributes to a more diverse gene pool. Nondisjunction occurs when the homologous pairs of chromosomes separate during anaphase I.
9. B. During the anaphase I of meiosis, the homologous chromosomes may remain attached to each other, and the two newly formed cells will have unequal chromosomal numbers. This process is known as *nondisjunction*. Diplotene, zygotene, diakinesis, and leptotene are phases of prophase I of the first meiotic division.
10. B. The formation of the cyclin B–CDK1 complex allows the cell to enter the mitotic cycle, that is, progress from G_2 phase into the M phase. The cell cycle cannot go from G_1 to G_2 directly; it has to first go through the S phase. Cyclins D and E are required to enter S from G_1. Cyclins A and B are both required for the entry from S phase into G_2 phase.

This page intentionally left blank

Extracellular Matrix

4

Cells of multicellular organisms congregate to form structural and functional associations, known as ***tissues***. Each of the four basic tissues of the body—epithelium, connective tissue, muscle, and nervous tissue—possesses specific, defined characteristics, which are detailed in subsequent chapters. However, all tissues are composed of **cells** and an **extracellular matrix (ECM)**, a complex of nonliving macromolecules manufactured by the cells and exported by them into the extracellular space, that is, the space between cells.

Some tissues, such as epithelium, form sheets of cells with only a scant amount of ECM. At the opposite extreme is connective tissue, composed mostly of ECM with a limited number of cells scattered throughout the matrix. Cells maintain their associations with the ECM by forming specialized junctions that hold them to the surrounding macromolecules. This chapter explores the nature of the ECM and its functions not only as it relates to the tissues that house it but also its relationship with the cells contained with it. Although it was initially believed that the ECM forms merely the skeletal elements of the tissue in which it resides, it is now known that it may also

- Modify the morphology and functions of cells
- Modulate the survival of cells
- Influence the development of cells
- Regulate the migration of cells
- Direct mitotic activity of the cells
- Form junctional associations with cells

The ECM of connective tissue proper, the most common connective tissue of the body, is composed of a hydrated gel-like **ground substance** with **fibers** embedded in it. Ground substance resists forces of compression, whereas fibers withstand tensile forces. The water of hydration permits the rapid exchange of nutrients and waste products carried by the extracellular fluid as it percolates through the ground substance (Fig. 4–1).

GROUND SUBSTANCE

Ground substance is an amorphous gel-like material composed of glycosaminoglycans, proteoglycans, and glycoproteins.

Ground substance is composed of **glycosaminoglycans (GAGs)**, **proteoglycans**, and **cell adhesive glycoproteins**. These three families of macromolecules form various interactions with each other, with fibers, and with the cells of connective tissue and epithelium (Fig. 4–2).

Glycosaminoglycans

GAGs are negatively charged, long, rod-like chains of repeating disaccharides that have the capability of binding large quantities of water.

GAGs are long, inflexible, unbranched polysaccharides composed of chains of repeating disaccharide units. One of the two repeating disaccharides is always an **amino sugar** (*N*-acetylglucosamine or *N*-acetylgalactosamine); the other one is typically a **uronic acid** (iduronic or glucuronic) (Table 4–1). Because the amino sugar is usually sulfated, and these sugars also have carboxyl groups projecting from them, they are negatively charged and thus attract cations, such as sodium (Na^+). A high sodium concentration in the ground substance attracts extracellular fluid, which (by hydrating the intercellular matrix) assists in the resistance to forces of compression. As these molecules come into close proximity to each other, their negative charges repel one another, which cause them to have a slippery texture, as evidenced by the slickness of mucus, vitreous humor of the eye, and synovial fluid.

All but one of the major GAGs of ECM are sulfated, each consisting of fewer than 300 repeating disaccharide units (see Table 4–1). The sulfated GAGs include **keratan sulfate**, **heparan sulfate**, **heparin**, **chondroitin 4-sulfate**, **chondroitin 6-sulfate**, and **dermatan sulfate**. These GAGs are usually linked covalently to protein molecules to form proteoglycans. The only nonsulfated GAG is **hyaluronic acid (hyaluronan)**, which may have as many as 10,000 repeating disaccharide units. It is a very large macromolecule (up to 10,000 kDa) that does *not* form covalent links to protein molecules (although proteoglycans do become attached to it via link proteins). It is interesting to note that all GAGs are synthesized within the Golgi apparatus by resident enzymes except for hyaluronic acid, which is synthesized as a free linear polymer at the cytoplasmic face of the plasma membrane by hyaluronan synthases. These enzymes are integral membrane proteins that not only catalyze the polymerization but also facilitate the transfer of the newly formed macromolecule into the ECM. It has been suggested that hyaluronic acid also has intracellular functions. Some of the newly released hyaluronic acid is endocytosed by some cells, especially during the cell cycle, where it has a role in maintaining

SUMMARY

Tissues of the body consist of cells and non-living materials, known as the **extracellular matrix (ECM)**, that surround these cells. The ECM of connective tissue is composed of **ground substance** and **fibers**, where ground substance is an amorphous gel-like material, consisting of **glycosaminoglycans**, **proteoglycans**, and **glycoproteins**. The fibers are proteins classified into two major categories, the nonelastic **collagen fibers** that are quite flexible and whose tensile strength exceeds that of stainless steel, and highly accommodating fibers, known as ***elastic fibers*** that may be stretched as much as 150% of their resting length before they break. Currently, approximately 25 different types of collagen fibers are known, depending on their amino acid composition. The ECM of specialized types of connective tissues differ from the previous description. In blood, the ECM is a fluid, whereas in bone, the ECM is calcified to form a hard solid substance. Some cells, such as epithelial cells and muscle cells, are separated from the connective tissue elements by a special type of ECM, known as the *basal lamina*.

KEY WORDS

Alpha chains; basal lamina; basement membrane; collagen; elastic fibers; elastin; extracellular matrix; fibrillin; fibronectin; gap region; glycoproteins; glycosaminoglycans; ground substance; hyaluronic acid; laminin; overlap region; proteoglycans; tropocollagen

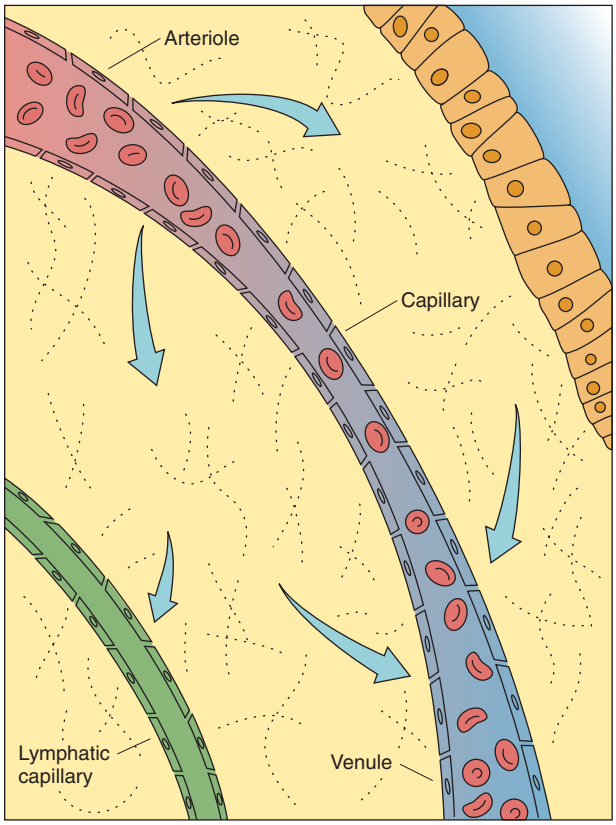


Figure 4–1 Schematic diagram of tissue fluid flow. Fluid from the higher pressure arterial ends of the capillary bed enters the connective tissue spaces and becomes known as *extracellular fluid*, which percolates through the ground substance. Some, but not all, of the extracellular fluid then reenters the blood circulatory system at the lower pressure venous end of the capillary bed and the venules. The extracellular fluid that did not reenter the blood vascular system will enter the even lower pressure lymphatic system which will eventually deliver it to the blood vascular system.

space and modulating microtubular activities during metaphase and anaphase stages of mitosis thus facilitating chromosomal movements. Additional intracellular roles may involve modulation of intracellular trafficking and influencing intracytoplasmic and intranuclear specific kinases.

Proteoglycans

Proteoglycans constitute a family of macromolecules; each is composed of a protein core to which GAGs are covalently bonded.

When sulfated GAGs form covalent bonds with a protein core, they form a family of macromolecules known as **proteoglycans**, many of which occupy huge domains. These large structures look like a bottle brush,

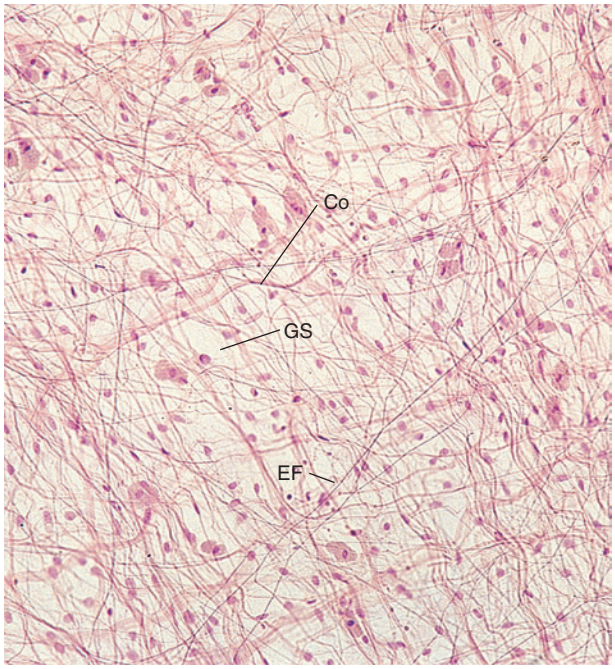


Figure 4–2 Light micrograph of areolar connective tissue, displaying cells, collagen fibers (Co), elastic fibers (EF), and ground substance (GS). Observe that, in this very loose type of connective tissue, the fibers, although interwoven, present a relatively haphazard arrangement, thus permitting the stretching of the tissue in any direction. The cells of areolar connective tissue are principally of three types, fibroblast, macrophages, and mast cells. The extensive extracellular spaces are occupied by ground substance composed mainly of glycosaminoglycans and proteoglycans, a large component of which is aggrecan aggregate, a highly hydrated macromolecule. (×132)

with the protein core resembling the wire stem and the various sulfated GAGs projecting from its surface in three-dimensional space, as do the bristles of the brush (Fig. 4–3).

Proteoglycans may be of various sizes, ranging from about 50,000 Da **(decorin** and **betaglycan)** to as many as 3 million Da **(aggrecan)**. The protein cores of proteoglycans, manufactured on the rough endoplasmic reticulum (RER), are transported to the Golgi apparatus, where resident enzymes covalently bind **bridge tetrasaccharides** (a series of four saccharides) to its serine side chains, and then the GAG is assembled by the addition of sugars one at a time. That is because the GAGs molecule cannot bind to the protein core by itself; instead it requires the tetrasaccharides to be able to do that. Sulfation, catalyzed by sulfotransferases and epimerization (rearrangement of various groups around the carbon atoms of the sugar units), also occurs in the Golgi apparatus.

Many proteoglycans, especially **aggrecan**, a macromolecule found in cartilage and connective tissue

TABLE 4–1 Types of Glycosaminoglycans

GAG	Molecular Mass (Da)	Repeating Disaccharides	Covalent Linkage to Protein	Location in Body
Hyaluronic acid	10^7–10^8	D-glucuronic acid-beta-1,3-*N*-acetyl-D-glucosamine	No	Most connective tissue, synovial fluid, cartilage, dermis
Keratan sulfate I and II	10,000–30,000	Galactose-beta-1,4-*N*-acetyl-D-glucosamine-6-SO_4	Yes	Cornea (keratan sulfate I), cartilage (keratan sulfate II)
Heparan sulfate	15,000–20,000	D-Glucuronic acid-beta-1,3-*N*-acetyl galactosamine L-Iduronic acid-2SO_4-beta-1,3-N-acety-D-galactosamine	Yes	Blood vessels, lung, basal lamina
Heparin (90%) (10%)	15,000–20,000	L-Iduronic acid-beta-1,4-sulfo-D-Glucosamine-6-SO_4 *D-Glucuronic acid-beta-1,4-N*-acetylglucosamine-6-SO_4	No	Mast cell granule, liver, lung, skin
Chondroitin 4-sulfate	10,000–30,000	D-Glucuronic acid-beta-1,3-*N*-acetylgalactosamine-6-SO_4	Yes	Cartilage, bone, cornea, blood vessels
Chondroitin 6-sulfate	10,000–30,000	D-Glucuronic acid-beta-1,3-*N*-acetylgalactosamine-6-SO_4	Yes	Cartilage, Wharton jelly, blood vessels
Dermatan sulfate	10,000–30,000	L-Iduronic acid-alpha-1,3-and *N*-acetylglucosamine-4-SO_4	Yes	Heart valves, skin, blood vessels

proper, attach to hyaluronic acid (see Fig. 4–3). The mode of attachment involves a noncovalent ionic interaction between the sugar groups of the hyaluronic acid and the core protein of the proteoglycan molecule. The connection is reinforced by small **link proteins** that form bonds with both the core protein of aggrecan as well as with the sugar groups of hyaluronic acid. Because hyaluronic acid may be as much as 20 μm in length, the result of this association is an aggrecan composite that occupies a very large volume and may have a molecular mass as large as several hundred million daltons. This immense molecule is responsible for the gel state of the ECM and acts as a barrier to fast diffusion of aqueous deposits, as when one observes the slow disappearance of an aqueous bubble after its subdermal injection.

CLINICAL CORRELATIONS

Many pathogenic bacteria, such as *Staphylococcus aureus,* secrete **hyaluronidase**, an enzyme that cleaves hyaluronic acid into numerous small fragments, thus converting the gel state of the ECM to a sol (liquid) state. The consequence of this reaction is to permit the rapid spread of the bacteria through the connective tissue spaces.

Functions of Proteoglycans

Proteoglycans have numerous functions. By occupying a large volume, they resist compression and retard the rapid movement of microorganisms and metastatic cells; however, in the same fashion, they facilitate normal cellular locomotion by permitting migrating cells to move into the space that these hydrated macromolecules occupied. In addition, in association with the basal lamina, they form molecular filters of varying pore sizes and charge distributions that selectively screen and retard macromolecules as they pass through them.

Proteoglycans also possess binding sites for certain signaling molecules, such as transforming growth factor-β. By binding these signaling molecules, proteoglycans can either impede their function by preventing the molecules from reaching their destinations or enhance their function by concentrating them in a specific location. Some proteoglycans, such as decorins, are required for the formation of collagen fibers; mutated mice that cannot produce decorins or produce defective decorins possess skins with reduced tensile strength.

Some proteoglycans, such as **syndecans**, instead of being released into the ECM, remain attached to the cell membrane. The core proteins of syndecans act as transmembrane proteins and are attached to the actin filaments of the cytoskeleton. Their extracellular moieties bind to components of the ECM, thus permitting the cell to become attached to macromolecular components of the matrix. In addition, syndecans of fibroblasts function as co-receptors because they bind fibroblast growth factor and present it to cell membrane fibroblast growth factor receptors in their vicinity.

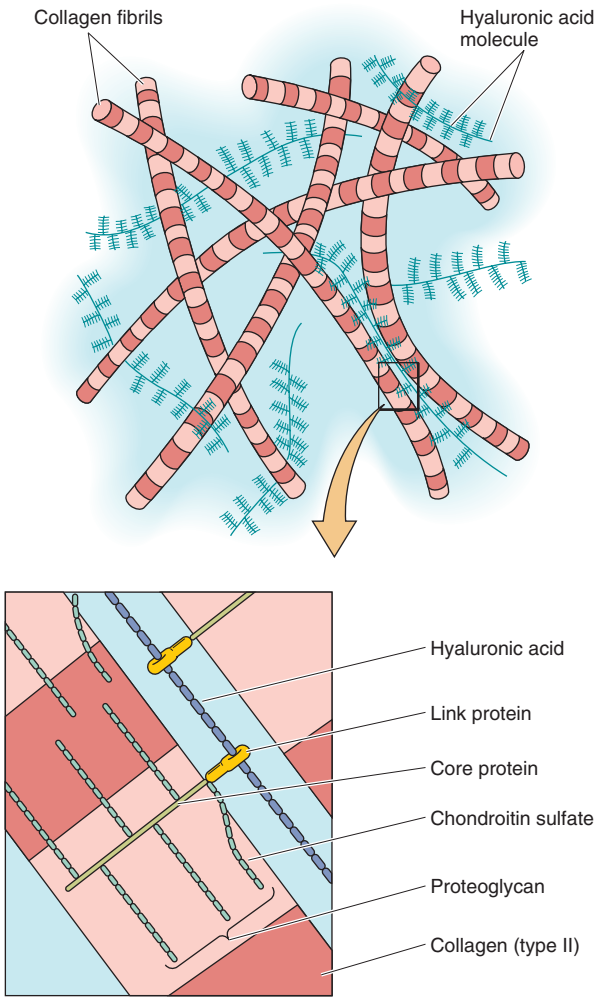


Figure 4–3 Schematic diagram of the association of aggrecan molecules with collagen fibers. *Inset* displays a higher magnification of the aggrecan molecule, indicating the core protein of the proteoglycan molecule to which glycosaminoglycans are attached. The core protein is attached to the hyaluronic acid by link proteins. (Modified from Fawcett DW. *Bloom and Fawcett's a Textbook of Histology*. 11th ed. Philadelphia: WB Saunders; 1986.)

Glycoproteins (Cell Adhesive Glycoproteins)

Cell adhesive glycoproteins have binding sites for several components of the ECM as well as for integrin molecules of the cell membrane that facilitate the attachment of cells to the extracellular matrix.

The ability of cells to adhere to components of the ECM is mediated to a great extent by **cell adhesive glycoproteins**. These large macromolecules have several domains, at least one of which usually binds to cell surface proteins called ***integrins***, one to collagen fibers, and one to proteoglycans. In this manner, adhesive glycoproteins fasten the various components of tissues to each other. The major types of adhesive glycoproteins are fibronectin, laminin, entactin, tenascin, chondronectin, and osteonectin.

Fibronectin is a large dimer composed of two similar polypeptide subunits, each about 220,000 Da, attached to one another at their carboxyl ends by disulfide bonds. Each arm of this V-shaped macromolecule has binding sites for various extracellular components (e.g., collagen, heparin, heparan sulfate, and hyaluronic acid) and for cell adhesive molecules, specific integrins, known as ***fibronectin receptors***, of the cell membrane. The region of the fibronectin that is specific for adhering to the fibronectin receptors has the three-residue sequence arginine, glycine, and aspartate, referred to as the ***RGD sequence***. This sequence of amino acids is characteristic of the integrin binding site in many adhesive glycoproteins. Fibronectin is produced mainly by connective tissue cells known as ***fibroblasts***. The actin components of the cytoskeleton of these cells and their associated myosin counterparts interact, placing tension on their plasmalemma. The integrin molecules relay the tensile forces to the newly exocytosed fibronectin molecules stretching them just enough to expose hidden binding sites that permit fibronectins to bind to each other, thus forming the fibronectin matrix.

Fibronectin is also present in blood as **plasma fibronectin**, where it facilitates wound healing, phagocytosis, and coagulation. Fibronectin may be temporarily attached to the plasma membrane as **cell-surface fibronectin**. Fibronectin marks migratory pathways for embryonic cells so that the migrating cells of the developing organism can reach their destination.

Laminin is a very large glycoprotein (950,000 Da) composed of three large polypeptide chains, A, B_1, and B_2. The B chains wrap around the A chain, forming a cross-like pattern of one long and three short chains. The three chains are held in position by disulfide bonds. The location of laminin is almost strictly limited to the basal lamina; therefore, this glycoprotein has binding sites for heparan sulfate, type IV collagen, entactin, and the cell membrane.

The sulfated glycoprotein **entactin** (also known as ***nidogen*** and is about 150,000 Da) binds to the laminin molecule where the three short arms of that molecule meet each other. Entactin also binds to type IV collagen, thus facilitating the binding of laminin to the collagen meshwork.

Tenascin is a large glycoprotein (250,000 to 300,000 Da) composed of six polypeptide chains held together by disulfide bonds. This macromolecule, which resembles an insect whose six legs project radially from a central body, has binding sites for the transmembrane proteoglycan syndecans and for fibronectin. Tenacin's distribution is usually limited to embryonic tissue, where it marks migratory pathways for specific cells.

Chondronectin and **osteonectin** (about 40,000 Da) are similar to fibronectin. The former has binding sites for type II collagen, chondroitin sulfates, hyaluronic acid, and integrins of chondroblasts and chondrocytes. Osteonectin possesses domains for type I collagen, proteoglycans, and integrins of osteoblasts and osteocytes. In addition, it may facilitate the binding of calcium hydroxyapatite crystals to type I collagen in bone.

FIBERS

Collagen and elastic fibers, the two major fibrous proteins of connective tissue, have distinctive biochemical and mechanical properties as a consequence of their structural characteristics.

The fibers of the ECM provide tensile strength and elasticity to this substance. Classical histologists have described three types of fibers on the basis of their morphology and reactivity with histological stains: **collagen**, **reticular**, and **elastic** (see Fig. 4–2). Although it is now known that reticular fibers are in fact a type of collagen fibers, many histologists retain the term *reticular fibers* not only for historical reasons but also for convenience when describing organs that possess large quantities of this particular collagen type.

Collagen Fibers: Structure and Function

Collagen fibers are composed of tropocollagen subunits whose α-chain amino acid sequences permit the classification of collagen into at least 35 different fiber types. There are at least four categories of collagen: fibril-forming, fibril-associated, network-forming, and transmembrane collagens.

The capability of the ECM to withstand compressive forces is due to the presence of the hydrated matrix formed by GAGs and proteoglycans. Tensile forces are resisted by fibers of the tough, firm, inelastic protein **collagen**. This family of proteins is very abundant, constituting about 20% to 25% of all the proteins in the body. Although at least 35 different types of collagen are known, depending on the amino acid sequence of their α-chains, only 10 of them are of interest in this textbook. Each α-chain is coded by a separate messenger ribonucleic acid. These different collagen types are located in specific regions of the body, where they serve various functions (Table 4–2). Collagens are classified into four categories: fibril-forming, fibril-associated, network-forming, and transmembrane collagens; the last category is also known as *collagen-like proteins*.

Fibril-forming collagen, as its classification implies, forms flexible fibers (Fig. 4–4) whose tensile

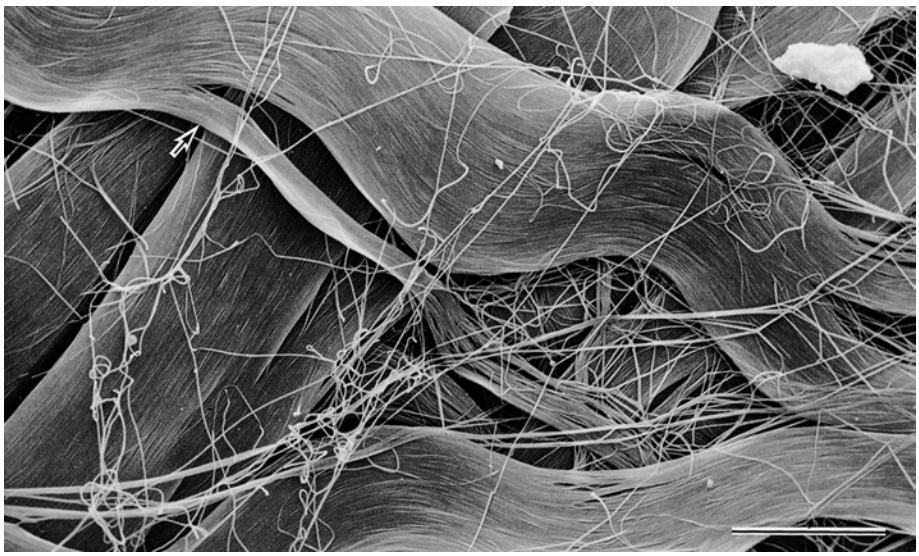


Figure 4–4 Scanning electron micrograph of collagen fiber bundles from the epineurium of the rat sciatic nerve. Note that the thick fiber bundles are interwoven, and they are arranged in an almost haphazard manner. Also, fiber bundles split into thinner bundles (or thinner bundles coalesce to form larger bundles). Moreover, each of the thick fiber bundles is composed of numerous fine fibrils that run a parallel course in each bundle. (×2034) (From Ushiki T, Ide C. Three-dimensional organization of the collagen fibrils in the rat sciatic nerve as revealed by transmission and scanning electron microscopy. *Cell Tissue Res.* 1990;260:175-184.)

TABLE 4–2 Major Types and Characteristics of Collagen

Molecular Type	Molecular Formula	Synthesizing Cells	Function	Location in Body
I (Fibril forming). Most common of all collagens	$[\alpha1(I)]_2\alpha2(I)$	Fibroblast, osteoblast, odontoblast, cementoblast	Resists tension	Dermis, tendon, ligaments, capsules of organs, bone, dentin, cementum
II (Fibril forming)	$[\alpha1(II)]_3$	Chondroblasts	Resists tension	Hyaline cartilage, elastic cartilage
III (Fibril forming) Also known as reticular fibers; they are highly glycosylated	$[\alpha1(III)]_3$	Fibroblast, reticular cell, smooth muscle cell, hepatocyte	Forms structural framework of spleen, liver, lymph nodes, smooth muscle, adipose tissue	Lymphatic system, spleen, liver, cardiovascular system, lung, skin
IV (Network forming) Do not display 67-nm periodicity and the α-chains retain their propeptides	$[\alpha1(IV)]_2\alpha2(IV)$	Epithelial cells, muscle cells, Schwann cells	Forms meshwork of the lamina densa of the basal lamina to provide support and filtration	Basal lamina
V (Fibril forming)	$[\alpha1(V)]_2\alpha2(V)$	Fibroblasts, mesenchymal cells	Associated with type I collagen, also with placental ground substance	Dermis, tendon, ligaments, capsules of organs, bone, cementum, placenta
VII (Network forming) They form dimers that assemble into anchoring fibrils	$[\alpha1(VII)]_3$	Epidermal cells	Forms anchoring fibrils that fasten lamina densa to underlying lamina reticularis	Junction of epidermis and dermis
IX (Fibril associated) Decorate the surface of type II collagen fibers	$[\alpha1(IX)\alpha2(IX)\alpha3(IX)]$	Chondroblasts and chondrocytes	Associates with type II collagen fibers	Cartilage
XI (Fibril forming) Occupies the center of type I collagen stabilizes cartilage matrix	$[\alpha1(XI)]_2\alpha2(II)$	Chondrocytes, fibroblasts	Associated with type I collagen as well as type II collagen fibers	Collagenous connective tissue; cartilage
XII (Fibril associated) Decorate the surface of type I collagen fibers	$[\alpha1(XII)]_3$	fibroblasts	Associates with type I collagen fibers	Tendons, ligaments, and aponeuroses
XVII (Collagen-like protein) A transmembrane protein, formerly known as bullous pemphigoid antigen	$[\alpha1(XVII)]_3$	Epithelial cells	?	Hemidesmosomes
XVIII (Collagen-like protein) Cleavage of its C-terminal forms endostatin and angiogenesis inhibitor	$[\alpha1(XVIII)]_3$	Endothelial cells	?	Lamina reticularis of the basement membrane

strength is greater than that of stainless steel of comparable diameter. Large collections of collagen fibers appear glistening white in the living individual; therefore, collagen fiber bundles are also referred to as *white fibers*. Collagen fibers of connective tissue are usually less than 10 μm in diameter and are colorless when unstained. Stained with hematoxylin and eosin, they appear as long, wavy, pink fiber bundles.

Electron micrographs of collagen fibers stained with heavy metals display cross-banding at regular intervals of 67 nm, a characteristic property of these fibers. These fibers are formed from parallel aggregates of thinner fibrils 10 to 300 nm in diameter and many micrometers in length (Figs. 4–5 and 4–6). The fibrils themselves are fashioned from a highly regular assembly of even smaller subunits, **tropocollagen (collagen)**

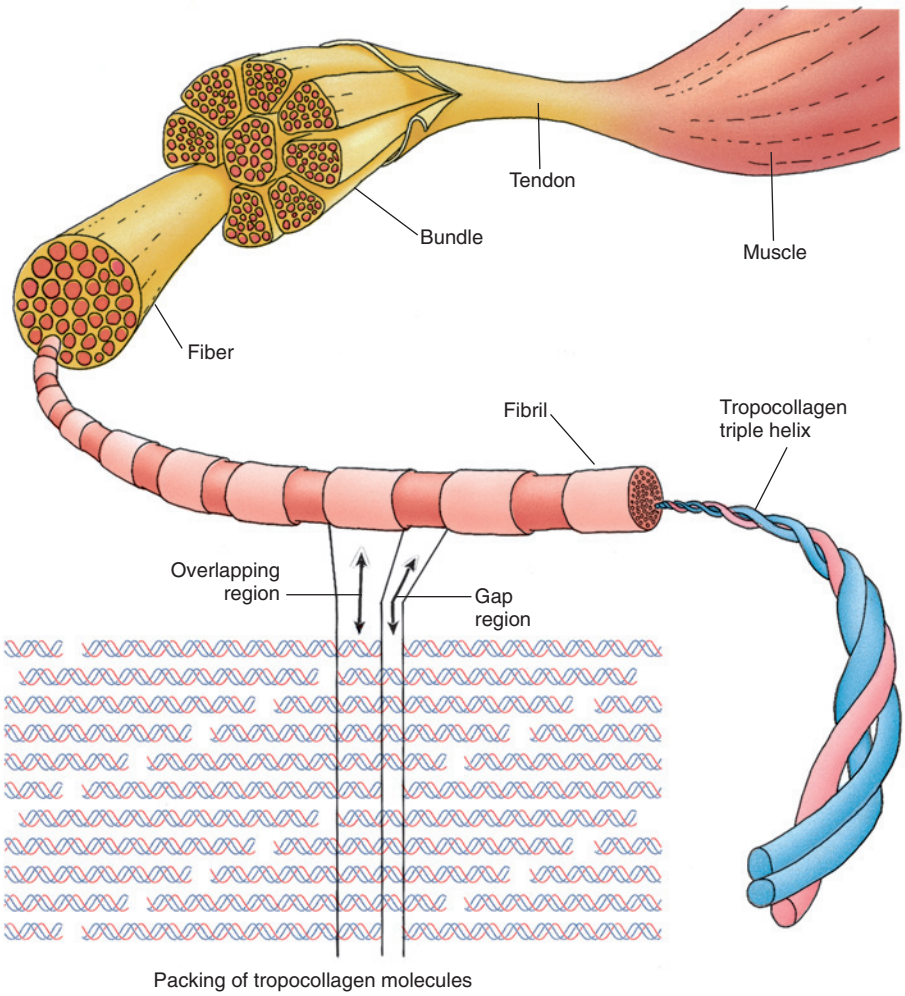


Figure 4–5 Schematic representation of the components of a collagen fiber. The ordered arrangement of the tropocollagen molecules gives rise to the gap and overlap regions, responsible for the 67-nm cross-banding of type I collagen. The gap region is the area between the head of one and the tail of the next tropocollagen molecule, whereas the overlap region is where the tail of one tropocollagen molecule overlaps the tail of a tropocollagen molecule in the row above or below that tropocollagen. In three dimensions, the overlap region coincides with numerous other overlap regions, and the gap regions coincide with numerous other gap regions. The heavy metals that are used in electron microscopy precipitate into the gap regions; therefore, the large number of these coinciding gaps are filled with heavy metal precipitates and are visible as the 67-nm cross banding. Type I collagen is composed of two identical α1(I) chains *(blue)* an oneα2(I) chain *(pink)*.

molecules, each about 280 nm long and 1.5 nm in diameter. Individual tropocollagen molecules are composed of three polypeptide chains, called ***α-chains***, wrapped around each other in a triple helical configuration.

Each α-chain possesses about 1000 amino acid residues. Every third amino acid is **glycine**, and most of the remaining amino acids are composed of **proline**, **hydroxyproline**, and **hydroxylysine**. It is believed that glycine, because of its small size, permits the close association of the three α-chains; the hydrogen bonds of hydroxyproline hold the three α-chains together; and hydroxylysine permits the formation of fibrils by binding the tropocollagen molecules to each other. The most common fibril-forming collagens are types I, II, III, V, and XI.

Fibril-associated collagens have been recognized as stabilizing collagens because they form molecular

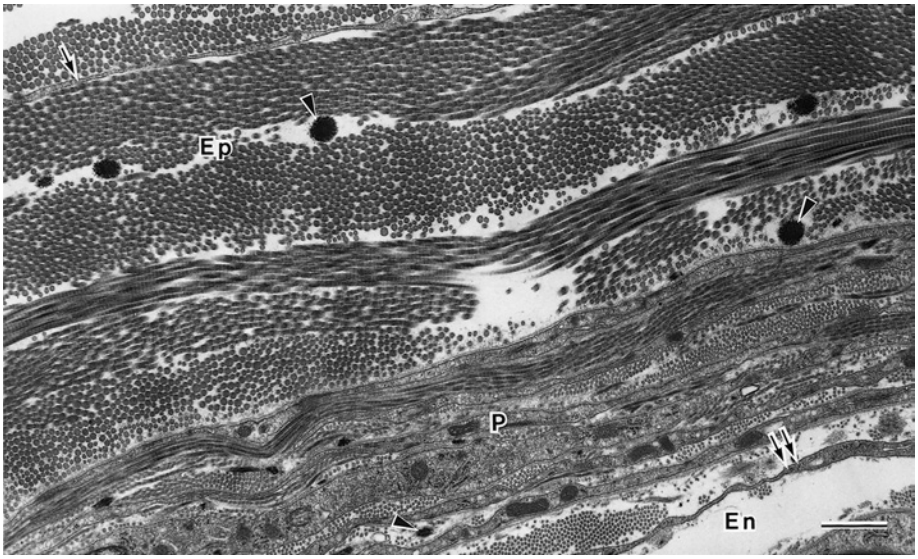


Figure 4–6 Electron micrograph of collagen fibers from the perineurium of the rat sciatic nerve (×22463). En, Endoneurium; Ep, epineurium; P, perineurium. (From Ushiki T, Ide C. Three-dimensional organization of the collagen fibrils in the rat sciatic nerve, as revealed by transmission and scanning electron microscopy. *Cell Tissue Res*. 1990;260:175-184.)

bridges between fibril-forming collagens and components of the ground substance. There are two types of fibril-associated collagens:

- **Type XII collagen** that binds to type I collagen of the dermis of the skin and connective tissues of the placenta
- **Type IX collagen** that binds to type II collagens of cartilage

Network-forming collagens are formed by epithelial cells and, unlike fibrous collagen types, are not exposed to **procollagen peptidase**, the enzyme that cleaves the telopeptides off the ends of the **procollagen molecules**. Therefore, instead of **tropocollagen molecules** that form the basic units of fibrous collagens, **procollagen** molecules form the basic unit of network-forming collagens. Procollagen units cannot associate to form fibers; instead they form **dimers** that assemble with other dimers to form an interlocking **network of thin three-dimensional sheets**. There are two types of network-forming collagens:

- **Type IV collagen** that forms the sheet-like lamina densa of the basal lamina
- **Type VII collagen** that aggregates in sheaves to form **anchoring fibers** whose function is to attach the basal lamina to the lamina reticularis of the basement membrane

Transmembrane collagens (also known as ***collagen-like proteins***) are integral proteins one of which, **type XVII**, functions in the adherence of the epidermis to the dermis. As such, these transmembrane collagens are components of the hemidesmosomes. There are three other types of transmembrane collagens, types XIII, XXIII, and XXV, whose functions are not as yet understood.

Collagen Synthesis

The synthesis of collagen occurs on the RER as individual preprocollagen chains (α-chains).

The synthesis of collagen occurs on the RER as individual **preprocollagen** chains (Fig. 4–7), which are α-chains possessing additional amino acid sequences, known as ***propeptides***, at both the amino and carboxyl ends. As a preprocollagen molecule is being synthesized, it enters the cisterna of the RER, where it is modified. First, the signal sequence directing the molecule to the RER is removed; then some of the proline and lysine residues are hydroxylated (by the enzymes peptidyl proline hydroxylase and peptidyl lysine hydroxylase) in a process known as *posttranslational modification* to form hydroxyproline and hydroxylysine, respectively. Subsequently, selected hydroxylysines are glycosylated by the addition of glucose and galactose.

Three preprocollagen molecules align with each other and assemble to form a tight helical configuration known as a ***procollagen molecule***. It is believed that the precision of their alignment is accomplished by the propeptides. Because these propeptides do not wrap around each other, the procollagen molecule resembles a tightly wound rope with frayed ends. The propeptides apparently have the additional function of keeping the procollagen molecules soluble, thus preventing their

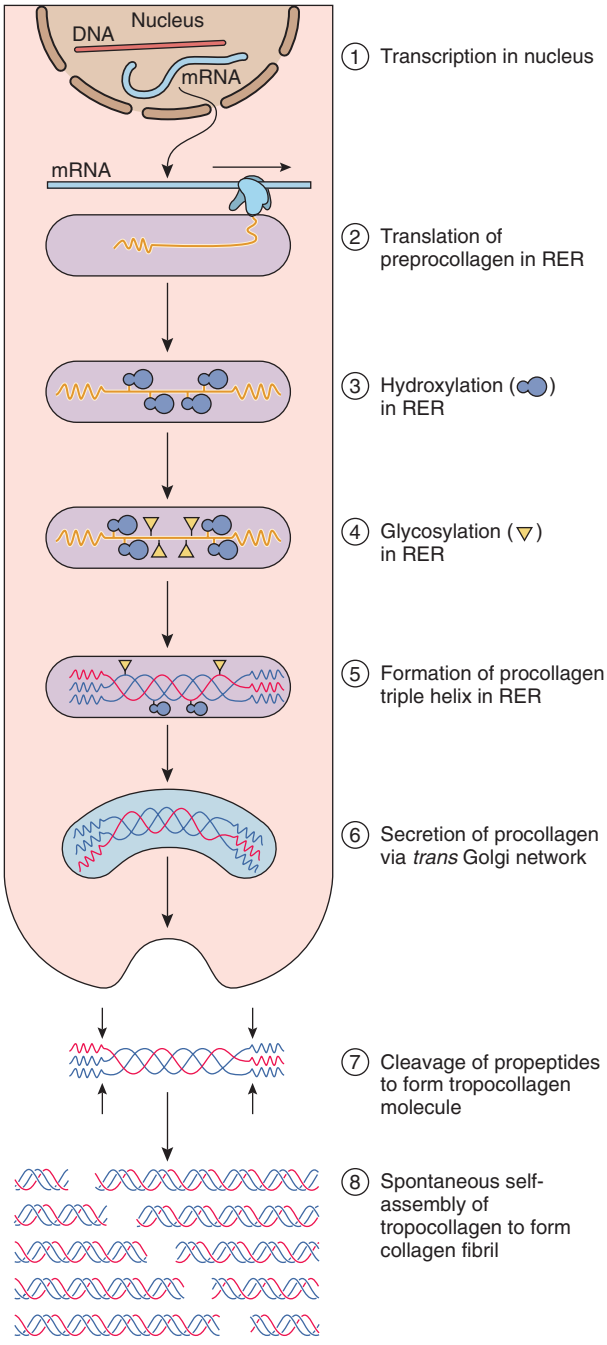


Figure 4–7 Schematic diagram of the sequence of events in the synthesis of type I collagen. Messenger RNA leaves the nucleus and attracts small and large subunits of ribosomes. As translation begins, the polysome complex translocates to the rough endoplasmic reticulum (RER), and the nascent alpha chains enter the lumen of the RER. Within the lumen, some proline and lysine residues of the alpha chains are hydroxylated, and the preprocollagen molecule is also glycosylated. Three alpha chains form a helical configuration, and the procollagen triple helix is formed. The procollagen is packaged and transferred to the Golgi complex, where further modification occurs. At the *trans*-Golgi network, the procollagen is packaged in clathrin-coated vesicles, and the procollagen is exocytosed. As the procollagen leaves the cell, the membrane-bound enzyme, procollagen peptidase, cleaves the propeptides from both the carboxyl- and the amino-end of procollagen, transforming it into tropocollagen. These newly formed macromolecules self-assemble into collagen fibrils.

spontaneous aggregation into collagen fibers within the cell.

The procollagen molecules leave the RER via transfer vesicles that transport them to the Golgi apparatus, where they are further modified by the addition of oligosaccharides. The modified procollagen molecules are packaged in the *trans*-Golgi network and are immediately ferried out of the cell.

As procollagen enters the extracellular environment, proteolytic enzymes, **procollagen peptidases**, endopeptidases located on the extracellular side of the plasmalemma, cleave the propeptides (removing a portion

CLINICAL CORRELATIONS

1. At the end of surgery, the cut surfaces of skin are carefully sutured; usually, a week or so later, the sutures are removed. The tensile strength of the dermis at that point is only about 10% that of normal skin. Within the next 4 weeks, the tensile strength increases to about 80% of normal, but in many cases, it never reaches 100%. The initial weakness is attributed to the formation of type III collagen during early wound healing, whereas the later improvement in tensile strength is due to scar maturation, when type III collagen is replaced by type I collagen.
2. Some individuals, especially blacks, are predisposed to an excessive accumulation of collagen during wound healing. In these patients, the scar forms an elevated growth known as a **keloid**.
3. Malformation of **type XVII** collagens, previously known as bullous pemphigoid antigen, result in blistering of the skin and mucous membranes due to incomplete adherence of the epithelium to the underlying connective tissue, a condition known as ***epidermolysis bullosa***.
4. Malformation of **types II** and **XI collagens** results in a condition known as ***Stickler syndrome***, a genetic condition that causes defects in the formation of bone and connective tissue proper. Depending on the degree of the mutation in the genes coding for types II and XI collagens as well as additional attributing mutations, the severity of Stickler syndrome may vary from no symptoms at all to serious malformations. These severe symptoms may include oral defects such as cleft of the palate, decrease in mandibular size, enlarged tongue; ocular defects such as retinal detachment, myopia, glaucoma, and malformed vitreous humor; defects in the auditory apparatus, resulting in partial or complete hearing loss; as well as early onset arthritis.

of the frayed ends) from both amino and carboxyl ends (see Fig. 4–7). The newly formed molecule is shorter (280 nm in length) and is known as a ***tropocollagen* (collagen) *molecule***. Tropocollagen molecules spontaneously self-assemble (see Fig. 4–7), in specific head-to-tail direction, into a regularly staggered array, fashioning fibrils that display a 67-nm banding representative of collagen types I, II, III, V, and XI (see Fig. 4–5). The formation and maintenance of the fibrillar structure are augmented by covalent bonds formed between lysine and hydroxylysine residues of neighboring tropocollagen molecules.

As the tropocollagen molecules self-assemble in a three-dimensional array, the spaces between the heads and tails of successive molecules in a single row line up as repeating **gap regions** (every 67 nm), not in adjoining but in neighboring rows (see Figs. 4–5 and 4–7). Similarly, the overlaps of heads and tails in neighboring rows are in register with one another as the **overlap regions**. Heavy metal stains used in electron microscopy preferentially deposit in the gap regions. Consequently, viewed in the electron microscope, collagen displays alternating dark and light bands; the dark bands represent the gap regions filled with heavy metal, and the light bands represent overlap regions, where the heavy metal cannot be deposited (see Fig. 4–6). The formation of some of the collagen types requires the presence of other collagens, such as type XI that forms the core of type I collagen.

The alignment of the collagen fibrils and fiber bundles is determined by the cells that synthesize them. The procollagen is released into folds and furrows of the plasmalemma, which act as molds that arrange the forming fibrils in the proper direction. The fibril orientation is further enhanced as the cells tug on the fibrils and physically drag them to fit the required pattern.

Fibrillar structure is absent in **types IV and VII collagen** because the propeptides are not removed from the procollagen molecule. Its procollagen molecules assemble into dimers, which then form a felt-like meshwork.

CLINICAL CORRELATIONS

Hydroxylation of proline residues requires the presence of vitamin C. In individuals with a deficiency of this vitamin, the α-chains of the tropocollagen molecules are unable to form stable helices, and the tropocollagen molecules are incapable of aggregating into fibrils. This condition, known as ***scurvy***, first affects connective tissues with high turnover of collagen, such as the periodontal ligament and gingiva (Fig. 4–8). Because these two structures are responsible for maintaining teeth in their sockets, the symptoms of scurvy include bleeding gums and loose teeth. If the vitamin C deficiency is prolonged, other sites are also affected. These symptoms may be alleviated by eating foods rich in vitamin C.

Deficiency of the enzyme **lysyl hydroxylase**, a genetic disorder known as ***Ehlers-Danlos syndrome***, results in abnormal cross-links among tropocollagen molecules. Individuals afflicted with this anomalous condition possess abnormal collagen fibers that result in hypermobile joints and hyperextensive skin. In many instances, the skin of affected patients is readily traumatized, and the patient is subject to dislocation of the affected joints.

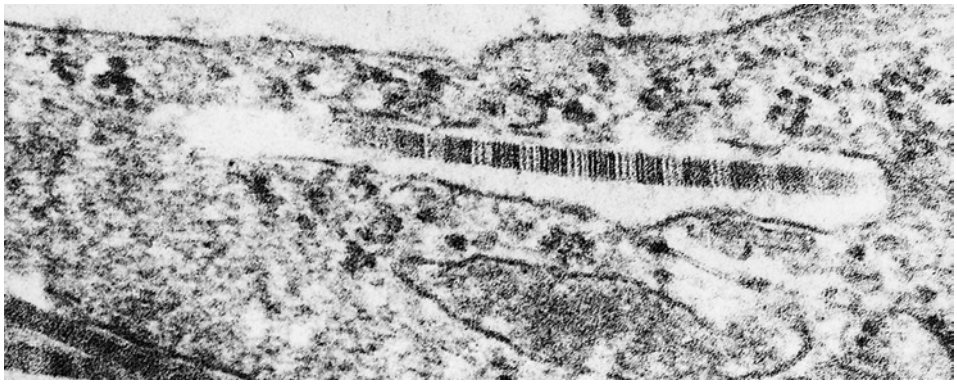


Figure 4–8 Degradation of type I collagen by fibroblasts. Collagen turnover is relatively slow in some regions of the body (e.g., bone, where it may be stable for as long as 10 years), whereas, in other regions, such as the gingiva and the periodontal ligament, the half-life of collagen may be a matter of weeks or months. Fibroblasts of the gingiva and periodontal ligament are responsible not only for the synthesis but also for the resorption of collagen. (From Ten Cate AR. *Oral Histology: Development, Structure, and Function*. 4th ed. St. Louis: Mosby-Year Book; 1994.)

Elastic Fibers

Elastic fibers, unlike collagen, are highly accommodating and may be stretched one and a half times their resting length without breaking. When the force is released, elastic fibers return to their resting length.

The elasticity of connective tissue is due, in great part, to the presence of **elastic fibers** in the ECM (Figs. 4–9 and 4–10; see Fig. 4–2). These fibers are usually slender, long, and branching in loose connective tissue, but they may form coarser bundles in ligaments and fenestrated sheets. Such bundles are found in the ligamentum flava of the vertebral column, and concentric sheets occur in the walls of larger blood vessels; in fact, elastic fibers constitute about 50% of the aorta by dry weight.

Elastic fibers are manufactured by fibroblasts of connective tissue as well as by smooth muscle cells of blood vessels. They are composed of **elastin**, **fibrillin-1 and fibulin-5**, and type VIII collagen.

- **Elastin** is a protein that is rich in glycine, lysine, alanine, valine, and proline but has no hydroxylysine. Elastin is derived from a soluble protein precursor, **tropoelastin**, that becomes insoluble due to the cross-linking of its lysine residues by the enzyme lysyl oxidase. In this manner, the elastin chains that are formed are held together because four lysine molecules, each belonging to a different elastin chain, form covalent bonds with each other to form what are known as ***desmosine cross-links***. These desmosine residues are highly deformable, and they impart a high degree of elasticity to elastic fibers to such an extent that these fibers may be stretched to about 150% of their resting lengths before breaking. After

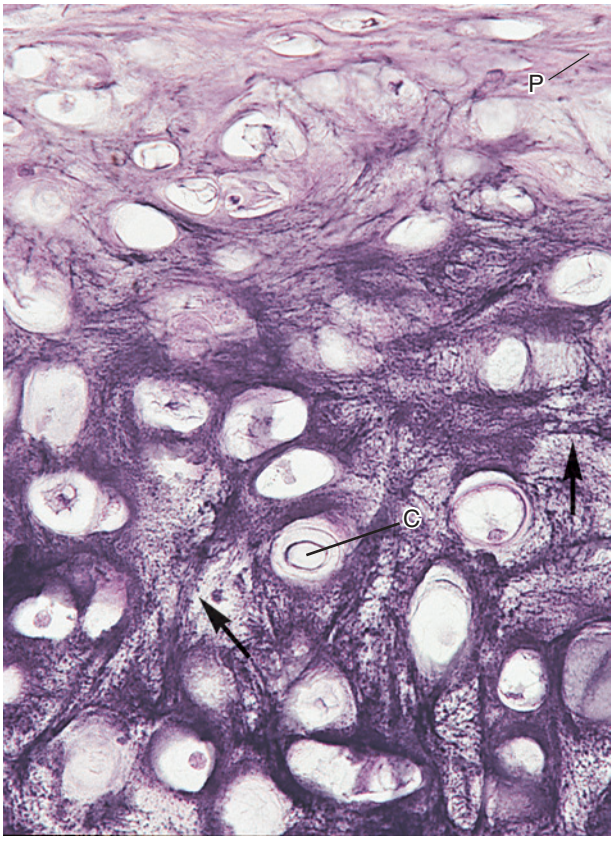


Figure 4–9 Note the presence of elastic fibers *(arrows)* in the matrix in this photomicrograph of elastic cartilage. The large chondrocytes of elastic cartilage occupy spaces, known as *lacunae*, in the proteoglycan-rich matrix. The large bundles of elastic fibers are clearly evident, and they appear to be arranged in a haphazard fashion. Observe that the thicker elastic fibers are composed of fine fibrils. C, Chondrocyte; P, perichondrium. (×270)

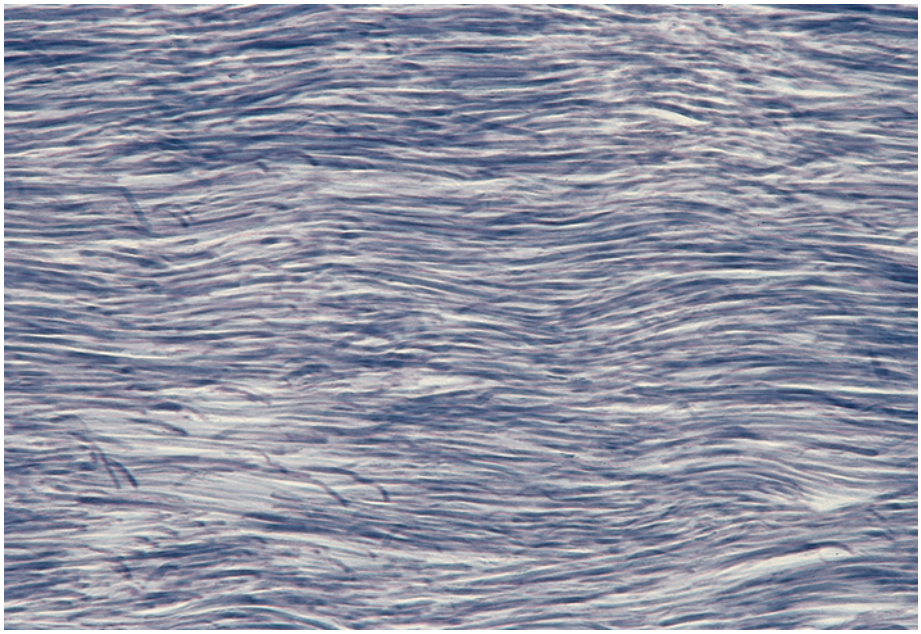


Figure 4–10 Dense, regular elastic connective tissue. Note that the elastic fibers are short and are arranged almost parallel with each other and that their ends are somewhat curled. Unlike collagen fibers of dense regular collagenous connective tissue, where the collagen fibrils and fibers closely parallel each other, elastic fibers appear somewhat misaligned. (×270)

being stretched, elastic fibers return to their resting length.

- The core of elastic fibers is composed of elastin and is surrounded by a sheath of **microfibrils**; each microfibril is about 10 nm in diameter and is composed of the glycoprotein **fibrillin-1** (Fig. 4–11). During the formation of elastic fibers, the fibrillin-1 molecules are elaborated first and are arranged head to tail in rod-shaped configuration to form a microfibril. A number of microfibrils gather to form a hollow cylinder, and the tropoelastin is deposited in the space surrounded by the microfibrils (Fig. 4–12). The soluble tropoelastin, which forms chemical bonds to fibrillin-1, is converted into **elastin** by the action of lysine oxidase.
- Integrin molecules of cells that synthesize elastic fibers bind to **fibulin-5**, a protein that has an affinity to itself as well as to tropoelastin and fibrillin-1. Recent investigations have demonstrated that fibulin-5 facilitates the formation of elastic fibers.
- **Type VIII collagen** has also been demonstrated to form a part of elastic fibers. Because collagen is inelastic, it is believed that type VIII collagen acts to limit the amount of stretching that elastic fibers are permitted to undergo.

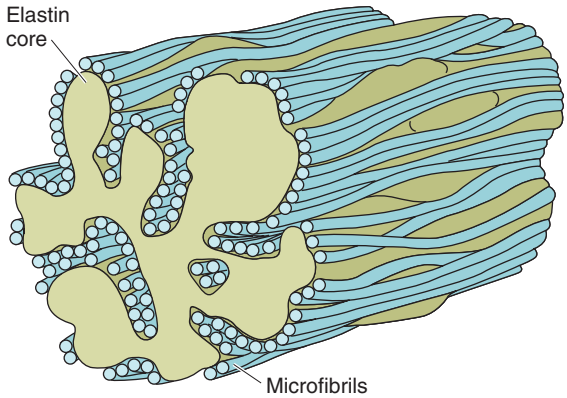


Figure 4–11 Schematic diagram of elastic fiber. Microfibrils surround the amorphous elastin.

CLINICAL CORRELATIONS

The integrity of elastic fibers depends on the presence of microfibrils. Patients with **Marfan syndrome** have a defect in the gene on chromosome 15 that codes for fibrillin; therefore, their elastic fibers do not develop normally. People who are severely affected with this condition are predisposed to fatal rupture of the aorta.

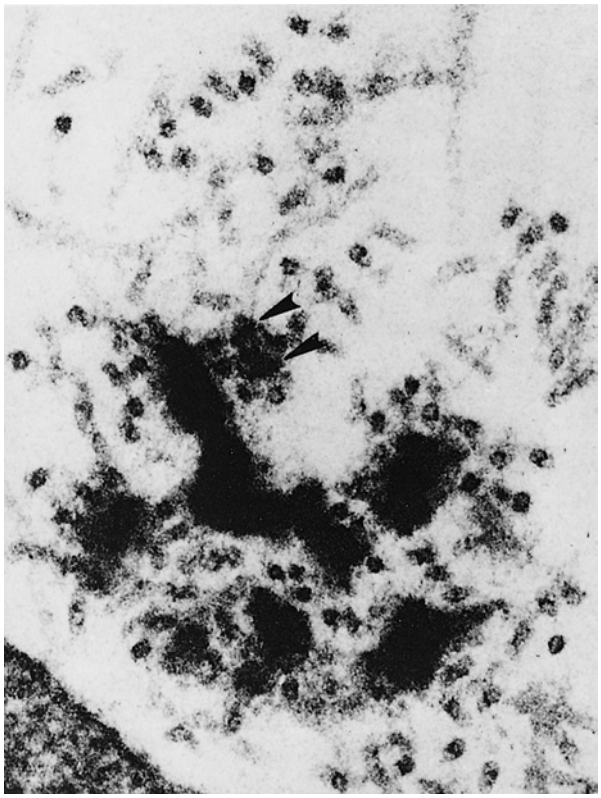


Figure 4–12 Electron micrograph of elastic fiber development. Note the presence of microfibrils surrounding the amorphous matrix of elastin as if a small space were to be delineated by slats of a picket fence *(arrowheads)*. These fibrillin-containing microfibrils are elaborated and released first, and then the manufacturing cell, be it fibroblasts of connective tissue proper or smooth muscle cells of a blood vessel, releases elastin into the space enclosed by the microfibrils. (From Fukuda Y, Ferrans VJ, Crystal RG. Development of elastic fibers of nuchal ligament, aorta, and lung of fetal and postnatal sheep: an ultrastructural and electron microscopic immunohistochemical study. *Am J Anat.* 1984;170: 597-629. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

BASEMENT MEMBRANE

The basement membrane seen with light microscopy is shown by electron microscopy to be composed of the basal lamina and lamina reticularis.

The interface between epithelium and connective tissue is occupied by a narrow, acellular region, the **basement membrane**, which is well stained by the periodic acid-Schiff reaction and by other histological stains that detect GAGs. A structure similar to the basement membrane, the **external lamina**, surrounds smooth and skeletal muscle cells, adipocytes, and Schwann cells.

The basement membrane, as visible by light microscopy, is better defined by electron microscopy as having two constituents: the **basal lamina**, elaborated by epithelial cells, and the **lamina reticularis**, manufactured by cells of the connective tissue (Fig. 4–13).

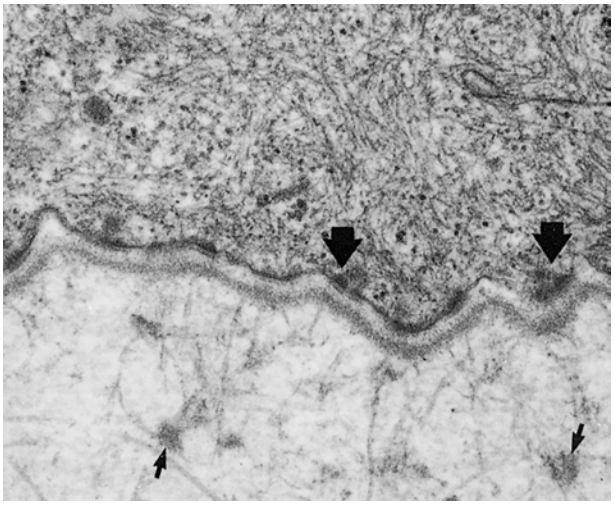


Figure 4–13 Electron micrograph of the basal lamina of the human cornea. Note the hemidesmosomes *(large arrows)* and the anchoring plaques among the anchoring fibrils *(small arrows)*. Observe that the basal cell membrane is clearly visible, and that the plaques of the hemidesmosomes are attached to the cytoplasmic surface of the basal plasmalemma. The dense, amorphous-appearing line that follows the contour of the basal plasma membrane is the lamina densa, and the clear area between it and the basal cell membrane is the lamina lucida. These two laminae constitute the epithelially derived basal lamina. The region that is located beneath the lamina densa is the connective tissue-derived lamina reticularis. (×50000) (From Albert D, Jakobiec FA. *Principles and Practice of Ophthalmology: Basic Sciences*. Philadelphia: WB Saunders; 1994.)

Basal Lamina

The basal lamina manufactured by the epithelium is composed of the lamina lucida and the lamina densa.

Electron micrographs of the **basal lamina** display its two regions: the **lamina lucida**, a 50-nm-thick electron-lucent region just beneath the epithelium, and the **lamina densa**, a 50-nm-thick electron-dense region (see Fig. 4–13; Figs. 4–14 and 4–15).

The **lamina lucida** is about 50 nm in width and consists mainly of the extracellular glycoproteins **laminin** and **entactin** as well as those moieties of **integrins** and **dystroglycans** (i.e., transmembrane laminin receptors, both discussed in the section on integrins and dystroglycans) that project from the epithelial cell membrane into the basal lamina. In rapidly frozen tissues, the lamina lucida is frequently absent, suggesting that it may be an artifact of fixation, and the lamina

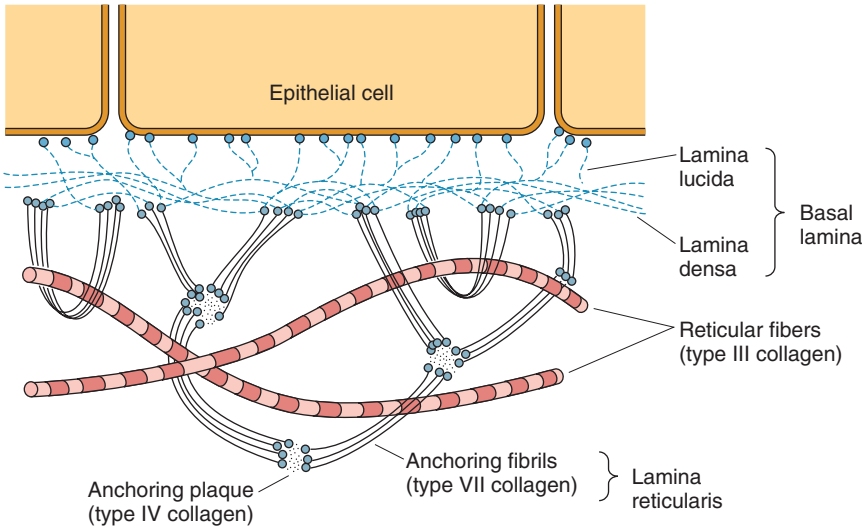


Figure 4–14 Schematic diagram of the basal lamina and the lamina reticularis. (Modified from Fawcett DW. *Bloom and Fawcett's a Textbook of Histology*. 12th ed. New York: Chapman and Hall; 1994.)

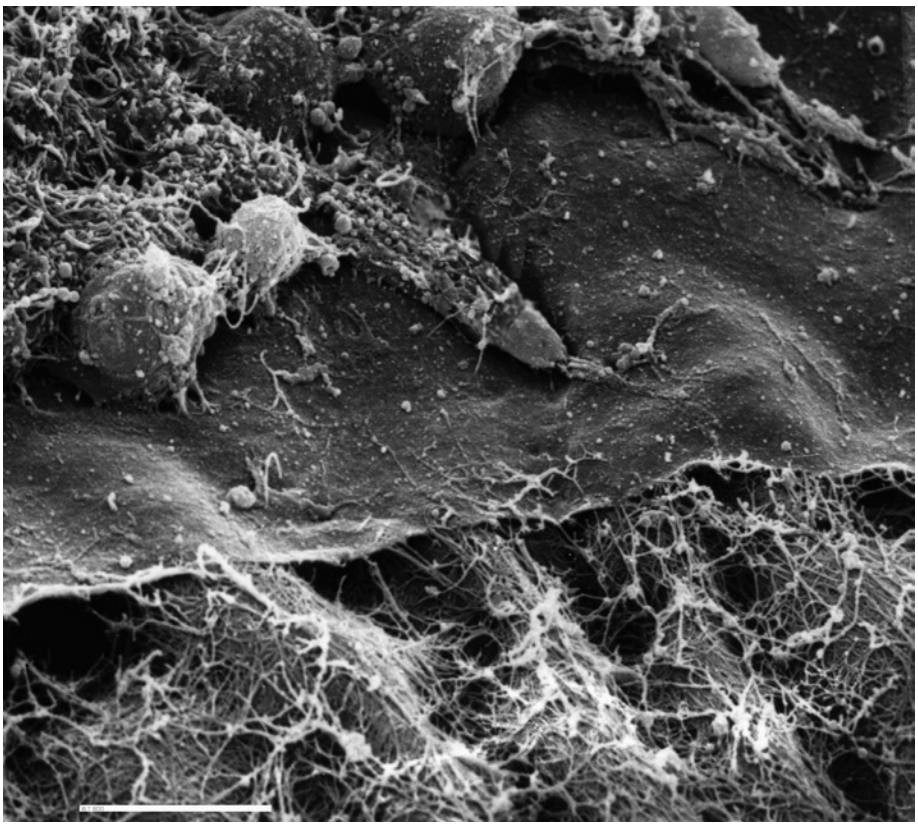


Figure 4–15 Image from a 6-day chick embryo cornea from which a portion of the epithelium has been removed, exposing epithelial cells on the underlying basement membrane. The membrane itself has been partially removed, revealing the underlying primary corneal stroma composed of orthogonally arrayed collagen fibrils. The *white bar* at the lower left is the 10-µm mark. (Courtesy Robert L. Trelstad.)

densa may be closer to the integrins and dystroglycans of the basal cell membrane than previously believed.

The **lamina densa** is also about 50 nm in thickness and comprises a meshwork of type IV collagen, which is coated on both the lamina lucida and lamina reticularis sides by the proteoglycan **perlecan**. The **heparan sulfate** side chains projecting from the protein core of perlecan form a polyanion. The lamina reticularis aspect of the lamina densa also possesses **fibronectin**.

Laminin has domains that bind to type IV collagen, heparan sulfate, and the integrins and dystroglycans of the epithelial basal cell membrane, thus anchoring the epithelial cell to the basal lamina. The basal lamina appears to be well anchored to the reticular lamina by several substances, including fibronectin, anchoring fibrils (type VII collagen), and microfibrils (fibrillin-1), all elaborated by fibroblasts of connective tissue (Fig. 4–16).

The basal lamina functions both as a molecular filter and as a flexible, firm support for the overlying epithelium. The filtering aspect is due not only to the type IV collagen, whose interwoven meshwork forms a physical filter of specific pore size, but also to the negative charges of its heparan sulfate constituent, which preferentially restricts the passage of negatively charged molecules. Additional functions of the basal lamina include facilitation of mitotic activity and cell differentiation, modulating cellular metabolism, assisting in the establishment of cell polarity, playing a role in the modification of the arrangement of the integral proteins localized in the basal cell membrane, and acting as a path for cellular migration, as in reepithelialization during wound repair or in the reestablishment of myoneural junctions during regeneration of motor nerves.

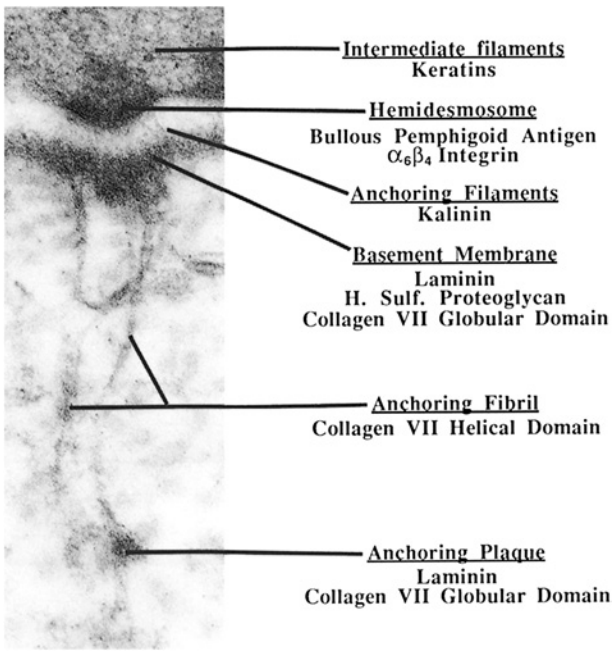


Figure 4–16 Electron micrograph of the basal lamina of the corneal epithelium. (×165000) (From Albert D, Jakobiec FA. *Principles and Practice of Ophthalmology: Basic Sciences*. Philadelphia: WB Saunders; 1994.)

Lamina Reticularis

The lamina reticularis is derived from the connective tissue component and is responsible for affixing the lamina densa to the underlying connective tissue.

The **lamina reticularis** (see Figs. 4–13, 4–14, and 4–16), a region of varying thickness (usually about 200 nm), is manufactured by fibroblasts and is composed of **types III**, **VII (anchoring fibrils)**, and **XVIII** and a slight amount of **type I collagen**; additionally, a slight amount of **microfibrils** is also present. The lamina reticularis is the interface between the basal lamina and the underlying connective tissue, and its thickness varies with the amount of frictional force on the overlying epithelium. Thus, it is quite thick in skin and very thin beneath the epithelial lining of the alveolus of the lung.

Type I and type III collagen fibers of the connective tissue loop into the lamina reticularis, where they interact with and are bound to the microfibrils and anchoring fibrils and type XVIII collagen of the lamina reticularis. Moreover, the basic groups of the collagen fibers form bonds with the acidic groups of the GAGs of the lamina densa. In addition, collagen binding domains and GAG domains of fibronectin further assist in anchoring the basal lamina to the lamina reticularis. Thus, the epithelial sheath is bound to the underlying connective tissue by these resilient, acellular interfaces, the basal lamina, and lamina reticularis.

INTEGRINS AND DYSTROGLYCANS

Integrins and dystroglycans are transmembrane glycoproteins that act as laminin receptors as well as organizers of basal lamina assembly.

Integrins are transmembrane proteins that are similar to cell membrane receptors in that they form bonds with ligands. However, unlike those of receptors, their cytoplasmic regions are linked to the cytoskeleton, and their ligands are not signaling molecules but structural members of the ECM, such as collagen, laminin, and fibronectin. Moreover, the association between an integrin and its ligand is much weaker than that between a receptor and its ligand. Integrins are much more numerous than receptors, thus compensating for the bond weakness and also permitting the migration of cells along a surface of ECM.

Integrins are heterodimers (~250,000 Da) composed of **α and β glycoprotein** chains whose carboxyl ends are linked to **talin**, **paxillin**, **vinculin**, and **α-actinin** of the cytoskeleton, which, in turn, form bonds with **actin filaments**. Their amino ends possess binding sites for macromolecules of the ECM (see Chapter 2, Fig. 2–32). Because integrins link the cytoskeleton to the ECM, they are also called ***transmembrane linkers***. The α-chain of the integrin molecule binds Ca^{2+} or Mg^{2+}, divalent cations necessary for the maintenance of proper binding with the ligand. In this fashion the integrin molecules form a plethora of **focal adhesions (anchoring junctions)** that help secure the epithelium to the basal lamina.

Many integrins differ in their ligand specificity, cellular distribution, and function. Some are commonly referred to as *receptors* for their ligand (e.g., laminin receptor, fibronectin receptor). Cells can modulate the affinity of their receptor for its ligand by regulating the availability of divalent cations, modifying the conformation of the integrin, or otherwise altering the integrin's affinity for the ligand. In this manner, cells are not locked into a particular position once their integrins bind to the macromolecules of the ECM but can release their integrin–ligand bonds and move away from that particular location.

In addition to their roles in adhesion, integrins function in transducing biochemical signals into intracellular events by activating second messenger system cascades. The versatility of integrins in biochemical transduction is evidenced by their ability to stimulate diverse signaling pathways, including mitogen-activated protein kinase, protein kinase C, and phosphoinositide pathways that lead to activation of the cell cycle, cell differentiation, cytoskeletal reorganization, regulation of gene expression, and even programmed cell death via apoptosis. Frequently, integrins have to be activated by **focal adhesion kinase**, a protein tyrosine kinase; otherwise, they cannot initiate their signaling functions.

Dystroglycans are glycoproteins that are also composed of two subunits, a **transmembrane β-dystroglycan** and an **extracellular α-dystroglycan**. The α-dystroglycan binds to the laminin of the basal lamina but at different sites than does the integrin molecule. The intracellular moiety of the β-dystroglycan binds to the actin-binding protein **dystrophin**, which, in turn, binds to α-actinin of the cytoskeleton.

Dystroglycans and integrins have significant roles in the assembly of basal laminae because embryos lacking in either or both of these glycoproteins are unable to form normal basal laminae.

CLINICAL CORRELATIONS

Individuals with the autosomal recessive disorder **leukocyte adhesion deficiency** are incapable of synthesizing the β-chain of the white blood cell integrins. Their leukocytes are incapable of adhering to the endothelial cells of blood vessels and thus cannot migrate to sites of inflammation. Patients with this disease have difficulty in fighting bacterial infections.

PATHOLOGICAL CONSIDERATIONS

See Figures 4–17 and 4–18.

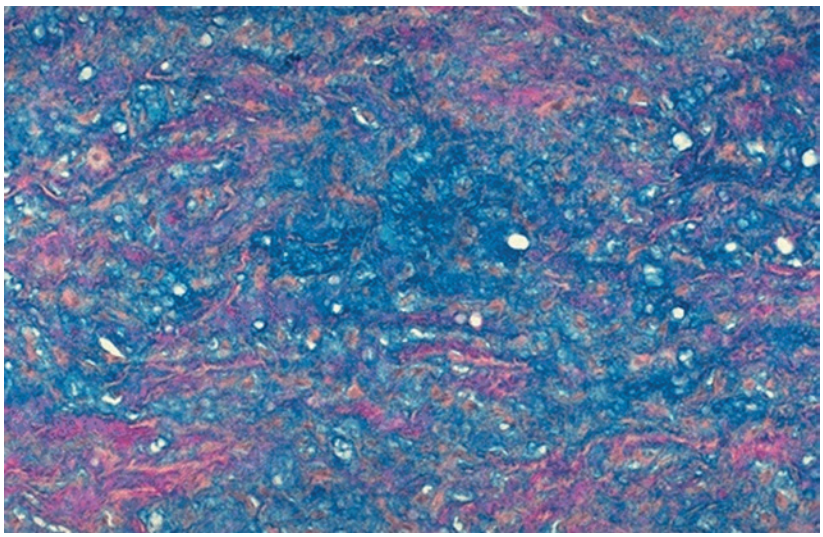


Figure 4–17 This mucin stain of the aortic media shows cystic medial degeneration. Pink elastic fibers, instead of running in parallel arrays, are seen here to be disrupted by pools of blue ground substance. This is typical for Marfan syndrome affecting connective tissues containing elastin. This causes the connective tissue weakness that explains the propensity for aortic dissection. (From Klatt EC. *Robbins and Cotran: Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010.)

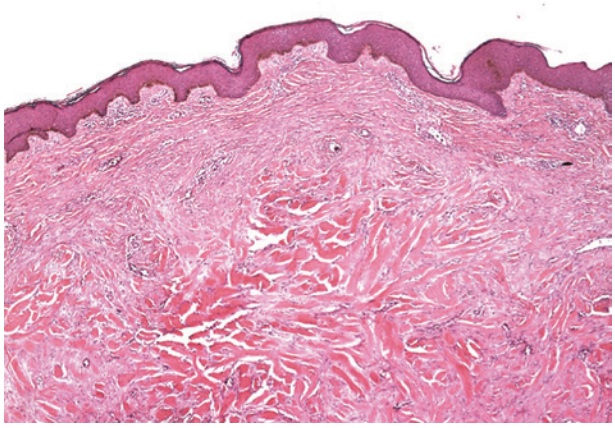
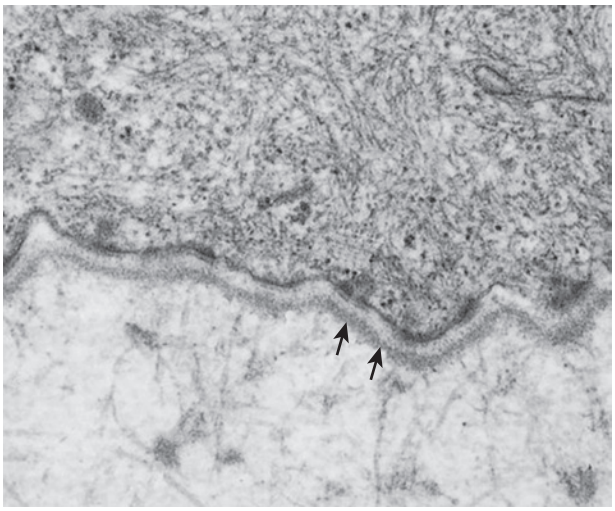


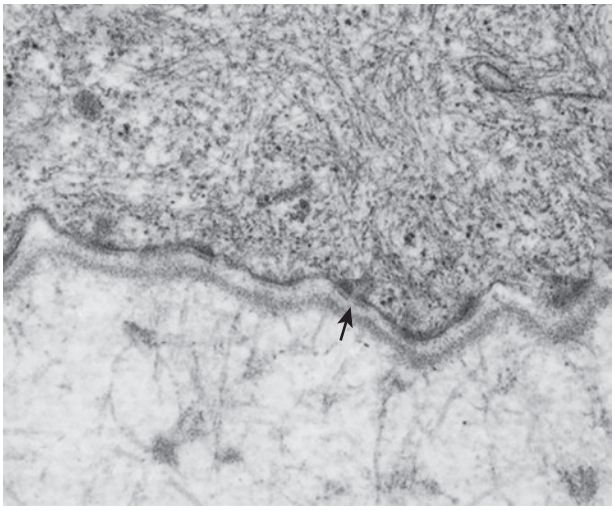
Figure 4–18 Microscopic image of a keloid. Note the thick collagen deposition in the dermis. (From Kumar V, et al. *Robbins and Cotran: Pathologic Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:110.)

REVIEW QUESTIONS

1. A patient whose symptoms include discolored, violet-colored skin with large blisters, fever, malaise, rapid heart rate, and a great deal of pain is diagnosed with necrotizing fasciitis. The rapid spread of *Staphylococus aureus* and group A streptococci that ravage the connective tissue of this patient is due to the ability of these organisms to destroy one of the components of the extracellular matrix of the skin. Which is this component?
   a. hyaluronic acid
   b. keratan sulfate
   c. dermatan sulfate
   d. heparan sulfate
   e. chondroitin-4-sulfate
2. An African-American patient had surgery and forms a very thick, elevated growth at the site of the skin incision. This elevated growth is known as a *keloid* and is due to the excessive accumulation of
   a. hyaluronic acid
   b. proteoglycans
   c. type I collagen
   d. elastic fibers
   e. oxytalan fibers
3. The tensile strength of the healing wound about a week after the cut surfaces of the skin are sutured to each other is about 10% of the normal tensile strength of skin. The initial weakness of the dermis during wound healing is due to the material elaborated by fibroblasts to close the wound in a very rapid fashion. This material is
   a. type I collagen
   b. type II collagen
   c. type III collagen
   d. type V collagen
   e. type VII collagen
4. A male patient is seen for the first time by his physician; the patient has very thin, slender legs and unusually long, slender fingers. The patient's history indicates that he has been diagnosed with a connective tissue disorder, known as *Marfan syndrome*. The mutation that causes this syndrome affects
   a. tropomyosin
   b. troponin
   c. microfibrils
   d. elastin
   e. fibrillin
5. A patient with leukocyte adhesion deficiency will have a/an
   a. autosomal dominant disorder
   b. sex-linked chromosomal disorder
   c. dysfunctional adhesins
   d. dysfunctional integrins
   e. dysfunctional cadherins
6. A 9-month-old baby is seen by her pediatrician, who notices a lack of weight gain in the infant as well as thin, knobby joints. The parents tell the physician that they are making their own baby food and that the child has been very irritable and cries a lot when she is crawling on the floor. The baby's gums appear to be overly red. The pediatrician suspects lack of vitamin C in the baby's diet and recommends that the parents switch to commercially prepared baby foods and urges them to offer the child citrus fruits. Additionally, the child is to receive high doses of vitamin C for 7 days and a lower dose for an additional 30 days. Vitamin C is necessary for the synthesis of normal
   a. elastin
   b. fibrillin
   c. tropocollagen
   d. troponin
   e. hyaluronic acid
7. A 12-year-old child complains to his physician that he frequently dislocates his joints. Upon examination, the physician notes that the patient has hyperextensive joints, has highly stretchable skin, and has kyphoscoliosis (hump back). The doctor should suspect
   a. scurvy
   b. Ehlers-Danlos syndrome
   c. Marfan syndrome
   d. cutis laxa
   e. mucopolysaccharidosis
8. 

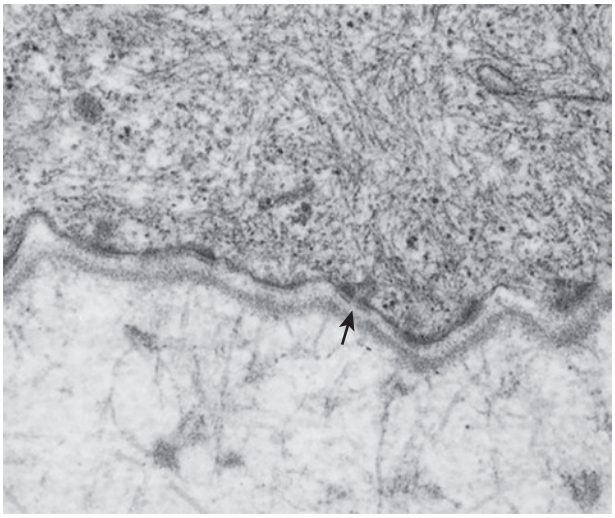
   The arrows point to an area that is rich in
   a. type I collagen
   b. type II collagen
   c. type III collagen
   d. type IV collagen
   e. type VII collagen

9.



The clear area indicated by the tip of the arrow is rich in
a. hyaluronic acid
b. fibronectin
c. laminin
d. perlecan
e. cadherins

10.



An elderly patient is seen by his dermatologist because he developed itching and blisters of his skin. If the blistering involves the region indicated by the *arrow* in this electron micrograph and if the patient is shown to have IgG along the same area indicated by the arrows, the patient is probably suffering from
a. dermatitis herpetiformis
b. atopic dermatitis
c. pemphigus vulgaris
d. pemphigus foliaceus
e. bullous pemphigoid

ANSWERS

1. A. Hyaluronic acid is a very large glycosaminoglycan that is not sulfated and is not bound to a protein core. It is able to bind a very large number of proteoglycans, thus forming a very large molecule with an enormous domain. This very large macromolecule complex is responsible for the "gel state" of the extracellular matrix. *Staphylococus aureus* and group A streptococci possess hyaluronidase that cleaves hyaluronic acid and converts the gel to a "sol (soluble) state," making it much easier for the microorganisms to spread very rapidly through the connective tissue element, destroying the tissues in its path. Some call these microorganisms, *flesh-eating bacteria*. The other four glycosaminoglycans are much smaller and, by binding to a protein core, form proteoglycans. They are not large enough by themselves to form a gel.
2. C. African Americans are especially predisposed to the excessive accumulation of type I collagen at the site of wound healing.
3. C. The first collagen to be formed by fibroblasts to effect initial closure of a wound is type III collagen (reticular fibers). This slender collagen type has a very weak tensile strength and is replaced by type I collagen within 4 weeks of the injury and, at that point, the tensile strength of the healed tissue is about 80% of the tensile strength of intact skin.
4. E. Marfan syndrome is due to a mutation that involves the gene that codes for the component of elastic fibers known as *fibrillin*. Individuals with this condition frequently have cardiovascular problems, including aortic aneurysm, high blood pressure, dislocation of the lens of the orb. Their collagen fibers, muscle fibers, and actin filaments are normal.
5. D. Leukocyte adhesion deficiency is an autosomal recessive disorder that results in the inability of white blood cells to synthesize the beta chains of their integrin molecules. Therefore, the leukocytes are incapable of adhering to the endothelial cells and are unable to migrate to the site of inflammation. Patients with this disease have difficulties resisting bacterial infections. Adhesins are surface antigens of many microorganisms that permit them to adhere to mammalian cell surfaces, whereas cadherins are responsible for cell–cell adhesions.
6. C. Vitamin C is necessary for the hydroxylation of proline residues. Hydroxyproline is required to hold the alpha chains of tropocollagen molecules to each other. The synthesis of elastin, fibrillin, and hyaluronic acid are not affected by vitamin C. Troponin is a protein present in skeletal and cardiac muscle and is not affected by the lack of vitamin C.
7. C. The doctor should suspect Ehlers-Danlos syndrome because individuals with this genetic disorder have very flexible joints, occasional kyphoscoliosis, and skin that can be stretched a couple of inches, and upon release, the skin returns to its previous position. A patient with scurvy would be recognized by bleeding gums and possible disfiguration of the long bones of the leg. Patients wtih Marfan syndrome are usually tall and thin and possess especially long fingers. Cutis laxa is characterized by stretchy skin that hangs loosely in folds. Patients with mucopolysaccharidosis die at a very young age.
8. D. The lamina densa of the basal lamina is formed mostly of a network of type IV collagen. Type I collagen is the most common collagen present in the body, and it forms thick bundles of collagen fibers. Type II collagen is present in the matrix of cartilage, and type III collagen is also known as *reticular fibers*, a thin fibrous collagen with many sugar groups that allow it to bind silver stain. Type VII collagen is located in the lamina reticularis of the basement membrane.
9. C. The clear area indicated by the tip of the arrow in this electron micrograph represents the lamina lucida component of the basal lamina. It is rich in laminin, a glycoprotein that facilitates adhesion between the basal lamina and epithelial cells. Fibronectin is located on the connective tissue side of basal lamina. Cadherins are transmembrane proteins that permit cells to contact and adhere to one another. Neither hyaluronic acid nor perlecans is located in this region.
10. E. Bullous pemphigoid is a relatively rare autoimmune condition occurring in elderly patients in which the patient produces IgG against his or her hemidesmosomes and separation of the epidermis from the dermis by the formation of blisters. Dermatitis herpetiformis is similar to bullous pemphigoid in that the vesicles form at the interface of the epidermis and dermis and is accompanied by itching. However, the condition begins at the highest points of the dermal ridges, and it is only later that the entire epidermal–dermal interface becomes involved. The main difference is that IgA rather than IgG is the antibody involved. Pemphigus vulgaris and pemphigus foliaceus are both blistering conditions; however, the blister is intraepidermal and involved autoantibodies against desmosomes. Atopic dermatitis is more prevalent in children than in adults and is usually located at the joints where flexion occurs. It is accompanied by edema, a thicker than normal epidermis, and excessive development of the rete apparatus.

This page intentionally left blank

Epithelium and Glands

5

The approximately 200 distinctly different types of cells composing the human body are arranged and cooperatively organized into four basic **tissues**. Groups of these tissues are assembled in various organizational and functional arrangements into **organs**, which carry out functions of the body. The four basic tissue types are **epithelium**, **connective tissue**, **muscle**, and **nervous tissue**. Each of these tissues and the cells and extracellular matrix that constitute them are covered in this and in the next few chapters.

EPITHELIAL TISSUE

Epithelial tissue is present in two forms: as (1) sheets of contiguous cells **(epithelia)** that cover the body on its external surface and line the body on its internal surface and (2) glands, which originate from invaginated epithelial cells.

Epithelia are derived from all three embryonic germ layers, although most of the epithelia are derived from ectoderm and endoderm. The ectoderm gives rise to the oral and nasal mucosae, the cornea, the epidermis of skin, and the glands of the skin and the mammary glands. The liver, pancreas, and lining of the respiratory and gastrointestinal tract are derived from the endoderm. The uriniferous tubules of the kidney, the lining of the male and female reproductive systems, the endothelial lining of the circulatory system, and the mesothelium of the body cavities develop from the mesodermal germ layer.

Epithelial tissues have numerous functions:

- **Protection** of underlying tissues of the body from abrasion and injury
- **Transcellular transport** of molecules across epithelial layers
- **Secretion** of mucus precursor, hormones, enzymes, and other molecules from various glands
- **Absorption** of material from a lumen (i.e., intestinal tract or certain kidney tubules)
- Control of movement of materials between body compartments via **selective permeability** of intercellular junctions between epithelial cells
- **Detection** of **sensations** via taste buds, retina of the eye, and specialized hair cells in the ear

Epithelium

Tightly bound contiguous cells forming sheets covering or lining the body are known as an epithelium.

The sheets of contiguous cells in the epithelium are tightly bound together by junctional complexes. Epithelia display little extracellular space and little extracellular matrix. Epithelium is separated from the underlying connective tissue by an extracellular matrix, the **basement membrane**, composed of the **basal lamina** and the **lamina reticularis** (discussed in Chapter 4), synthesized by the epithelial cells and cells of the connective tissue. Because epithelium is avascular, the adjacent supporting connective tissue through its capillary beds supplies nourishment and oxygen via diffusion through the basement membrane.

Classification of Epithelial Membranes

Cell arrangement and morphology are the bases of classification of epithelium.

Epithelial membranes are classified according to the number of cell layers between the basal lamina and the free surface and by the morphology of the surface-most epithelial cells (Table 5–1). If the membrane is composed of a single layer of cells, it is called ***simple epithelium***; if it is composed of more than one cell layer, it is called ***stratified epithelium*** (Fig. 5–1). The morphology of the cells may be squamous (flat), cuboidal, or columnar when viewed in sections taken perpendicular to the basement membrane. Stratified epithelia are classified by the morphology of the cells in their superficial layer only. In addition to these two major classes of epithelia, which are further identified by cellular morphology, there are two other distinct types: pseudostratified and transitional (see Fig. 5–1).

Simple Squamous Epithelium

Simple squamous epithelium is formed of a single layer of flat cells.

Simple squamous epithelium is composed of a single layer of tightly packed, thin, or low-profile polygonal cells. When viewed from the surface, the epithelial sheet looks much like a tile floor with a centrally placed bulging nucleus in each cell (Fig. 5–2A). Viewed in section, however, only some cells display nuclei because the plane of section frequently does not encounter the nucleus. Simple squamous epithelia line pulmonary alveoli, compose the loop of Henle and the parietal layer of Bowman capsule in the kidney, and form the endothelial lining of blood and lymph vessels as well as

SUMMARY

Epithelial tissue is present in two forms: sheets of contiguous cells, known as ***epithelia***, that cover a structure or line a cavity and **glands** that originate from epithelial cells. Epithelia are classified into two categories, **simple**, consisting of only a single layer of cells, and **stratified**, composed of two or more cell layers. Epithelia are always separated from connective tissue by a thin, acellular layer, known as the ***basement membrane***. Epithelia are further classified according to the shape of the cells that form the surface layer (in stratified epithelia, they are the cells farthest from the basement membrane), namely **squamous**, **cuboidal**, and **columnar**. Two additional categories are **pseudostratified** and **transitional epithelia**. Epithelial cells form specialized junctions with each other, **desmosomes**, **occluding junctions**, **adhering junctions**, **gap junctions**, as well as with the basement membrane, **hemidesmosomes**. **Glands** manufacture secretions, which they release. There are two major types of glands, those that possess ducts and release their products onto a surface, known as ***exocrine glands***, and those that release their substance into the extracellular matrix, known as ***endocrine glands***.

KEY WORDS

Autocrine; cilia; columnar; cuboidal; diffuse neuroendocrine; endocrine glands; exocrine glands; keratinized; microvilli; nonkeratinized; paracrine; pseudostratified; simple epithelium; squamous; stratified epithelium; transitional

TABLE 5–1 Classification of Epithelia

Type	Shape of Surface Cells	Sample Locations	Functions
Simple			
Simple squamous	Flattened	*Lining:* pulmonary alveoli, loop of Henle, parietal layer of Bowman capsule, inner and middle ear, blood and lymphatic vessels, pleural and peritoneal cavities	Limiting membrane, fluid transport, gaseous exchange, lubrication, reducing friction (thus aiding movement of viscera), lining membrane
Simple cuboidal	Cuboidal	Ducts of many glands, covering of ovary, form kidney tubules	Secretion, absorption, protection
Simple columnar	Columnar	*Lining:* oviducts, ductuli efferentes of testis, uterus, small bronchi, much of digestive tract, gallbladder, and large ducts of some glands	Transportation, absorption, secretion, protection
Pseudostratified	All cells rest on basal lamina but not all reach epithelial surface; surface cells are columnar	*Lining:* most of trachea, primary bronchi, epididymis and ductus deferens, auditory tube, part of tympanic cavity, nasal cavity, lacrimal sac, male urethra, large excretory ducts	Secretion, absorption lubrication, protection, transportation
Stratified			
Stratified squamous (nonkeratinized)	Flattened (with nuclei)	*Lining:* mouth, epiglottis, esophagus, vocal folds, vagina	Protection, secretion
Stratified squamous (keratinized)	Flattened (without nuclei)	Epidermis of skin	Protection
Stratified cuboidal	Cuboidal	*Lining:* ducts of sweat glands	Absorption, secretion
Stratified columnar	Columnar	Conjunctiva of eye, some large excretory ducts, portions of male urethra	Secretion, absorption, protection
Transitional	Dome-shaped (relaxed), flattened (distended)	*Lining:* urinary tract from renal calyces to urethra	Protection, distensible

the mesothelium of the pleural, pericardial, and peritoneal cavities.

Simple Cuboidal Epithelium

Simple cuboidal epithelium is composed of a single layer of cells shaped like truncated hexagonal solids.

A single layer of polygon-shaped cells constitutes **simple cuboidal epithelium** (see Fig. 5–2A). When viewed in a section cut perpendicular to the surface, the cells present a square profile with a centrally placed round nucleus. Simple cuboidal epithelia make up the ducts of many glands of the body, form the covering of the ovary, and compose many kidney tubules.

Simple Columnar Epithelium

Simple columnar epithelium is composed of a single layer of tall cells shaped like hexagonal solids.

The cells of **simple columnar epithelium** appear much like those of simple cuboidal epithelium in a surface view; when viewed in longitudinal section, however, they are tall, rectangular cells whose ovoid nuclei are usually located at the same level in the basal half of the cell (see Fig. 5–2B). Simple columnar epithelium lines much of the digestive tract, gallbladder, and large ducts of glands. Simple columnar epithelium may exhibit a striated border, or **microvilli** (narrow, finger-like cytoplasmic processes), projecting from the apical surface of the cells. The simple columnar epithelium that lines the uterus, oviducts, ductuli efferentes, and small bronchi is ciliated. In these organs, **cilia** (hair-like structures) project from the apical surface of the columnar cells into the lumen.

Stratified Squamous (Nonkeratinized) Epithelium

Stratified squamous (nonkeratinized) epithelium is composed of several layers of cells, the surface-most layer of which possess nuclei.

Stratified squamous (nonkeratinized) epithelium is thick; because it is composed of several layers of cells,

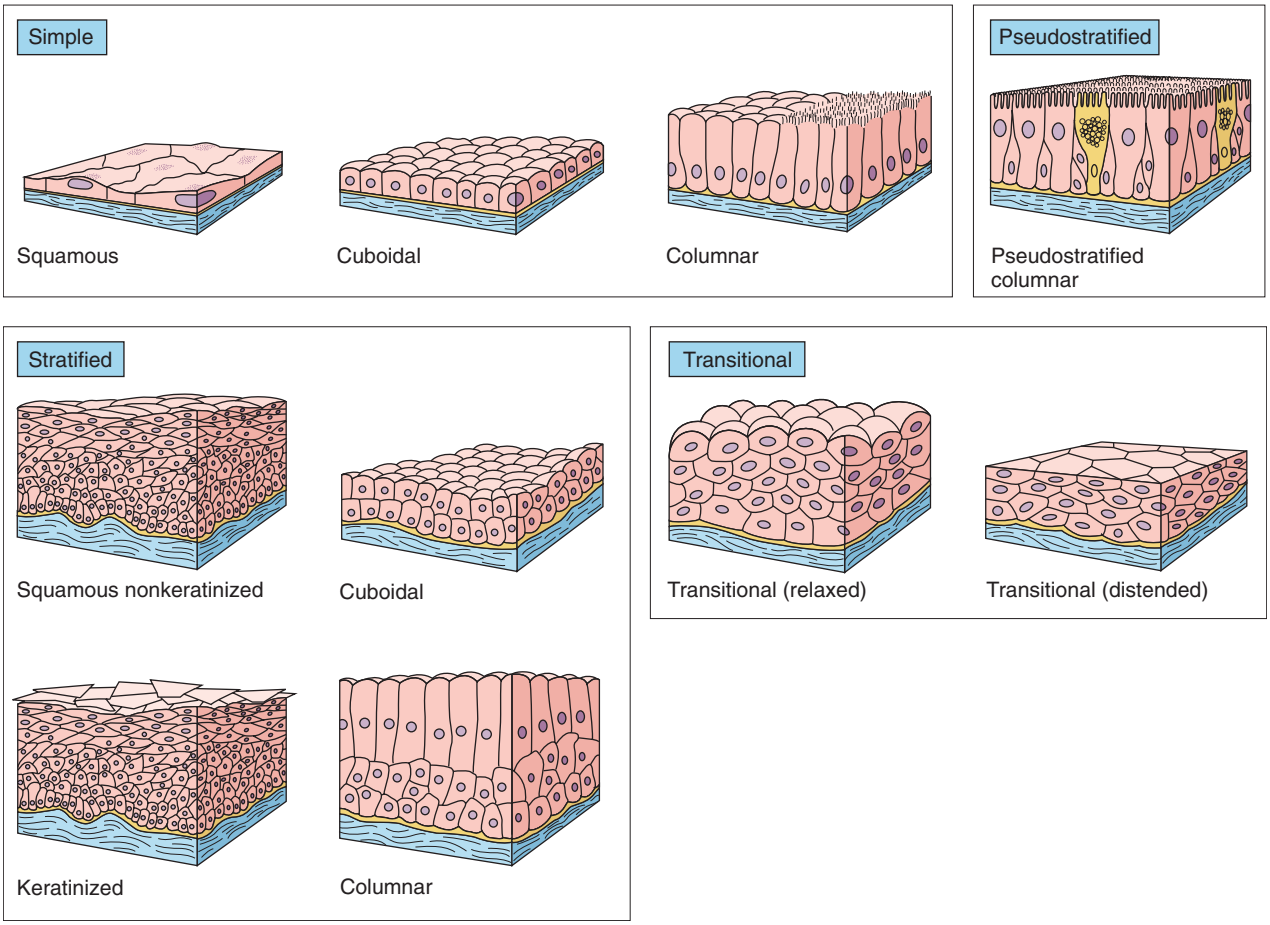


Figure 5–1 Types of epithelia.

only the deepest layer is in contact with the basal lamina (Fig. 5–3A). The most basal (deepest) cells of this epithelium are cuboidal in shape, those located in the middle of the epithelium are polymorphous, and the cells composing the free surface of the epithelium are flattened (squamous)—hence the name *stratified squamous*. Because the surface cells are nucleated, this epithelium is called *nonkeratinized*. It is usually wet and is found lining the mouth, oral pharynx, esophagus, true vocal folds, and vagina.

Stratified Squamous (Keratinized) Epithelium

Stratified squamous (keratinized) epithelium is distinct in that the layers of cells composing the free surface are dead, are non-nucleated, and are filled with keratin.

Stratified squamous (keratinized) epithelium is similar to stratified squamous (nonkeratinized) epithelium, except that the superficial layers of the epithelium are composed of dead cells whose nuclei and cytoplasm have been replaced with keratin (see Fig. 5–3B). This epithelium constitutes the epidermis of skin, a tough layer that resists friction and is impermeable to water.

It should be noted that there is another category of stratified squamous epithelium, namely ***stratified squamous parakeratinized epithelium***. This epithelium is similar to stratified squamous keratinized; however, many of its surface-most cells retain their nuclei, though they are **pyknotic**. Thus, its degree of keratinization of this epithelium is more than that of nonkeratinized and less than that of keratinized stratified squamous epithelium. The epithelium of both the hard palate and gingiva are stratified squamous parakeratinized.

Stratified Cuboidal Epithelium

Stratified cuboidal epithelium, which contains only two layers of cuboidal cells, lines the ducts of sweat glands (see Fig. 5–3C).

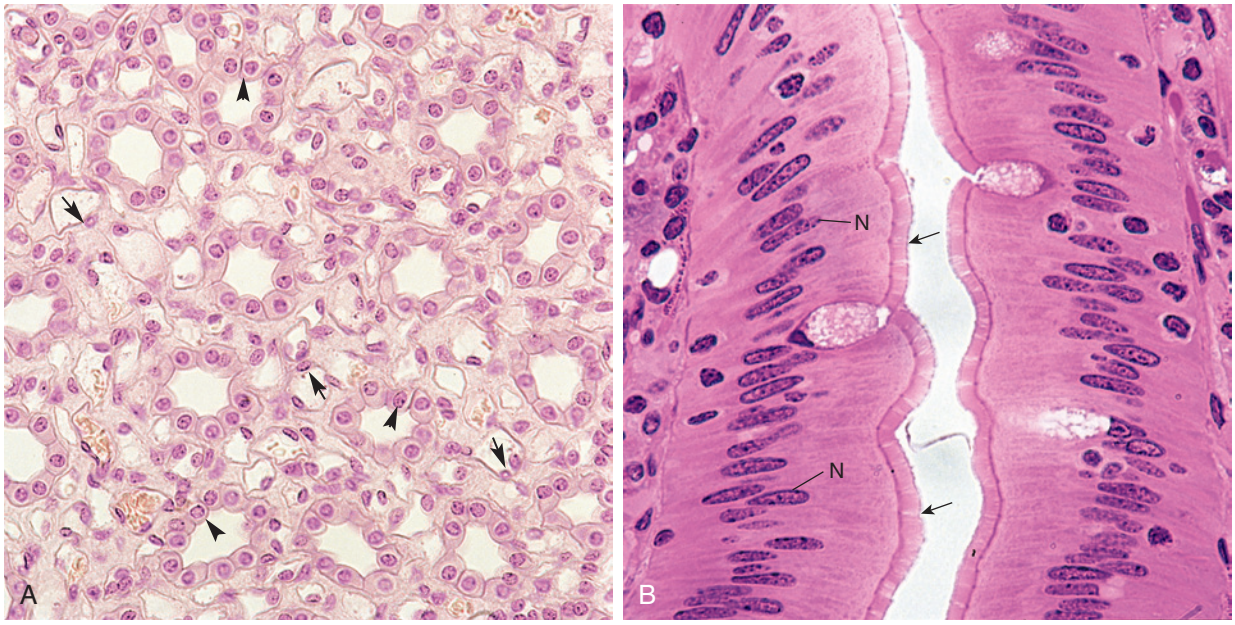


Figure 5–2 Light micrographs of simple epithelia. **A,** Simple squamous epithelium *(arrows)*. Note the morphology of the cells and their nuclei. Simple cuboidal epithelium *(arrowheads)*. Note the round, centrally placed nuclei (×270). **B,** Simple columnar epithelium. Observe the oblong nuclei (N) and the striated border *(arrows)*(×540).

Stratified Columnar Epithelium

Stratified columnar epithelium consists of more than one layer of cells; the superficial layer is columnar in shape.

Stratified columnar epithelium is composed of a low polyhedral to cuboidal deeper layer in contact with the basal lamina and a superficial layer of columnar cells. This epithelium is found only in a few places in the body, namely, the conjunctiva of the eye, some large excretory ducts, and regions of the male urethra.

Transitional Epithelium

Transitional epithelium consists of several layers of cells with the surface layer being large and dome shaped.

Transitional epithelium received its name because it was erroneously believed to be in transition between stratified columnar and stratified squamous epithelia. This epithelium is now known to be a distinct type located exclusively in the urinary system, where it lines the urinary tract from the renal calyces to the urethra.

Transitional epithelium is composed of many layers of cells; those located basally are either low columnar or cuboidal cells. Polyhedral cells compose several layers above the basal cells. The most superficial cells of the empty bladder are large, are occasionally binucleated, and exhibit rounded dome tops that bulge into the lumen (see Fig. 5–3D). These dome-shaped cells become flattened, and the epithelium becomes thinner when the bladder is distended with urine.

Pseudostratified Columnar Epithelium

Pseudostratified columnar epithelium only appears to be stratified; all cells of this epithelium are in contact with the basal lamina.

As the name implies, **pseudostratified columnar epithelium** appears to be stratified, but it is actually composed of a single layer of cells. All of the cells in pseudostratified columnar epithelium are in contact with the basal lamina, but only some cells reach the surface of the epithelium (Fig. 5–4). Cells not extending to the surface usually have a broad base and become narrow at their apical end. Taller cells reach the surface and possess a narrow base in contact with the basal lamina and a broadened apical surface. Because the cells of this epithelium are of different heights, their nuclei are located at different levels, giving the impression of a stratified epithelium, even though it is composed of a single layer of cells. Pseudostratified columnar epithelium is found in the male urethra, epididymis, and larger excretory ducts of glands.

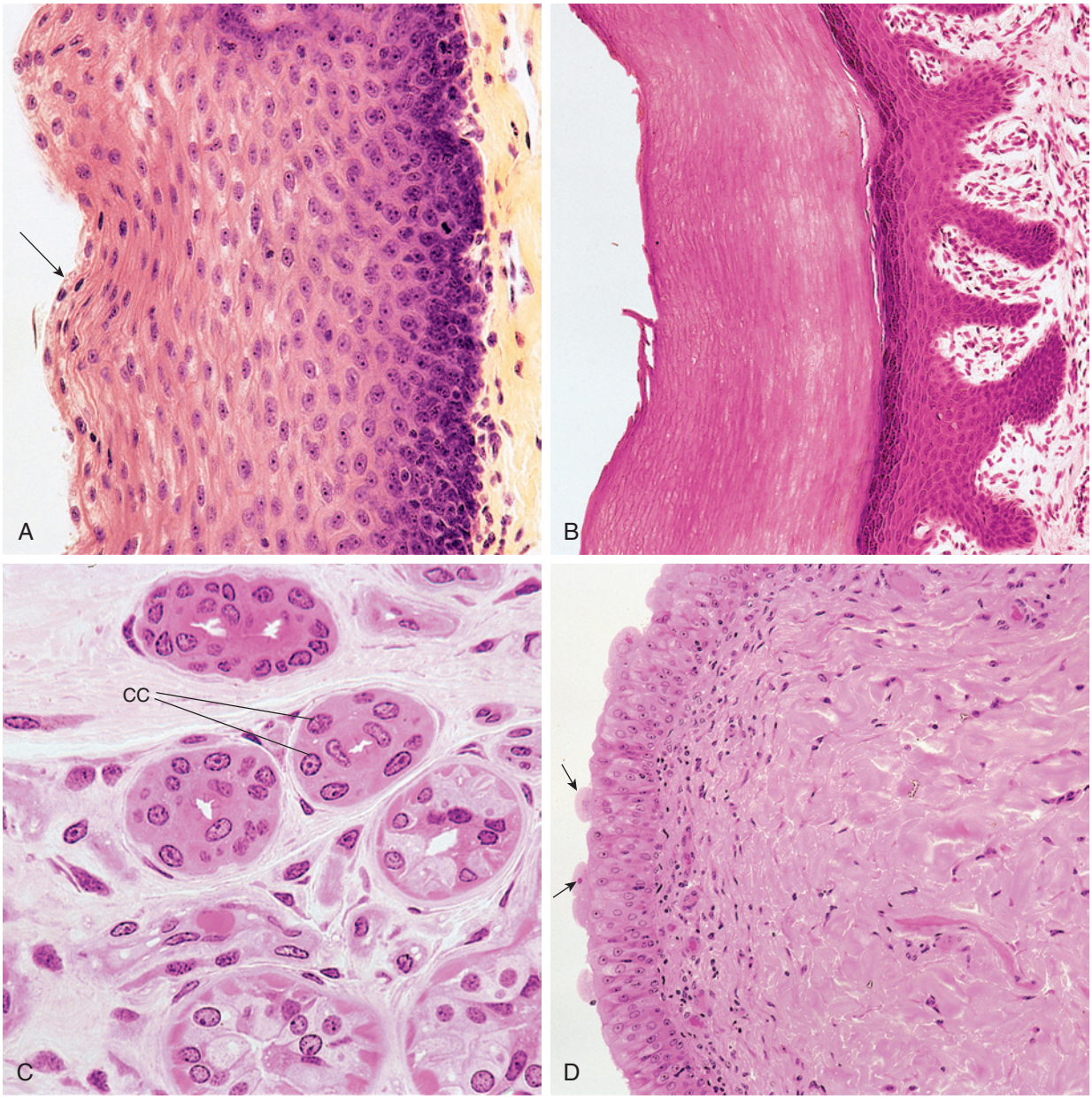


Figure 5–3 Light micrographs of stratified epithelia. **A,** Stratified squamous nonkeratinized epithelium. Observe the many layers of cells and flattened (squamous), nucleated cells in the top layer *(arrow)* (×509). **B,** Stratified squamous keratinized epithelium (×125). **C,** Stratified cuboidal epithelium of the duct of a sweat gland (CC) (×509). **D,** Transitional epithelium. Observe that the surface cells facing the lumen of the bladder are dome-shaped *(arrows)*, which characterize transitional epithelium (×125).

The most widespread type of pseudostratified columnar epithelium is **ciliated**, having cilia on the apical surface of the cells that reach the epithelial surface. Pseudostratified ciliated columnar epithelium is found lining most of the trachea and primary bronchi, the auditory tube, part of the tympanic cavity, the nasal cavity, and the lacrimal sac.

Polarity and Cell-Surface Specializations

Epithelial cell polarity and cell-surface specializations are related to cellular morphology and function.

Most epithelial cells have distinct morphological, biochemical, and functional domains and thus commonly display a polarity that may be related to one or all of

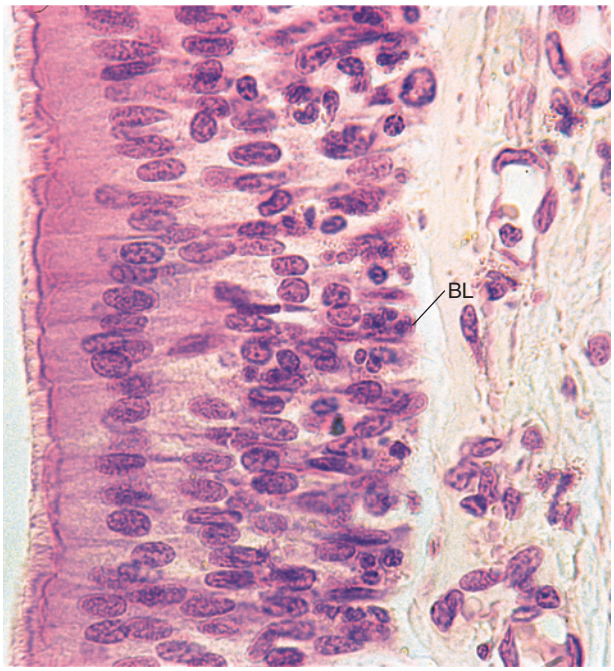


Figure 5–4 Light micrograph of pseudostratified epithelia. This type of epithelium appears to be stratified; however, all of the epithelial cells in this figure stand on the basal lamina (BL) (×540).

these differences. Such polarized cells, for instance, possess an **apical domain** that faces a lumen and a **basolateral domain** whose basal component is in contact with the basal lamina. Because these regions are distinct functionally, each may have surface modifications and specializations related to that function. For example, the apical surfaces of many epithelial cells possess microvilli or cilia, whereas their basolateral regions may exhibit many types of junctional specializations and intercellular interdigitations. The apical and basolateral domains are separated from each other by tight junctions that encircle the apical aspect of the cell.

Apical Domain

The apical domain represents the free surface of the epithelial cells.

The **apical domain**, the region of the epithelial cell facing the lumen, is rich in ion channels, carrier proteins, ATPase (adenosine triphosphatase, transmembrane ATPase), glycoproteins, and hydrolytic enzymes as well as **aquaporins**, channel-forming proteins that function in regulation of water balance. It also is the site where regulated secretory products are delivered for release. Several surface modifications are necessary for the apical domain of an epithelium to carry out its many functions. These include microvilli with associated glycocalyx and, in some cases, stereocilia, cilia, and flagella.

Microvilli

Microvilli are small finger-like cytoplasmic projections emanating from the free surface of the cell into the lumen.

When observed by electron microscopy, absorptive columnar (and cuboidal) epithelial cells exhibit closely packed **microvilli**, which are cylindrical, membrane-bound projections of the cytoplasm emanating from the apical (luminal) surface of these cells (Fig. 5–5). Microvilli represent the **striated border** of the intestinal absorptive cells and the **brush border** of the kidney proximal tubule cells observed by light microscopy.

In less active cells, microvilli may be sparse and short; in intestinal epithelia, where the major function is transport and absorption, they are crowded and 1 to 2 μm in length, thus greatly increasing the surface area of the cells. Each microvillus contains a core of 25 to 30 **actin filament** bundle, whose members are cross-linked to each other by a number of actin-binding proteins, such as **espin**, **fascin**, **villin**, and **fimbrin**. The plus ends of the actin filaments are attached to an amorphous region, composed mostly of villin, at the tip of the microvillus, whereas the minus ends of the actin filaments are embedded in and attached to the terminal web. The **terminal web** is a complex of **actin** and **spectrin** molecules as well as **intermediate filaments** located at the cortex of the epithelial cells (Figs. 5–6, 5–7, and 5–8). At regular intervals, **myosin-I** and **calmodulin** connect the peripheral members of the actin filaments to the plasma membrane of the microvillus, giving it support. Additionally, **tropomyosin** and **myosin II** are located in the terminal web, and, as these two proteins act on the actin filaments and the apical aspect of the cell contracts, the microvilli spread apart, providing more space for molecular transport at the cell apex. Epithelia not functioning in absorption or transport may exhibit microvilli without cores of actin filaments.

Light microscopy of epithelia stained for carbohydrates reveals the glycocalyx, evident in electron micrographs as an amorphous, fuzzy coating over the luminal surface of the microvilli. The **glycocalyx** represents carbohydrate residues attached to the transmembrane proteins of the plasmalemma. These glycoproteins function in protection and cell recognition (see Chapter 2).

Stereocilia (not to be confused with cilia) are long microvilli found only in the epididymis and on the sensory hair cells of the cochlea (inner ear). It is believed that these nonmotile structures are unusually rigid because of their core of actin filaments. These actin filaments are held together by **fimbrin**. **Ezrin** and **villin-2** attach the peripheral-most members of the actin

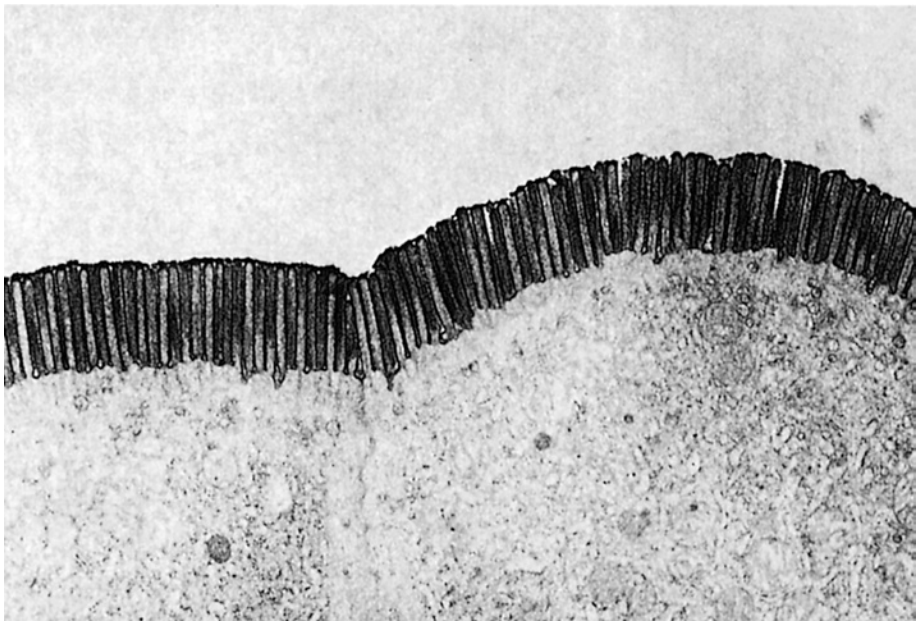


Figure 5–5 Electron micrograph of microvilli of epithelial cells from the small intestine (×2800). (From Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

filament bundle to the cell membrane of the stereocilia, and there is no villin at the tip of the stereocilia where the plus ends of the actin filaments terminate. The minus ends of the actin filaments terminate in the terminal web. In the epididymis, they probably function in increasing the surface area; in the hair cells of the ear, they function in signal generation.

Cilia

Cilia are of two types: long, motile, hair-like structures emanating from the apical cell surface (whose core is composed of a complex arrangement of microtubules known as the axoneme*) and primary cilia that are similar but are nonmotile.*

Motile Cilia. **Motile cilia** (called *cilia* in this textbook) are hair-like projections (diameter, 0.2 µm; length, 7 to 10 µm) that emanate from the surface of certain epithelial cells. In the ciliated epithelia of the respiratory system (e.g., trachea and bronchi) and in the oviduct, there may be hundreds of cilia in orderly arrays on the luminal surface of each cell.

Cilia are specialized to function in propelling mucus and other substances over the surface of the epithelium via rapid rhythmic oscillations. Cilia of the respiratory tree, for example, move mucus and debris toward the oropharynx, where it may be swallowed or expectorated. Cilia of the oviduct move the fertilized ovum toward the uterus.

Electron microscopy reveals that cilia possess a specific internal structure that is consistently conserved throughout the plant and animal kingdoms (Figs. 5–9 and 5–10). The core of the cilium contains a complex of uniformly arranged microtubules called the ***axoneme***. The axoneme is composed of a constant number of longitudinal microtubules arranged in a consistent 9 + 2 organization (Fig. 5–10B). Two centrally placed microtubules **(singlets)** are evenly surrounded by nine **doublets** of microtubules. The two microtubules located in the center of the core are separated from each other, each displaying a circular profile in cross-section, composed of 13 protofilaments. Each of the nine doublets is composed of two subunits. In cross-section, **subunit A** is a microtubule composed of 13 protofilaments, exhibiting a circular profile. **Subunit B** possesses 10 protofilaments, exhibits an incomplete circular profile in cross-section, and shares three protofilaments of subunit A.

Several elastic protein complexes are associated with the axoneme. **Radial spokes** project from subunit A of each doublet inward toward the **central sheath** surrounding the two singlets. Neighboring doublets are connected by **nexin**, another elastic protein, extending from subunit A of one doublet to subunit B of the adjacent doublet (see Fig. 5–9).

The microtubule-associated protein **dynein** has ATPase activity and is known as ***ciliary dynein arm***. Two of these dynein arms, an inner and an outer, radiate from subunit A of one doublet toward subunit B of the neighboring doublet. These dynein arms are arranged at 24-nm intervals along the length of subunit A, so that subunit A resembles a millipede and its numerous

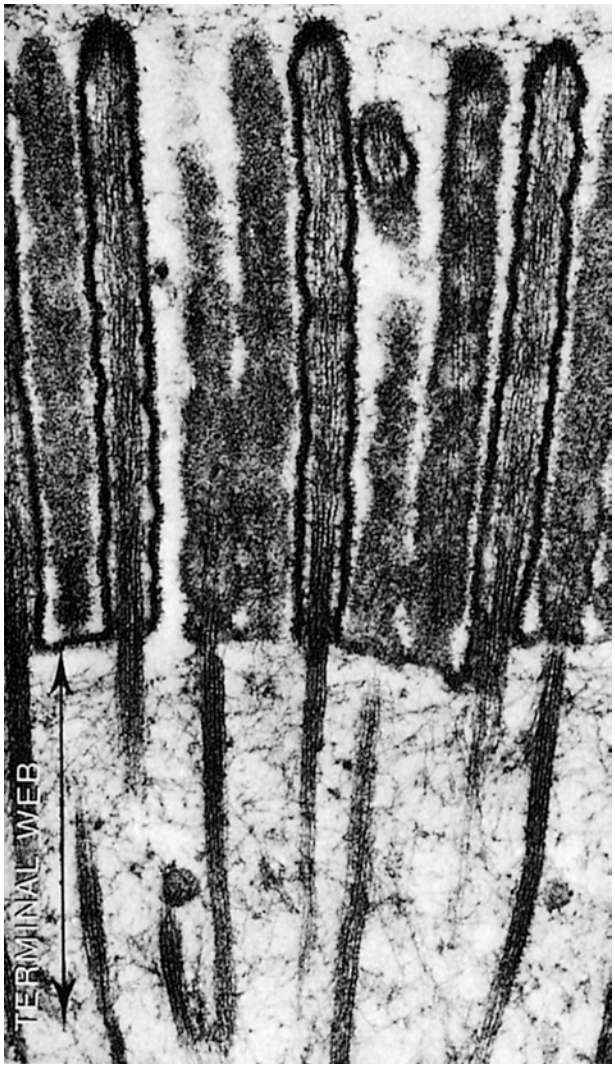


Figure 5–6 High-magnification electron micrograph of microvilli (×60800). (From Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

bilaterally symmetrical legs. Dynein ATPase, by hydrolyzing ATP, provides the energy for the ciliary bending. Movement of the cilia is initiated by the dynein arms transiently attaching to specific sites on the protofilaments of the adjacent doublets, sliding them toward the tip of the cilium. However, nexin, an elastic protein extending between adjacent doublets, restrains this action to some degree, thus translating the sliding movement into a bending motion. As the cilium bends, an energy-requiring process, the elastic protein complex is stretched. When the dynein arms release their hold on the B subunit, the elastic protein complex returns to its original length, snapping the cilium back to its straight position (which does not require energy), effecting movement of material at the tip of the cilium. An additional protein, **tektin**, which resembles a rod and is approximately 2 to 3 nm in diameter, assembles head to tail to form a "rod" along the entire doublet microtubule. This **tektin rod** is located at the junction of microtubules A and B, close to the outer dynein arm, and acts as a support for the doublet and prevents the cilium from bending too far.

CLINICAL CORRELATIONS

Kartagener syndrome results from hereditary defects in the ciliary dynein that would normally provide the energy for ciliary bending. Thus, ciliated cells without functional dynein are prohibited from functioning. Persons having this syndrome are susceptible to lung infections because their ciliated respiratory cells fail to clear the tact of debris and bacteria. Additionally, males with this syndrome are sterile because their sperm are immotile.

The 9 + 2 microtubule arrangement within the axoneme continues throughout most of the length of the cilium except at its base, where it is attached to the basal body (see Fig. 5–9). The morphology of the **basal body** is similar to that of a centriole, in that it is composed of nine **triplets** and no singlets. The basal body and its associated components are responsible for the adherence of the cilium to the cell and also for the ability of the cilia to beat in a uniform fashion and in the identical direction.

Basal bodies develop from **procentriole organizers**. As tubulin dimers are added, the procentriole lengthens to form the nine triplet microtubules characteristic of the basal body, where the third microtubule is added to microtubule B and is known as ***microtubule C*** and is also composed of 10 protofilaments and shares 3 of microtubule B's protofilaments. After formation, the basal body migrates to the apical plasmalemma and gives rise to a cilium. Nine doublet microtubules develop from the nine triplets of the basal body, and a pair of central microtubules, the two **singlets**, forms to give the cilium's axoneme its characteristic 9 + 2 microtubule arrangement. The junction of the axoneme and the basal body is known as the ***transition zone***. Arising from this transition zone and attached to microtubule C is the **alar sheath**, a fibrous-like semipermeable membrane that forms an upside down tent-like cover whose tip encloses microtubule C and whose base attaches to the cell membrane around the region where the cilium emerges from the cell, effectively separating the cytoplasm of the cilium from that of the cell. Two additional structures are associated with the basal body, and these are the basal foot and the striated rootlet. The **basal foot** is responsible for orienting the cilia so that all cilia face and beat in the same direction, whereas the

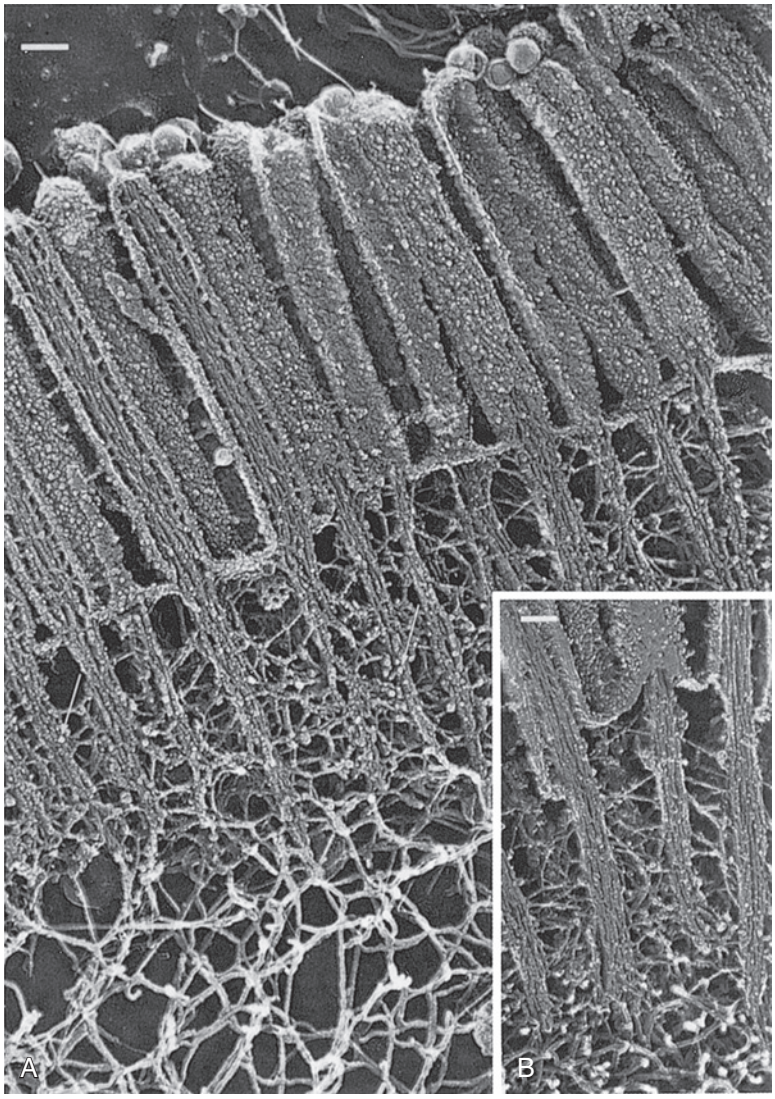


Figure 5–7 Electron micrograph of the terminal web and microvillus. Observe that the actin filaments of the microvilli are attached to the terminal web (**A,** ×83060; **B,** *inset*, ×66,400). (From Hirokana N, Tilney LG, Fujiwara K, Heuser JE. Organization of actin, myosin, and intermediate filaments in the brush border of intestinal epithelial cells. *J Cell Biol*. 1982;94:425-443. Reprinted with the permission of The Rockefeller University Press.)

striated rootlet is believed to anchor the basal body into the apical cytoplasm. Because cilia (both motile and primary as well as flagella) are living, they require the constant transport of various substances in and out of their cytoplasm. Generally, this movement is known as ***axonemal transport***, but, within cilia, it is referred to as ***intraciliary transport*** (***intraflagellar transport*** in flagella); if it occurs from the basal body toward the tip of the cilium, it is known as ***anterograde intraciliary transport;*** in the opposite direction, it is known as ***retrograde intraciliary transport***. The material to be transported, such as tubulin molecules, is referred to as ***cargo,*** and the transport of cargo is performed by carrier proteins known as ***raft proteins***, which are ferried in the anterograde direction by **kinesin-2** and in the retrograde direction by **dynein-2** along the outer aspect (facing the plasmalemma of the cilia) of the axoneme microtubules. The change of direction from anterograde to retrograde intraciliary transport occurs at the distal tip of the cilia and depends on the presence

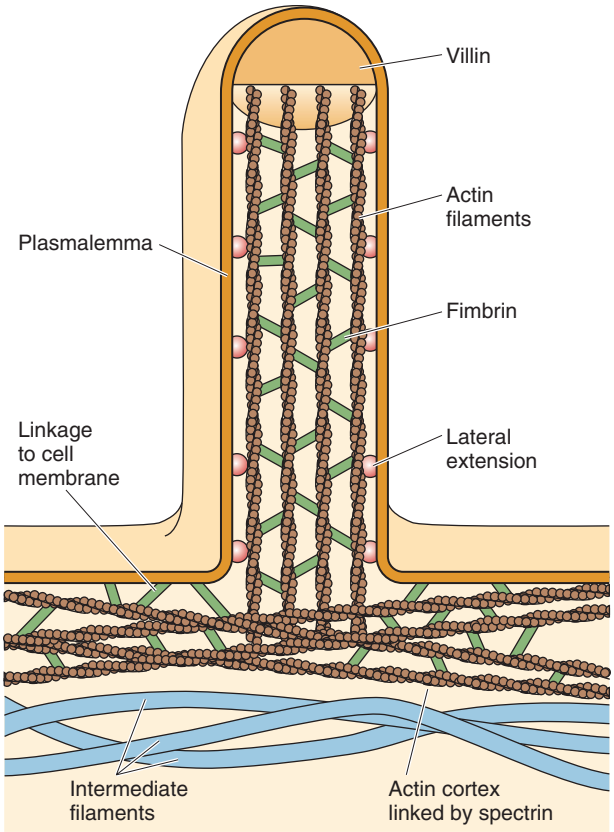


Figure 5–8 Schematic diagram of the structure of a microvillus.

of the enzyme **intestinal cell kinase (ICK)**, which phosphorylates a subunit of kinesin-2, which functions not only in intraciliary transport but also in ciliogenesis, the formation of cilia. In the absence of ICK, intraciliary transport ceases, and ciliogenesis does not occur in the proper fashion.

CLINICAL CORRELATIONS

Individuals with **Majewski polydactyly syndrome**, a recessive autosomal defect, have been shown to have abnormally developed chondrocyte cilia. These individuals display abnormal chondrogenesis as well as abnormal osteogenesis, resulting in shortened limbs, narrowed thoracic cage, fused fingers and toes, genital malformations, cardiovascular problems, and respiratory insufficiency, among numerous other congenital defects. It appears that the chondrocyte cilia of these individuals are foreshortened and display a bulbous expansion, and their **intraciliary retrograde transport** was disrupted.

Primary Cilia. **Primary cilia** are nonmotile and are noted to be present on most mammalian cells that are not participating in the cell cycle, that is, they are in the G_o state. These cells possess a single primary cilium that functions in surveying its immediate environment and in eliciting the cell to respond to changes in that milieu.

Because primary cilia are nonmotile, their morphology differs from those of motile cilia in that axoneme of primary cilia have no central singlets and lack the dynein arms, central sheath, and radial spokes. They do have the nine doublets that resemble those of the motile cilia's axoneme. However, primary cilia display the presence of a protein complex, known as ***BBSome complex* (Bardet-Biedl syndrome protein complex)** at the junction of the basal body with the basal foot. The BBSome complex, in a similar fashion to COP I, COP II, and clathrin, forms a membrane coat that sorts, binds to, and ferries membrane proteins (e.g., somatostatin or other membrane-bound receptors), into the cytoplasm of primary cilia to be inserted into their plasmalemma.

The **basal foot** serves a similar role in primary cilia as it does in motile cilia; it is responsible for insuring that primary cilia of all cells in the region are aligned in the same direction, thus experiencing the same conditions.

CLINICAL CORRELATIONS

A number of genetic disorders that involve primary and motile cilia are known as **ciliopathies**. These involve various mutations that result in disturbances in the intraciliary transport and may be lethal during fetal life or early in postnatal development. Some of these mutations involve the ***MKS1* gene (Meckel syndrome, type 1)** that codes for **MKS1 protein**. This protein, in conjunction with another protein known as **meckelin** are associated with the basal body and are essential for ciliogenesis and its mutant form is responsible for the lethal genetic ciliopathy known as **Meckel syndrome**. The symptoms of this condition include aberrant formation of the central nervous system, defects in hepatogenesis, polydactyly, and the formation of renal cysts that cause an enormous enlargement of the kidneys.

Flagella

The only cells in the human body that possess **flagella** are the spermatozoa. The structure of flagella is discussed in Chapter 21, which covers the male reproductive system.

Basolateral Domain

The basolateral domain includes the basal and lateral aspects of the cell membrane.

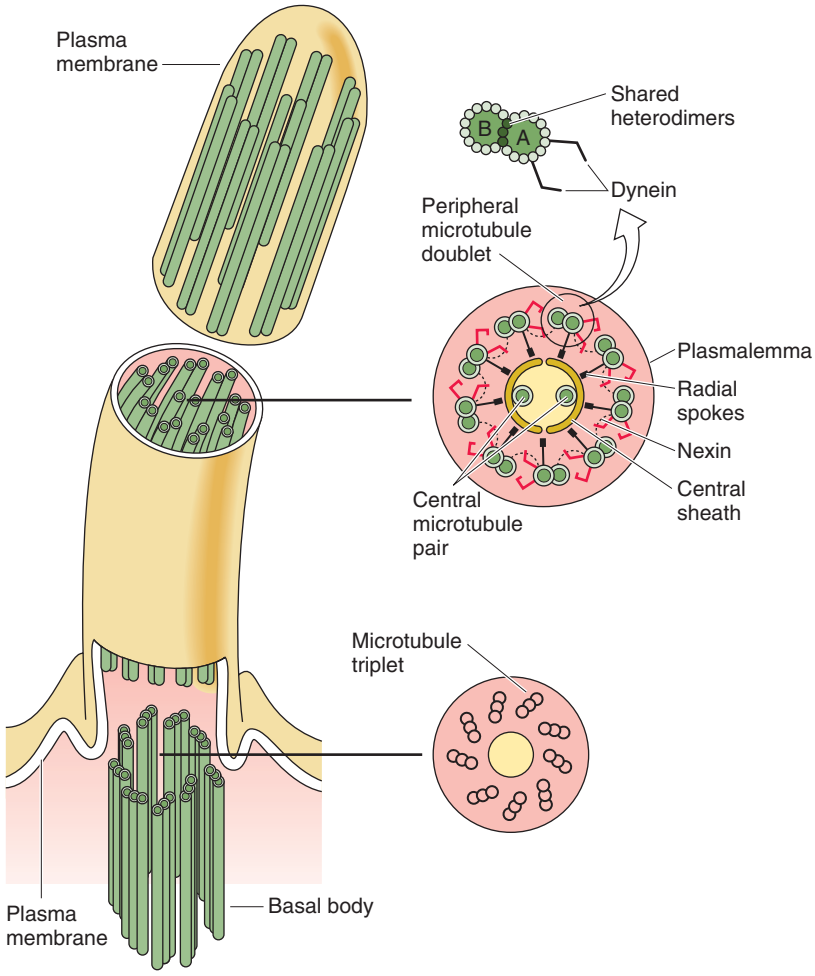


Figure 5–9 Schematic diagram of the microtubular arrangement of the axoneme in the cilium.

The **basolateral domain** may be subdivided into two regions: the lateral plasma membrane and the basal plasma membrane. Each region possesses its own junctional specializations and receptors for hormones and neurotransmitters. In addition, these regions are rich in Na^+-K^+ ATPase and ion channels and are sites for constitutive secretion.

Lateral Membrane Specializations

Lateral membrane specializations reveal the presence of junctional complexes.

Light microscopy reveals zones, called ***terminal bars***, where epithelial cells are in contact and, presumably, attach to each other. Especially notable in the apical region of the simple columnar epithelium lining the gut, terminal bars were once thought to be composed of an amorphous intercellular cement substance. Horizontal sections through the terminal bars showed that they were continuous around the entire circumference of each cell, indicating that these cells were attached to every adjacent cell. Electron microscopy has revealed that terminal bars are in fact composed of intricate **junctional complexes** that possess **cell adhesion molecules** that assist in holding contiguous epithelial cells to each other. Junctional complexes may be classified into three types, schematically depicted in Figure 5–11:

- **Occluding junctions** function in joining cells to form an impermeable barrier, preventing material from taking an intercellular route in passing across the epithelial sheath
- **Anchoring junctions** function in maintaining cell-to-cell or cell-to-basal lamina adherence
- **Communicating junctions** function in permitting movement of ions or signaling molecules between cells, thus coupling adjacent cells both electrically and metabolically

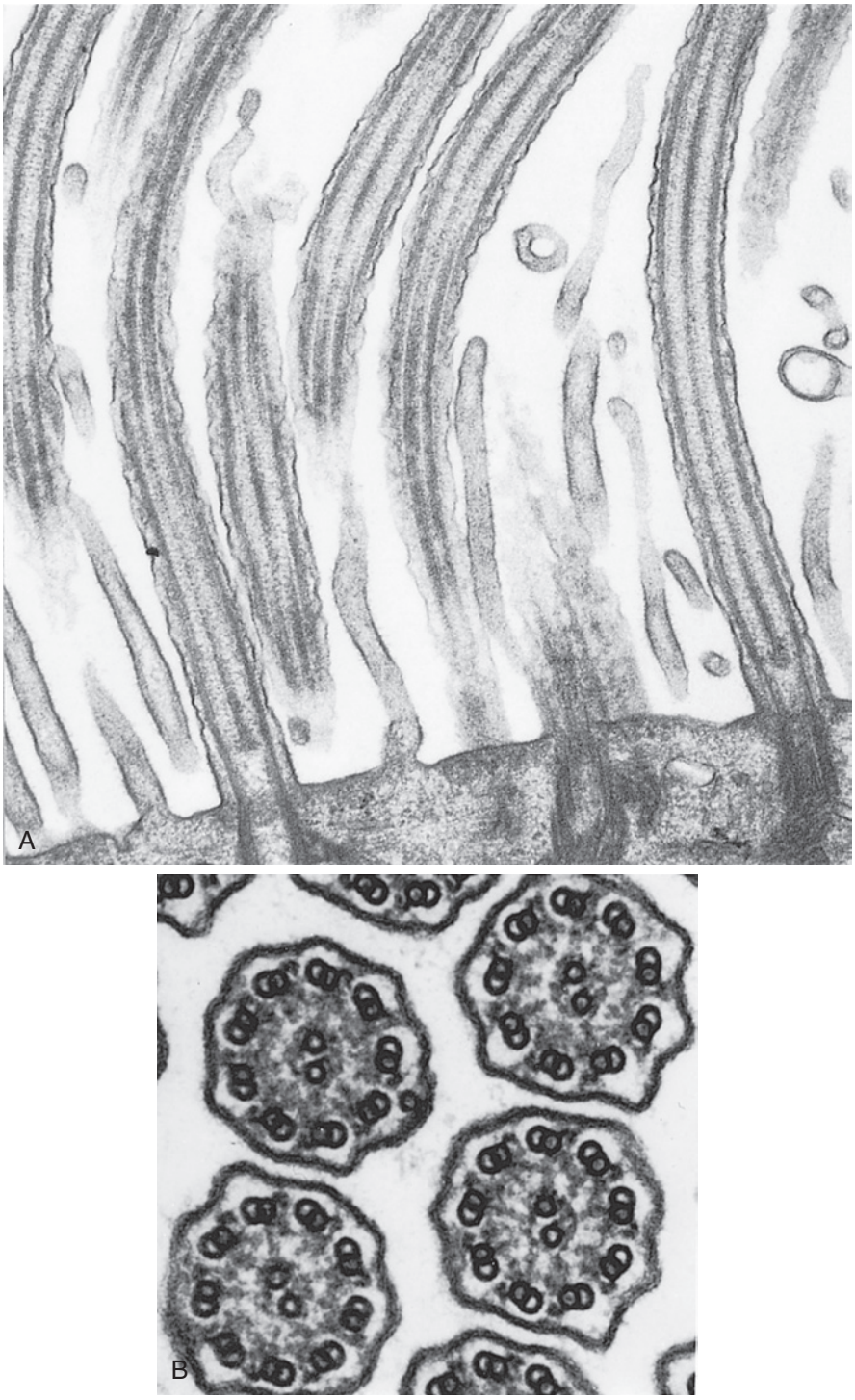
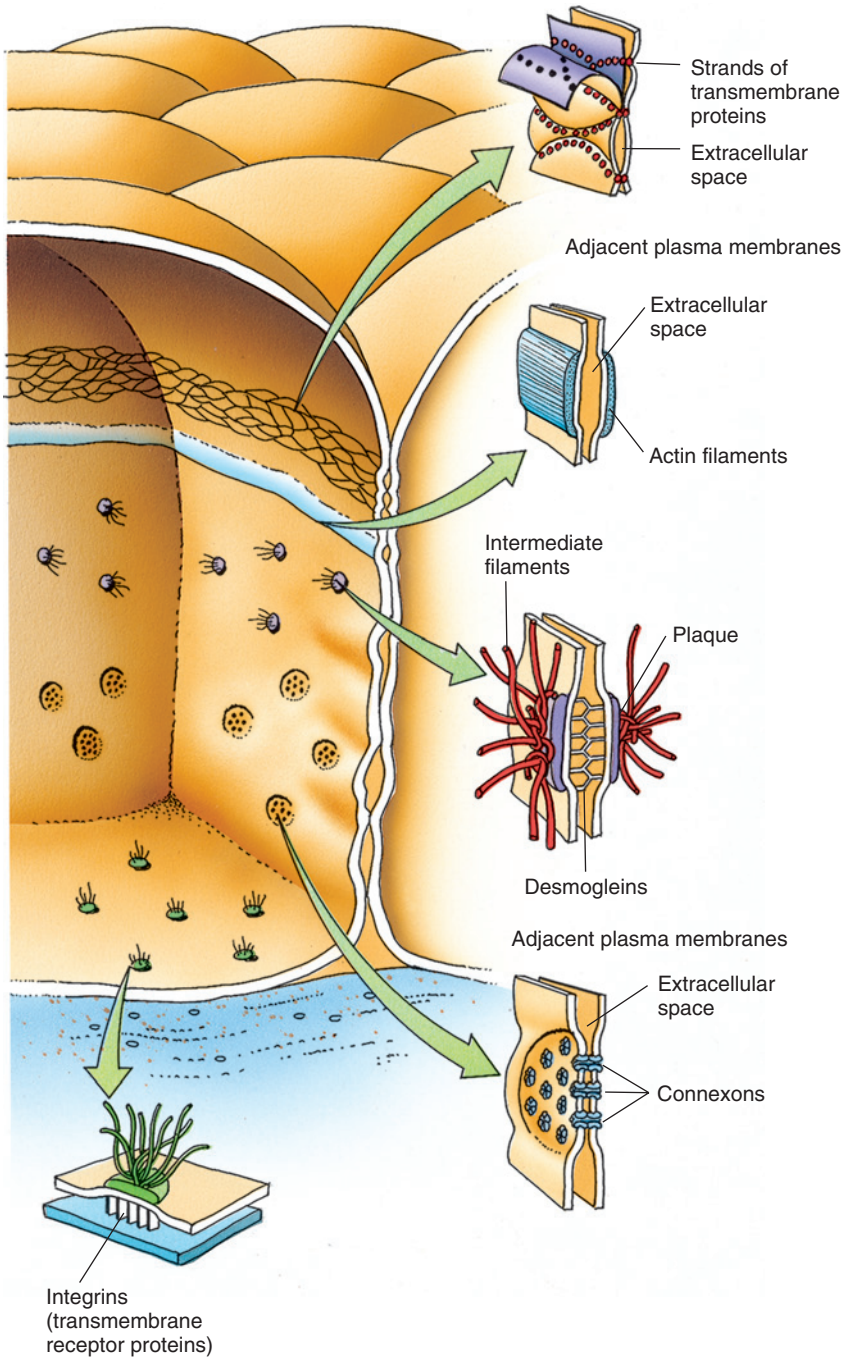


Figure 5–10 Electron micrographs of cilia. **A,** Longitudinal section of cilia (×36000). **B,** Cross-sectional view demonstrating microtubular arrangement in cilia (×88000). (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)



Zonulae occludentes
Extend along entire circumference of the cell. Prevent material from taking paracellular route in passing from the lumen into the connective tissues.

Zonulae adherentes
Basal to zonulae occludentes. E-cadherins bind to each other in the intercellular space and to actin filaments, intracellularly.

Maculae adherentes
E-cadherins are associated with the plaque; intermediate filaments form hairpin loops.

Gap junctions
Communicating junctions for small molecules and ions to pass between cells. Couple adjacent cells metabolically and electrically.

Hemidesmosomes
Attach epithelial cells to underlying basal lamina.

Figure 5–11 Schematic diagram of junctional complexes, gap junctions, and hemidesmosomes.

Zonulae Occludentes

Zonulae occludentes, the only type of occluding junction, prevent movement of membrane proteins and function to prevent intercellular movement of water-soluble molecules.

Also known as *tight junctions*, **zonulae occludentes** (**zonula occludens** is the singular form) are located between adjacent plasma membranes and are the most apically located junction between the cells of the epithelia (see Fig. 5–11). They form a belt-like junction that encircles the entire circumference of the cell.

Fasciae adherentes are analogous junctions, but they do not encircle the cell; instead, they are ribbon-like fusions where overlapping endothelial cells of blood vessels fuse with each other.

In electron micrographs, the adjoining cell membranes approximate each other; their outer leaflets fuse, then diverge, and then fuse again several times within a distance of 0.1 to 0.3 μm (Fig. 5–12). At the fusion sites, the extracellular moieties of the four types of transmembrane proteins (**claudins**, **occludins**, **nectins**, and **junctional adhesive molecules [JAMs]**) of the two membranes bind to each other, thus forming a seal occluding the intercellular space. Freeze-fracture analysis of cell membranes at the zonulae occludentes displays a "quilted" appearance of anastomosing strands, known as ***tight junction strands***, on the P-face and a

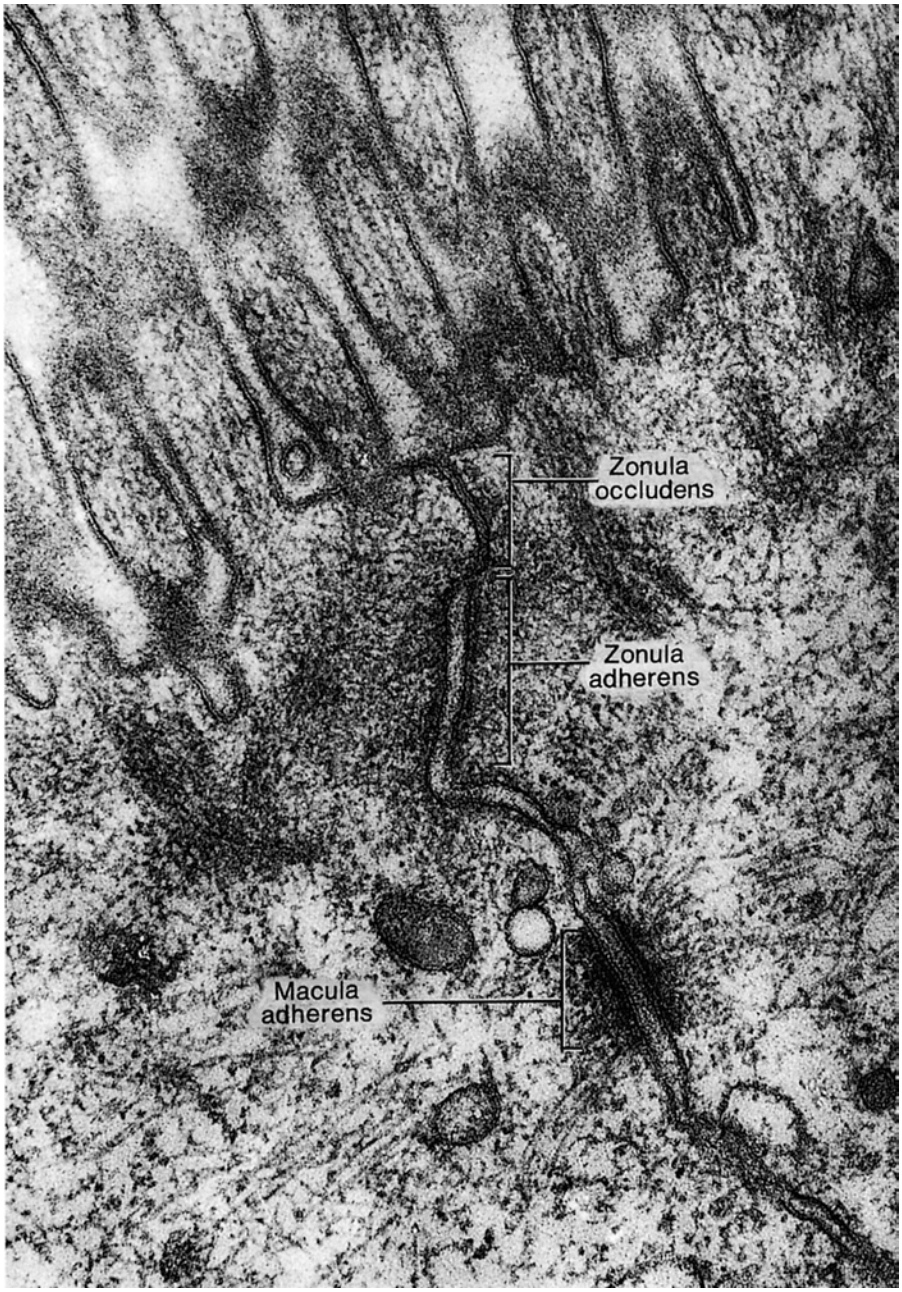


Figure 5–12 Electron micrograph of the junctional complex. (From Fawcett DW. *The Cell*. 2nd ed. Philadelphia: WB Saunders; 1981.)

Figure 5–13 Freeze-fracture replica displaying the tight junction (zonula occludens) in a guinea pig's small intestine. The P-face of the microvillar membrane (M) possesses fewer intramembrane particles than the P-face of the lateral cell membrane (L). *Arrows* point to free terminal ridge–shaped protrusions. A desmosome (D) is shown (×60,000). (From Trier JS, Allan CH, Marcial MA, Madara JL. Structural features of the apical and tubulovesicular membranes of rodent small intestinal tuft cells. *Anat Rec*. 1987;219:69-77. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

corresponding network of **grooves** on the E-face (Fig. 5–13). These tight junction strands are the extracellular components of the transmembrane proteins of the two adjacent membranes that contact each other to obliterate the extracellular space between the two cells.

Although all four transmembrane proteins participate in the formation of the tight junction, it appears that **claudins** have a more active role than the other three because these are the proteins that are probably responsible for the obliteration of the intercellular space by forming the greatest portion of the tight junction strands described earlier. The roles of nectins, occludins, and JAMs are as yet not known. Because claudins are calcium independent, they do not form strong cell adhesions. As a result, their contact must be reinforced by **cadherins** as well as by the cytoplasmic zonula occludens proteins, such as **ZO-1**, **ZO-2**, **ZO-3**, and **afadin**. These four proteins bind to the cytoplasmic moieties of the transmembrane proteins participating in the formation of the zonula occludens and bind to the actin filaments of the cell's cytoskeleton, thus providing stability to the tight junction.

Tight junctions function in two ways; they (1) prevent the movement of membrane proteins from the apical domain to the basolateral domain (and vice versa) and (2) fuse plasma membranes of adjacent cells to each other to prohibit water-soluble molecules from passing between cells. Depending on the numbers and patterns of these *sealing strands* in the zonula, some tight junctions are said to be "tight," whereas others are "leaky." These terms reflect the efficiency of the epithelial cells in maintaining the integrity of the epithelial barrier between two adjacent body compartments.

Zonulae Adherentes

Zonulae adherentes, one of the four types of anchoring junctions, are belt-like junctions that assist adjoining cells to adhere to one another.

Zonulae adherentes (singular: **zonula adherens**), one of the four types of **anchoring junctions** of epithelial cells, are part of the junctional complex and located just basal to the zonulae occludentes, and, as their name implies, also encircle the cell (see Fig. 5–11). The intercellular space of 15 to 20 nm between the outer leaflets of the two adjacent cell membranes is occupied by the extracellular moieties of **E-cadherins** (see Fig. 5–12). These Ca^{2+}-dependent integral proteins of the cell membrane are also known as **transmembrane linker proteins**. Their intracytoplasmic aspect binds to a specialized region of the cell web, specifically bundles of actin filaments that run parallel to and along the cytoplasmic aspect of the cell membrane. The actin filaments are attached to each other by **α-actinin**. They are also attached to the intracytoplasmic moieties of the E-cadherins by the anchor proteins **vinculin** and **catenins** (see Chapter 2). If calcium ions are present, the extracellular region of the cadherins of one cell forms bonds with those of the adjoining cell participating in the formation of the zonula adherens. Then this junction not only joins the cell membranes to each other but also links the

cytoskeleton of the two cells via the transmembrane linker proteins.

Fascia adherens is similar to zonula adherens but does not go around the entire circumference of the cell. Instead of being belt-like, it is ribbon-like. Cardiac muscle cells, for example, are attached to each other at their longitudinal terminals via the fascia adherens.

Desmosomes (Maculae Adherentes)

Desmosomes, one of the four types anchoring junctions, are weld-like junctions along the lateral cell membranes that help resist shearing forces.

Desmosomes are the last of the three components of the junctional complex. These spot-weld–like junctions also appear to be randomly distributed along the lateral cell membranes of simple epithelia and throughout the cell membranes of stratified squamous epithelia, especially in the epidermis.

Two disk-shaped **attachment plaques**, the **inner** and **outer dense plaques** (combined dimensions of ~400 × 250 × 10 nm) are located opposite each other on the cytoplasmic aspects of the plasma membranes of each of the adjacent epithelial cells (Fig. 5–14; see Fig. 5–11). The extracellular space between the two cells is approximately 30 nm in width and is occupied by the extracellular regions of **desmocollins** and **desmogleins**, the **cadherins** (**transmembrane proteins** whose name comes from *calcium-dependent adhesion*) that project from both cell membranes into this space. In the presence of calcium ions, the extracellular portions of these cadherins contact each other and form bonds that attach these adjacent cells to one another. This extracellular contact is evident with electron microscopy as an electron-dense line and is known as the ***extracellular core***.

The **outer dense plaque** is closely adhering to the cytoplasmic aspect of the plasmalemma. This plaque is composed of the glycoproteins **plakoglobins** and **plakophilins** held together by proteins known as ***desmoplakins***. The intracellular components of the desmocollins and desmogleins contact and are stabilized by the plakoglobins and plakophilins. Additionally, the desmoplakins contact the **keratin intermediate filaments** located somewhat deeper in the cytoplasm, and this region of contact is visible with the electron microscope and forms the **inner dense plaque**. This inner plaque stabilizes the outer plaque, which, in turn, stabilizes the extracellular core. It is in this fashion that the desmosome is able to maintain the adherence of the two cells to each other, as if they were spot welded to each other; it should be appreciated that the formation of a desmosome requires the interaction of *two* cells.

Gap Junctions

Gap junctions, also called nexus, communicating junctions, *or* gap junction channel, *are regions of intercellular communication.*

CLINICAL CORRELATIONS

1. Some people produce autoantibodies against desmosomal proteins, especially those in the skin, resulting in a skin disease called ***pemphigus vulgaris***. Binding of the autoantibodies to desmosomal proteins disrupts cell adhesion, leading to widespread blistering and consequent loss of extracellular fluids; if untreated, this condition leads to death. Treatment with systemic steroids and immunosuppressive agents usually controls the condition.
2. **Naxos syndrome**, a genetic anomaly prevalent in the Greek islands in the vicinity of Naxos as well as in regions of the Middle East, is due to malformed **plakoglobins** and **desmoplakins**, and results in the keratoderma of the palms of the hands and soles of the feet, in wooly hair, and in a cardiomyopathy involving right ventricular arrhythmia. A similar condition that affects the left ventricles and prevalent in parts of the Indian subcontinent and Ecuador is known as **Carvajal syndrome**. Both conditions have a high degree of mortality, but patients' lives may be prolonged by the administration of antiarrhythmic medications and, at the end stages of the disease, a heart transplant.

Gap junctions are widespread in epithelial tissues throughout the body as well as in cardiac muscle cells, smooth muscle cells, and neurons but not skeletal muscle cells. They differ from the occluding and anchoring junctions, in that they mediate intercellular communication by permitting the passage of various small molecules between adjacent cells. The intercellular cleft at the gap junction is narrow and constant at about 2 to 4 nm.

Gap junctions are built by six closely packed transmembrane channel-forming proteins (**connexins**) that assemble to form channel-structures called ***connexons*** (**hemichannels**), aqueous pores through the plasma membrane that juts out about 1.5 nm into the intercellular space (see Fig. 5–11). Two connexons, one in each adjacent cell, precisely aligned with each other to be able to fuse with one another, form a **gap junction**. Presently, it is believed that there may be more than 20 different connexins that can assemble into many different arrays of connexons that may be related to their specific function; therefore, there are **homotypic** gap junctions, where both connexons of the gap junction are identical to each other or **heteromeric**, where the two connexons of the gap junction are not identical to each other. Each gap junction may be formed by clusters of a few to many thousands of connexons, known as ***gap junction plaques***. When a connexon of one plasma

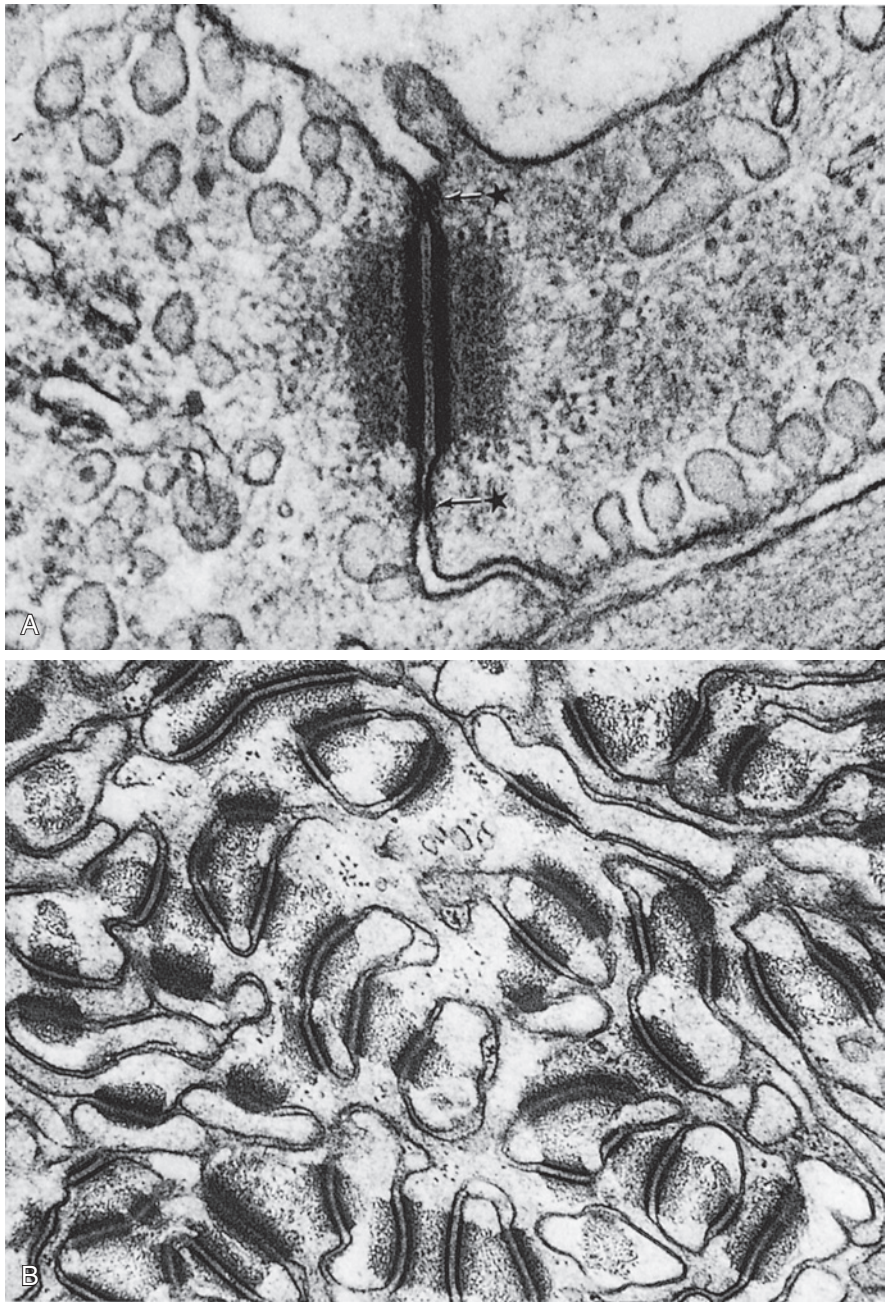


Figure 5–14 Electron micrograph of a desmosome. Observe the dense accumulation of intracellular intermediate filaments inserting into the plaque of each cell. (From Fawcett DW. *The Cell*. 2nd ed. Philadelphia: WB Saunders; 1981.)

membrane is in register with its counterpart of the adjacent plasma membrane, the two connexons fuse, forming a functional intercellular hydrophilic communication channel (Fig. 5–15). With a diameter of 1.5 to 2.0 nm, the hydrophilic channel permits the passage of ions, amino acids, vitamins, small second messenger molecules (e.g., cyclic adenosine monophosphate), certain hormones, and molecules smaller than 490 Da in size. At the same time, the channel created by the fused connexons thwarts the escape of the transiting material from the channel and thus entering the space between the cells.

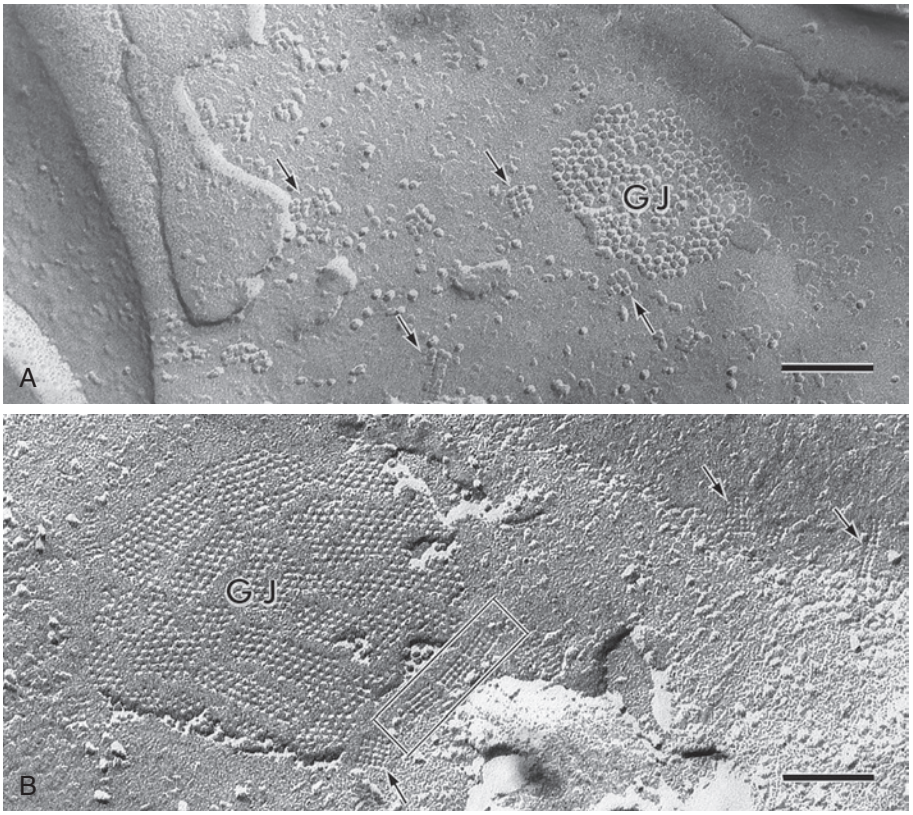


Figure 5–15 Electron micrographs of freeze-fracture replica showing the intramembrane particles of the astrocyte. **A,** Protoplasmic fracture face. Orthogonal arrays of particles (OAP) *(arrows)* are observed near the gap junction (GJ). Note the differences between OAP and GJ particles in shape (*square* and *circle*), size (30 nm^2 and 45 nm^2 in average), and arrangement (*orthogonal* and *hexagonal*). **B,** Ectoplasmic fracture face. Corresponding pits of OAP are oriented into columns *(arrows)* near the GJ pits. Three OAP show gathering (*squared*). Scale bar = 0.1 µm. (From Yakushigawa H, Tokunaga Y, Inanobe A, et al. A novel junction-like membrane complex in the optic nerve astrocyte of the Japanese macaque with a possible relation to a potassium channel. *Anat Rec*. 1998;250:465-474. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Gap junctions are regulated and may be opened or closed rapidly. Although the opening and closing mechanism is not fully understood, it has been shown experimentally that a decrease in cytosolic pH or an increase in cytosolic Ca^{2+} concentrations closes gap junctions. Conversely, high pH or low Ca^{2+} concentration opens the channels. In addition, gap junctions exhibit different properties with diverse channel permeabilities in different cells.

Gap junctions exhibit many diverse functions within the body, including cellular sharing of molecules for coordinating physiological continuity within a particular tissue. For example, when glucose is needed in the bloodstream, the nervous system stimulates liver cells (hepatocytes) to initiate glycogen breakdown. Because not all hepatocytes are individually stimulated, the signal is dispersed to other hepatocytes via gap junctions, thus coupling the hepatocytes. Gap junctions also function in electrical coupling of cells (i.e., in heart muscle and in smooth muscle cells of the gut during peristalsis), thus coordinating the activities of these cells. Also, gap junctions are important during embryogenesis in coupling the cells of the developing embryo electrically and in distributing informational molecules throughout the migrating cell masses, thus keeping them coordinated in the proper development pathway. Mutations in the genes coding for connexins may cause an X-linked form of Charcot-Marie-Tooth syndrome.

Basal Surface Specializations

Basal surface specializations include the basal lamina, plasma membrane infoldings, and hemidesmosomes.

Three important features mark the basal surface of epithelia: the basal lamina, plasma membrane infoldings,

CLINICAL CORRELATIONS

1. Mutations in connexin genes have been linked to a genetically based **nonsyndromic deafness** and to **erythrokeratodermia variabilis**, a skin disorder. In addition, dysfunctional migration of neural crest cells during development have been linked to mutations in the connexin genes, resulting in defects in the formation of the pulmonary vessels of the heart.
2. A number of **cardiac arrhythmias** have been attributed to gap junction anomalies. In certain diseased ventricular cardiac myocardial cells, the *number* of **gap junction plaques** and the *cellular locations* of these plaques were different from those of healthy ventricular myocardial cells. Specifically, the gap junction plaques instead of being in the ends of the cells were located in the lateral plasma membranes.

and hemidesmosomes, which anchor the basal plasma membrane to the basal lamina. The basal lamina is an extracellular supporting structure secreted by an epithelium and is located at the boundary between the epithelium and the underlying connective tissue. The structure and appearance of the basal lamina are discussed in Chapter 4.

Plasma Membrane Infoldings

Infoldings of the basal plasma membrane increase the surface area available for transport.

The basal surface of some epithelia, especially those involved in ion transport, possesses multiple finger-like infoldings of the basal plasma membranes, which increase the surface area of the plasmalemma and partition the mitochondria-rich basal cytoplasm. The mitochondria provide the energy required for active transport of ions in establishing osmotic gradients to ensure the movement of water across the epithelium, such as those of the kidney tubules. The compactness of the infolded plasma membranes coupled with the arrangement of the mitochondria within the infoldings gives a striated appearance when viewed with the light microscope; this is the origin of the term ***striated ducts*** for certain ducts of the pancreas and salivary glands.

Hemidesmosomes

Hemidesmosomes attach the basal cell membrane to the underlying basal lamina; they have strong adhesive properties.

Hemidesmosomes are of two types, **type I hemidesmosome (classical type of hemidesmosome)** and **type II hemidesmosome**, but both resemble half desmosomes and serve to attach firmly and for a long period of time the basal cell membrane to the basal lamina (Fig. 5–16; see Fig. 5–11).

Type I hemidesmosome is more complex than type II and is present in the basal layers of cells of the stratified squamous epithelia as well as on the basal plasma membranes of the cells comprising pseudostratified epithelia. Type I hemidesmosomes have a number of component parts.

The intracellular components, starting at the plasmalemma, include:

- A large number of **α6β4 integrin molecules** in close proximity to each other
- **Tonofilaments** (**keratin-5** and **keratin-14 intermediate filaments**)
- **Plakin proteins** (**bullous pemphigoid antigen 230 [BP230]** and **plectin**) that bind to tonofilaments as well as to the intracytoplasmic aspects of α6β4 integrin molecules
- **Erbin** molecules facilitating the binding of the α6β4 integrin molecules to BP230
- **CD 151** (**cluster of differentiation protein 151**) that binds intracellularly to the **α6** component of the integrin molecules and recruits other α6β4 integrin molecules to the region to ensure a proper concentration of these integrins for the formation of a hemidesmosome

All of the above structures, together, form a dense **intracytoplasmic plaque** that is visible with the electron microscope.

The extracellular components, starting at the plasmalemma, include:

- The extracellular moieties of the **α6β4 integrin molecules**
- **Bullous pemphigoid antigen 180 (BP180)**, a transmembrane protein (also referred to as *type XVII collagen*), whose intracellular moiety binds to the *intracellular portion* of the **α6** component of the integrin molecules as well as to **plectin**. (Extracellularly, it binds to the extracellular component of the **α6** portion of the integrin molecules and to the **laminin** and **type IV collagen** of the **basal lamina**.)

Type II hemidesmosomes are less complex than type I, and they are present in the basal plasma membranes of the simple columnar epithelial lining of the small and large intestines. Just as in type I hemidesmosomes, the most numerous components of type II hemidesmosomes are a large concentration of **α6β4 integrin molecules**. The intracellular moieties of these integrin molecules attach to **plectin,** which then form bonds with **keratin-8** and **keratin-18** intermediate filaments, thereby forming a dense **intracellular plaque** similar to that of the type I hemidesmosome.

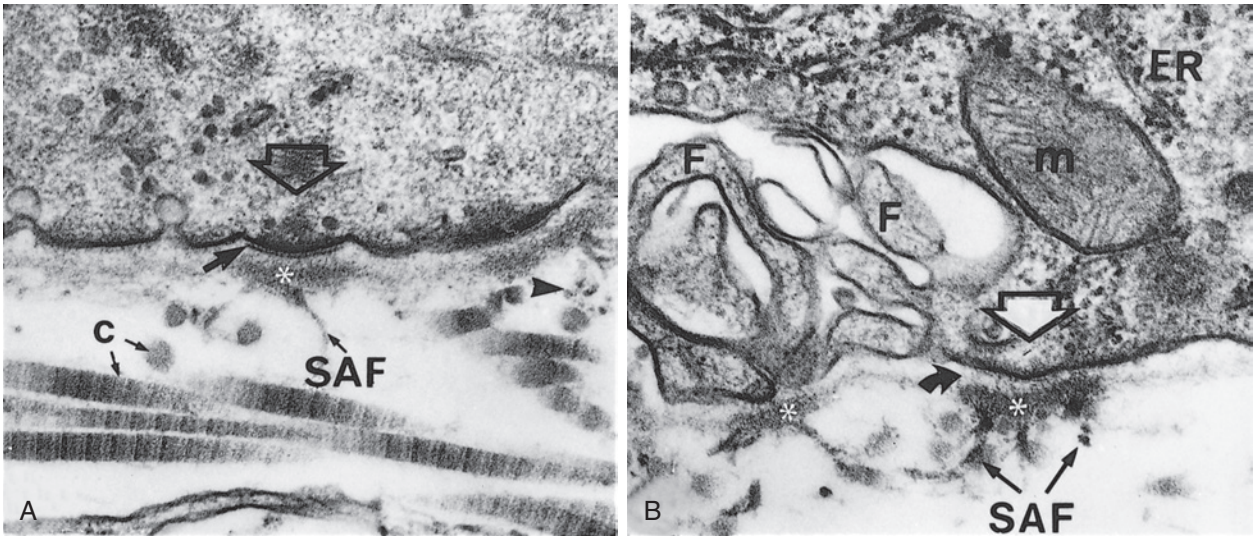


Figure 5–16 Electron micrograph of hemidesmosomes illustrating the relationship of striated anchoring fibers (SAF), composed of type VII collagen, with the lamina densa and type III collagen of the lamina reticularis. c, Collagen fibers; ER, rough endoplasmic reticulum; F, cell extensions; m, mitochondria. *Open arrowheads* indicate the cytoplasmic aspect of hemidesmosomes; *asterisk* (*) indicates SAF plaque. (From Clermont Y, Xia L, Turner JD, Hermo L. Striated anchoring fibrils–anchoring plaque complexes and their relation to hemidesmosomes of myoepithelial and secretory cells in mammary glands of lactating rats. *Anat Rec*. 1993;237:318-325. Reprinted by permission of Wiley-Liss, Inc., subsidiary of John Wiley & Sons, Inc.)

Both type I and type II hemidesmosomes require the presence of calcium ions to maintain an attachment to the basal lamina. Because most of these cells will move at one time or another, the cells have access to complex and well regulated molecular pathways, whereby they are able to modulate the extracellular calcium ion concentration in the vicinity of the hemidesmosome and, in that fashion, affix the cell to or release it from the basal lamina.

Focal Adhesions

Focal adhesions are anchoring junctions that attach the basal cell membranes of epithelial cells to the basal lamina; however, these are relatively transient and weak attachments.

Focal adhesions are present in clusters on the basal cell membranes of epithelial cells and form fairly weak anchoring junctions with the basal lamina and participate in the cell signaling pathways by acting as receptors for signaling molecules. Each focal adhesion is formed by clusters of α and β integrins whose extracellular moieties form weak interactions with laminin, type IV collagen, and fibronectin molecules of the basal lamina. The intracytoplasmic moieties of the α and β integrins bind to the actin-binding proteins vinculin, α-actinin, paxillin, and talin, which, by binding to actin filaments, anchor the integrins into the cytoplasm. These focal adhesions can be modified by intracellular and extracellular molecular signals severing the attachment of the α and β integrins to the basal lamina as well as to the actin filaments, thus freeing the cell from its attachment and permitting it to migrate from its previous location.

Renewal of Epithelial Cells

Cells constituting the epithelial tissues generally exhibit a high turnover rate, which is related to their location and function. The time frame for cell renewal remains constant for a particular epithelium.

Cells of the epidermis, for example, are constantly being renewed at the basal layer by cell division. From here, the cells begin their migration from the germinal layer to the surface, being keratinized on their route until they reach the surface, die, and are sloughed, the total event taking approximately 28 days. Other epithelial cells are renewed in a shorter period of time.

Cells lining the small intestine are replaced every 4 to 6 days by regenerative cells in the base of the crypts. The new cells then migrate to the tips of the villi, die, and are sloughed. Still other epithelia, for example, are

renewed periodically until adulthood is reached; subsequently, the cell population remains for life. Even then, however, when a large number of cells are lost because of injury or acute toxic destruction, cell proliferation is triggered, and the cell population is restored.

CLINICAL CORRELATIONS

Each epithelium within the body has its own unique characteristics, location, and cell morphology, all of which are related to function. In certain pathological conditions, the cell population of an epithelium may undergo **metaplasia**, transforming it into another epithelial type.

Pseudostratified ciliated columnar epithelium of the bronchi of heavy smokers may undergo **squamous metaplasia**, transforming it into stratified squamous epithelium. This change impairs function, but the process may be reversed when the pathological insult (smoking) is removed.

Tumors that arise from epithelial cells may be benign (nonmalignant) or malignant. Malignant tumors arising from epithelia are called ***carcinomas***; those arising from glandular epithelial cells are called ***adenocarcinomas***. It is interesting to note that cancers in adults are most often adenocarcinomas and, after age 45 years, about 90% are of epithelial cell origin. However, in children younger than 10 years of age, epithelial-derived cancers are the least prevalent type of cancer.

GLANDS

Glands originate from epithelial cells that leave the surface where they developed and penetrate into the underlying connective tissue, manufacturing a basal lamina that envelop them. The secretory units, along with their ducts, are the **parenchyma** of the gland, whereas the **stroma** of the gland represents the elements of the connective tissue that invade and support the parenchyma.

Glandular epithelia manufacture their product intracellularly by synthesis of macromolecules that are usually packaged and stored in vesicles called ***secretory granules***. The secretory product may be a polypeptide hormone (e.g., from the pituitary gland); a waxy substance (e.g., from the ceruminous glands of the ear canal); a mucinogen (e.g., from the goblet cells); or milk, a combination of protein, lipid, and carbohydrates from the mammary glands. Other glands (e.g., sweat glands) secrete little besides the exudate that they receive from the bloodstream. In addition, cells of striated ducts (e.g., those of the major salivary glands) act as ion pumps that modify the substances produced by their secretory units.

Glands are classified into two major groups on the basis of the method of distribution of their secretory products:

1. **Exocrine glands** secrete their products via ducts onto the external or internal epithelial surface from which they originated.
2. **Endocrine glands** are **ductless**, having lost their connections to the originating epithelium, and thus secrete their products into the blood or lymphatic vessels for distribution.

Many cell types secrete signaling molecules called ***cytokines***, which perform the function of cell-to-cell communication. Cytokines are released by **signaling cells** and act on **target cells**, which possess receptors for the specific signaling molecule. (Hormone signaling is discussed in detail in Chapter 2.)

Depending on the distance the cytokine must travel to reach its target cell, its effect may be one of the following:

- **Autocrine**. The signaling cell is its own target; thus, the cell stimulates itself.
- **Paracrine**. The target cell is located in the vicinity of the signaling cell; thus, the cytokine does not have to enter the vascular system for distribution to its target.
- **Endocrine**. The target cell and signaling cell are far from each other; thus, the cytokine has to be transported either by the blood or by the lymph vascular system.

Glands that secrete their products via a **constitutive secretory pathway** do so continuously, releasing their secretory products immediately without storage and without requiring a prompt by signaling molecules. Glands that exhibit a **regulated secretory pathway** concentrate and store their secretory products until the proper signaling molecule for its release is received (see Chapter 2, Figs. 2–20 and 2–22).

Exocrine Glands

Exocrine glands secrete their products via a duct to the surface of their epithelial origin.

Exocrine glands are classified according to the nature of their secretion, their mode of secretion, and the number of cells (unicellular or multicellular). Many exocrine glands in the digestive, respiratory, and urogenital systems secrete substances that are described as mucous, serous, or mixed (both) types.

Mucous glands secrete **mucinogens**, large glycosylated proteins that, upon hydration, swell to become a thick, viscous, gel-like protective lubricant known as **mucin**, a major component of **mucus**. Examples of mucous glands include goblet cells and the minor salivary glands of the hard and soft palates.

Serous glands (Fig. 5–17), such as the pancreas, secrete an enzyme-rich watery fluid.

Mixed glands contain acini (secretory units) that produce mucous secretions as well as acini that produce serous secretions; in addition, some of their mucous acini possess **serous demilunes**, a group of cells that secrete a serous fluid (it is now believed that serous demilunes are artifacts of fixation; see Chapter 18). The sublingual and submandibular glands are examples of mixed glands (Fig. 5–18).

Cells of exocrine glands exhibit three different mechanisms for releasing their secretory products: (1) merocrine, (2) apocrine, and (3) holocrine (Fig. 5–19). The release of the secretory product of **merocrine glands** (e.g., parotid gland) occurs via exocytosis; as a result, neither cell membrane nor cytoplasm becomes a part of the secretion. Although many investigators question the existence of the apocrine mode of secretion, historically, it was believed that in **apocrine glands** (e.g., lactating mammary gland), a small portion of the apical cytoplasm is released along with the secretory product. In **holocrine glands** (e.g., sebaceous gland), as a secretory cell matures, it dies and becomes the secretory product.

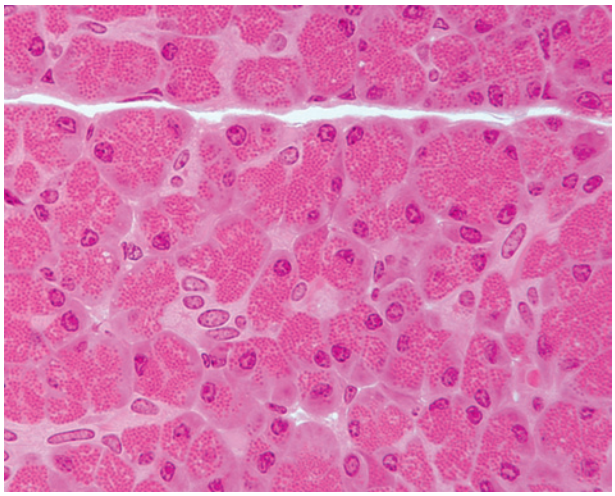


Figure 5–17 Serous gland. Light micrograph of a plastic-embedded monkey pancreas. (×540)

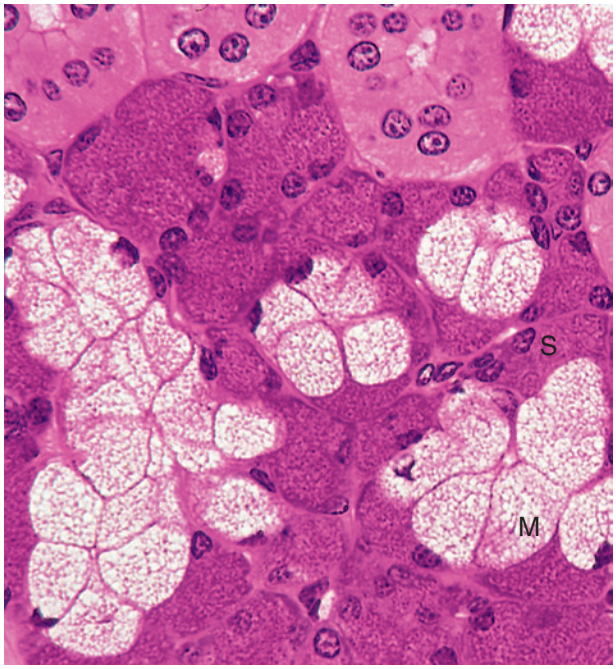


Figure 5–18 Light micrograph of the monkey submandibular gland. (×540)

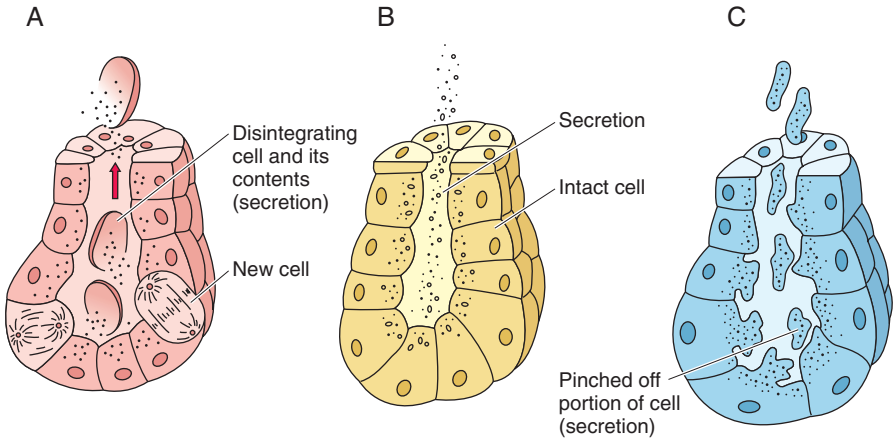


Figure 5–19 Schematic diagram of modes of secretion. **A,** Holocrine; **B,** merocrine; **C,** apocrine.

Figure 5–20 Light micrograph of goblet cells (GC) in the epithelial lining of the monkey ileum (×540).

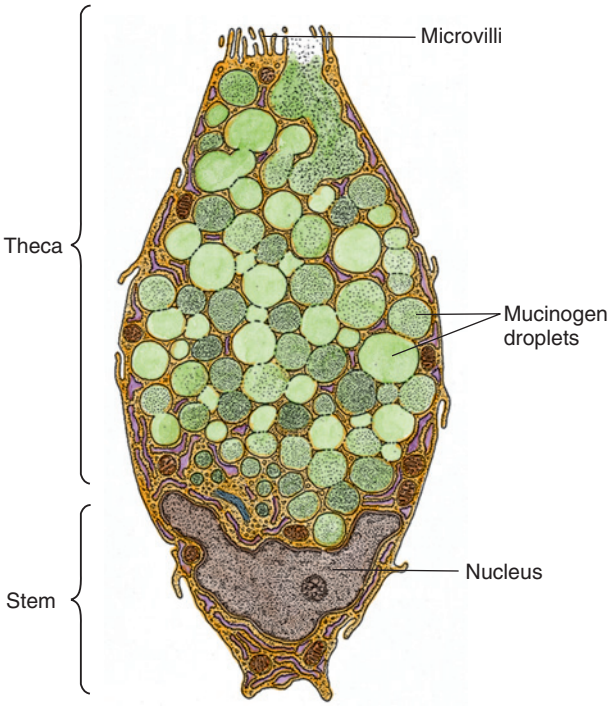


Figure 5–21 Schematic diagram of the ultrastructure of a goblet cell illustrating the tightly packed secretory granules of the theca. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

Unicellular Exocrine Glands

Unicellular exocrine glands represent the simplest form of exocrine gland.

Unicellular exocrine glands, represented by isolated secretory cells in an epithelium, are the simplest form of exocrine gland. The primary example is the **goblet cell**, which are dispersed individually in the epithelia lining the digestive tract and portions of the respiratory tract (Figs. 5–20 and 5–21). The secretions released by goblet cells protect the linings of these tracts.

Goblet cells derive their name from their shape, that of a wine goblet (Fig. 5–22). Their thin **basal region** sits on the basal lamina, whereas their expanded apical portion, the **theca**, faces the lumen of the digestive tube or respiratory tract. The theca is filled with membrane-bound secretory droplets filled with **mucinogen**, which displace the cytoplasm to the cell's periphery and the nucleus toward its base. The process of mucinogen release is regulated and stimulated by chemical irritation and parasympathetic innervation, resulting in exocytosis of the entire secretory contents of the cell, thus lubricating and protecting the epithelial sheet.

Multicellular Exocrine Glands

Multicellular exocrine glands exist as organized clusters of secretory units.

Multicellular exocrine glands consist of clusters of secretory cells arranged in varying degrees of organization. These secretory cells do not act independently but instead function as secretory organs. Multicellular glands may have a simple structure, exemplified by the glandular epithelium of the uterus and gastric mucosa, or a complex structure composed of various types of secretory units and organized in a compound branching fashion.

Because of their structural arrangement, multicellular glands are subclassified according to the organization of their secretory and duct components as well as according to the shape of their secretory units (Fig. 5–23).

Multicellular glands are classified as **simple** if their ducts do not branch and **compound** if their ducts do branch. They are further categorized according to the morphology of their secretory units as ***tubular***, ***acinar*** (sometimes also referred to as ***alveolar***, resembling a grape), or ***tubuloacinar*** (Fig. 5–24).

Larger multicellular glands are surrounded by a collagenous connective tissue **capsule**, which sends **septa**—strands of connective tissue—into the gland, subdividing it into smaller compartments known as ***lobes*** and ***lobules***. Vascular elements, nerves, and ducts use the connective tissue septa to enter and exit

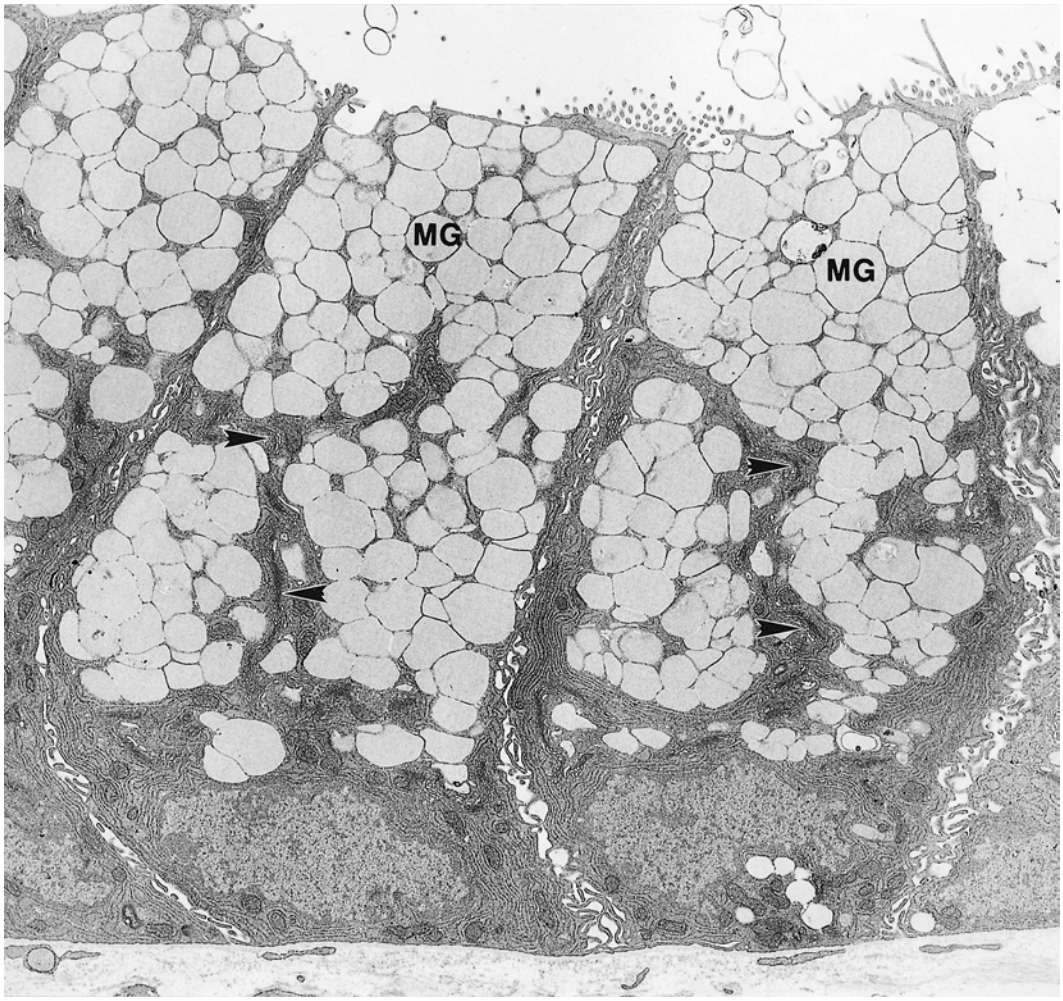


Figure 5–22 Electron micrograph of goblet cells from the colon of the rabbit. Note the presence of several Golgi complexes *(arrowheads)* and the numerous, compactly packed mucinogen granules (MGs) that occupy much of the apical portion of the cells (×9114). (From Radwan KA, Oliver MG, Specian RD. Cytoarchitectural reorganization of rabbit colonic goblet cells during baseline secretion. *Am J Anat*. 1990;198:365-376. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

the gland. In addition, the connective tissue elements provide structural support for the gland.

Acini of many multicellular exocrine glands, such as sweat glands and major salivary glands, possess **myoepithelial cells** that share the basal lamina of the acinar cells. Although myoepithelial cells are of epithelial origin, they have some characteristics of smooth muscle cells, particularly contractility. These cells exhibit small nuclei and sparse fibrillar cytoplasm radiating out from the cell body, wrapping around the acini and some of the small ducts (Fig. 5–25; see Fig. 5–24). Their contractions assist in expressing secretions from the acini and from some small ducts.

Endocrine Glands

Endocrine glands are without ducts, and their secretory products are thus released directly into the bloodstream or the lymphatic system.

Endocrine glands release their secretions, **hormones**, into blood or lymphatic vessels for distribution to target organs. The major endocrine glands of the body include the suprarenal (adrenal), pituitary, thyroid, parathyroid, and pineal glands as well as the ovaries, placenta, and testes.

The islets of Langerhans and the interstitial cells of Leydig are unusual because they are composed of

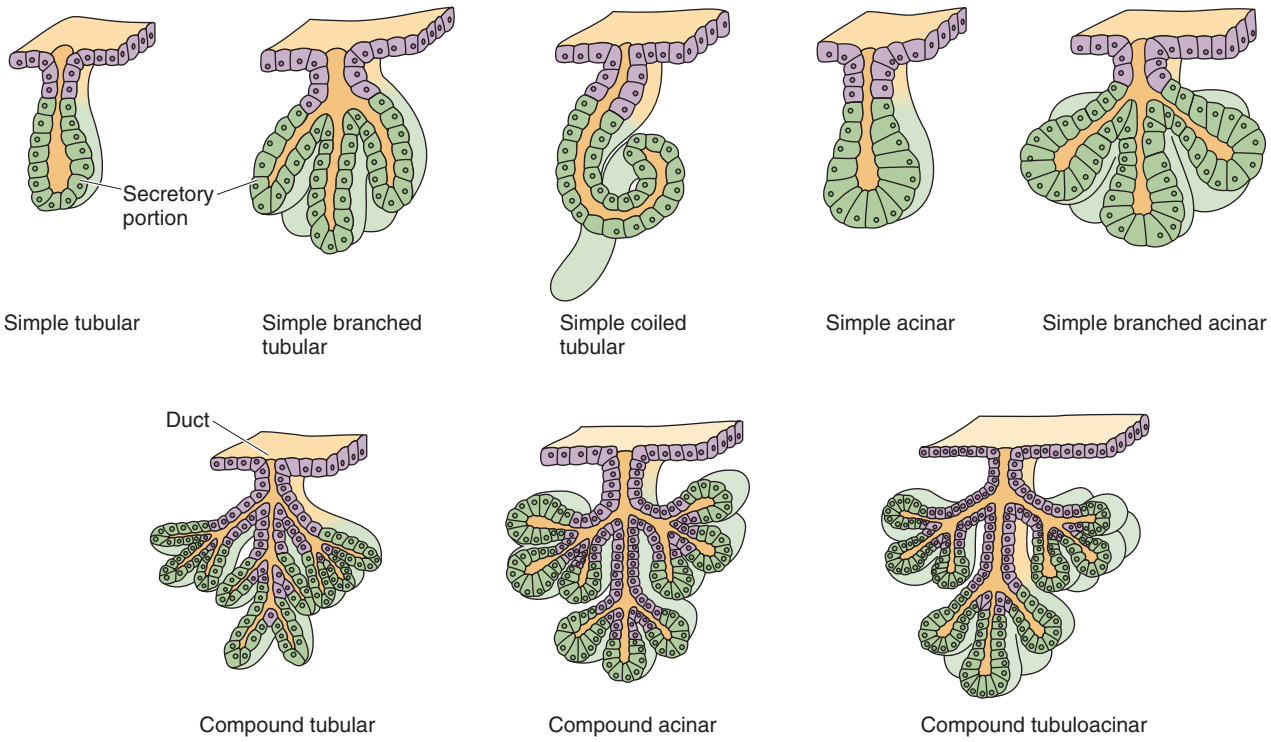


Figure 5–23 Schematic diagram of the classification of multicellular exocrine glands. *Green* represents secretory portion; *lavender* represents the duct portion of the gland.

clusters of cells ensconced within the connective tissue stroma of other organs (the pancreas and the testes, respectively). Hormones secreted by endocrine glands include peptides, proteins, modified amino acids, steroids, and glycoproteins. Because of their complexity and important role in regulating bodily processes, the endocrine glands are discussed in detail in Chapter 13.

The secretory cells of endocrine glands are organized either in **cords** of cells or in a **follicular** arrangement. In the **cord** type, the most common arrangement, cells form anastomosing cords around capillaries or blood sinusoids. The hormone to be secreted is stored intracellularly and is released upon the arrival of the proper signaling molecule or neural impulse. Examples of the cord type of endocrine gland include the suprarenal gland, anterior lobe of the pituitary gland, and the parathyroid gland.

In the **follicle** type of endocrine gland, secretory cells **(follicular cells)** form follicles that surround a cavity that receives and stores the secreted hormone. When a release signal is received, the stored hormone is resorbed by the follicular cells and released into the connective tissue to enter the blood capillaries. An example of a follicle type of endocrine gland is the thyroid gland.

Some glands of the body are mixed; for example, the parenchyma contains both exocrine and endocrine secretory units. In these mixed glands (e.g., pancreas, ovary, and testes), the exocrine portion of the gland secretes its product into a duct, whereas the endocrine portion of the gland secretes its product into the bloodstream.

Diffuse Neuroendocrine System

The diffuse neuroendocrine system functions to produce paracrine and endocrine hormones.

Widespread throughout the digestive tract and in the respiratory system are endocrine cells interspersed among other secretory cells. These cells, members of the **diffuse neuroendocrine system (DNES)**, manufacture various paracrine and endocrine hormones (Fig. 5–26). Because these cells are capable of taking up precursors of amines and decarboxylating amino acids, they were also called **APUD cells** (*a*mine *p*recursor *u*ptake and *d*ecarboxylation). At one time, some of these cells were called ***argentaffin*** and ***argyrophil cells*** because of the way they stained with silver salts. This entire cell group is now called ***DNES cells*** and is described in greater detail in Chapter 17.

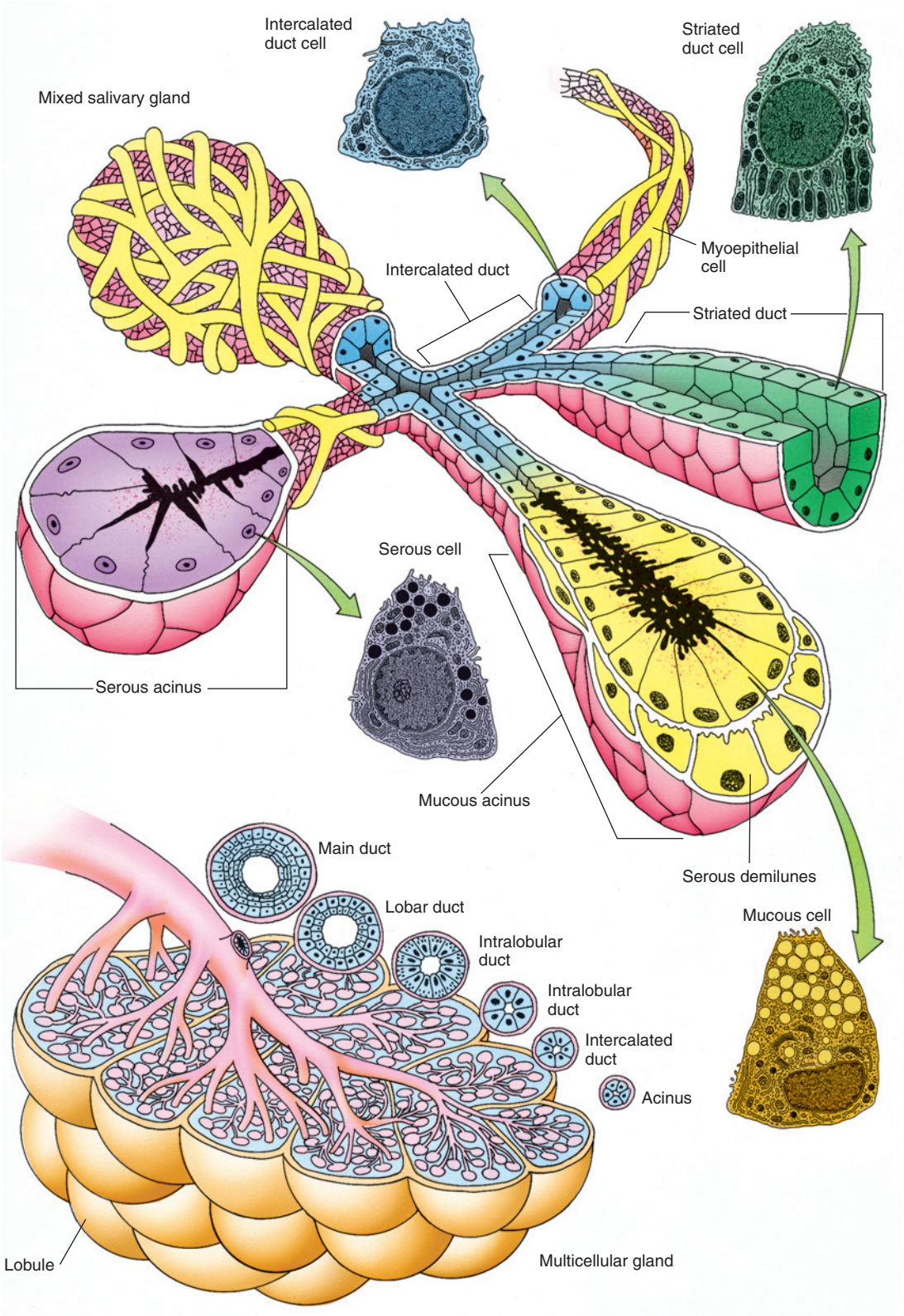


Figure 5–24 Schematic diagram of a salivary gland displaying its organization, secretory units, and system of ducts.

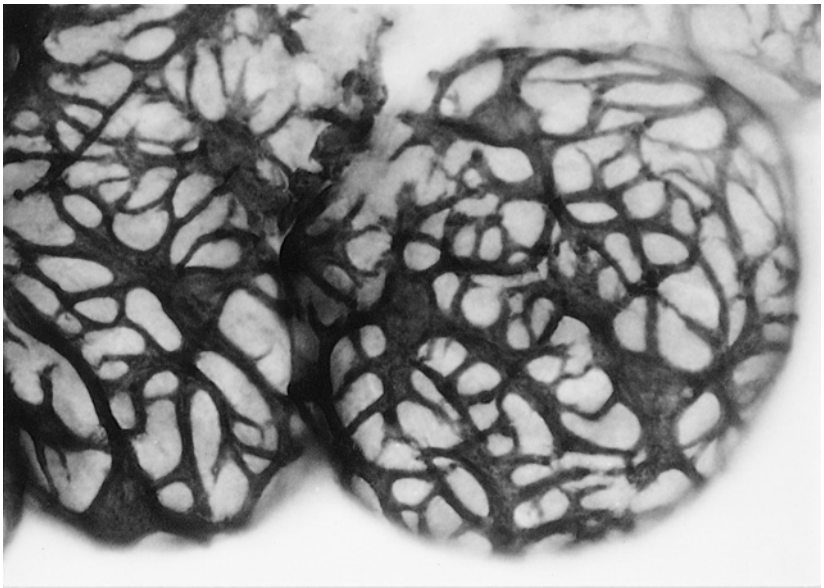


Figure 5–25 Light micrograph of myoepithelial cells immunostained for actin. Myoepithelial cells surround the acini (×640). (From Satoh Y, Habara Y, Kanno T, Ono K. Carbamylcholine-induced morphological changes and spatial dynamics of $[Ca^{2+}]c$ in Harderian glands of guinea pigs: calcium-dependent lipid secretion and contraction of myoepithelial cells. *Cell Tissue Res*. 1993;274:1-14.)

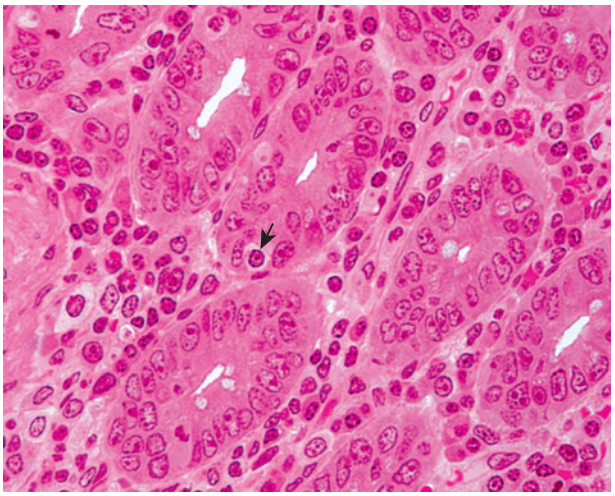


Figure 5–26 Diffuse neuroendocrine system (DNES) cell. Note the pale-staining DNES cell *(arrow)* located in the mucosa of the ileum (×540).

PATHOLOGICAL CONSIDERATIONS

See Figures 5–27 through 5–29.

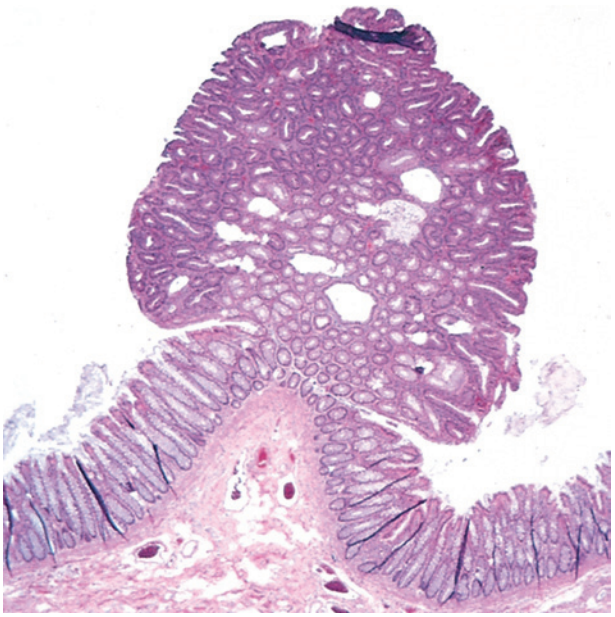


Figure 5–27 A colonic polyp that is jutting into the lumen of the colon. Note that it is connected to the mucosa by a slender stalk. (From Kumar V, et al. *Robbins and Cotran: Pathologic Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015.)

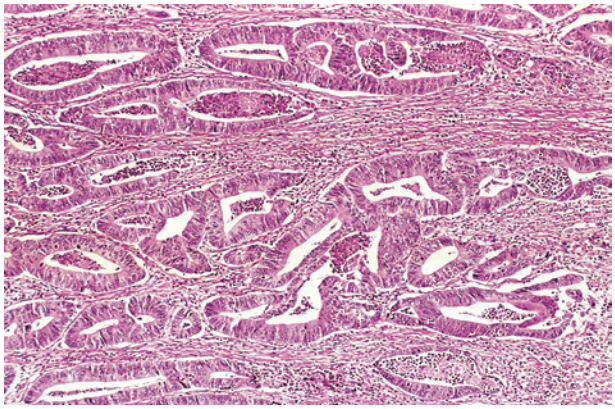


Figure 5–28 Adenocarcinoma (glandular carcinoma) of the colon. Observe that the malignant glands that appear in this photomicrograph are irregular in morphology and appear completely different from the normal glands of a healthy colon. (From Kumar V, et al. *Robbins and Cotran: Pathologic Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:269.)

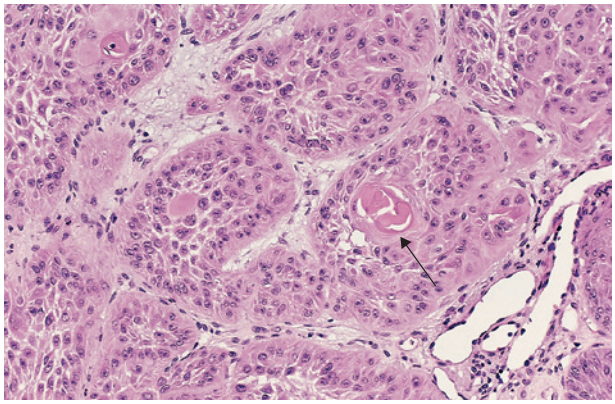
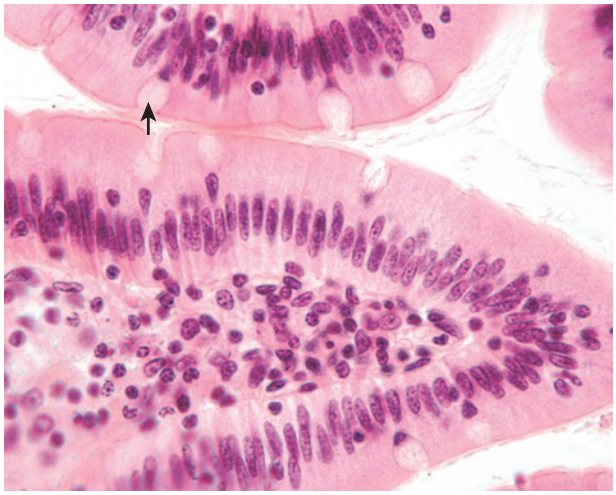
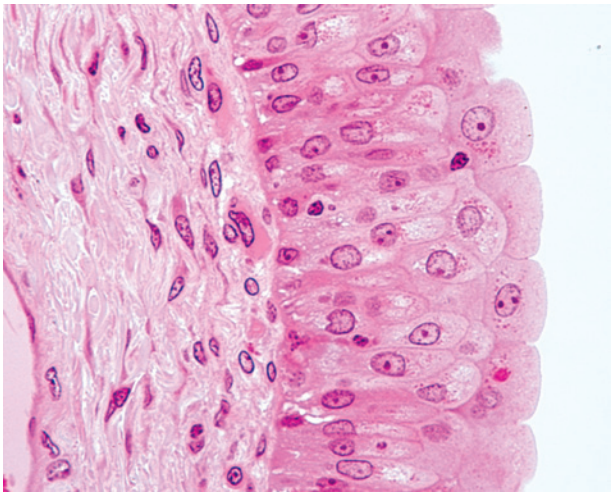


Figure 5–29 Note that this photomicrograph of a squamous cell carcinoma of the skin displays a keratin pearl *(arrow)*. (From Kumar V, et al. *Robbins and Cotran: Pathologic Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:269.)

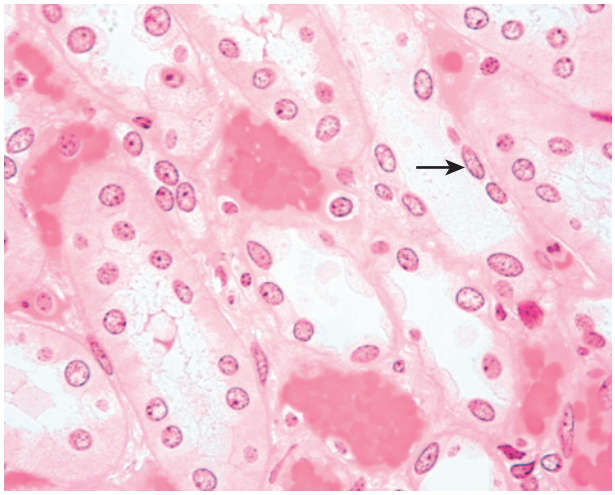
REVIEW QUESTIONS

1. The apical and basolateral surfaces of a polarized cell maintain their integrity due to the presence of
   a. gap junction
   b. zonula adherens
   c. fascia adherens
   d. zonula occludens
   e. fascia occludens
2. An elderly patient is seen by her physician for dry mouth. It appears that her parotid glands no longer produce saliva. Which is the correct classification of the parotid glands?
   a. apocrine
   b. holocrine
   c. sebaceous
   d. mixed
   e. serous
3. The urinary bladder needs to stretch (distend) and then return to its original shape. Which of the following epithelia is associated with that organ?
   a. transitional
   b. simple cuboidal
   c. simple columnar
   d. simple squamous
   e. pseudostratified columnar
4. A 10-year-old boy presents with peroneal muscular atrophy (Charcot-Marie-Tooth syndrome). He has weakness in his leg and atrophied calf and hand muscles. Additionally, he has lost some heat, pain, and cold sensation in his feet and hands. This very common hereditary neuropathy is the result of a mutation in the gene coding for
   a. cadherins
   b. integrins
   c. dystroglycans
   d. connexins
   e. occludins
5. The principal product manufactured by goblet cells is known as
   a. mucin
   b. mucus
   c. mucinogen
   d. theca
   e. mucosa
6. 

   The cell at the tip of the *arrow* manufactures and releases
   a. mucinogen
   b. mucinase
   c. mucus
   d. mucilage
   e. mucin
7. 

   This is a healthy region of a patient with cancer of the
   a. esophagus
   b. skin
   c. urinary bladder
   d. gallbladder
   e. cervix

8.



The tissue at the tip of the pointer is classified as
a. stratified squamous epithelium
b. loose connective tissue
c. dense irregular collagenous connective tissue
d. simple squamous epithelium
e. stratified cuboidal epithelium

ANSWERS

1. D. Zonulae occludentes maintain the integrity of the basolateral cell membranes by restricting the movement of their integral proteins. Gap junctions function in intercellular communication, zonulae and fasciae adherents assist in the maintenance of intercellular adhesion, and fasciae occludentes restrict the movement of materials in and out of a lumen.
2. E. The parotid glands produce a purely serous (watery) saliva. The term *apocrine* refers to glands that release a part of their cytoplasm with their secretory product (some consider the mammary glands to be apocrine glands), whereas cells of the holocrine glands die and become the secretory product. Sebaceous glands (oil glands of the skin) are holocrine glands. Mixed glands, such as the submandibular and sublingual glands, produce a secretion composed of both serous and mucous components.
3. A. The urinary bladder expands as it becomes filled with urine, and it is the transitional epithelium that maintains the integrity of its lining.
4. D. Mutations in connexin 32 gene result in the development of X-linked Charcot-Marie-Tooth syndrome. Fortunately, this condition is not life threatening. The other proteins are not affected by this mutation.
5. C. Goblet cells manufacture a glycoprotein known as *mucinogen* which, when released into an aqueous environment, becomes hydrated and is known as *mucin*. When mucin is mixed with other substances, such as dust particle in the respiratory system lumina, it is known as *mucus*. The theca is the expanded portion of the goblet cell that is filled with mucinogen containing secretory granules. Mucosa is the region that lines the lumen of an organ.
6. D. This image is from the duodenum, and the arrow is pointing to a goblet cell that manufactures and releases mucinogen. When mucinogen contacts water, it becomes hydrated to form mucin, a substance that is intermingled with other substances in the digestive canal and becomes known as *mucus*. Mucinase is an enzyme that hydrolyses mucins, whereas mucilage is a substance manufactured from plant material that is applied to mucous membranes for its soothing properties.
7. C. The specimen depicted in this photomicrograph is composed of transitional epithelium overlying a dense irregular collagenous connective tissue. Therefore, this is from the urinary bladder. The esophagus is lined by a stratified squamous nonkeratinized epithelium, whereas the cervix and the gall bladder are both lined by a simple columnar epithelium. The intravaginal portion of the cervix is covered by a stratified squamous epithelium. The epidermis of the skin is stratified squamous keratinized epithelium.
8. D. The specimen depicted in this photomicrograph is from the medulla of the kidney, and the various tubules located in that region are sectioned in a random fashion. The arrow points to flat nuclei of the simple squamous epithelium. Note that the stem of the arrow, located in the lumen of a thin limb of Henle loop, is near the flat nuclei of the simple squamous epithelium of the opposite side of the wall of the thin loop.

Connective Tissue

6

Connective tissue, as the name implies, forms a continuum with epithelial tissue, muscle, and nervous tissue as well as with other components of connective tissues to maintain a functionally integrated body. Most connective tissues originate from **mesoderm**, the middle germ layer of the embryonic tissue. From this layer, the multipotential cells of the embryo ***(mesenchymal cells)***, develop, although, in certain areas of the head and neck, mesenchyme also develops from neural crest cells of the developing embryo and is known as *ectomesenchyme*. Mesenchymal cells migrate throughout the body, giving rise to the connective tissues and their cells, including those of bone, cartilage, tendons, capsules, blood and hemopoietic cells, and lymphoid cells (Fig. 6–1).

Mature connective tissue is classified as **connective tissue proper**, the major subject of this chapter, or **specialized connective tissue** (i.e., cartilage, bone, and blood), detailed in Chapters 7 and 10.

Connective tissue is composed of cells and extracellular matrix (ECM) consisting of ground substance and fibers (Figs. 6–2 and 6–3). The cells are the most important components in some connective tissues, whereas fibers are the most important component in other connective tissue types. For example, fibroblasts are the most important cell component of loose connective tissue because these cells manufacture and maintain the fibers and ground substance composing the ECM. In contrast, fibers are the most important component of tendons and ligaments. In still other connective tissues, the ground substance is the most important component because it is where certain specialized connective tissue cells carry out their functions. Thus, all three components are critical to the role of connective tissue function in the body.

FUNCTIONS OF CONNECTIVE TISSUE

Although many functions are attributed to connective tissue, its primary functions include

- Providing structural **support**
- Serving as a **medium for exchange** of nutrients and waste products as well as signaling molecules
- Aiding in the **defense**, **protection**, and **repair** of the body
- Forming a site for **storage of fat**

Bones, cartilage, and the ligaments holding the bones together, as well as the tendons attaching muscles to bone, act as **support**. Similarly, the connective tissue that forms the capsules encasing organs and the stroma forming the structural framework within organs has support functions. Connective tissue also functions as a **medium for exchange** of metabolic waste, nutrients, and oxygen between the blood and many of the cells of the body.

The functions of **defense** and **protection** are carried out by (1) the body's phagocytic cells, which engulf and destroy cellular debris, foreign particles, and microorganisms; (2) the body's immunocompetent cells, which produce antibodies against antigens; and (3) certain cells that produce pharmacological substances that help in controlling inflammation. Connective tissues also help protect the body by forming a physical barrier to invasion and dissemination of microorganisms. **Repair** is performed mostly by fibroblasts that manufacture fibrous connective tissue and by cells of bone that mend broken or fractured bones.

EXTRACELLULAR MATRIX

The **extracellular matrix (ECM)**, a nonliving material, is composed of ground substance and fibers, designed to resist compressive and stretching forces. The components of the ECM are **ground substance** and **fibers**, as described in Chapter 4, and the reader is directed to that chapter to review their features.

CELLULAR COMPONENTS

The cells in connective tissues are grouped into two categories: **fixed cells** and **transient cells** (see Fig. 6–1).

Fixed cells are a resident population of cells that have developed and remain in place within the connective tissue, where they perform their functions. The fixed cells are a stable and long-lived population that include

- Fibroblasts
- Adipose cells
- Pericytes
- Mast cells
- Macrophages

Additionally, some authors consider certain of the macrophages (e.g., Kupffer cells of the liver) to be fixed connective tissue cells.

Transient cells (free or wandering cells) originate mainly in the bone marrow and circulate in the bloodstream. Upon receiving the proper stimulus or signal, these cells leave the bloodstream and migrate into the connective tissue to perform their specific functions.

SUMMARY

Connective tissue not only forms the structural support of the body but also is a site for fat storage, provides the cells and macromolecules that defend the body by mounting an **inflammatory** and/or **immune response**, and is the medium for the exchange of the cells' waste products with nutrients and oxygen. Its cells are grouped into two components, those that remain residents, known as ***fixed cells***, and those that migrate in and out of connective tissue, referred to as ***transient cells***. The major component of connective tissue is its **extracellular matrix (ECM)**, whereas cells are usually distributed sparsely throughout the ECM. Connective tissue is classified into three major groups: **embryonic connective tissue**, **connective tissue proper**, and **specialized connective tissue**.

KEY WORDS

Adipose cells; adipose tissue; connective tissue; dense connective tissue; extracellular matrix; extravasated leukocytes; fibroblasts; fixed cells; loose connective tissue; macrophages; mast cells; mesenchymal connective tissue; mucous connective tissue; pericytes; plasma cells; reticular tissue; transient cells

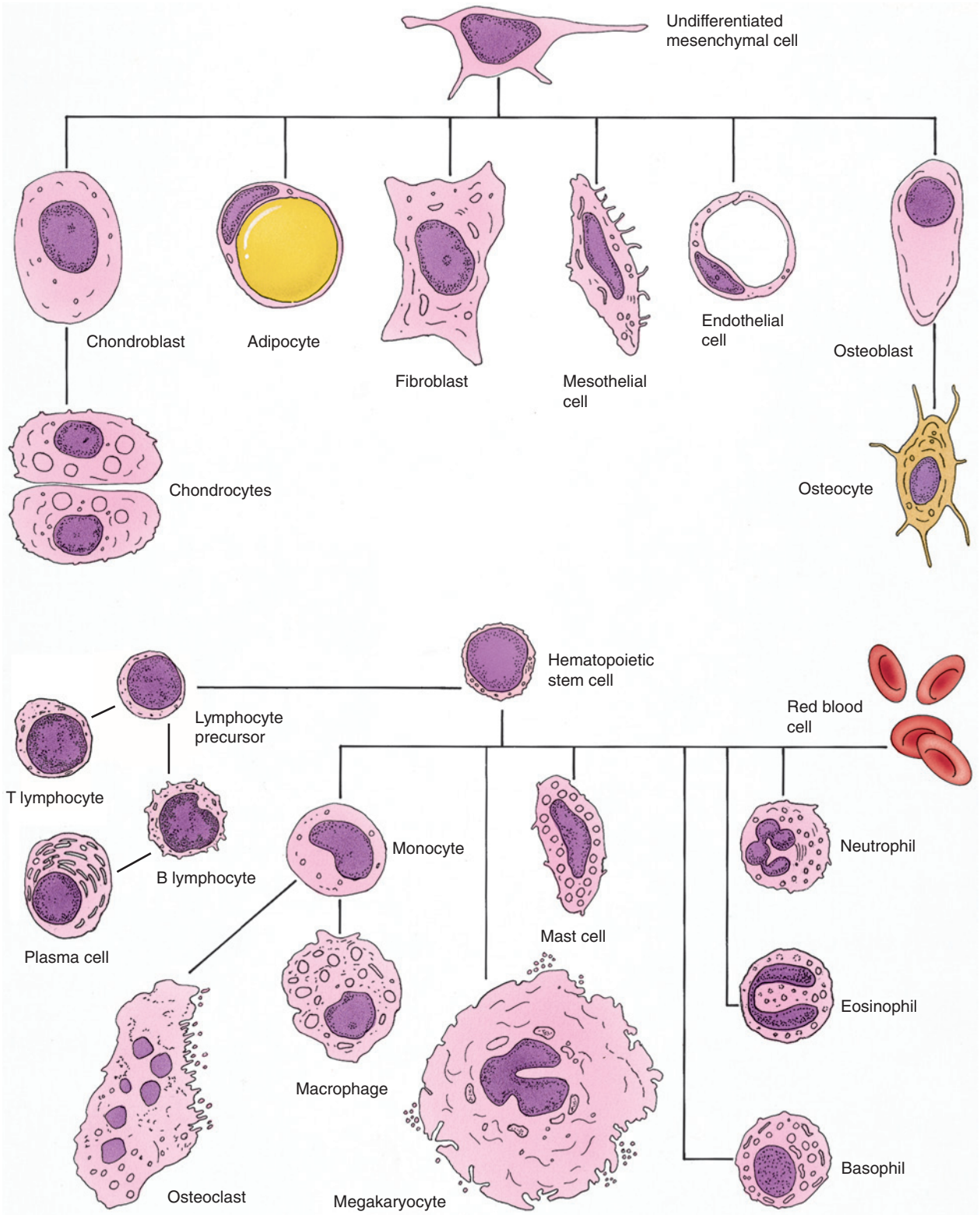


Figure 6–1 Schematic diagram of the origins of the cells of connective tissue. Cells are not drawn to scale.

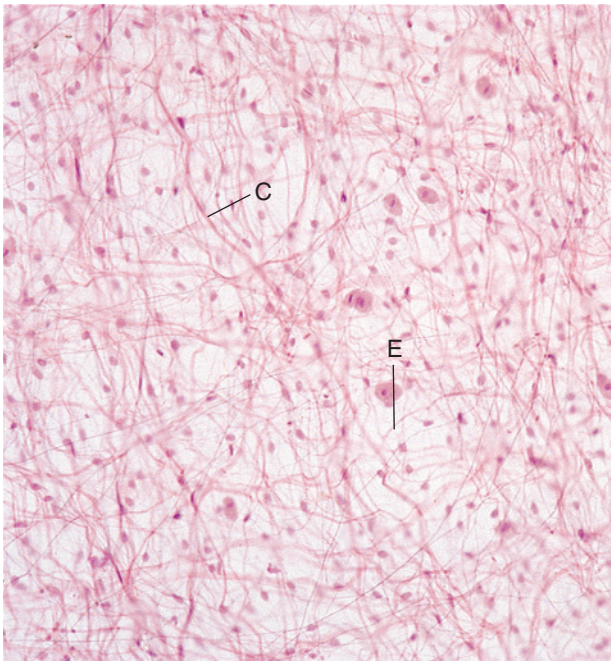


Figure 6–2 Light micrograph of loose (areolar) connective tissue displaying collagen (C) and elastic (E) fibers and some of the cell types common to loose connective tissue (×132).

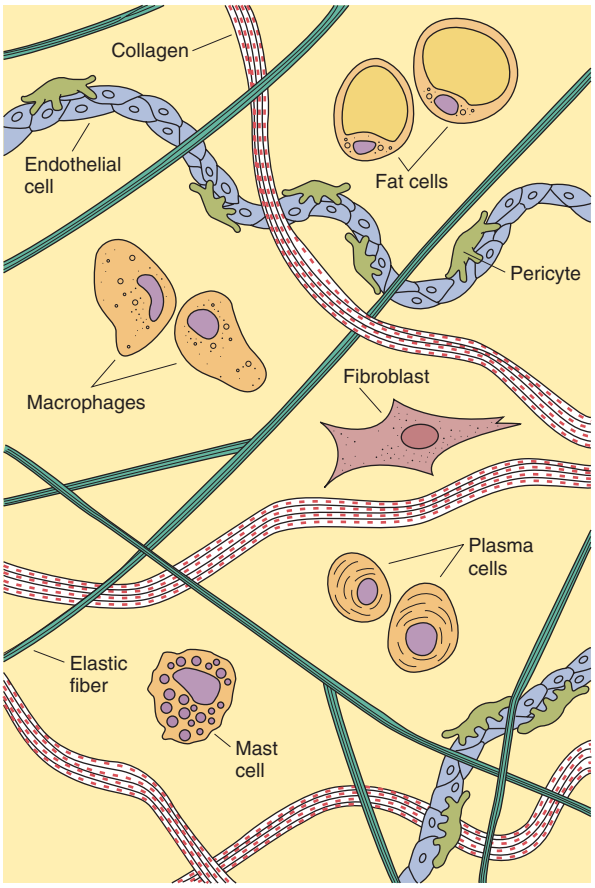


Figure 6–3 Schematic diagram illustrating the cell types and fiber types in loose connective tissue. Cells are not drawn to scale.

Because most of these motile cells are usually short-lived, they must be replaced continually from a large population of stem cells. Transient cells include

- Plasma cells
- Lymphocytes
- Neutrophils
- Eosinophils
- Basophils
- Monocytes
- Macrophages

Fixed Connective Tissue Cells

The four connective tissue cell types that are clearly fixed are described in this section. Macrophages, which exhibit some fixed and some transient properties, are discussed later in the section on macrophages.

Fibroblasts

Fibroblasts, the most abundant cell type in the connective tissue, are responsible for the synthesis of almost all of the extracellular matrix.

Fibroblasts, the most abundant and most widely distributed resident cells of connective tissue, are derived from undifferentiated mesenchymal cells and synthesize the ECM of connective tissue (see Fig. 6–1). Fibroblasts are the least specialized of the cells making up connective tissue and may even be represented by several different functioning populations within certain areas of the body. Because mature and immature fibroblasts may exist side by side, the immature cells, difficult to distinguish from mesenchymal cells, depending upon the signal proteins present, may differentiate into other cell members of connective tissue (i.e., fat cells, osteoblasts, chondroblasts, and myofibroblasts).

Fibroblasts may occur in either an active state or a quiescent state. Some histologists differentiate between them, calling the quiescent cells ***fibrocytes***; however, because the two states are transitory, the term *fibroblast* is used in this text.

Active fibroblasts often reside in close association with collagen bundles, where they lie parallel to the long axis of the fiber (Fig. 6–4). Such fibroblasts are elongated, fusiform cells possessing pale-staining cytoplasm, which is often difficult to distinguish from collagen when stained with hematoxylin and eosin (see Fig. 6–18). The most obvious portion of the cell is the darker-stained, large, granular, ovoid nucleus containing

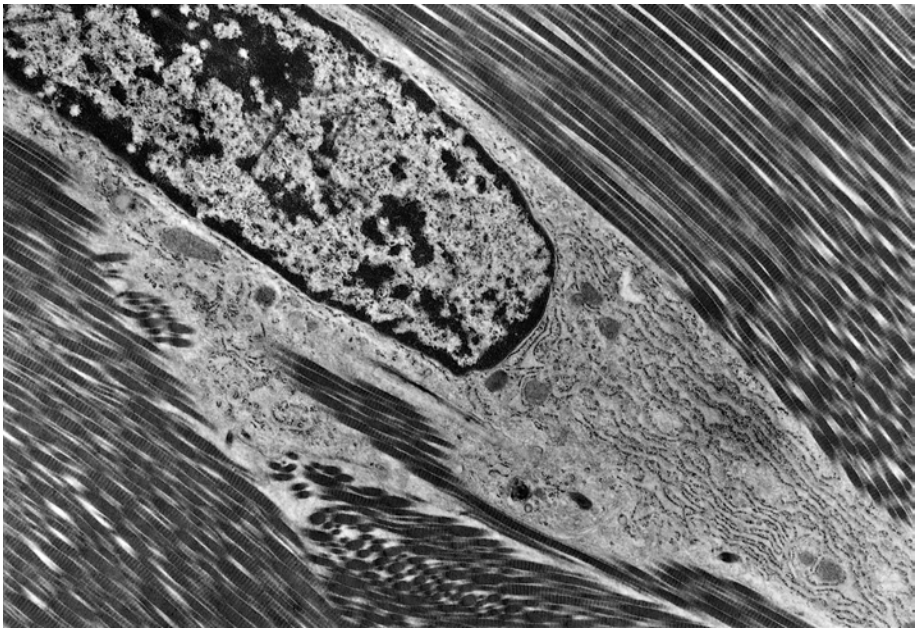


Figure 6–4 Electron micrograph displaying a portion of a fibroblast and the packed collagen fibers in rat tendon. Observe the heterochromatin in the nucleus and the rough endoplasmic reticulum in the cytoplasm. Banding in the collagen fibers may also be observed. (From Ralphs JR, Benjamin M, Thornett A. Cell and matrix biology of the suprapatellar in the rat: a structural and immunocytochemical study of fibrocartilage in a tendon subject to compression. *Anat Rec.* 1991;231:167-177. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

a well-defined nucleolus. Electron microscopy reveals a prominent Golgi apparatus and abundant rough endoplasmic reticulum (RER) in the fibroblast, especially when the cell is actively manufacturing matrix, as in wound healing. Actin and α-actinin are localized at the periphery of the cell, whereas myosin is present throughout the cytoplasm.

Inactive fibroblasts are smaller and more ovoid and possess an acidophilic cytoplasm. Their nucleus is smaller, elongated, and more deeply stained. Electron microscopy reveals sparse amounts of RER but an abundance of free ribosomes.

CLINICAL CONSIDERATIONS

Although considered to be fixed cells in the connective tissues, fibroblasts are capable of some movement. Fibroblasts seldom undergo cell division but may do so during wound healing. These cells, however, may differentiate into adipose cells, chondrocytes (during formation of fibrocartilage), and osteoblasts (under pathological conditions).

Myofibroblasts

Myofibroblasts are modified fibroblasts that demonstrate characteristics similar to those of both fibroblasts and smooth muscle cells.

Histologically, fibroblasts and **myofibroblasts** are not easily distinguished by routine light microscopy. Electron microscopy, however, reveals that myofibroblasts have bundles of actin filaments and myosin and dense bodies similar to those of smooth muscle cells. Additionally, the surface profile of the nucleus resembles that of a smooth muscle cell. Myofibroblasts differ from smooth muscle cells in that an external lamina (basal lamina) is absent. Myofibroblasts represent transitional modifications of fibroblasts as a result of being contacted by signaling molecules within the regional intercellular matrix. Myofibroblasts are abundant in areas undergoing wound healing where they function in wound contraction.

Pericytes

Pericytes surround endothelial cells of capillaries and small venules and technically reside outside of the connective tissue compartment because they possess their own basal lamina.

Pericytes (also known as *perivascular cells* and *adventitial cells*), derived from undifferentiated mesenchymal cells, partly surround the endothelial cells of capillaries and small venules (see Fig. 6–3). These multipotential perivascular cells are outside of the connective tissue compartment because they are surrounded by their own basal lamina, which may be fused with that of the endothelial cells. Pericytes possess characteristics of endothelial cells and smooth muscle cells in that they contain actin, myosin, and tropomyosin, suggesting that they may function in contraction. Under certain conditions, they may differentiate into other cells, including vascular smooth muscle cells, endothelial cells, and fibroblasts. Pericytes are discussed more fully in Chapter 11.

Adipose Cells

Adipose cells are fully differentiated cells that function in the synthesis, storage, and release of fat.

Fat cells, or **adipocytes**, are derived from undifferentiated fibroblast-like mesenchymal cells (Fig. 6–5; see Figs. 6–1 and 6–3), although, under certain conditions, histologists believe that fibroblasts may give rise to adipose cells. Adipose cells are fully differentiated and do not undergo cell division. They function in the synthesis and storage of triglycerides, as well as in producing hormones known as ***adipokines*** (see section on white adipose tissue). There are two types of fat cells, which constitute two types of adipose tissue. Cells with

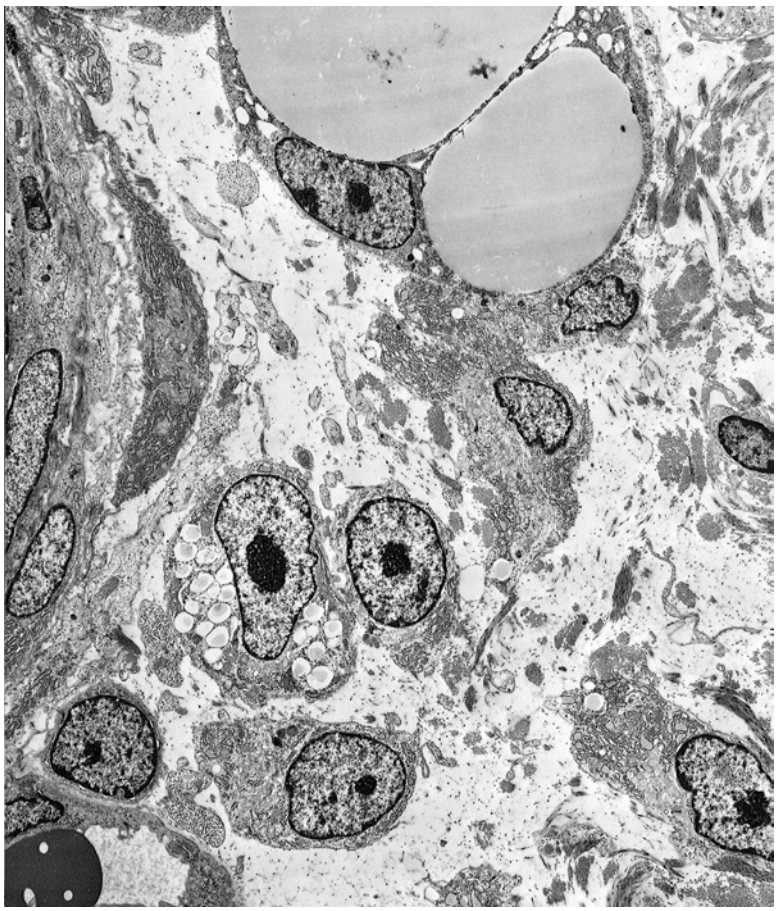


Figure 6–5 Electron micrograph of adipocytes in various stages of maturation in rat hypodermis. Observe the adipocyte at the top of the micrograph with its nucleus and cytoplasm crowded to the periphery by the fat droplet. (From Hausman GJ, Campion DR, Richardson RL, Martin RJ. Adipocyte development in the rat hypodermis. *Am J Anat.* 1981;161:85-100. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

a single, large lipid droplet, called ***unilocular fat cells***, form **white adipose tissue**, and cells with multiple, small lipid droplets, called ***multilocular fat cells***, form **brown adipose tissue**. White fat is much more abundant than brown fat. As discussed in the following the distribution and histophysiology of the two types of fat tissue differ. Here we describe the histologic characteristics of the adipocytes themselves.

Unilocular adipocytes are large spherical cells, up to 120 μm in diameter, that become polyhedral when crowded into adipose tissue (Fig. 6–6). Unilocular fat cells continually store fat in the form of a single droplet, which enlarges so much that the cytoplasm and nucleus are displaced peripherally against the plasma membrane, thus giving these cells a "signet ring" profile when viewed by light microscopy. Electron micrographs reveal a small Golgi complex situated adjacent to the nucleus, only a few mitochondria, and sparse RER but an abundance of free ribosomes. That the fat droplet is not bound by a membrane is clear in electron micrographs but unclear in light micrographs. The external surfaces of the plasma membranes are enveloped by a basal lamina–like substance and possess receptors for glucocorticoids, growth hormone, insulin, and norepinephrine whose function is to regulate free fatty acid and glycerol transport into and out of the cell. Minute pinocytotic vesicles, whose function is unclear, have been noted on the surface of the plasma membrane. During fasting, the cell surface becomes irregular, having pseudopod-like projections.

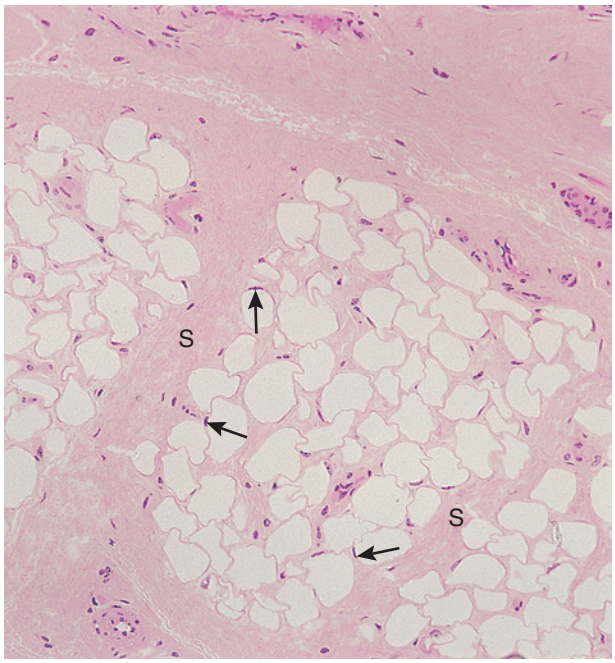


Figure 6–6 Light micrograph of white adipose tissue from monkey hypodermis (×132). The lipid was extracted during tissue processing. Note how the cytoplasm and nuclei *(arrows)* are crowded to the periphery. Septa (S) divide the fat into lobules.

Multilocular adipocytes contrast with unilocular adipocytes in several ways. First, brown fat cells are smaller and more polygonal than white fat cells. Moreover, because the brown fat cell stores fat in several small droplets rather than a single droplet, the spherical nucleus is not squeezed up against the plasma membrane. Multilocular fat cells contain many more mitochondria but fewer free ribosomes than unilocular fat cells (Fig. 6–7). Although brown fat cells lack RER, they do have smooth endoplasmic reticulum (SER).

Unilocular fat cells are found throughout the body in loose connective tissue and are concentrated along blood vessels. They may also accumulate into masses, forming adipose tissue.

Mast Cells

Mast cells arise from bone marrow stem cells and function in mediating the inflammatory process and immediate hypersensitivity reactions.

Mast cells, among the largest of the fixed cells of the connective tissue, are 20 to 30 μm in diameter. They are ovoid and possess a centrally placed, spherical nucleus (Fig. 6–8). Unlike the three types of fixed cells discussed earlier, mast cells probably derive from precursors in the bone marrow (see Fig. 6–1).

The presence of numerous granules in the cytoplasm, ranging in size from 0.3 to 0.8 μm in diameter, is the identifying characteristic of mast cells (Fig. 6–9). Electron microscopic studies of the granules reveal differences in size and form and display variations in ultrastructure even within the same cell. Otherwise, the cytoplasm is unremarkable because it contains several mitochondria, a sparse number of RER profiles, and a relatively small Golgi complex.

Mast cell granules contain **heparin**, **histamine** (or **chondroitin sulfates**), **neutral proteases** (tryptase, chymase, and carboxypeptidases), **aryl sulfatase** (as well as other enzymes, such as β-glucuronidase, kininogenase, peroxidase, and superoxide dismutase), **eosinophil chemotactic factor (ECF)**, and **neutrophil chemotactic factor (NCF).** These pharmacological agents that are present in the granules are referred to as the ***primary mediators*** (also known as ***preformed mediators***). In addition to the pharmacological agents in the granules, mast cells synthesize a number of mediators from membrane arachidonic acid precursors. These newly synthesized mediators include **leukotrienes (C_4, D_4, and E_4)**, **thromboxanes** (**TXA_2** and **TXB_2**), and **prostaglandins** (PGD_2). A number of other **cytokines** are also released that are *not derived from* arachidonic acid precursors, such as **platelet-activating factor (PAF)**, **bradykinins**, **interleukins**

Figure 6–7 Multilocular fat cells (brown fat) in the bat (×11000). Note the numerous mitochondria dispersed throughout the cell. (From Fawcett DW. *An Atlas of Fine Structure. The Cell*. Philadelphia: WB Saunders; 1966.)

(**IL-4**, **IL-5**, **IL-6**), and **tumor necrosis factor-alpha (TNF-α).** All of these newly synthesized mediators are formed at the time of their release and are collectively referred to as ***secondary mediators*** (or ***newly synthesized mediators***). These pharmacological agents, whether primary or secondary, function in the immune system by the initiation of an **inflammatory response** (see later).

Mast Cell Development and Distribution

Because basophils and mast cells share some characteristics, it was once believed that mast cells were basophils that had left the bloodstream to perform their tasks in the connective tissues. It is now known that basophils and mast cells are different cells and have different precursors (see Fig. 6–1). Mast cell precursors probably originate in the bone marrow as myeloid stem cells, circulate in the blood for a short time, and then enter the connective tissues, where they differentiate into mast cells and acquire their characteristic cytoplasmic granules. These cells have a life span of less than a few months and, occasionally, undergo cell division.

Mast cells are located throughout the body in the connective tissue proper, where they are concentrated

Figure 6–8 Light micrograph of mast cells *(arrows)* in monkey connective tissue (×540). The granules within the mast cells contain histamine and other preformed pharmacological agents.

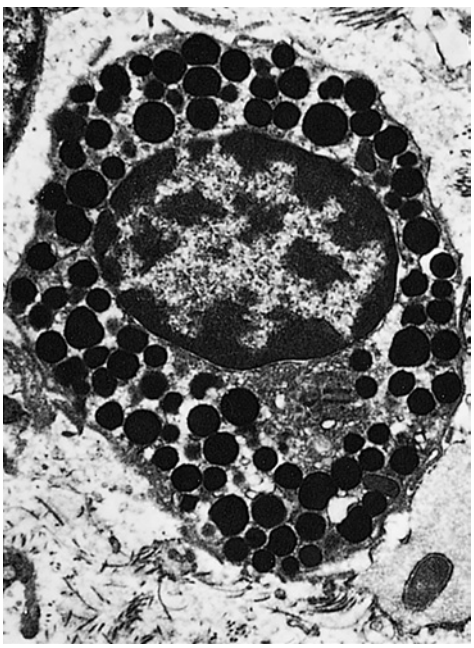


Figure 6–9 Electron micrograph of a mast cell in the rat (×5500). Observe the dense granules filling the cytoplasm. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

along small blood vessels and are known as ***connective tissue mast cells***. They also are present in the subepithelial connective tissue of the respiratory and digestive systems and are called ***mucosal mast cells***. Mast cells in connective tissue contain mostly heparin in their granules, whereas those located in the alimentary tract mucosa contain chondroitin sulfate instead of heparin. Because both **heparin** and **chondroitin sulfate** are sulfated glycosaminoglycans, mast cell granules stain metachromatically with toluidine blue (i.e., toluidine blue stains the granules purple), a characteristic feature of these cells. It is interesting to note that the central nervous system is devoid of mast cells, most probably to prevent swelling of the brain and spinal cord.

The reason for the existence of the two diverse populations of mast cells is not understood. Further, it has been determined that mast cells vary in phenotype, morphology, histochemistry, mediator content, and response. Thus, phenotypically different mast cell populations are thought to function differently in health and disease. For example, mucosal mast cells release histamine to facilitate the activation of parietal cells of the stomach to produce hydrochloric acid.

Mast Cell Activation and Degranulation

Mast cells possess high-affinity cell-surface Fc receptors **(FcεRI)** for immunoglobulin E (IgE). As indicated previously, mast cells function in the immune system by initiating an inflammatory response known as the ***immediate hypersensitivity reaction*** (whose systemic form, known as an ***anaphylactic reaction***, may have lethal consequences)**.** This response commonly is induced by foreign molecules (antigens) such as bee venom, pollen, and certain drugs, as follows:

1. The first exposure to any of these antigens elicits formation of IgE antibodies, which bind to the FcεRI receptors of the plasmalemma of mast cells, thereby **sensitizing** these cells.
2. On subsequent exposure to the *same* antigen, the antigen binds to the IgE on the mast cell surface, causing cross-linking of the bound IgE antibodies and clustering of the receptors (Fig. 6–10).
3. Cross-linking and clustering activate membrane-bound **receptor coupling factors**, which, in turn, initiate at least two independent processes, the release of **primary mediators** from the granules and synthesis and release of the **secondary mediators** from arachidonic acid precursors as well as from other cytoplasmic or membrane lipid sources.
4. The release of preformed mediators is accomplished by the activation of **adenylate cyclase**, the enzyme responsible for the conversion of adenosine triphosphate (ATP) to **cyclic adenosine monophosphate (cAMP)**.

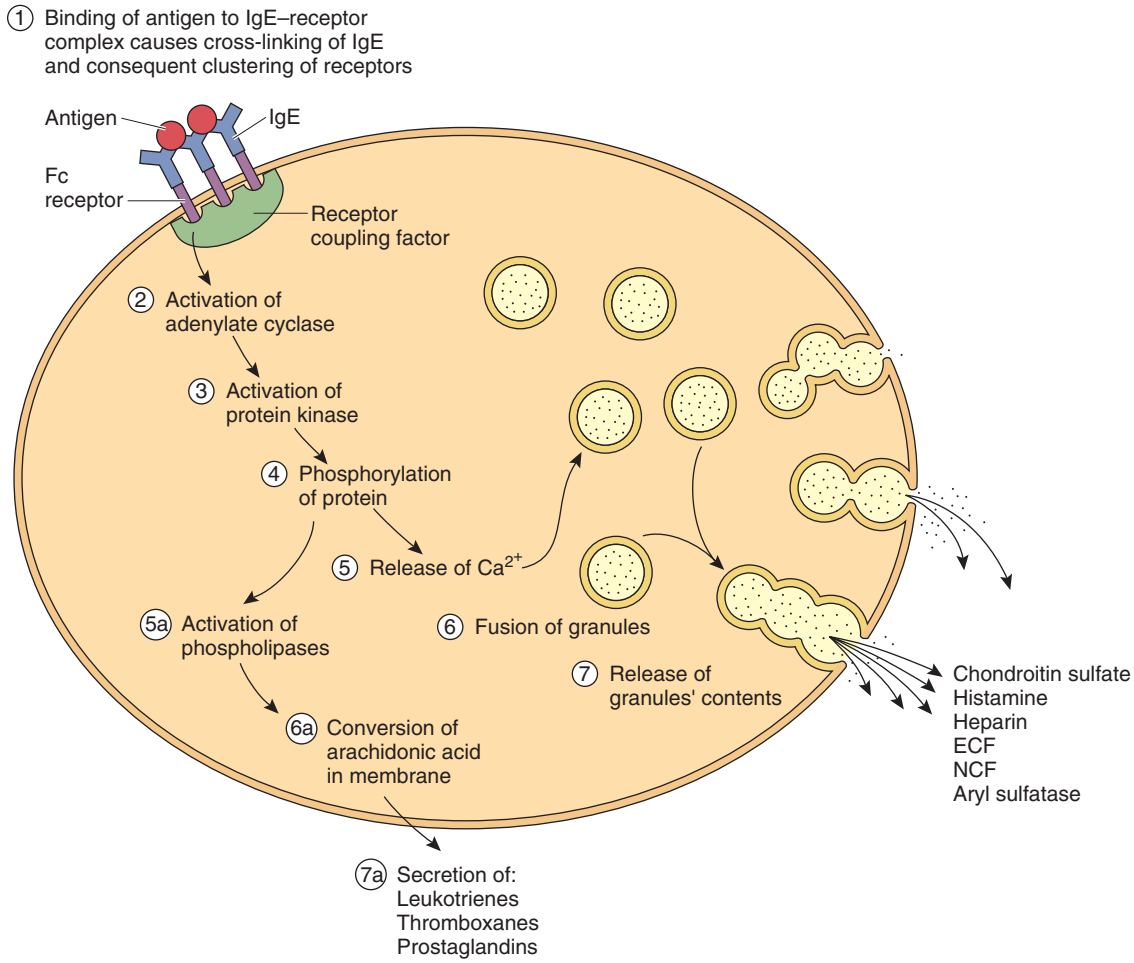


Figure 6–10 Schematic diagram illustrating the binding of antigens and cross-linking of immunoglobulin E (IgE)–receptor complexes on the mast cell plasma membrane. This event triggers a cascade that ultimately results in the synthesis and release of leukotrienes and prostaglandins as well as in degranulation, thus releasing histamine, heparin, eosinophil chemotactic factor (ECF), and neutrophil chemotactic factor (NCF).

5. This increase in cAMP levels activates release of calcium ion (Ca^{2+}) from intracellular storage sites and facilitates an influx from extracellular sources. The resulting increase in cytosolic Ca^{2+} causes the secretory granules to fuse with one another as well as with the cell membrane. These processes lead to **degranulation**, the release of the granule contents, namely histamine, heparin, neutral proteases, aryl sulfatase and other enzymes, ECF, and neutrophil chemotactic factor.
6. Cross-linking of the membrane-bound IgE also activates **phospholipase A_2**, which acts on membrane phospholipids to form **arachidonic acid**.
7. Arachidonic acid is converted into the secondary mediators **leukotrienes C_4, D_4,** and **E_4, prostaglandin D_2,** and **thromboxane A_2**. Additionally, the mast cell releases other newly formed pharmacological agents and cytokines**.** It is important to note that these secondary mediators are *not* stored in the mast cell granules but are manufactured and immediately released.

Table 6–1 lists the sources and activities of the principal primary and secondary mediators released from mast cells.

The primary and secondary mediators released by mast cells during immediate hypersensitivity reactions initiate the inflammatory response, activate the body's defense system by attracting leukocytes to the site of inflammation, and modulate the degree of inflammation.

TABLE 6–1 Principal Primary and Secondary Mediators Released by Mast Cells

Substance	Type of Mediator	Source	Action
Histamine	Primary	Granule	Increases vascular permeability; vasodilation; smooth muscle contraction of bronchi; increases mucus production
Heparin	Primary	Granule (of C.T. mast cells)	Anticoagulant; binds to and inactivates histamine
Chondroitin sulfate	Primary	Granule (of mucosal mast cells)	Binds to and inactivates histamine
Aryl sulfatase	Primary	Granule	Inactivates leukotriene C_4, thus limiting the inflammatory response
Neutral proteases	Primary	Granule	Protein cleavage to activate complement (especially C3a); increases inflammatory response
Eosinophil chemotactic factor	Primary	Granule	Attracts eosinophils to site of inflammation
Neutrophil chemotactic factor	Primary	Granule	Attracts neutrophils to site of inflammation
Leukotrienes C_4, D_4, and E_4	Secondary	Membrane lipid	Vasodilator; increases vascular permeability; bronchial smooth muscle contractant
Prostaglandin D_2	Secondary	Membrane lipid	Causes contraction of bronchial smooth muscle; increases mucus secretion; vasoconstriction
Thromboxane A_2	Secondary	Membrane lipid	Causes platelet aggregation; vasoconstriction
Bradykinins	Secondary	Formed by activity of enzymes located in granules	Causes vascular permeability and is responsible for pain sensation
Platelet-activating factor	Secondary	Activated by phospholipase A_2	Attracts neutrophils and eosinophils; causes vascular permeability and contraction of bronchial smooth muscle

Sequence of Events in the Inflammatory Response

1. **Histamine** causes vasodilation and increases the vascular permeability of blood vessels in the vicinity. It also causes bronchiospasm and increases mucus production in the respiratory tract.
2. Complement components leak out of blood vessels and are cleaved by **neutral proteases** to form additional agents of inflammation.
3. **ECF** attracts eosinophils to the site of inflammation. These cells phagocytose antigen–antibody complexes, destroy any parasites present, and limit the inflammatory response.
4. **NCF** attracts neutrophils to the site of inflammation. These cells phagocytose and kill microorganisms, if present.
5. **Leukotrienes C_4, D_4,** and **E_4** increase vascular permeability and cause bronchiospasms. They are several thousand times more potent than histamine in their vasoactive effects.
6. **Prostaglandin D_2** causes bronchiospasm and increases secretion of mucus by the bronchial mucosa.
7. **PAF** (platelet activating factor) causes greater vascular permeability.
8. **Thromboxane A_2** is a vigorous platelet aggregating mediator and also causes vasoconstriction. It is quickly transformed into **thromboxane B_2**, its inactive form.
9. **Bradykinin** is a powerful vascular dilator that causes vascular permeability. It is also responsible for pain.

Macrophages

Macrophages belong to the mononuclear phagocytic system and are subdivided into two groups of cells: phagocytes and antigen-presenting cells.

As noted earlier, some macrophages behave as fixed cells and some as transient cells. Because macrophages are active phagocytes, they function in removing cellular debris and in protecting the body against foreign invaders.

Macrophages measure about 10 to 30 µm in diameter and are irregularly shaped (Fig. 6–11). Their cell surface is uneven, varying from short, blunt projections to finger-like filopodia. More active macrophages have pleats and folds in their plasma membranes as a consequence of cell movement and phagocytosis. Their cytoplasm is basophilic and contains many small vacuoles and small dense granules. The eccentric nucleus of macrophages is smaller and more darkly stained than

CLINICAL CORRELATIONS

1. People with **hay fever** attacks experience the effects of **histamine** being released by the mast cells of the nasal mucosa, which causes localized edema from increased permeability of the small blood vessels. The swelling of the mucosa results in feeling "stuffed up" and hinders breathing.
2. People with **asthma** attacks have difficulty breathing as a result of bronchiospasm caused by **leukotrienes** released in the lungs.
3. Because degranulation of mast cells usually is a localized phenomenon, the typical inflammatory response is mild and site specific. However, a risk also exists for **hyperallergic persons** who may experience a systemic and severe immediate hypersensitivity reaction (**systemic anaphylaxis**) following a secondary exposure to an allergen (i.e., insect stings, antibiotics, or other antigens). This reaction (**anaphylactic shock**) may occur within seconds to a few minutes, including shortness of breath, decreasing blood pressure, and the symptoms of shock, which may result in the person's death (in a matter of a few hours) if left untreated. Persons susceptible to this condition often wear a medical emergency bracelet informing the first responder giving assistance to get him or her immediate medical attention.
4. **Mastocytosis** is a rare condition in which the patient possesses too many mast cells. The most common form of this disease is a skin disorder, known as ***urticaria pigmentosa***, a condition limited to the skin in neonates that exhibits itself, upon irritation, as hives or splotchy reddish-brown discoloration of the skin. This condition is caused by an autosomal point mutation that alters a transmembrane protein on mast cell precursors, which become hypersensitive to the chemokine, known as *mast cell growth factor*. This hypersensitivity to mast cell growth factor greatly enhances the proliferative ability of these cells, resulting in an increased number of mast cells in the dermis.

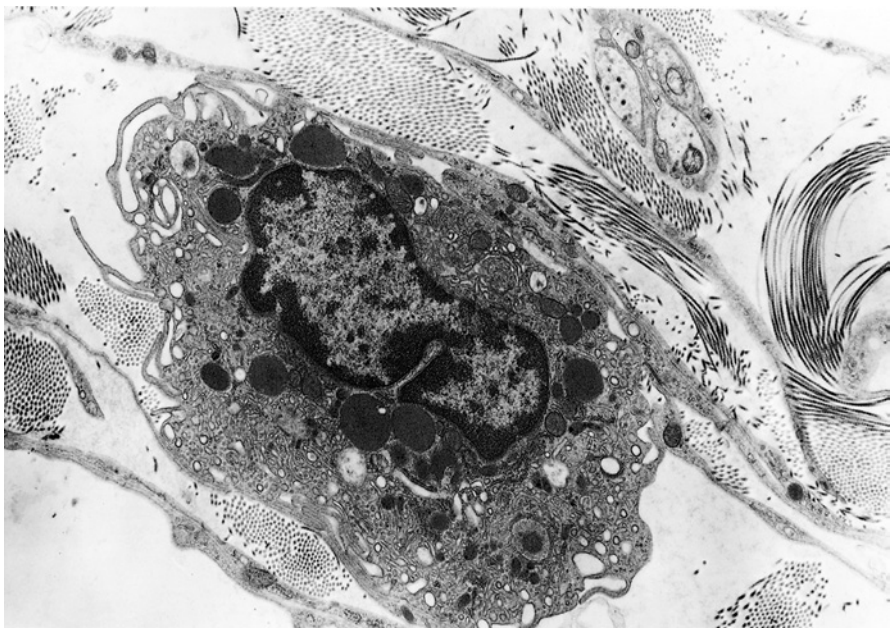


Figure 6–11 Electron micrograph of a macrophage in the rat epididymis. (From Flickinger CJ, Herr CJ, Sisak JR, Howards SS. Ultrastructure of epididymal interstitial reactions following vasectomy and vasovasostomy. *Anat Rec.* 1993;235:61-73. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

that of fibroblasts, and it usually does not display nucleoli. The macrophage nucleus is somewhat distinctive, in that it is ovoid and usually indented on one side, so that it resembles a kidney. Electron microscopic studies demonstrate a well-developed Golgi apparatus, prominent RER, and an abundance of lysosomes, which appear as small, dense granules in light micrographs.

As young macrophages mature, they increase in size, and there are concomitant increases in RER profiles,

Golgi complex, microtubules, lysosomes, microfilaments, and protein synthesis.

Macrophage Development and Distribution

Histologists once believed that macrophages were derived from precursor cells in the **reticuloendothelial system**, which included nonphagocytic cells such as reticulocytes. This classification has been replaced with the **mononuclear phagocyte system.** All members of the mononuclear phagocyte system arise from a common stem cell in the bone marrow, possess lysosomes, are capable of phagocytosis, and display FcεRI receptors and receptors for complement.

Monocytes develop in the bone marrow and circulate in the blood. At the proper signal, they leave the bloodstream by migrating through the endothelium of capillaries or venules. In the connective tissue compartment, they mature into macrophages, which normally have a life span of about 2 months. Macrophages arise from monocytes, activated by the macrophage colony stimulating factor (see Chapter 10 and Table 10–6.)

Macrophages localized in certain regions of the body were given specific names before their origin was completely understood. Thus, **Kupffer cells** of the liver (Fig. 6–12), **dust cells** of the lung, **Langerhans cells** of the skin, **monocytes** of the blood, and **macrophages** of the connective tissue, spleen, lymph nodes, thymus, and bone marrow are all members of the mononuclear phagocyte system and possess similar morphology and functions. Additionally, **osteoclasts** of bone and **microglia** of the brain, although morphologically different, belong to the mononuclear phagocyte system.

Under chronic inflammatory conditions, macrophages congregate, greatly enlarge, and become polygonal **epithelioid cells.** When the particulate matter to be disposed of is excessively large, several to many macrophages may fuse to form a **foreign-body giant cell**, a giant multinucleated macrophage.

Macrophages residing in the connective tissues were previously called ***fixed macrophages***, and those that developed as a result of an exogenous stimulus and migrated to the particular site were called ***free macrophages.*** These names have been replaced by the more descriptive terms ***resident macrophages*** and ***elicited macrophages***, respectively.

Macrophage Function

Macrophages phagocytose foreign substances and damaged and senescent cells as well as cellular debris; they also assist in the initiation of the immune response.

Macrophages phagocytose senescent, damaged, and dead cells and cellular debris and digest the ingested material through the action of hydrolytic enzymes in their lysosomes (see Chapter 2). Macrophages also assist in defense of the body by phagocytosing and destroying foreign substances, including microorganisms. During the immune response, factors released by lymphocytes activate macrophages, increasing their

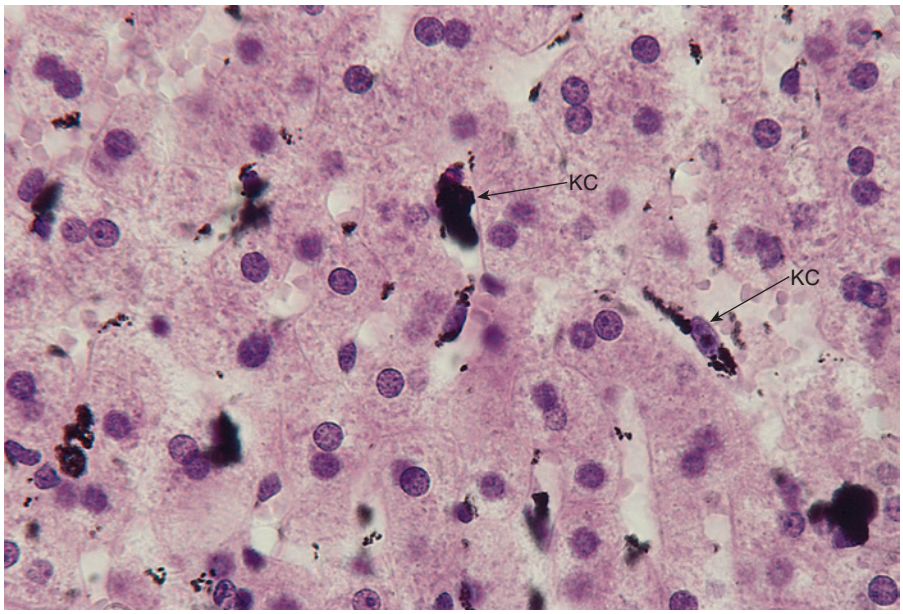


Figure 6–12 Light micrograph of liver of an animal injected with India ink demonstrating the presence of cells known as Kupffer cells (KC) that preferentially phagocytose the ink (×540).

phagocytic activity. **Activated macrophages** vary considerably in shape, possess microvilli and lamellipodia, and exhibit more locomotion compared with inactivated macrophages. Macrophages also play a key role in presenting antigens to lymphocytes and are therefore also known as ***antigen-presenting cells***. They do this by phagocytosing foreign proteins; cleaving them into short peptide sequences, known as *epitopes*; complexing these epitopes with their major histocompatibility complex molecules (major histocompatibility complex [MHC] I or MHC II); placing them on their cell membranes; and presenting them to T cells (see Chapter 12 for a more complete description of this process).

Transient Connective Tissue Cells

All of the transient connective tissue cells are derived from precursors in the bone marrow (see Fig. 6–1). All of these cells are discussed in more detail in other chapters.

Plasma Cells

Plasma cells are derived from B lymphocytes and manufacture antibodies.

Although **plasma cells** are scattered throughout the connective tissues, they are present in greatest numbers in areas of chronic inflammation and where foreign substances or microorganisms have entered the tissues. These differentiated cells, which are derived from B lymphocytes that have interacted with antigen, produce and secrete antibodies and are responsible for **humorally mediated immunity** (see Chapters 10 and 12). Plasma cells are large, ovoid cells, 20 µm in diameter, with an eccentrically placed nucleus that have a relatively short life span of 2 to 3 weeks. Their cytoplasm is intensely basophilic as a result of a well-developed RER with closely spaced cisternae (Fig. 6–13). Only a few mitochondria are scattered between the profiles of RER. Electron micrographs also display a large juxtanuclear Golgi complex and a pair of centrioles (Figs. 6–14 and 6–15). These structures are located in the pale-staining regions adjacent to the nucleus in light micrographs. The spherical nucleus possesses heterochromatin radiating out from the center, giving it a characteristic "clock face" or "spoke wheel" appearance under the light microscope.

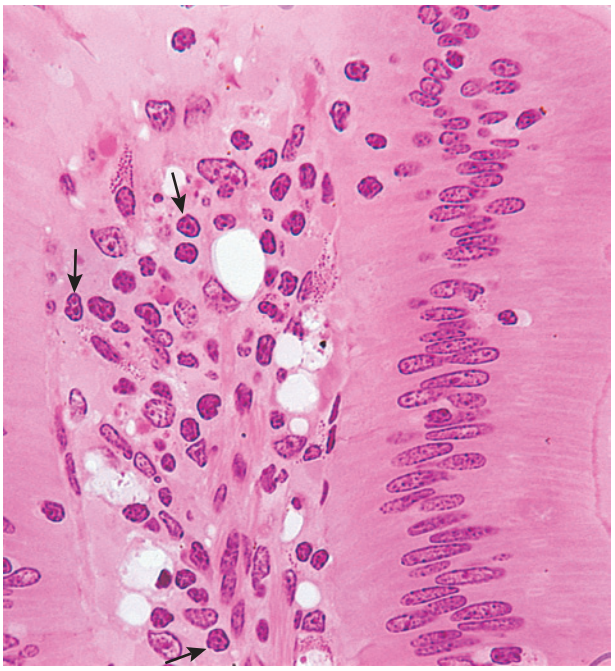


Figure 6–13 Light micrograph of plasma cells in the lamina propria of the monkey jejunum (×540). Observe the "clock face" nucleus *(arrows)* and clear perinuclear zone.

Leukocytes

Leukocytes exit the bloodstream during inflammation, invasion by foreign elements, and immune responses to perform various functions.

Leukocytes are white blood cells that circulate in the bloodstream. However, they frequently migrate through the capillary walls to enter the connective tissues, especially during inflammation, when they carry out various functions. See Chapter 10 for more comprehensive discussions and the summary in Table 10–3.

Monocytes is discussed in the section on macrophages.

Neutrophils phagocytose and digest bacteria in areas of acute inflammation, resulting in formation of **pus**, an accumulation of dead neutrophils and debris. See Chapter 10 for a more detailed discussion.

Eosinophils, similar to neutrophils, are attracted to areas of inflammation by leukocyte chemotactic factors. Eosinophils combat parasites by releasing cytotoxins. They also are attracted to sites of allergic inflammation, where they moderate the allergic reaction and phagocytose antibody-antigen complexes. See Chapter 10 for a more detailed discussion.

Basophils (similar to mast cells) release preformed and newly synthesized pharmacological agents that initiate, maintain, and control the inflammatory process. See Chapter 10 for a more detailed discussion.

Lymphocytes (T lymphocytes, B lymphocytes, natural killer cells) are present only in small numbers in most connective tissue, except at sites of chronic inflammation, where they are abundant. Chapter 10 describes leukocytes in more detail, and Chapter 12 discusses lymphocytes in their roles as immune cells.

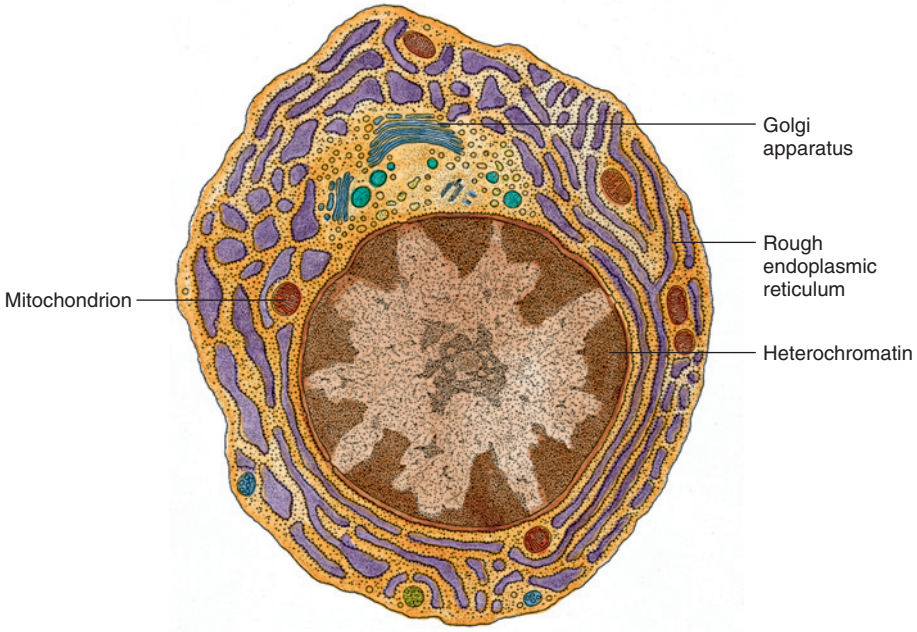


Figure 6–14 Drawing of a plasma cell from an electron micrograph. The arrangement of heterochromatin gives the nucleus the "clock face" appearance. (From Lentz TL. *Cell Fine Structure: an Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

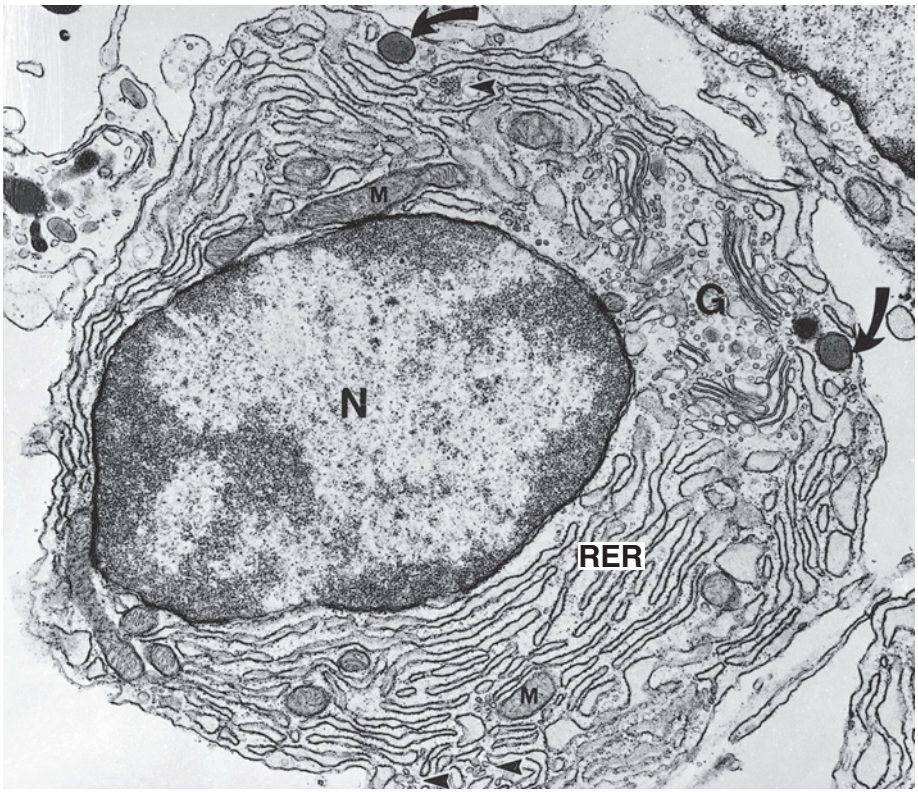


Figure 6–15 Electron micrograph of a plasma cell from the lamina propria of the rat duodenum displaying abundant rough endoplasmic reticulum (RER) and prominent Golgi complex (×10300). *Arrowheads* represent small vesicles; *arrows* represent dense granules. G, Golgi apparatus; M, mitochondria; N, nucleus. (From Rambourg A, Clermont Y, Hermo L, Chretien M. Formation of secretion granules in the Golgi apparatus of plasma cells in the rat. *Am J Anat.* 1988;184:52-61. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

TABLE 6–2 Classification of Connective Tissues

A. Embryonic connective tissues
   1. Mesenchymal connective tissue
   2. Mucous connective tissue
B. Connective tissue proper
   1. Loose (areolar) connective tissue
   2. Dense connective tissue
      a. Dense irregular connective tissue
      b. Dense regular connective tissue
         • Collagenous
         • Elastic
   3. Reticular tissue
   4. Adipose tissue
C. Specialized connective tissue
   1. Cartilage
   2. Bone
   3. Blood

CLASSIFICATION OF CONNECTIVE TISSUE

As noted earlier, connective tissue is classified into connective tissue proper—the major subject of this chapter—and specialized connective tissue, embracing cartilage, bone, and blood. The third recognized category of connective tissue is **embryonic connective tissue**. Table 6–2 summarizes the major classes of connective tissue and their subclasses.

Embryonic Connective Tissue

Embryonic connective tissue includes both mesenchymal tissue and mucous tissue.

Mesenchymal connective tissue is present only in the embryo and consists of mesenchymal cells in a gel-like, amorphous ground substance containing scattered reticular fibers. **Mesenchymal cells** possess an oval nucleus exhibiting a fine chromatin network and prominent nucleoli. The sparse, pale-staining cytoplasm extends small processes in several directions. Mitotic figures are frequently observed in mesenchymal cells because they give rise to most of the cells of loose connective tissue. It is generally believed that most, if not all, of the mesenchymal cells, once scattered throughout the embryo, are eventually depleted and do not exist as such in the adult, except in the pulp of teeth. In adults, however, pluripotential pericytes, which reside along capillaries, can differentiate into certain other cells of connective tissue.

Mucous tissue is a loose, amorphous connective tissue exhibiting a jelly-like matrix primarily composed of hyaluronic acid and sparsely populated with type I and type III collagen fibers and fibroblasts. This tissue, also known as ***Wharton jelly***, is found only in the umbilical cord and subdermal connective tissue of embryos.

Connective Tissue Proper

The four recognized types of connective tissue proper (**loose**, **dense**, **reticular connective tissues**, and **adipose tissue**) differ in their histology, location, and functions**.**

Loose (Areolar) Connective Tissue

Loose (areolar) connective tissue is composed of a loose arrangement of fibers and dispersed cells embedded in a gel-like ground substance.

Loose connective tissue, also known as ***areolar connective tissue***, fills in the spaces of the body just deep to the skin, lies below the mesothelial lining of the internal body cavities, is associated with the adventitia of blood vessels, and surrounds the parenchyma of glands. The loose connective tissue of mucous membranes (as in the alimentary canal) is called the ***lamina propria***.

Loose connective tissue is characterized by abundant **ground substance** and extracellular fluid (tissue fluid) housing the fixed connective tissue cells: **fibroblasts**, **adipose cells**, **macrophages**, and **mast cells** as well as some **undifferentiated cells.** Also scattered throughout the ground substance are loosely woven **collagen**, **reticular**, and **elastic fibers.** Coursing in this amorphous tissue are small nerve fibers as well as blood vessels, which supply the cells with oxygen and nutrients.

Because this tissue lies immediately beneath the thin epithelia of the digestive and respiratory tracts, it is here that the body first attacks antigens, bacteria, and other foreign invaders. Therefore, loose connective tissue contains many transient cells responsible for inflammation, allergic reactions, and the immune response. These cells, which originally circulate in the bloodstream, are released from blood vessels in response to an inflammatory stimulus. Pharmacological agents released by mast cells increase the permeability of small vessels so that excess plasma enters the loose connective tissue spaces, causing it to become swollen.

Dense Connective Tissue

Dense connective tissue contains a greater abundance of fibers and fewer cells than loose connective tissue.

Dense connective tissue contains most of the same components found in loose connective tissue, except that it has many more fibers and fewer cells. The orientation and the arrangements of the bundles of collagen fibers in this tissue make it resistant to stress. When the collagen fiber bundles are arranged randomly, the tissue is called ***dense irregular connective tissue.*** When

CLINICAL CORRELATIONS

1. Under normal circumstances, extracellular fluid returns to the blood capillaries or enters lymph vessels to be returned to the blood. A potent and prolonged inflammatory response, however, causes accumulation of excess tissue fluid within loose connective tissue beyond what can be returned via the capillaries and lymph vessels. This results in gross swelling, or **edema**, in the affected area. Edema can result from excessive release of histamine and leukotrienes C_4 and D_4, which all increase capillary permeability, as well as from the obstruction of venous or lymphatic vessels.
2. **Sarcomas**, unlike carcinomas that have epithelial origins, are cancers that arise from cells of the connective tissues, that is, from cells whose origins were mesenchymal cells. Therefore, sarcomas include cancers of bone, or osteosarcomas; cancers of muscle, that is, leiomyosarcomas and rhabdomyosarcomas; cancers of cartilage, chondrosarcomas; cancers of adipose cells, liposarcomas; cancers of fibrous connective tissues, fibrosarcomas; as well as cancers of the endothelial cells of blood vessels, angiosarcomas.

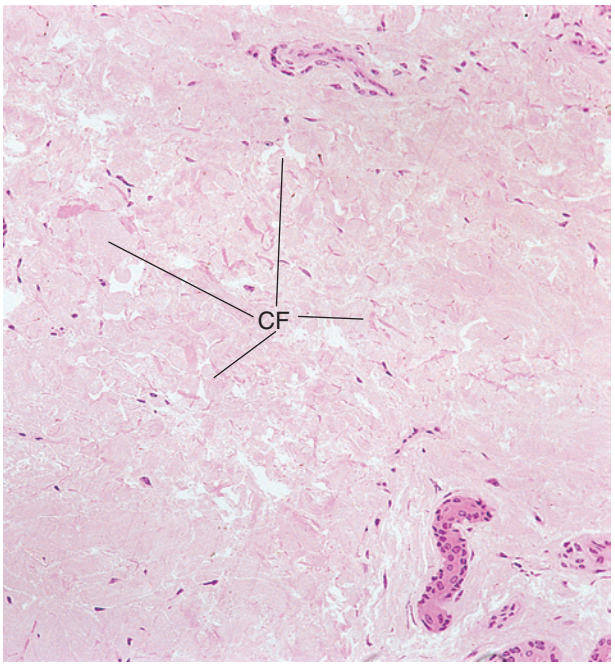


Figure 6–16 Light micrograph of dense, irregular, collagenous connective tissue from monkey skin (×132). Observe the many bundles of collagen (CF) in random orientation.

fiber bundles of the tissue are arranged in parallel or organized fashion, the tissue is called ***dense regular connective tissue***, which is divided into **collagenous** and **elastic** types.

Dense irregular connective tissue contains mostly coarse collagen fibers interwoven into a meshwork that resists stress from all directions (Fig. 6–16). The collagen bundles are packed so tightly that space is limited for ground substance and cells. Fine networks of elastic fibers are often scattered about the collagen bundles. Fibroblasts, the most abundant cells of this tissue, are located in the interstices between collagen bundles. Dense irregular connective tissue constitutes the dermis of skin; the sheaths of nerves; and the capsules of spleen, testes, ovary, kidney, and lymph nodes.

Dense regular collagenous connective tissue is composed of coarse collagen bundles densely packed and oriented into parallel cylinders or sheets that resist tensile forces (Fig. 6–17). Because of the tight packing of the collagen fibers, little space can be occupied by ground substance and cells. Thin, sheet-like fibroblasts are located between bundles of collagen with their long axes parallel to the bundles. Tendons (Figs. 6–17 and 6–18), ligaments, and aponeuroses are examples of dense regular collagenous connective tissue.

Dense regular elastic connective tissue possesses coarse branching elastic fibers with only a few collagen fibers forming networks. Scattered throughout the interstitial spaces are fibroblasts. The elastic fibers

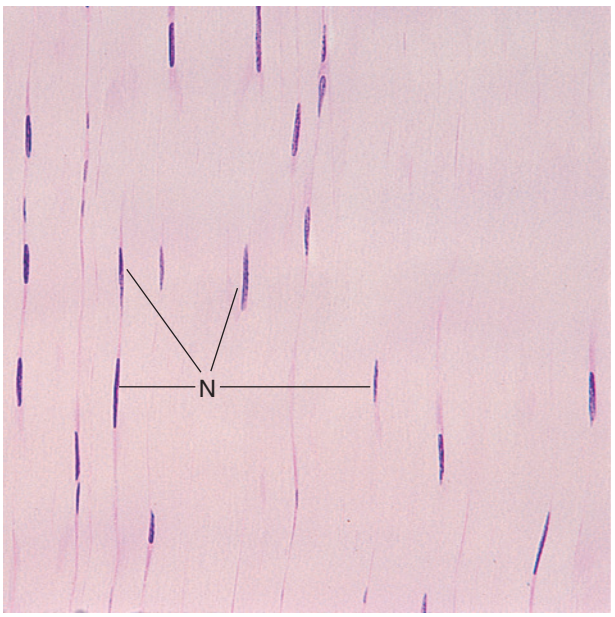


Figure 6–17 Light micrograph of dense regular collagenous connective tissue from monkey tendon (×270). Note the ordered, parallel array of collagen bundles and the elongated nuclei (N) of the fibroblasts lying between collagen bundles.

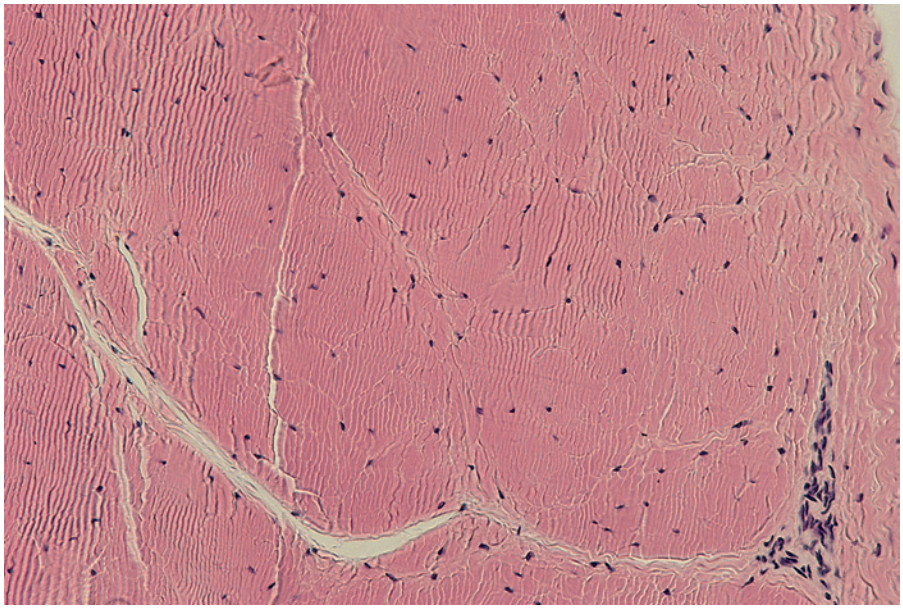


Figure 6–18 Light micrograph of a cross section of monkey tendon. The scattered small black structures represent nuclei of fibroblasts (×270).

are arranged parallel to one another and form either thin sheets or fenestrated membranes. The latter are present in large blood vessels, ligamenta flava of the vertebral column, and the suspensory ligament of the penis.

Reticular Tissue

Type III collagen is the major fiber component of **reticular tissue.** The collagen fibers form mesh-like networks interspersed with fibroblasts and macrophages (Fig. 6–19). It is the fibroblasts that synthesize the type III collagen. Reticular tissue forms the architectural framework of liver sinusoids, adipose tissue, bone marrow, lymph nodes, spleen, smooth muscle, and the islets of Langerhans.

Adipose Tissue

Adipose tissue is classified into two types according to whether it is composed of **unilocular** or **multilocular** adipocytes. Other differences between the two types of adipose tissue are color, vascularity, and metabolic activity.

White (Unilocular) Adipose Tissue

Each **unilocular** fat cell contains a single lipid droplet, giving the adipose tissue composed of such cells a white color. (In a person whose diet is especially rich in foods containing carotenoids, such as carrots, this adipose tissue is yellow.) **White adipose tissue** is heavily

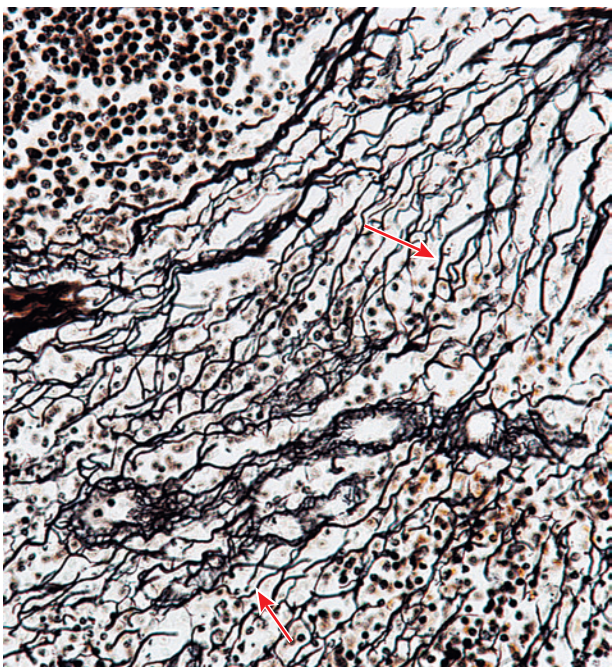


Figure 6–19 Light micrograph of reticular tissue (stained with silver) displaying the networks of reticular fibers (×270). Many lymphoid cells are interspersed between the reticular fibers *(arrows)*.

supplied with blood vessels, which form capillary networks throughout the tissue, and with nerve fibers. The blood vessels and nerve fibers gain access via connective tissue **septa** that partition the fat into **lobules** that resemble clusters of grapes closely packed together (see Fig. 6–6). Slender connective tissue elements, derived from the septa, surround each adipocyte and bring capillaries and nerve fibers into the immediate vicinity of the cells. The plasma membranes of the unilocular adipose cells contain receptors for several substances, including **insulin**, **growth hormone**, **norepinephrine**, and **glucocorticoids**, that facilitate the uptake and release of free fatty acids and glycerol, thereby regulating the metabolic state of the body's fatty acid levels. Additionally, white adipose tissue also produces endocrine hormones. Adipocyte constitute only about half of the cells in adipose tissue; the remainder of the cells are composed of macrophages, mast cells, fibroblasts, and lymphoid elements that reside in the connective tissue **(stroma)**.

Unilocular fat is present in the subcutaneous layers throughout the body. It also occurs in masses in characteristic sites influenced by sex and age. In men, fat is stored in the neck, in the shoulders, about the hips, and in the buttocks. As men age, the abdominal wall becomes an additional storage area. In women, fat is stored in the breasts, buttocks, hips, and lateral aspects of the thighs. Additionally, fat is stored in both sexes in the abdominal cavity about the omental apron and the mesenteries.

Storage and Release of Fat by Adipose Cells

During digestion, fats are broken down in the duodenum by **pancreatic lipase** into **fatty acids** and **glycerol**. The intestinal epithelium absorbs these substances and reesterifies them in the SER to **triglycerides**, which then are surrounded by proteins to form **chylomicrons**. Chylomicrons are released into the extracellular space at the basolateral membranes of the surface absorptive cells, enter the lacteals of the villus, and are carried by the lymph to the bloodstream. Additionally, very-low-density lipoprotein (VLDL), which is synthesized by the liver, and albumin-bound fatty acids are present in the bloodstream.

Once in the capillaries of adipose tissue, VLDL, fatty acids, and chylomicrons are exposed to **lipoprotein lipase** (manufactured and released by fat cells and delivered to blood vessels), which breaks them down into free fatty acids and glycerol (Fig. 6–20). The fatty acids enter the connective tissue and diffuse through the cell membranes of adipocytes. These cells then combine their own glycerol phosphate with the imported fatty acids to form triglycerides, which are added to the forming lipid droplets within the adipocytes until needed. Adipose cells can convert glucose and amino acids into fatty acids when stimulated by insulin.

Norepinephrine is released from nerve endings of postganglionic sympathetic neurons in the vicinity of fat cells. Also, during strenuous exercise, **epinephrine** and **norepinephrine** are released from the suprarenal medulla. These two hormones bind to their respective receptors of the adipocyte plasmalemma, activating **adenylate cyclase** to form **cAMP**, a second messenger, resulting in activation of two enzymes, **adipose triglyceride lipase (ATL)**, which converts triglycerides to diglycerides, and **hormone-sensitive lipase (HSL)**, which completes the hydrolysis, forming glycerol and fatty acids. It appears that in primates, including humans, **natriuretic peptides**, formed by cardiac muscle cells, can also induce ATL and HSL activities. The fatty acids and glycerol leave the adipocyte, enter the ECM, and, once there, gain entry into the bloodstream.

Adipokines Produced by White Adipose Tissue

As indicated previously, adipose tissue plays a major role in controlling the body's fatty acid equilibrium. When food is readily available, the body stores the bulk of its free fatty acids as triglycerides in fat cells of adipose tissue, and, when food is not available, the triglyceride depot is degraded into fatty acids and glycerol and is released for the body's use. This control mechanism is performed by macromolecules known as ***adipokines***, although some of these molecules *do not* act on the immune system and are probably better designated as **hormones**. However, the collective term *adipokine* will be used here for all of these macromolecules whether or not they act as hormones. Not all adipokines are produced by adipose cells; some are released by macrophages that reside in the stroma of adipose tissue. Adipocytes manufacture and release leptin, adiponectin, and **retinol-binding protein-4 (RBP-4)**, whereas resistin, TNF-α, and interleukin-6 are derived from macrophages. Interestingly, chronic inflammation is always present in the adipose tissues of obese individuals, and the inflammatory agents that are released act as chemoattractants that result in the influx of macrophages.

A number of additional factors have been discovered, such as visfatin, apelin, and chemerin, but their roles in fatty acid homeostasis are not yet understood and will not be discussed here.

Leptin

Leptin serum levels, a protein that is manufactured and released by adipocytes, evidence a diurnal rhythm, in that it is lowest in the afternoon and highest in the midmorning. Leptin acts to reduce appetite by binding to **neuropeptide Y**, inhibiting this signaling molecule from triggering the appetite control center of the hypothalamus to induce the feeling of hunger. Instead, leptin binds to leptin receptors of the hypothalamus to induce

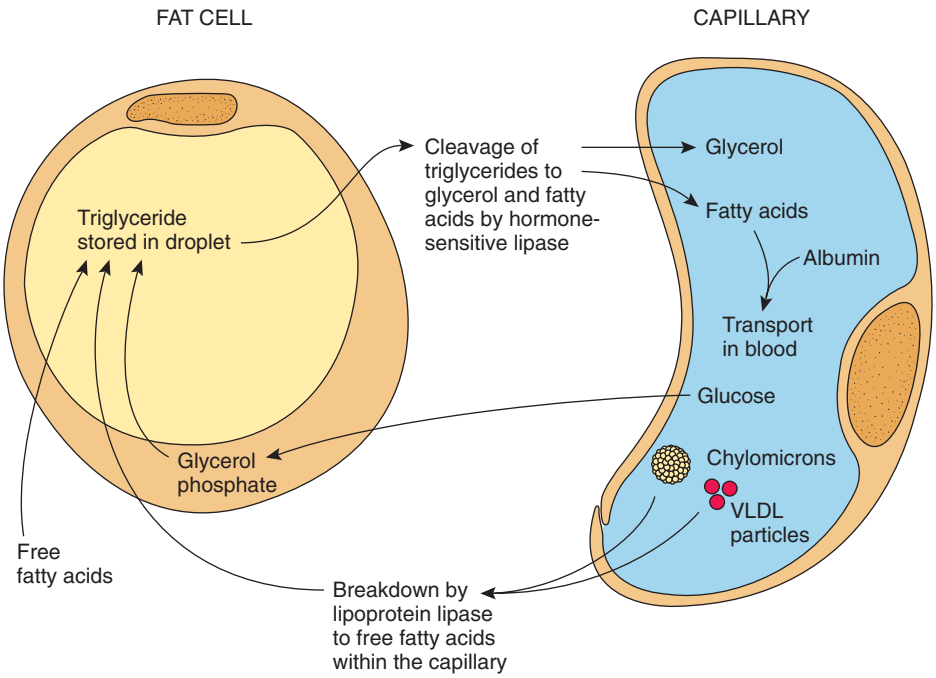


Figure 6–20 Schematic diagram of the transport of lipid between a capillary and an adipocyte. Lipids are transported in the bloodstream in the form of chylomicrons and very-low-density lipoproteins (VLDLs). The enzyme lipoprotein lipase, manufactured by the fat cell and transported to the capillary lumen, hydrolyzes the lipids to fatty acids and glycerol. Fatty acids diffuse into the connective tissue of the adipose tissue and into the lipocytes, where they are reesterified into triglycerides for storage. When required, triglycerides stored within the adipocyte are hydrolyzed by *hormone-sensitive lipase* into fatty acids and glycerol. These then enter the connective tissue spaces of adipose tissue and from there into a capillary, where they are bound to albumin and transported in the blood. Glucose from the capillary can be transported to adipocytes, which can manufacture lipids from carbohydrate sources.

ATL and HSL to breakdown triglycerides and release fatty acids from the adipocytes. Leptin also induces an increased level of **α-melanocyte-stimulating hormone**, which also acts on the appetite control center to suppress appetite. Interestingly, even though obese individuals have a much higher leptin serum concentration than lean individuals that should decrease their appetite, there appears to be a **leptin resistance** in obese people, and they do not respond to the higher leptin levels.

CLINICAL CORRELATION

There appears to be a genetic basis in some cases of obesity. Mutations in the gene responsible for the coding for **leptin** produce an inactive form of that hormone. Because leptin regulates the appetite center of the hypothalamus, people who either do not produce leptin or who produce a biologically inactive form of this hormone have a voracious appetite, leading to an almost uncontrollable weight gain.

Adiponectin

Adiponectin is also a protein hormone produced by adipocytes, and it circulates in the blood in various combinations as multimers, that is, individual adiponectin molecules form small weight trimers to an association of as many as 18 individual molecules, forming heavy octadecamers. The plasma concentration of adiponectin is inversely proportional to the amount of adipose tissue an individual possesses. Obese individuals have low plasma levels of adiponectin, whereas lean individuals have high plasma adiponectin levels. Adiponectin octadecamers increase the **insulin sensitivity** of skeletal muscle cells, thereby causing these cells to internalize glucose and oxidize fatty acids. Moreover, adiponectin octadecamers activate hepatic **adenosine monophosphate (AMP)-activated protein kinase**, which represses glucose release and increases gluconeogenesis by the liver. Additionally, adiponectin trimers act on the appetite control center of the hypothalamus to repress hunger.

Retinol-Binding Protein-4

RBP-4, a protein product of adipocytes, interferes with the ability of insulin to effect glucose uptake by skeletal muscle cells and to amplify glucose production and release by hepatocytes. Obese individuals possess increased **insulin resistance** believed to be effected by RBP-4, and this adipokine may be partly responsible for the presence of chronic inflammation and fatty liver disease in these individuals.

Resistin

Resistin is a product of macrophages in humans (although it is synthesized by adipocytes in rodents). This peptide hormone, similar to RBP-4, is partly responsible for the establishment of **insulin resistance** in obese individuals. This peptide is similar in structure to adiponectin, and it also circulates in the multimeric form, where six monomers join each other to form hexamers. Resistin represses hepatic **AMP-activated protein kinase**, thereby elevating glucose release by the liver, resulting in hyperglycemia and contributing to obesity, chronic inflammation, and type II diabetes mellitus.

Tumor Necrosis Factor-α

TNF-α, a product of macrophages in the stroma of adipose tissue of obese individuals, has two major functions in the body's fatty acid homeostasis. It is the primary cause of insulin resistance, and it also interferes with fatty acid oxidation by hepatocytes.

Interleukin-6

Interleukin-6, another product of macrophages in the stroma of adipose tissue of obese individuals, also has two functions in the body's fatty acid homeostasis. It also contributes to insulin resistance and induces glucose uptake and fatty acid oxidation by skeletal muscle cells.

Table 6–3 shows the characteristics of adipokines of human white adipose tissue.

Brown (Multilocular) Adipose Tissue

Brown adipose tissue (brown fat) is composed of **multilocular** fat cells, which store fat in multiple droplets. This tissue may appear tan to reddish brown because of its extensive vascularity and the cytochromes present in its abundant mitochondria (see Fig. 6–7).

Multilocular adipose tissue has a lobular organization and vascular supply similar to those of a gland. Brown fat tissue is very vascular because the vessels are located near the adipocytes. Unmyelinated nerve fibers enter the tissue, with the axons ending on the blood vessels as well as on fat cells, whereas in white fat tissue, the neurons end on the blood vessels only.

Although it has long been known that multilocular fat is found in many mammalian species, especially those that hibernate, and in the infants of most mammals, it was unclear whether multilocular fat exists in adult humans. However, in newborn humans, brown fat is located in the neck region and interscapular region. As humans mature, the fat droplets in brown fat cells coalesce and form into one droplet (similar to the droplets in white fat cells), and the cells become more similar to those in unilocular fat tissue. Thus, although adults appear to contain unilocular fat only, there is evidence that they also possess brown fat. This feature can be demonstrated in some of the wasting diseases of older people, in which multilocular fat tissue forms again and in the same areas as in a newborn.

CLINICAL CORRELATIONS

1. Obesity increases the risks for many health problems, including non–insulin-dependent diabetes mellitus, as well as problems involving the cardiovascular system. In adults, obesity develops in two ways. **Hypertrophic obesity** results from the accumulation and storage of fat in unilocular fat cells, which may increase their size by as much as four times. **Hypercellular obesity**, as the name implies, results from an overabundance of adipocytes. This type of obesity usually is severe.

   Although mature adipocytes do not divide, their precursors proliferate in early postnatal life. There is substantial evidence that overfeeding newborn infants for a few weeks may actually increase the number of adipocyte precursors, leading to an increase in the number of adipocytes and setting the stage for hypercellular obesity in the adult. Overweight infants are at least three times more likely to exhibit obesity as adults than infants of average weight. Presently, it is understood that persons exhibiting severe obesity also exhibit an increase in the adipocyte population, although it is not understood how this recruitment is driven.
2. **Cachexia** is one of the wasting disorders that accompanies cancers of the digestive tract and displays severe loss of muscle and fat tissues, resulting in unmanageable weight loss and a high degree of mortality. Interestingly, the loss of fat tissue is due not to the death of white adipocytes but to the reduction of the fat droplet within each adipocyte. It is believed that this loss of lipids is due to increased lipase activity that depletes the triglyceride deposits. Apparently, the malignant cells release certain factors that result in cardiac insufficiency that may cause an increase in the release of natriuretic peptides that enhance the production of both ATL and HSL, resulting in the depletion of the triglyceride content of unilocular fat cells.

TABLE 6–3 Adipokines of Human White Adipose Tissue

Adipokine	Molecular Weight (kDa)*	Cells of Origin	Function in Fatty Acid Metabolism
Leptin	16	Adipocytes	Reduces appetite
Adiponectin	30	Adipocytes	Increases insulin sensitivity of skeletal muscle cells; decreases glucose release by the liver
Retinol-binding protein-4	21	Adipocytes	Increases insulin resistance; amplifies glucose production and release by the liver
Resistin	12.5	Macrophages	Increases insulin resistance; amplifies glucose production and release by the liver
Tumor necrosis factor-α	17	Macrophages	Primary cause of insulin resistance; interferes with fatty acid oxidation by hepatocytes
Interleukin-6	21	Macrophages	Increases insulin resistance; induces glucose uptake and fatty acid oxidation by skeletal muscle cells

*Monomeric form.

Brown adipose tissue is associated with production of body heat because of the large number of mitochondria in the multilocular adipocytes composing this tissue. These cells can oxidize fatty acids at up to 20 times the rate of white fat, increasing body heat production threefold in cold environments. Sensory receptors in the skin send signals to the temperature-regulating center of the brain, resulting in the relaying of sympathetic nerve impulses directly to the brown fat cells. The neurotransmitter norepinephrine activates the enzyme that cleaves triglycerides into fatty acids and glycerol, initiating heat production by oxidation of fatty acids in the mitochondria. **Thermogenin**, a transmembrane protein located on the inner membrane of mitochondria, permits backflow of protons instead of using them for synthesis of ATP; as a result of uncoupling oxidation from phosphorylation, the proton flow generates energy that is dispersed as heat.

Histogenesis of Adipose Tissue

It is believed that adipose cells are derived from undifferentiated embryonic stem cells that develop into **preadipocytes**, cells that, under the influence of a series of activating factors, differentiate into adipocytes.

The predominant view is that adipose tissue develops via two separate processes. In **primary fat formation**, which occurs early in fetal life, groups of **epithelioid precursor cells**, probably preadipocytes, are distributed at certain locations in the developing fetus; in these tissues, lipid droplets begin to accumulate in the form of brown adipose tissue. Near the end of fetal life, other **fusiform precursor cells** differentiate in many areas of the connective tissues within the fetus and begin to accumulate lipids that coalesce into the single droplet in each cell, thus forming the unilocular fat cells found in adults. The latter process has been named ***secondary fat formation.*** It should be understood, however, that brown adipose tissue is present in embryos, but white adipose tissue appears after birth only.

CLINICAL CORRELATIONS

1. Tumors of the adipose tissues may be benign or malignant. **Lipomas** are common benign tumors of adipocytes, whereas **liposarcomas** are malignant tumors of adipocytes. The latter form most commonly in the leg and in retroperitoneal tissues, although they may form anywhere in the body. The tumor cells may resemble either unilocular adipocytes or multilocular adipocytes, another indication that adult humans do indeed possess the two kinds of adipose tissue.
2. Brown fat has been demonstrated to be present in small quantities in adults in the neck as well as in the region of the clavicle. Interestingly, the amount of brown fat detected was inversely proportional to the degree to which an individual is overweight. That is, thin young men had more brown fat than did overweight young men.

PATHOLOGICAL CONSIDERATIONS

See Figures 6–21 and 6–22.

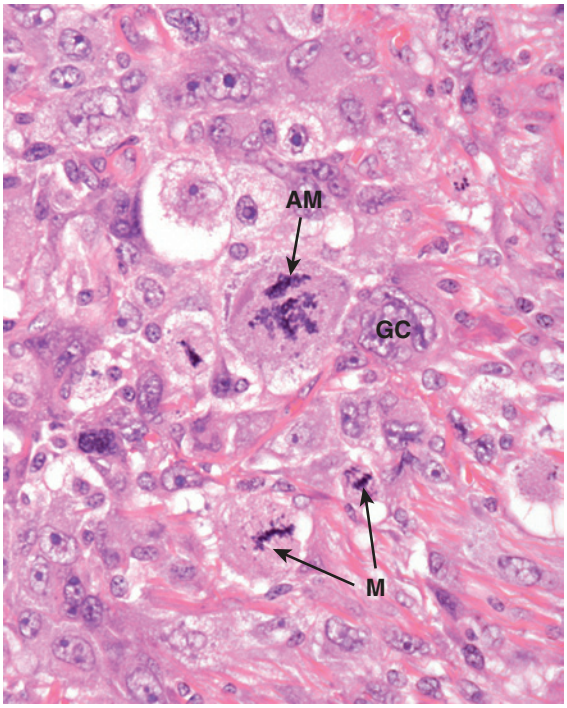


Figure 6–21 Photomicrograph of a highly pleomorphic sarcoma. Note the presence of some very large cells (GC) as well as numerous mitotic figures (M), some of which are atypical (AM). (From Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:295, with permission.)

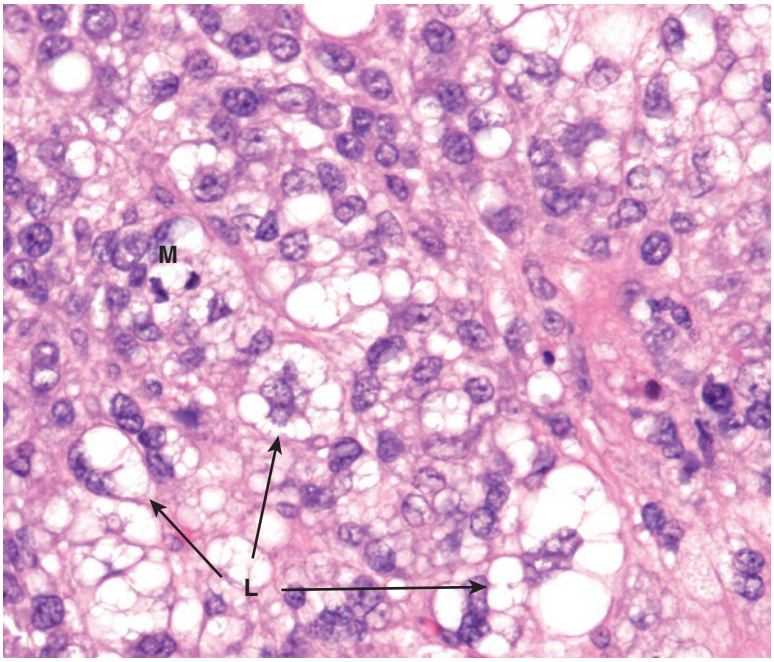


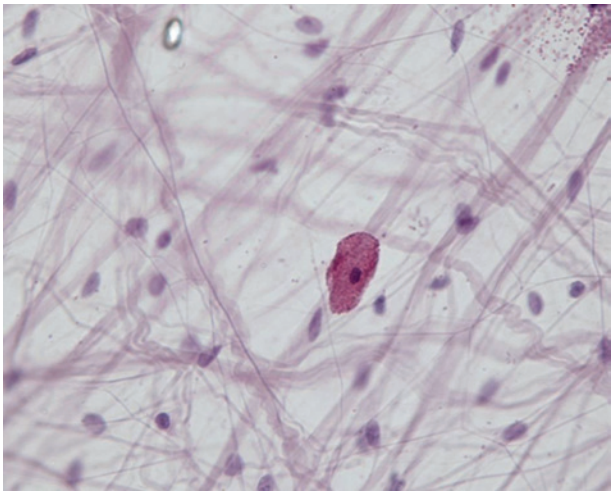
Figure 6–22 Liposarcomas are characterized by vacuolated tumor cells, known as lipoblasts (Ls). Note the presence of a cell undergoing mitosis (M). (From Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:294, with permission.)

REVIEW QUESTIONS

Questions 1–5 refer to the following scenario:
A patient goes to the emergency department because his arm started to swell up after being stung by a wasp.

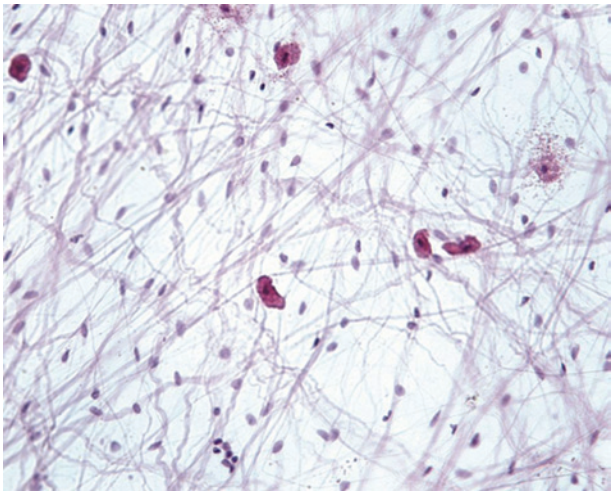
1. IgE receptors are present on which cells of this patient?
   a. eosinophils
   b. mast cells
   c. T lymphocytes
   d. plasma cells
   e. natural killer cells
2. Which of the following cells synthesized the immunoglobulin E (IgE) that occupied the IgE receptors?
   a. eosinophils
   b. monocytes
   c. T lymphocytes
   d. plasma cells
   e. natural killer cells
3. Prostaglandins released by the cells that possess IgE receptors were
   a. synthesized in the cytosol
   b. stored in vesicles
   c. synthesized from cholesterol
   d. synthesized from arachidonic acid precursors
   e. modified in the Golgi apparatus
4. The exocytosis of histamine from the granules of the cells that possessed IgE receptors was due to the increased intracellular concentration of calcium. The process of calcium release was initiated by a trigger mechanism that depended on the
   a. formation of IgE receptors
   b. activation of adenylate cyclase
   c. secretion of leukotrienes
   d. release of preformed mediators
   e. decrease in cyclic AMP levels
5. In order to eliminate possible parasites that entered the patient's connective tissue compartment when he was stung by the wasp, certain cells were recruited to the site of the lesion. Which of the following are those cells?
   a. eosinophils
   b. monocytes
   c. T lymphocytes
   d. plasma cells
   e. natural killer cells

6.



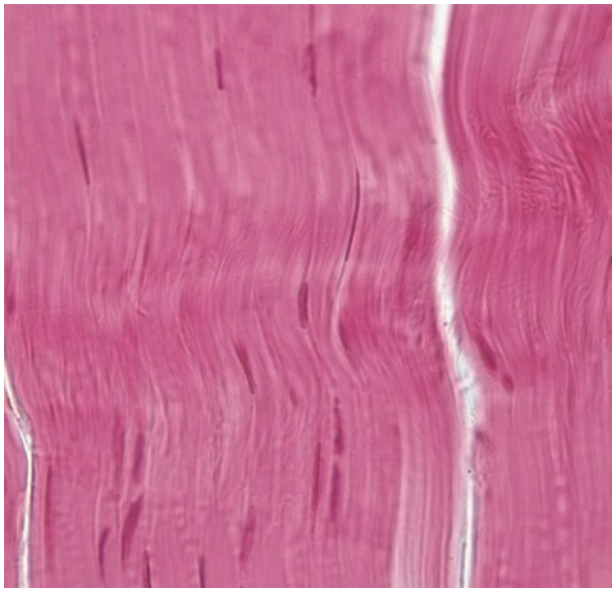
The large red cell in the middle of the field is responsible for which of the following conditions?
a. anaphylactic shock
b. osteoblastoma
c. leukemia
d. chondroblastoma
e. fibroma

7.



Classify the tissue presented in the accompanying photomicrograph.
a. dense regular collagenous connective tissue
b. dense irregular collagenous connective tissue
c. loose connective tissue
d. dense regular elastic connective tissue
e. mucoid connective tissue

8.



A patient enters the emergency department after she fell. The physician determined that she tore a tissue in her leg. The following photomicrograph represents the torn tissue. What did this patient tear?

a. dermis of the skin
b. epidermis of the skin
c. ligamentum nuchae
d. tendon
e. peripheral nerve bundle

9.

A patient enters the emergency department after she fell. The physician determined that she tore a tissue in her leg. The following photomicrograph represents the torn tissue. Identify the cells whose nuclei are visible in this photomicrograph.

a. fibroblasts
b. Schwann cells
c. smooth muscle cells
d. mesenchymal cells
e. macrophages

10. In some individuals undergoing a violent allergic response, the smooth muscles of the bronchioles may undergo prolonged contraction resulting in possible respiratory distress. Which of the following pharmacologic agent released by mast cells is responsible for contraction of the bronchiolar smooth muscles?

a. heparin
b. chondroitin sulfate
c. aryl sulfatase
d. prostaglandin
e. thromboxane A_2

ANSWERS

1. B. The cells that possess IgE receptors are mast cells and basophils. The other cells on the list possess various receptors but not receptors for IgE.
2. D. Plasma cells are derived from B lymphocytes, and they have a tremendous capacity for synthesizing immunoglobulins, including IgE. None of the other cells on the list are capable of manufacturing IgE.
3. D. Prostaglandins manufactured by mast cells were synthesized in situ from membrane arachidonic acid precursors. Prostaglandins were not synthesized in the cytosol from cholesterol or modified in the Golgi apparatus or stored in vesicles.
4. B. The release of histamine, one of the preformed mediators, from the granules of these cells was due to the activation of the adenylate cyclase system resulting in the increased intracellular concentration of cyclic AMP that causes an increase in the intracellular concentration of calcium. IgE, not IgE receptors, is synthesized by plasma cells. Leukotrienes also are released as a result of adenylate cyclase activation.
5. A. Eosinophils are attracted to the site of the wasp sting due to the release of eosinophil chemotactic factor. These cells are able to destroy parasites because the specific granules of eosinophils contain major basic protein, a substance that digests the pellicle that protects many parasites. Monocytes become macrophages after they leave the bloodstream and enter the connective tissue compartment but are ineffective against parasites. T lymphocytes, plasma cells, and natural killer cells cannot combat parasites.
6. A. Mast cells can release enough of their pharmacological agents in a systemic fashion in hyperallergic individuals, causing generalized edema and constriction of bronchiolar smooth muscle resulting in circulatory and respiratory shock. If the insult is overpowering, it is referred to as *anaphylactic shock*, and the patient may die. Osteoblastoma is a disease in which foci of bone, manufactured by osteoblasts, not mast cells, are formed within connective tissues. Leukemia is a disease of the blood where white blood cells, not mast cells, proliferate. Chondroblastoma are benign tumors, resembling embryonic cartilage, occurring within the epiphyseal plate of long bones. Fibromas are benign tumors of fibrous connective tissue
7. C. The photomicrograph presents a section of loose connective tissue (areolar connective tissue). The classification should be recognized because the fibers (both elastic and type III collagen) are very loosely scattered throughout the field and the tissue is highly cellular.
8. D. The photomicrograph represents a tendon (dense regular collagenous connective tissue). The dermis is dense irregular connective tissue, and the epidermis is stratified squamous keratinized epithelium. The ligamentum nuchae is dense regular elastic tissue. Although the photomicrograph of a tendon resembles that of the longitudinal section of a peripheral nerve bundle, the characteristic waviness of nerve bundles is not present.
9. A. Dense regular collagenous connective tissue has fibroblasts arranged in parallel rows with each other. Schwann cells are located in peripheral nerves, where they form myelin sheaths. Mesenchymal cells and macrophages are very seldom located in dense regular connective tissue. Smooth muscle has a somewhat different appearance than does tendon.
10. D. Prostaglandin is one of the secondary mediators released by mast cells, and it functions in vasoconstriction, constriction of bronchiolar smooth muscle, and increasing mucus secretion. Heparin is an anticoagulant and inactivates histamine as does chondroitin sulfate. Aryl sulfatase inactivates leukotriene C_4, thereby limiting the inflammatory response. Thromboxane A_2 is a vasoconstrictor that also causes platelet aggregation.

Cartilage and Bone

7

Cartilage and bone are both specialized connective tissues. Cartilage possesses a firm pliable matrix that resists mechanical stresses. Bone matrix, however, is one of the hardest tissues of the body, and it, too, resists stresses placed on it. Both of these connective tissues have cells that are specialized to secrete the matrix in which, subsequently, the cells become trapped. Although cartilage and bone have many varied functions, some of the functions are similar and related. Both are involved in supporting the body because they are intimately associated in the skeletal system. Most of the long bones of the body are formed first in the embryo as cartilage, which then acts as a template that is later replaced by bone; this process is referred to as ***endochondral bone formation***. Most of the flat bones are formed within preexisting membranous sheaths; thus, this method of osteogenesis is known as ***intramembranous bone formation***.

CARTILAGE

Cartilage possesses cells called ***chondrocytes***, which occupy small cavities called ***lacunae*** within the **extracellular matrix** that they secreted. The substance of cartilage is neither vascularized nor supplied with nerves or lymphatic vessels; however, the cells receive their nourishment from blood vessels of surrounding connective tissues by diffusion through the matrix. The extracellular matrix is composed of **glycosaminoglycans** and **proteoglycans**, which are intimately associated with the collagen and elastic fibers embedded within the matrix. The flexibility and resistance of cartilage to compression permit it to function as a shock absorber, and its smooth surface permits almost friction-free movement of the joints of the body as it covers the articulating surfaces of the bones.

There are three types of cartilage, a classification based on the types of fibers present in the matrix (Fig. 7–1 and Table 7–1):

- **Hyaline cartilage** contains **type II** collagen fibers in its matrix; it is the most abundant cartilage in the body and serves many functions.
- **Elastic cartilage** contains, in addition to the **type II** collagen fibers, an abundance of elastic fibers scattered throughout its matrix, giving it more pliability.
- **Fibrocartilage** possesses dense, coarse **type I** collagen fibers in its matrix, allowing it to withstand strong tensile forces.

The **perichondrium** is a dense irregular collagenous connective tissue sheath covering that overlies most cartilage. It has an outer fibrous layer and inner cellular layer whose cells secrete cartilage matrix. The perichondrium is vascular, and its vessels supply nutrients to the cells of cartilage. In areas where the cartilage has no perichondrium (e.g., the articular surfaces of the bones forming a joint), the cartilage cells receive their nourishment from the synovial fluid that bathes the joint surfaces.

Hyaline Cartilage

Hyaline cartilage, the most abundant cartilage in the body, forms the template for endochondral bone formation.

Hyaline cartilage, a bluish-gray, semitranslucent, pliable substance, is the most abundant cartilage of the body. It is located in the nose and larynx, on the ventral ends of the ribs where they articulate with the sternum, in the tracheal rings and bronchi, and on the articulating surfaces of the movable joints of the body. Also, it is this cartilage that forms the cartilage template of the long bones during embryonic development and constitutes the epiphyseal plates of growing long bones (see Table 7–1).

Histogenesis and Growth of Hyaline Cartilage

Cells responsible for hyaline cartilage formation differentiate from mesenchymal cells.

In the region where cartilage is to form, individual mesenchymal cells retract their processes, round up, and congregate in dense masses called ***chondrification centers***. These cells, probably under the influence of a small molecule called ***kartogenin***, differentiate into **chondroblasts** and commence secreting aggrecans and type II collagen, the typical cartilage matrix, around themselves. As this process continues, the chondroblasts become entrapped in their own matrix in small individual compartments called ***lacunae.*** Chondroblasts that are surrounded by this matrix are referred to as ***chondrocytes*** (Fig. 7–2). These cells are still capable of cell division, forming a cluster of two to four or more cells in a lacuna. These groups are known as ***isogenous groups*** and represent one, two, or more cell divisions from an original chondrocyte (see Fig. 7–1). As the cells of an isogenous group manufacture matrix, they are pushed away from each other, forming separate lacunae

SUMMARY

Cartilage and **bone** are specialized connective tissues that form the skeletal system, and their principal function is to support the body. Both tissues possess dedicated cells that secrete and maintain the **specific matrices** associated with them. Cartilage is flexible and is classified into three types, **hyaline**, **elastic**, and **fibrocartilage**, depending on the fibers in the matrix that they possess. The cells of cartilage are **chondrogenic cells**, **chondroblasts**, and **chondrocytes**. Unlike the matrix of cartilage that of bone is hard because it becomes **calcified** shortly after the bone matrix is elaborated.

Even though bone is one of the hardest substances in the body, it is a dynamic tissue that reacts to the changing stresses that are placed on it. Bone is the primary structural framework for support and protection of the organs of the body. Bones also serve as levers for the muscles attached to them, thereby multiplying the force of the muscles to attain movement. Bone is a reservoir for several minerals of the body, especially of calcium. Bone is of two types, c**ompact bone** and **cancellous (spongy) bone**. It contains hollow spaces known as the ***marrow cavity***, which houses **bone marrow**, a hemopoietic organ. The cells of bone include **osteoprogenitor cells**, **osteoblasts**, and **osteocytes**, as well as the bone marrow-derived **osteoclasts**.

KEY WORDS

Bone; bone marrow; cancellous bone; chondroblasts; chondrocytes; chondrogenic cells; compact bone; elastic cartilage; fibrocartilage; hyaline cartilage; marrow cavity; osteoblasts; osteoclasts; osteocytes; osteoprogenitor cells

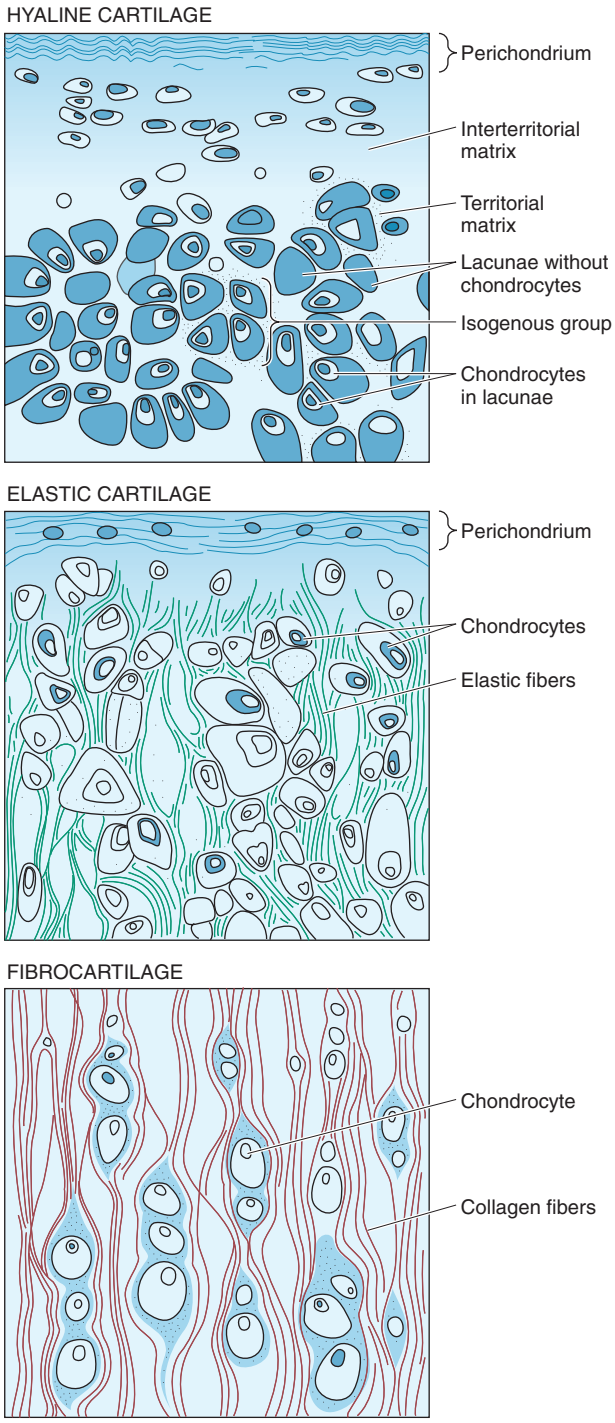


Figure 7–1 Diagram of the types of cartilage.

and thus enlarging the cartilage from within. This type of growth is called ***interstitial growth***.

Mesenchymal cells at the periphery of the developing cartilage differentiate to form fibroblasts. These cells manufacture a dense irregular collagenous connective tissue, the **perichondrium**, responsible for the growth and maintenance of the cartilage. The perichondrium has two layers, an **outer fibrous layer** composed of type I collagen, fibroblasts, and blood vessels and an **inner cellular layer** composed mostly of **chondrogenic cells**. The chondrogenic cells undergo division and differentiate into chondroblasts, which begin to elaborate matrix. In this way, cartilage also grows by adding to its periphery a process called ***appositional growth***.

Interstitial growth occurs only in the early phase of hyaline cartilage formation. Articular cartilage lacks a perichondrium and increases in size by interstitial growth only. This type of growth also occurs in the **epiphyseal plates** of long bones, where the lacunae are arranged in a longitudinal orientation parallel to the long axis of the bone; therefore, interstitial growth serves to lengthen the bone. The cartilage in the remainder of the body grows mostly by apposition, a controlled process that may continue during the life of the cartilage.

CLINICAL CORRELATIONS

1. Mesenchymal cells located within the chondrification centers are induced to become secreting chondroblasts by their attachments and the chemistry of the surrounding extracellular matrix. Also, if chondroblasts are removed from their secreted cartilage matrix and are grown in a monolayer in a low-density substrate, they will cease to secrete cartilage matrix containing type II collagen. Instead, they will become fibroblast-like and start secreting type I collagen.
2. It has been demonstrated in animal experiments that mice with arthritis-like knee joints when treated with kartogenin developed more cartilage and were able to move their joints freely and without pain.

Cartilage Cells

Three types of cells are associated with cartilage: chondrogenic cells, chondroblasts, and chondrocytes (see Fig. 7–2).

Chondrogenic cells are spindle-shaped, narrow cells that are derived from mesenchymal cells. They possess an ovoid nucleus with one or two nucleoli. Their cytoplasm is sparse, and electron micrographs of chondrogenic cells display a small Golgi apparatus, a few mitochondria, some profiles of **rough endoplasmic reticulum (RER)**, and an abundance of free ribosomes. These cells can differentiate into both chondroblasts and osteoprogenitor cells.

Chondroblasts are derived from two sources: **mesenchymal cells** located within the center of

TABLE 7–1 Cartilage Types, Characteristics, and Locations

Type of Cartilage	Identifying Characteristics	Perichondrium	Location
Hyaline	Type II collagen, basophilic matrix, chondrocytes usually arranged in groups	Perichondrium present in most places. Exceptions: articular cartilages and epiphyses	Articular ends of long bones, nose, larynx, trachea, bronchi, ventral ends of ribs
Elastic	Type II collagen, elastic fibers	Perichondrium present	Pinna of ear, walls of auditory canal, auditory tube, epiglottis, cuneiform cartilage of larynx
Fibrocartilage	Type I collagen, acidophilic matrix, chondrocytes arranged in parallel rows between bundles of collagen, always associated with dense regular collagenous connective tissue or hyaline cartilage	Perichondrium absent	Intervertebral disks, articular disks, pubic symphysis, insertion of some tendons

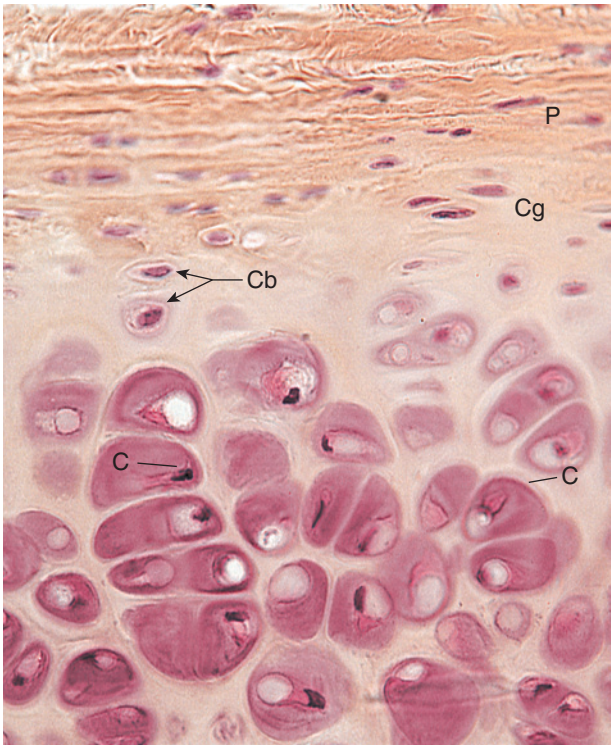


Figure 7–2 Light micrograph of hyaline cartilage (×270). Observe the large ovoid chondrocytes (C) trapped in their lacunae. Directly above them are the elongated chondroblasts (Cb), and at the very top is the perichondrium (P) and the underlying chondrogenic (Cg) cell layer.

chondrification and **chondrogenic cells** of the inner cellular layer of the perichondrium (as in appositional growth). Chondroblasts are plump, basophilic cells that display the organelles required for protein synthesis. Electron micrographs of these cells demonstrate a rich network of RER, a well-developed Golgi complex, numerous mitochondria, and an abundance of secretory vesicles.

Chondrocytes are chondroblasts that are surrounded by matrix. Those near the periphery are ovoid, whereas those deeper in the cartilage are more rounded, with a diameter of 10 to 30 μm. Histological processing creates artifactual shrinkage and distortion of the cells. Chondrocytes display a large nucleus with a prominent nucleolus and the usual organelles of protein-secreting cells. Young chondrocytes have a pale-staining cytoplasm with many mitochondria, an elaborate RER, a well-developed Golgi apparatus, and glycogen. Older chondrocytes, which are relatively quiescent, display a greatly reduced complement of organelles, with an abundance of free ribosomes. Thus, these cells can resume active protein synthesis if they revert to chondroblasts.

Matrix of Hyaline Cartilage

The matrix of hyaline cartilage is composed of type II collagen, proteoglycans, glycoproteins, and extracellular fluid.

The semitranslucent blue-gray matrix of hyaline cartilage contains up to 40% of its dry weight in collagen. In addition, it contains proteoglycan aggregates (mostly in the form af aggrecans), glycoproteins (mostly chondronectin), and extracellular fluid. Because the refractive index of the collagen fibrils and that of the ground substance are nearly the same, the matrix appears to be an amorphous, homogeneous mass with the light microscope.

The matrix of hyaline cartilage contains primarily **type II collagen**, but types IX, X, and XI and other minor collagens are also present in small quantities. Type II collagen does not form large bundles, although

the bundle thickness increases with distance from the lacunae. Fiber orientation appears to be related to the stresses placed on the cartilage. For example, in articular cartilage, the fibers near the surface are oriented parallel to the surface, whereas deeper fibers seem to be oriented in curved columns.

The matrix is subdivided into two regions: the **territorial matrix**, around each lacuna, and the **interterritorial matrix** (see Fig. 7–1). The territorial matrix, a 50-µm-wide band, is poor in collagen and rich in chondroitin sulfate, which contributes to its basophilic and intense staining with periodic acid–Schiff (PAS) reagent. The bulk of the matrix is **interterritorial matrix**, which is richer in type II collagen and poorer in proteoglycans than the territorial matrix.

A small region of the matrix, 1 to 3 µm thick, immediately surrounding the lacuna, is known as the ***pericellular capsule***. It displays a fine meshwork of collagen fibers embedded in a basal lamina–like substance. These fibers may represent some of the other minor collagens present in hyaline cartilage; it has been suggested that the pericellular capsule may protect chondrocytes from mechanical stresses.

Cartilage matrix is rich in **aggrecans**, large proteoglycan molecules composed of protein cores to which glycosaminoglycan molecules (chondroitin 4-sulfate, chondroitin 6-sulfate, and heparan sulfate) are covalently linked (see Fig. 4–3). As many as 100 to 200 aggrecan molecules are linked noncovalently to hyaluronic acid, forming huge aggrecan composites that can be 3 to 4 µm long. The abundant negative charges associated with these exceedingly large proteoglycan molecules attract cations, predominantly Na^+ ions, which, in turn, attract water molecules. In this way, the cartilage matrix becomes hydrated to such an extent that up to 80% of the wet weight of cartilage is water, accounting for the ability of cartilage to resist forces of compression.

Not only do hydrated proteoglycans fill the interstices among the collagen fiber bundles, but also their glycosaminoglycan side chains form electrostatic bonds with the collagen. Thus, the ground substance and fibers of the matrix form a cross-linked molecular framework that resists tensile forces.

Cartilage matrix also contains the adhesive glycoprotein **chondronectin**. This large molecule, similar to fibronectin, has binding sites for type II collagen, chondroitin-4-sulfate, chondroitin-6-sulfate, hyaluronic acid, and integrins (transmembrane proteins) of chondroblasts and chondrocytes. Chondronectin thus assists these cells in maintaining their contact with the fibrous and amorphous components of the matrix.

Histophysiology of Hyaline Cartilage

The smoothness of hyaline cartilage and its ability to resist forces of both compression and tension are essential to its function at the articular surfaces of joints. Because cartilage is avascular, nutrients and oxygen must diffuse through the water of hydration present in the matrix. The inefficiency of such a system necessitates a limit on the width of cartilage. There is a constant turnover in the proteoglycans of cartilage that changes with age. Hormones and vitamins also exert influence over the growth, development, and function of cartilage. Many of these substances also affect skeletal formation and growth (Table 7–2).

TABLE 7–2 Effects of Hormones and Vitamins on Hyaline Cartilage

Hormone	Effects
Thyroxine, testosterone, and somatotropin (via insulin-like growth factors)	Stimulate cartilage growth and matrix formation
Cortisone, hydrocortisone, and estradiol	Inhibit cartilage growth and matrix formation
Vitamin	
Hypovitaminosis A	Reduces width of epiphyseal plates
Hypervitaminosis A	Accelerates ossification of epiphyseal plates
Hypovitaminosis C	Inhibits matrix synthesis and deforms architecture of epiphyseal plate; leads to scurvy
Absence of vitamin D, resulting in deficiency in absorption of calcium and phosphorus	Proliferation of chondrocytes is normal but matrix does not become calcified properly; results in rickets

CLINICAL CORRELATIONS

Hyaline cartilage degenerates when the chondrocytes hypertrophy and die, and the matrix begins to calcify. This process is a normal and integral part of endochondral bone formation; however, it is also a natural process of aging, often resulting in less mobility and pain in the joints.

Cartilage regeneration is usually poor, except in children. Chondrogenic cells from the perichondrium enter the defect and form new cartilage. If the defect is large, the cells form dense connective tissue to repair the scar.

Elastic Cartilage

Elastic cartilage greatly resembles hyaline cartilage, except that its matrix and perichondrium possess elastic fibers.

Elastic cartilage is located in the pinna of the ear, the external and internal auditory tubes, the epiglottis, and the larynx (cuneiform cartilage). Because of the presence of elastic fibers, elastic cartilage is somewhat yellow and is more opaque than hyaline cartilage in the fresh state (see Table 7–1).

In most respects, elastic cartilage is identical to hyaline cartilage and is often associated with it. The outer fibrous layer of the perichondrium is rich in elastic fibers. The matrix of elastic cartilage possesses abundant fine-to-coarse branching elastic fibers interspersed with type II collagen fiber bundles, giving it much more flexibility than the matrix of hyaline cartilage (Fig. 7–3). Chondrocytes of elastic cartilage are more abundant and larger than those of hyaline cartilage. The matrix is not as ample as in hyaline cartilage, and the elastic fiber bundles of the territorial matrix are larger and coarser than those of the interterritorial matrix.

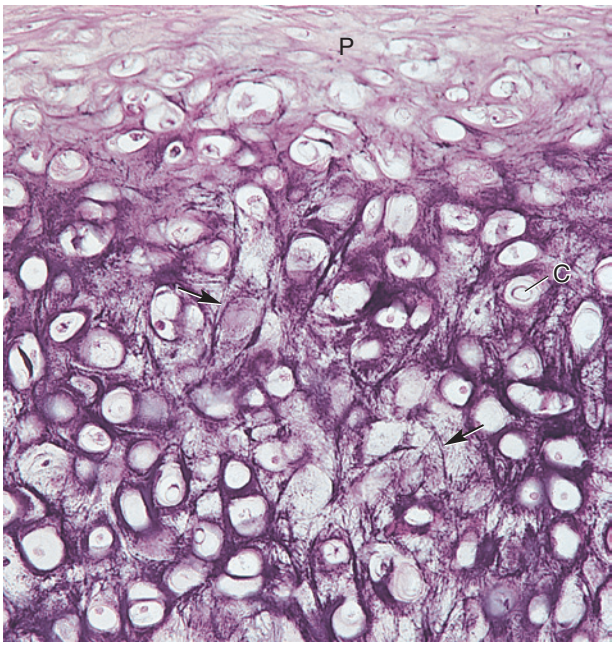


Figure 7–3 Light micrograph of elastic cartilage (×132). Observe the perichondrium (P) and the chondrocytes (C) in their lacunae (shrunken from the walls because of processing), some of which contain more than one cell, evidence of interstitial growth. Elastic fibers *(arrows)* are scattered throughout.

Fibrocartilage

Fibrocartilage, unlike hyaline and elastic cartilage, does not possess a perichondrium, and its matrix possesses type I collagen.

Fibrocartilage is present in intervertebral disks, in the pubic symphysis, in articular disks, and attached to bone. It is associated with hyaline cartilage and with dense connective tissue, which it resembles. Unlike the other two types of cartilage, fibrocartilage does not possess a perichondrium. It displays a scant amount of matrix (rich in chondroitin sulfate and dermatan sulfate) and exhibits bundles of type I collagen, which stain acidophilic (Fig. 7–4). Chondrocytes are often aligned in alternating parallel rows with the thick, coarse bundles of collagen, which parallel the tensile forces attendant on this tissue (see Table 7–1).

Chondrocytes of fibrocartilage usually arise from fibroblasts that begin to manufacture proteoglycans. As the ground substance surrounds the fibroblast, the cell becomes incarcerated in its own matrix and differentiates into a chondrocyte.

Intervertebral disks represent an example of the organization of fibrocartilage. They are interposed between the hyaline cartilage coverings of the articular surface of successive vertebrae. Each disk contains a gelatinous center, called the ***nucleus pulposus***, which

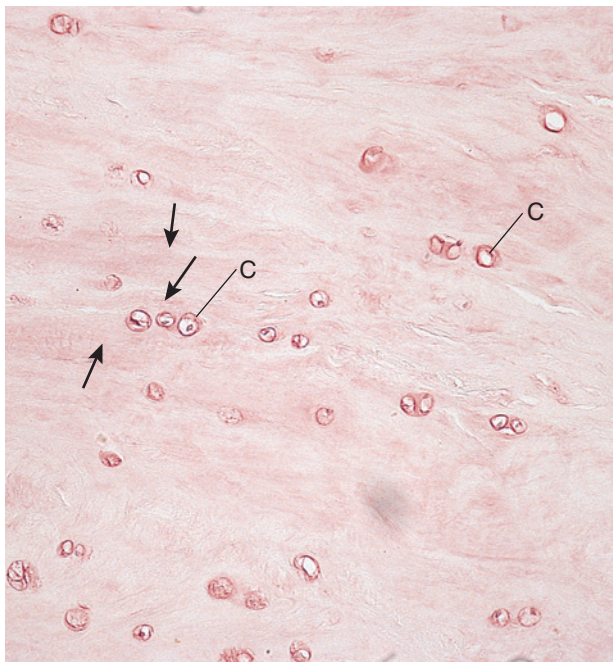


Figure 7–4 Light micrograph of fibrocartilage (×132). Note alignment of the chondrocytes (C) in rows interspersed with thick bundles of collagen fibers *(arrows)*.

is composed of cells, derived from the notochord, lying within a hyaluronic acid–rich matrix. These cells disappear by the 20th year of life. Much of the nucleus pulposus is surrounded by the **annulus fibrosus**, layers of fibrocartilage whose type I collagen fibers run vertically between the hyaline cartilages of the two vertebrae. The fibers of adjacent lamellae are oriented obliquely to each other, providing support to the gelatinous **nucleus pulposus**. The annulus fibrosus provides resistance against tensile forces, whereas the nucleus pulposus resists forces of compression.

CLINICAL CORRELATIONS

A ruptured disk refers to a tear or break in the laminae of the annulus fibrosus through which the gel-like nucleus pulposus extrudes. This condition occurs more often on the posterior portions of the intervertebral disks, particularly in the lumbar portion of the back, where the disk may dislocate, or slip. A "slipped disk" leads to severe, intense pain in the lower back and extremities because the displaced disk compresses the lower spinal nerves.

BONE

Bone is a specialized connective tissue whose extracellular matrix is calcified, incarcerating the cells that secreted it.

Although **bone** is one of the hardest substances of the body, it is a dynamic tissue that constantly changes shape in relation to the stresses placed on it. For example, pressures applied to bone lead to its resorption, whereas tension applied to it results in development of new bone. In applying these facts, an orthodontist is able to remodel the bone of the dental arches by moving and straightening the teeth to correct malocclusion, thus providing the patient with a more natural and pleasing smile.

Bone is the primary structural framework for support and protection of the organs of the body, including the brain and spinal cord and the structures within the thoracic cavity, namely the lungs and heart. The bones also serve as levers for the muscles attached to them, thereby multiplying the force of the muscles to attain movement. Bone is a reservoir for several minerals of the body; for example, it stores about 99% of the body's calcium. Bone contains a central cavity, the **marrow cavity**, which houses the **bone marrow**, a hemopoietic organ.

Bone is covered on its external surface, except at synovial articulations, with a **periosteum**, which consists of an outer layer of dense fibrous connective tissue and an inner cellular layer containing **osteoprogenitor (osteogenic) cells** as well as occasional **osteoblasts**. Bundles of collagen fibers from the periosteum, known as ***Sharpey fibers***, are embedded into the outer surface of bone, thereby securing the periosteum to the bone surface. The central cavity of a bone is lined with **endosteum**, a specialized thin connective tissue composed of a monolayer of **osteoprogenitor cells** and **osteoblasts**. The cells of bone are described in more detail in the following paragraphs.

Bone is composed of cells lying in an extracellular matrix that has become calcified. The calcified matrix is composed of fibers and ground substance. The fibers constituting bone are primarily type I collagen. The ground substance is rich in proteoglycans with chondroitin sulfate and keratan sulfate side chains. In addition, glycoproteins, such as osteonectin, osteocalcin, osteopontin, and bone sialoprotein, are present.

The cells of bone include **osteoprogenitor cells**, which differentiate into **osteoblasts**. Osteoblasts are responsible for secreting the matrix. When these cells are surrounded by matrix, they become quiescent and are known as *osteocytes*. The spaces that osteocytes occupy are known as *lacunae* (Fig. 7–5). Osteoclasts, multinucleated giant cells derived from fused bone marrow precursors, are responsible for bone resorption and remodeling.

Because bone is such a hard tissue, two methods are employed to prepare it for study. **Decalcified sections**

Figure 7–5 Light micrograph of decalcified compact bone (×540). Osteocytes (Oc) may be observed in lacunae (L). Also note the osteon (Os), the osteoprogenitor cells (Op), and the cementing lines (Cl).

can be prepared by decalcifying the bone in an acid solution to remove the calcium salts. The tissue can then be embedded, sectioned, and routinely stained for study. **Ground sections** are prepared by sawing the bone into thin slices followed by grinding the sections with abrasives between glass plates. When the section is sufficiently thin for study with light microscope, it is mounted for study.

Each system has disadvantages. In decalcified sections, osteocytes are distorted by the decalcifying acid bath; in ground sections, the cells are destroyed, and the lacunae and canaliculi are filled in with bone debris.

Bone Matrix

Bone matrix has inorganic and organic constituents.

Inorganic Component

The inorganic constituents of bone are crystals of calcium hydroxyapatite, composed mostly of calcium and phosphorus.

The inorganic portion of bone, which constitutes about 65% of its dry weight, is composed mainly of calcium and phosphorus along with other components, including bicarbonate, citrate, magnesium, sodium, and potassium. Calcium and phosphorus exist primarily in the form of **hydroxyapatite crystals** [$Ca_{10}(PO_4)_6(OH)_2$], but calcium phosphate is also present in an amorphous form. Hydroxyapatite crystals (40 nm in length by 25 nm in width and 1.5 to 3 nm in thickness) are arranged in an ordered fashion along the type I collagen fibers; they are deposited into the gap regions of the collagen but also are present along the overlap region. The free surface of the crystals is surrounded by amorphous ground substance. The surface ions of the crystals attract H_2O molecules, thus forming a **hydration shell** around the crystals, which permits ion exchange with the extracellular fluid.

Bone is one of the hardest and strongest substances in the body. Its hardness and strength are due to the association of hydroxyapatite crystals with collagen. If bone is decalcified (i.e., all of the mineral is removed from the bone), it still retains its original shape but becomes so flexible so that it can be bent like a piece of tough rubber. If the organic component is extracted from bone, the mineralized skeleton still retains its original shape, but it becomes extremely brittle and can be fractured with ease.

Organic Component

The predominant organic component of bone is type I collagen.

The **organic component** of bone matrix constitutes approximately 35% of the dry weight of bone; it includes fibers that are almost exclusively type I collagen.

Collagen comprises about 80% to 90% of the organic component of bone. It is formed in large (50 to 70 nm in diameter) bundles displaying the typical 67-nm periodicity. Type I collagen in bone is highly cross-linked, which prevents it from being easily extracted.

The fact that bone matrix stains with PAS reagent and displays slight metachromasia indicates the presence of sulfated glycosaminoglycans, predominantly chondroitin sulfate and keratan sulfate. These form small proteoglycan molecules with short protein cores to which the glycosaminoglycans are covalently bound. The proteoglycans are noncovalently bound via link proteins to hyaluronic acid, forming very large **aggrecan composites**. The abundance of collagen, however, causes the matrix to be acidophilic.

Several glycoproteins are also present in the bone matrix. These appear to be restricted to bone and include **osteocalcin** (which binds to hydroxyapatite) and **osteopontin**, which also binds to hydroxyapatite but has additional binding sites for other components as well as for integrins present on osteoblasts and osteoclasts. Vitamin D stimulates the synthesis of these glycoproteins. **Bone sialoprotein**, another matrix protein, has binding sites for matrix components and integrins of osteoblasts and osteocytes, suggesting its involvement in the adherence of these cells to bone matrix.

Cells of Bone

The cells of bone are osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts.

Osteoprogenitor Cells

Osteoprogenitor cells are derived from embryonic mesenchymal cells and retain their ability to undergo mitosis.

Osteoprogenitor cells are located in the inner cellular layer of the periosteum, lining haversian canals, and in the endosteum (see Fig. 7–5). These cells, derived from embryonic mesenchyme, remain in place throughout postnatal life and can undergo mitotic division and have the potential to differentiate into osteoblasts. Moreover, under certain conditions of low oxygen tension, these cells may differentiate into chondrogenic cells. Osteoprogenitor cells are spindle-shaped and have a pale-staining oval nucleus; their scant pale-staining cytoplasm displays sparse RER and a poorly developed Golgi apparatus but an abundance of free ribosomes. These cells are most active during the period of intense bone growth.

Osteoblasts

Osteoblasts not only synthesize the organic matrix of bone but also possess receptors for parathyroid hormone.

Bone morphogenic protein-6 (BMP-6)—as well as, to some extent, BMP-2 and BMP-4—and **transforming**

growth factor-β (TGF-β) induce osteoprogenitor cells to differentiate into **osteoblasts**, cells that manufacture and release the organic components of **bone matrix (osteoid)**, namely type I collagen and some type V collagen, glycoproteins, and proteoglycans. Osteoblasts also synthesize the **receptor for the activation of nuclear factor kappa B ligand (RANKL)**, **macrophage colony-stimulating factor (M-CSF)**, **alkaline phosphatase**, **insulin-like growth factor-1 (IGF-1) receptors**, and **parathyroid hormone (PTH) receptors** as well as alkaline phosphatase, all of which they place on their cell membranes. These cells also manufacture and release a number of additional macromolecules, such as

- **Osteocalcin**, a signaling molecule responsible for mineralization of bone
- **Osteonectin**, a glycoprotein that assists in the binding of calcium hydroxyl apatite crystals to collagen
- **Osteopontin**, assists in the formation of the sealing zone of osteoclasts (see section on the morphology of osteoclasts)
- **Bone sialoprotein**, which assists osteoblasts to adhere to the bone matrix
- **Osteoprotegerin (OPG)**, a glycoprotein that can bind to RANKL and thus interfere with osteoclast formation

Osteoblasts are located on the surface of the bone in a sheet-like arrangement of cuboidal to columnar cells (Fig. 7–6). When actively secreting matrix, they exhibit a basophilic cytoplasm.

The organelles of osteoblasts are polarized so that the nucleus is located away from the region of secretory activity, which houses secretory granules believed to contain matrix precursors. The contents of these vesicles stain pink with PAS reagent.

Electron micrographs exhibit abundant RER, a well-developed Golgi complex (Fig. 7–7A), and numerous secretory vesicles containing flocculent material that accounts for the PAS-staining pink vacuoles observed in the light microscope. Osteoblasts extend short processes that make contact with those of neighboring osteoblasts as well as long processes that make contact with processes of osteocytes. Although these processes form **gap junctions** with one another, the number of gap junctions between osteoblasts is much fewer than those between osteocytes. As osteoblasts exocytose their secretory products, each cell surrounds itself with the bone matrix that it has just produced; when this occurs, the incarcerated cell is referred to as an *osteocyte*, and the space it occupies is known as a ***lacuna***. Most of the bone matrix becomes calcified; however, osteoblasts as well as osteocytes are always separated from the calcified substance by a thin noncalcified layer known as the ***osteoid*** (uncalcified bone matrix).

Surface osteoblasts that cease to form matrix revert to a more flattened-shaped quiescent state and are called ***bone-lining cells***. Although these cells appear to be similar to osteoprogenitor cells, they are most likely incapable of dividing but can be reactivated to the secreting form with the proper stimulus.

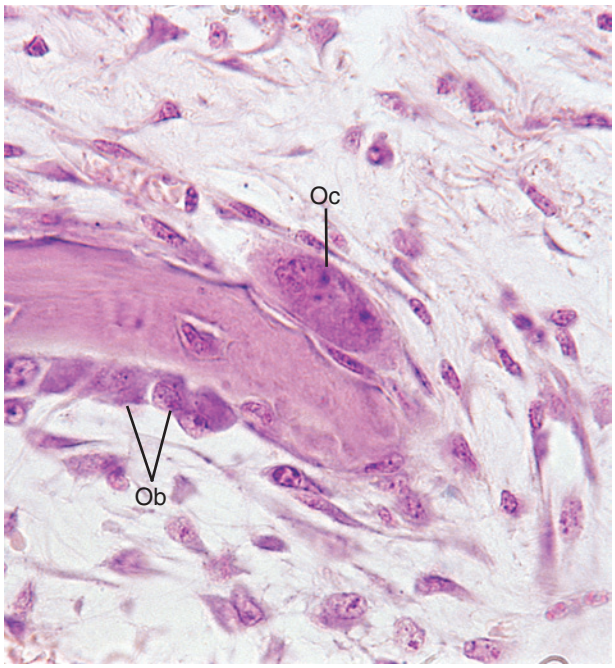


Figure 7–6 Light micrograph of intramembranous ossification (×540). Osteoblasts (Ob) line the bony spicule where they are secreting osteoid onto the bone. Osteoclasts (Oc) may be observed housed in Howship lacuna.

Osteoblasts have several factors on their cell membranes, the most significant of which are integrins and **PTH receptors**. When PTH binds to these receptors, it stimulates osteoblasts to secrete **RANKL**, a factor that induces the differentiation of preosteoclasts into osteoclasts. Also osteoblasts secrete an **osteoclast-stimulating factor**, which activates osteoclasts to resorb bone. Osteoblasts also secrete enzymes responsible for removing osteoid so that osteoclasts can make contact with the mineralized bone surface.

Osteocytes

Osteocytes are mature bone cells derived from osteoblasts that became trapped in their lacunae.

Osteocytes are mature bone cells, transformed from osteoblasts under the influence of two transcription factors, **Cbfa1/Runx2** and **osterix**, both of which appear to be dependent on BMP-2 to be expressed. As osteoblasts transform into osteoclasts, they no longer express alkaline phosphatase on their cell membranes, and they become flat, lenticular-shaped cells trapped in their **lacunae** within the calcified bony matrix (see

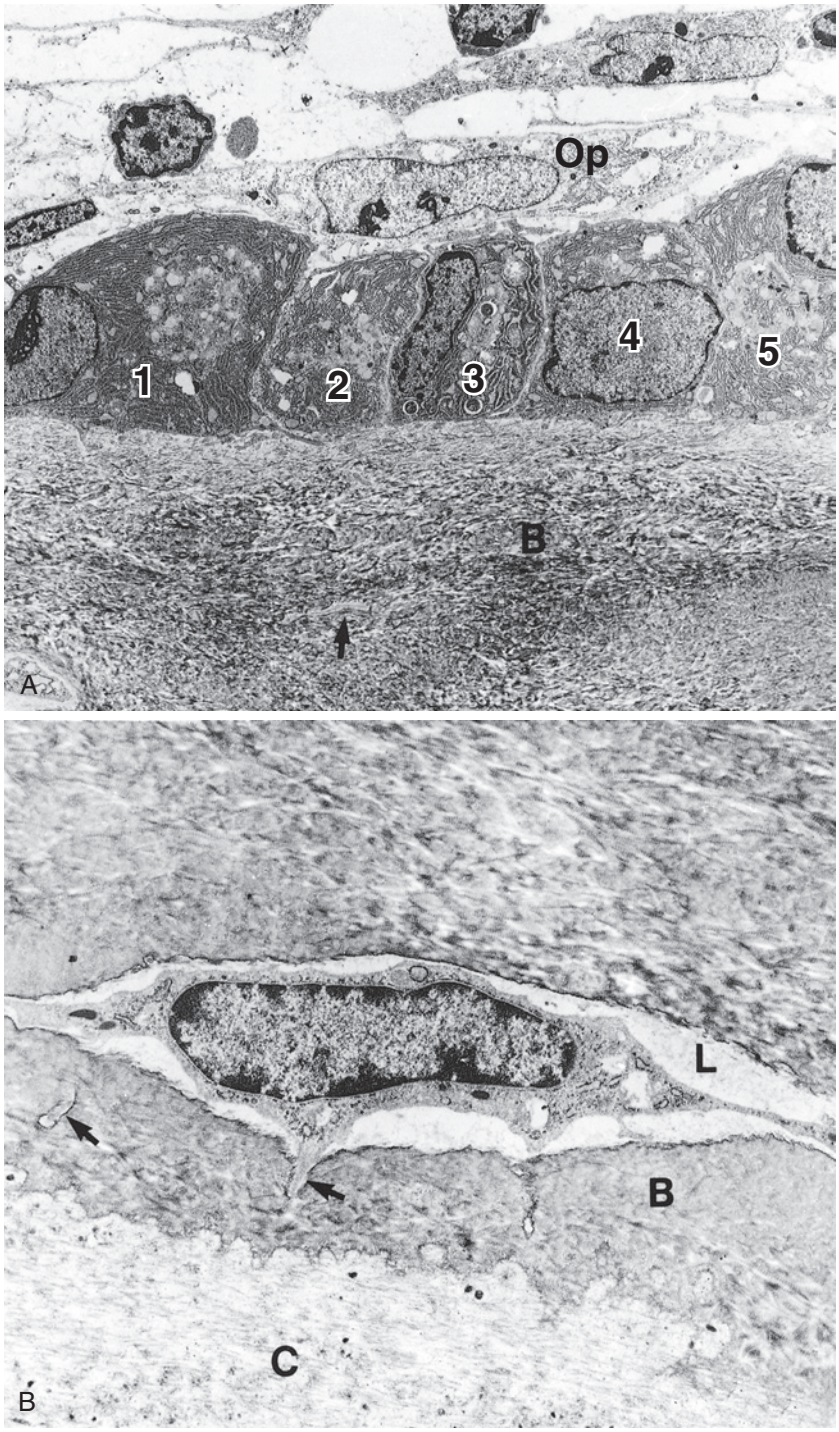


Figure 7–7 Electron micrograph of bone-forming cells. **A,** Observe the five osteoblasts (numbered 1 to 5) lined up on the surface of bone (B) displaying abundant rough endoplasmic reticulum. The *arrow* indicates the process of an osteocyte in a canaliculus. The cell with the elongated nucleus lying above the osteoblasts is an osteoprogenitor cell (op) (×2500). **B,** Note the osteocyte in its lacuna (L) with its processes extending into canaliculi (×1000). B, bone; C, cartilage. (From Marks SC Jr, Popoff SN. Bone cell biology: the regulation of development, structure, and function in the skeleton. *Am J Anat.* 1988;183:1-44. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

CLINICAL CORRELATIONS

1. Osteoblast cell membranes are rich in the enzyme **alkaline phosphatase**. During active bone formation, these cells secrete high levels of alkaline phosphatase, elevating the levels of this enzyme in the blood. Thus, the clinician can monitor bone formation by measuring the blood alkaline phosphatase level.
2. Individuals who have the type of mutations in the genes that code for TGF-β, where the factor is always active, have the rare autosomal dominant inherited disorder known as ***Camurati-Engelmann disease***. This ailment usually manifests itself in late adolescence or early adulthood and is characterized by an excess of and highly active osteoblasts, resulting in very thick bones of the extremities, although, in some patients, the bones of the skull and the hip may also be involved. The individual experiences intense pain in the bones and muscles of the leg. Additionally, the muscles of the extremities may be weakened and that with the additional factor of pain causes many affected individuals to "waddle" as they walk.

Figs. 7–5 and 7–7B). There are as many as 20,000 to 30,000 osteocytes per mm^3 of bone. Radiating out in all directions from the lacuna are narrow, tunnel-like spaces **(canaliculi)** that house cytoplasmic processes of the osteocyte. These processes make contact with similar processes of neighboring osteocytes, forming **gap junctions** through which ions and small molecules can move between the cells. The canaliculi also contain extracellular fluid carrying nutrients and metabolites, which nourish the osteocytes.

Osteocytes conform to the shape of their lacunae. Their nucleus is flattened, and their cytoplasm is poor in organelles, displaying scant RER and a greatly reduced Golgi apparatus. Although osteocytes appear to be inactive cells, they secrete substances necessary for bone maintenance. These cells have also been implicated in **mechanotransduction**, in that they respond to stimuli that place tension on bone by releasing **cyclic adenosine monophosphate (cAMP)**, **osteocalcin**, and **insulin-like growth factor (IGF)**. The release of these factors facilitates the recruitment of **osteoprogenitor cells** to assist in the remodeling of the skeleton (adding more bone) not only during growth and development but also during the long-term redistribution of forces acting on the skeleton. An example of such remodeling is evident in the comparison of male and female skeletons, in which the muscle attachments of the male skeleton are usually better defined than those of the female skeleton.

The interval between the osteocyte plasmalemma and the walls of the lacuna and canaliculi, known as the ***periosteocytic space***, is occupied by extracellular fluid. Considering the extensive network of the canaliculi and the sheer number of osteocytes present in the skeleton of an average person, the volume of the periosteocytic space and the surface area of the walls have been calculated to be a staggering 1.3 L and as much as 5000 m^2, respectively. It has been suggested that the 1.3 L of extracellular fluid occupying the periosteocytic space is exposed to as much as 20 g of exchangeable calcium that can be resorbed from the walls of these spaces. The resorbed calcium gains access to the bloodstream and ensures the maintenance of adequate blood calcium levels.

CLINICAL CORRELATIONS

Although bone is constantly remodeled during the individual's life, and osteocytes have a very long life span of approximately 25 years, these cells undergo apoptosis as an individual ages, so that, by the time the person is about 80 years old, 75% of the bone's osteocyte population is dead. As osteocytes undergo apoptosis and disintegrate into apoptotic bodies, they release RANKL, a cytokine that stimulates the formation and activation of osteoclasts. Young osteocytes release TGF-β, which suppresses bone resorption; however, as these cells age, they release increasingly reduced quantities of TGF-β, thereby permitting an increase in osteoclastic activity, resulting in increased bone resorption. Thus, the aging individual loses bone mass as a result of age-related osteocytic alterations.

In response to low-calcium levels in the extracellular fluid in their lacunae, osteocytes secrete **sclerostin**, a paracrine hormone that inhibits bone formation and encourages bone resorption, thereby elevating blood-calcium levels.

Osteoclasts

Osteoclasts are multinucleated cells originating from granulocyte-macrophage progenitors and play a role in bone resorption.

The precursor of the **osteoclast** originates in the bone marrow. Osteoclasts have receptors for **osteoclast-stimulating factor**, **colony-stimulating factor-1**, OPG, **receptor for activation of nuclear factor kappa B (RANK)**, and **calcitonin**, among others. Osteoclasts are responsible for resorbing bone, and, after they finish doing so, these cells probably undergo apoptosis.

Morphology of Osteoclasts

Osteoclasts are large, motile, multinucleated cells 150 µm in diameter; they contain up to 50 nuclei and have an acidophilic cytoplasm (see Fig. 7–6). Osteoclasts have a bone marrow precursor in common with monocytes, termed ***cells of the mononuclear-phagocyte system***.

Osteoblasts secrete four signaling molecules that regulate the differentiation of osteoclasts. The first of these signals, the M-CSF, binds to a receptor on the osteoclast precursor, inducing it to proliferate and to express the **RANK** on the osteoclast precursor. Another signaling molecule, **RANKL**, bound to the osteoblast cell membrane, binds to the **RANK** receptor on the osteoclast precursor cell membrane, inducing the precursor cell to differentiate into the multinucleated osteoclast, activating it, and enhancing bone resorption. The second osteoblast-derived signaling molecule, the growth factor **interleukin-6 (IL-6)**, facilitates the recruitment and differentiation of osteoclasts. The third signaling molecule released by the osteoblast is **interleukin-1 (IL-1)**, which induces osteoclast precursors to proliferate; the fourth signaling molecule, **OPG**, a member of the **tumor necrosis factor receptor (TNFR)** family, can serve as a decoy by interacting with RANKL, thus prohibiting it from binding to the macrophage and inhibiting osteoclast formation. Therefore, RANKL, RANK, and OPG regulate bone metabolism and osteoclastic activity. **Osteoprotegerin** is produced not only by osteoblasts but also by cells of many other tissues, including the cardiovascular system, lung, kidney, intestines, hematopoietic cells, and in immune cells. Therefore, it is not surprising that its expression is modulated by various cytokines, peptides, hormones, drugs, and so on. In bone, OPG not only inhibits the differentiation of osteoclasts but also suppresses the osteoclast's bone resorbing capabilities.

Osteoclasts occupy shallow depressions on the bone surface, called ***Howship lacunae*** **(resorption bays)**, that identify regions of bone resorption. An osteoclast active in bone resorption may be subdivided into four morphologically recognizable regions:

1. **Basal zone**, located farthest from the Howship lacuna, houses most of the organelles, including the multiple nuclei and their associated Golgi complexes and centrioles. Mitochondria, RER, and polysomes are distributed throughout the cell but are more numerous near the ruffled border.
2. **Ruffled border** is the portion of the cell that is directly involved in resorption of bone. Its finger-like processes are active and dynamic, changing their configuration continually as they project into the resorption compartment, known as the ***subosteoclastic compartment***. The cytoplasmic aspect of the ruffled border plasmalemma displays a regularly spaced, bristle-like coat that increases the thickness of the plasma membrane of this region. As resorption progresses and the subosteoclastic compartment increases in size, it becomes known as *Howship lacuna*.
3. **Clear zone** is the region of the cell that immediately surrounds the periphery of the ruffled border. It is organelle-free but contains many actin filaments that form an **actin ring** and appear to function in helping integrins of the clear zone plasmalemma maintain contact with the bony periphery of the Howship lacuna. In fact, the plasma membrane of this region is so closely applied to the bone that its integrin molecules, in concert with **osteopontin**, form the **sealing zone** of the subosteoclastic compartment. Thus, the clear zone isolates the subosteoclastic compartment from the surrounding region, establishing a microenvironment whose contents may be modulated by cellular activities. For the osteoclast to be able to resorb bone, the actin ring must first be formed, and its formation may be facilitated by **RANKL**. Then the ruffled border is formed, whose finger-like processes increase the surface area of the plasmalemma in the region of bone resorption, facilitating the resorptive process.
4. **Vesicular zone** of the osteoclast consists of numerous endocytotic and exocytotic vesicles that ferry lysosomal enzymes and metalloproteinases into the subosteoclastic compartment and the products of bone degradation into the cell (Fig. 7–8). The vesicular zone is between the basal zone and the ruffled border.

Mechanism of Bone Resorption

Before osteoclasts are able to resorb bone, they must be activated by the osteoblast-derived osteoclast stimulating factor. Next, the osteoblasts must resorb the osteoid that separates them from the calcified bony surface, and then the osteoblasts have to migrate from that surface. Activated osteoclasts occupy the newly freed bone surface, and they begin to display the four zones described previously. The sealing zone is established, isolating the subosteoclastic compartment from the external milieu, and the ruffled border is formed. The finger-like processes express aquaporins, proton pumps, and Cl^- channels on their plasmalemmae.

Within the cytosol of the osteoclasts, the enzyme carbonic anhydrase catalyzes the intracellular formation of carbonic acid (H_2CO_3) from carbon dioxide and water. Carbonic acid dissociates within the cells into H^+ ions and bicarbonate ions, HCO_3^-. The bicarbonate ions, accompanied by Na^+ ions, cross the plasmalemma of the basal zone, where they act to buffer any hydrochloric acid that may escape from the subosteoclastic compartment, and any excess bicarbonate will enter nearby capillaries. Proton pumps in the plasmalemma of the ruffled border of the osteoclasts actively transport

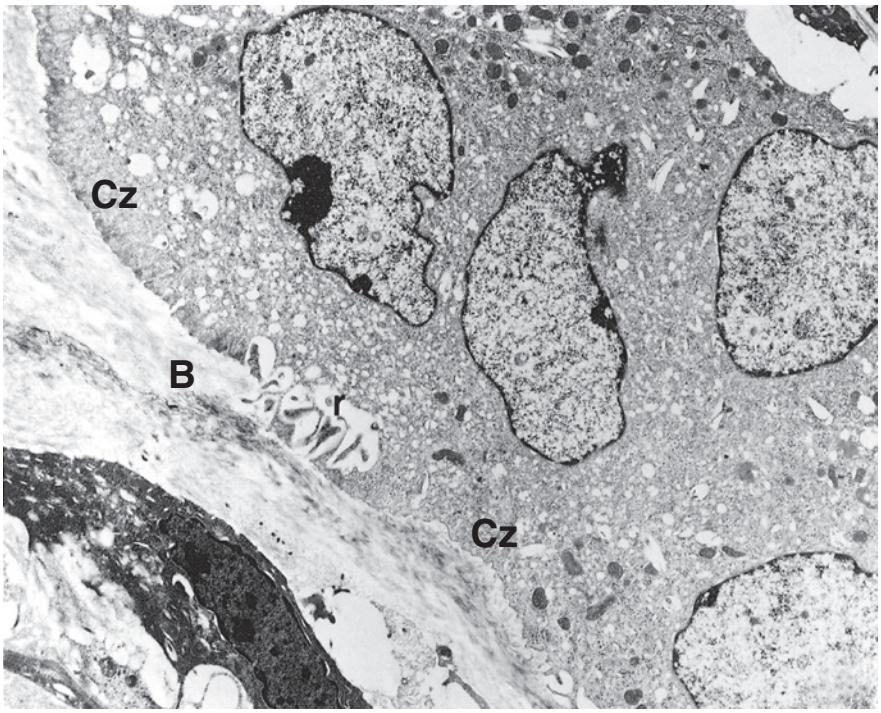


Figure 7–8 Electron micrograph of an osteoclast. Note the clear zone (Cz) on either side of the ruffled border (B) of this multinucleated cell. (From Marks SC Jr, Walker DG. The hematogenous origin of osteoclasts. Experimental evidence from osteopetrotic [microphthalmic] mice treated with spleen cells from beige mouse donors. *Am J Anat.* 1981;161:1-10. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

H^+ ions into the subosteoclastic compartment, and Cl^- ions follow passively and combined with the H^+ ions form HCl, thus reducing the pH of the microenvironment. Additionally, aquaporins of the ruffled border plasmalemma permit the flow of water from the cell into the subosteoclastic compartment. The inorganic component of the bone matrix is dissolved as the environment becomes acidic; the liberated minerals enter the osteoclast cytoplasm to be delivered to nearby capillaries.

Lysosomal hydrolases; **cathepsin K**; and **matrix metalloproteinases**, such as **collagenase** and **gelatinase**, are secreted by osteoclasts into the subosteoclastic compartment to degrade the organic components of the decalcified bone matrix. The degradation products are endocytosed by the osteoclasts and further broken down into amino acids, monosaccharides, and disaccharides, which then are released into nearby capillaries (Fig. 7–9).

Hormones of the Endocrine System That Control Bone Resorption

The bone-resorbing activity of osteoclasts is regulated by two hormones, **PTH** and **calcitonin**. PTH is released from the **parathyroid gland** when calcium blood levels fall below approximately 8.8 mg/dL (in adults). It acts in an indirect fashion by binding to PTH receptors on osteoblasts, and those cells respond by releasing factors noted previously to recruit and activate osteoclasts to resorb bone, thereby increasing blood calcium levels. Calcitonin, released by **C cells (parafollicular cells)** of the **thyroid gland**, has an opposite effect. When calcium blood levels are above approximately 10.5 mg/dL (in adults), calcitonin is released and binds directly to calcitonin receptors on osteoclasts and cause them to

CLINICAL CORRELATIONS

Osteopetrosis, not to be confused with osteoporosis, is a genetic disorder in which osteoclasts do not possess ruffled borders, possibly because of the presence of a mutated gene that codes for IL-6. Consequently, these osteoclasts cannot resorb bone, and persons with osteopetrosis display increased bone density. Individuals with this disease may exhibit anemia resulting from decreased marrow space, as well as blindness, deafness, and cranial nerve involvement because of impingement of the nerves caused by narrowing of the foramina.

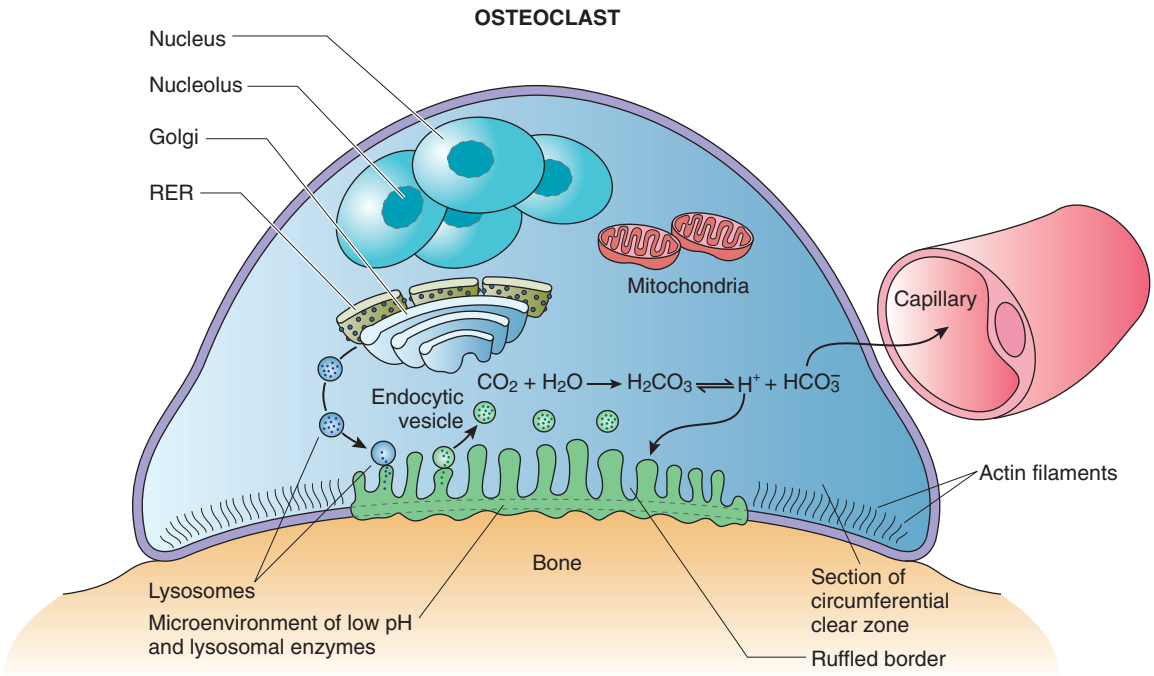


Figure 7–9 Schematic diagram illustrating osteoclastic function. RER, Rough endoplasmic reticulum. (From Gartner LP, Hiatt JL, Strum JM. *Cell Biology and Histology [Board Review Series]*. Philadelphia: Lippincott Williams & Wilkins; 1998:100.)

undergo apoptosis, thereby increasing blood calcium levels.

CLINICAL CORRELATIONS

Some postmenopausal women with signs of osteoporosis are prescribed calcitonin to reduce their osteoclastic activity and thereby retard bone loss. However, recent investigations have determined that calcitonin use may increase the incidence of cancer; therefore, in early 2013, the US Food and Drug Administration temporarily restricted the use of calcitonin until further study clarifies the situation.

Bone Structure

Bones are classified according to their anatomical shape: long, short, flat, irregular, and sesamoid.

Bones are classified according to their shape:

- **Long bones** display a shaft located between two heads (e.g., tibia).
- **Short bones** have more or less the same width and length (e.g., carpal bones of the wrist).
- **Flat bones** are flat, thin, and plate-like (e.g., bones forming the brain case of the skull).
- **Irregular bones** have an irregular shape that does not fit into the other classes (e.g., sphenoid and ethmoid bones within the skull).
- **Sesamoid bones** develop within tendons, where they increase the mechanical advantage for the muscle (e.g., patella) across a joint.

Gross Observation of Bone

Gross observations of the femur (a long bone) cut in longitudinal section reveal two different types of bone structure. The very dense bone on the outside surface is **compact bone**, whereas the porous portion lining the marrow cavity is **cancellous** or **spongy bone** (Fig. 7–10). Closer observation of the spongy bone reveals branching bony **trabeculae** and **spicules** jutting out from the internal surface of the compact bone into the marrow cavity. There are no haversian systems in spongy bone; instead, the lamellae of bone are arranged in an apparently irregular fashion. These lamellae of bone contain lacunae housing osteocytes that are nourished by diffusion from the marrow cavity, which is filled with **bone marrow** and which exists as two types: **red bone marrow**, in which blood cells are forming, and **yellow bone marrow**, composed of mostly fat.

The shaft of a long bone is called the ***diaphysis***, and the articular ends are called the ***epiphyses*** (singular, *epiphysis*). In a person who is still growing, the diaphysis is separated from each epiphysis by the **epiphyseal plate** of cartilage. The articular end of the bone is enlarged and sculpted to articulate with its bony

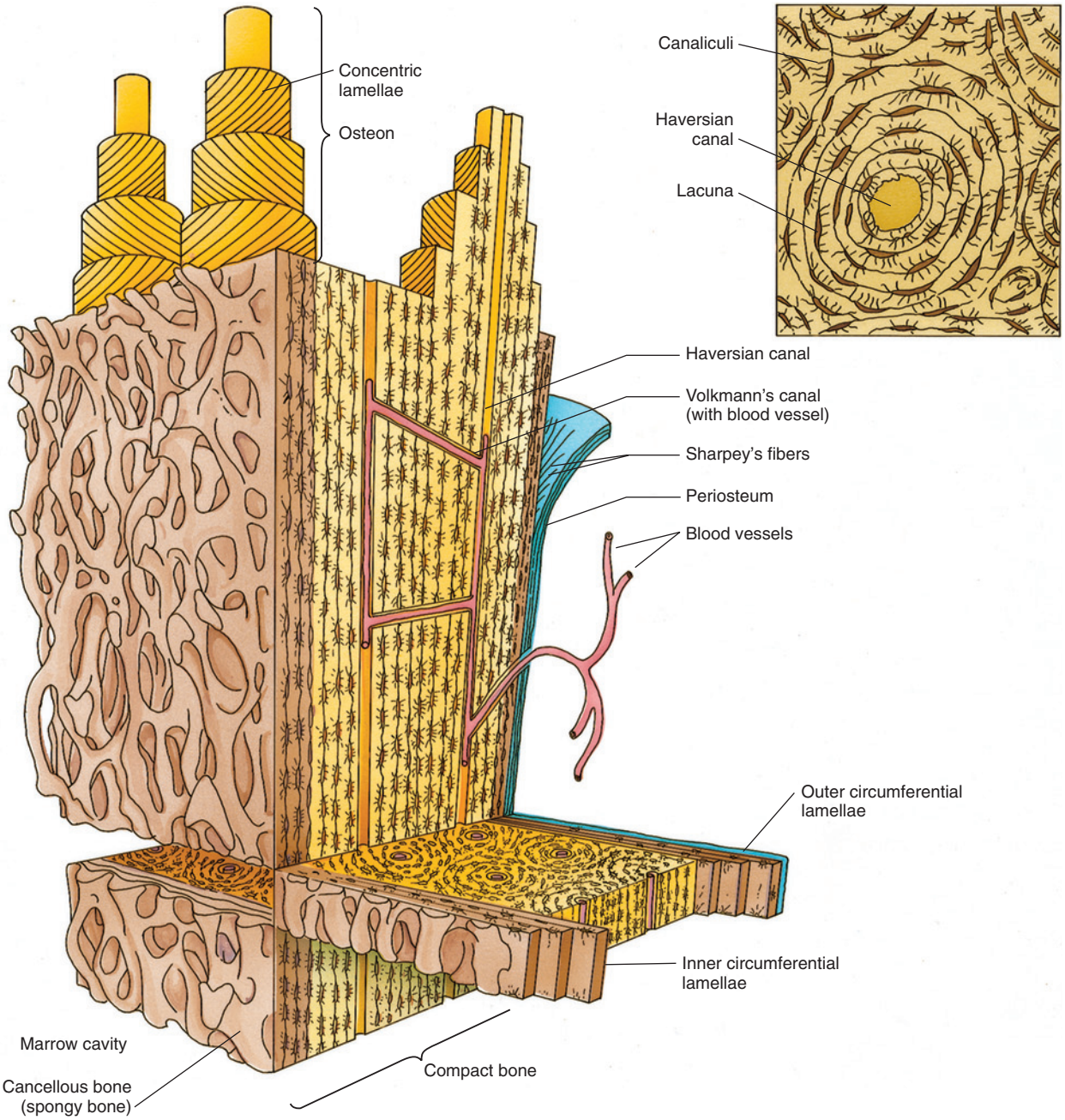


Figure 7–10 Diagram of bone illustrating compact cortical bone, osteons, lamellae, Volkmann canals, haversian canals, lacunae, canaliculi, and spongy bone.

counterpart of the joint. The surface of the articulating end is covered with only a thin layer of compact bone overlying spongy bone. On top of this is the highly polished articular hyaline cartilage, which reduces friction as it moves against the articular cartilage of the bony counterpart of the joint. The area of transition between the epiphyseal plate and the diaphysis is called the ***metaphysis***, where columns of spongy bone are located. It is from the epiphyseal plate and the metaphysis that bone grows in length.

The diaphysis is covered by a **periosteum**, except where tendons and muscles insert into the bone. There

is no periosteum on the surfaces of bone covered by articular cartilage. Periosteum is also absent from sesamoid bones (e.g., patella), which are formed within tendons and function to increase the mechanical advantage across a joint. As stated previously, the periosteum is a noncalcified, dense, irregular, collagenous connective tissue covering the bone on its external surface and inserting into it via **Sharpey fibers** (see Fig. 7–10). Periosteum is composed of two layers. The **outer fibrous layer** helps distribute vascular and nerve supply to bone, whereas the **inner cellular layer** possesses osteoprogenitor cells and occasional osteoblasts.

The flat bones of the skull develop by a different method than most of the long bones of the body. The inner and outer surfaces of the calvaria **(skull cap)** possess two relatively thick layers of compact bone called the ***inner*** and ***outer tables***, which surround the spongy bone **(diploë)** sandwiched between them. The outer table possesses a periosteum, identified as the **pericranium**, whereas, internally, the inner table is lined with **dura mater**, whose outer leaflet serves as a periosteum for the inner table as well as a protective covering for the brain.

Bone Types Based on Microscopic Observations

Microscopically, bone is classified as either primary (immature) or secondary (mature) bone.

Microscopic observations reveal two types of bone: primary bone (or immature or woven bone) and secondary bones (or mature or lamellar bone).

Primary bone is immature, in that it is the first bone to form during fetal development and during bone repair. It has abundant osteocytes and irregular bundles of collagen, which are later replaced and organized as secondary bone, except in certain areas (e.g., at sutures of the calvaria, insertion sites of tendons, and bony alveoli surrounding the teeth). The mineral content of primary bone is also much less than that of secondary bone.

Secondary bone is mature bone composed of parallel or concentric bony lamellae, where each lamella is 3 to 7 μm thick. Osteocytes in their lacunae are dispersed at regular intervals between, or occasionally within, lamellae. **Canaliculi**, housing osteocytic processes, connect neighboring lacunae with one another, forming a network of intercommunicating channels that facilitate the flow of nutrients, hormones, ions, and waste products to and from osteocytes. In addition, osteocytic processes within these canaliculi make contact with similar processes of neighboring osteocytes and form gap junctions, permitting these cells to communicate with each other.

Because the matrix of secondary bone is more calcified, it is stronger than primary bone. In addition, the collagen fibers of secondary bone are arranged so that they parallel each other within a given lamella.

Lamellar Systems of Compact Bone

There are four lamellar systems in compact bone: outer circumferential lamellae, inner circumferential lamellae, osteons, and interstitial lamellae.

Compact bone is composed of wafer-like thin layers of bone, **lamellae**, that are arranged in lamellar systems and are especially evident in the diaphyses of long bones. These lamellar systems are the outer circumferential lamellae, inner circumferential lamellae, osteons (haversian canal systems), and interstitial lamellae.

Outer and Inner Circumferential Lamellae

The **outer circumferential lamellae** are just deep to the periosteum, forming the outermost region of the diaphysis, and contain Sharpey fibers anchoring the periosteum to the bone (see Fig. 7–10).

The **inner circumferential lamellae**, analogous to but not as extensive as outer circumferential lamellae, completely encircle the marrow cavity. Trabeculae of spongy bone extend from the inner circumferential lamellae into the marrow cavity, interrupting the endosteal lining of the inner circumferential lamellae.

Haversian Canal System (Osteon) and Interstitial Lamellae

The bulk of compact bone is composed of an abundance of **haversian canal systems (osteons)**; each system is composed of cylinders of lamellae, concentrically arranged around a vascular space known as the ***haversian canal*** (Fig. 7–11; see Fig. 7–10). Frequently, osteons bifurcate along their considerable length. Each osteon is bounded by a thin **cementing line**, composed mostly of calcified ground substance with a scant amount of collagen fibers (see Fig. 7–5).

Collagen fiber bundles are parallel to each other within a lamella but are oriented almost perpendicular to those of adjacent lamellae. This arrangement is possible because the collagen fibers follow a helical arrangement around the haversian canal within each lamella but are pitched differently in adjacent lamellae.

Each haversian canal, lined by a layer of osteoblasts and osteoprogenitor cells, houses a neurovascular bundle with its associated connective tissue. Haversian canals of adjacent osteons are connected to each other by **Volkmann canals** (Fig. 7–12; see Fig. 7–10), vascular spaces that are oriented oblique to or perpendicular to haversian canals.

The diameter of haversian canals varies from approximately 20 to about 100 μm. During the formation of osteons, the lamella closest to the cementing line is the first one to be formed. As additional lamellae are added to the system, the diameter of the haversian canal is reduced, and the thickness of the osteon wall increases. Because nutrients from blood vessels of the haversian canal must traverse canaliculi to reach osteocytes, a rather inefficient process, most osteons possess 4 to 20 lamellae only.

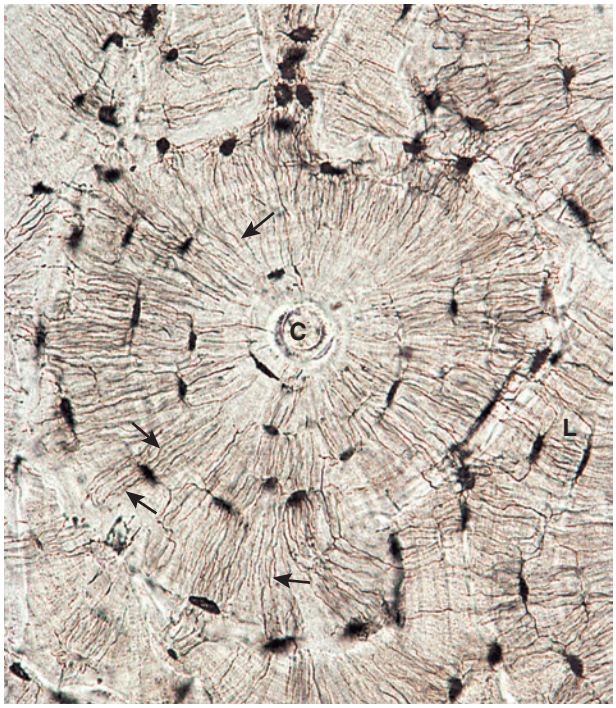


Figure 7–11 Light micrograph of nondecalcified ground bone (×270). Observe the haversian system containing the haversian canal (C) and concentric lamellae with lacunae with their canaliculi *(arrows)*.

As bone is being remodeled, osteoclasts resorb osteons, and osteoblasts replace them. Remnants of osteons remain as irregular arcs of lamellar fragments, known as ***interstitial lamellae***, surrounded by osteons. Similar to osteons, interstitial lamellae are also surrounded by cementing lines.

Histogenesis of Bone

Bone formation during embryonic development may be of two types: **intramembranous** and **endochondral**. Bone that is formed by either of the two methods is identical histologically. The first bone formed is primary bone, which is later resorbed and replaced by secondary bone. Secondary bone continues to be resorbed throughout life, although at a slower rate.

Intramembranous Bone Formation

Intramembranous bone formation occurs within mesenchymal tissue.

Most flat bones are formed by **intramembranous bone formation**. This process occurs in a richly vascularized mesenchymal tissue, whose cells make contact with each other via long processes.

Mesenchymal cells, under the influence of **Cbfa1/Runx2** and **osterix**, differentiate into **osteoblasts** that

Figure 7–12 Light micrograph of decalcified compact bone (×162). Several osteons (Os) are displayed with their concentric lamellae (l). A Volkmann canal (V) is also displayed. The dark staining structures scattered throughout represent nuclei of osteocytes (Oc).

secrete **bone matrix**, forming a network of **spicules** and **trabeculae** whose surfaces are populated by these cells (Figs. 7–13 and 7–14). This region of initial osteogenesis is known as the ***primary ossification center***. As expected in primary bone, the type I collagen fibers of these developing spicules and trabeculae are oriented in a random fashion. Calcification quickly follows osteoid formation, and osteoblasts trapped in their matrices become osteocytes. The processes of these osteocytes contact one another and are also surrounded by forming bone, establishing a system of canaliculi. Continuous mitotic activity of mesenchymal cells provides a supply of undifferentiated **osteoprogenitor cells**, which form osteoblasts.

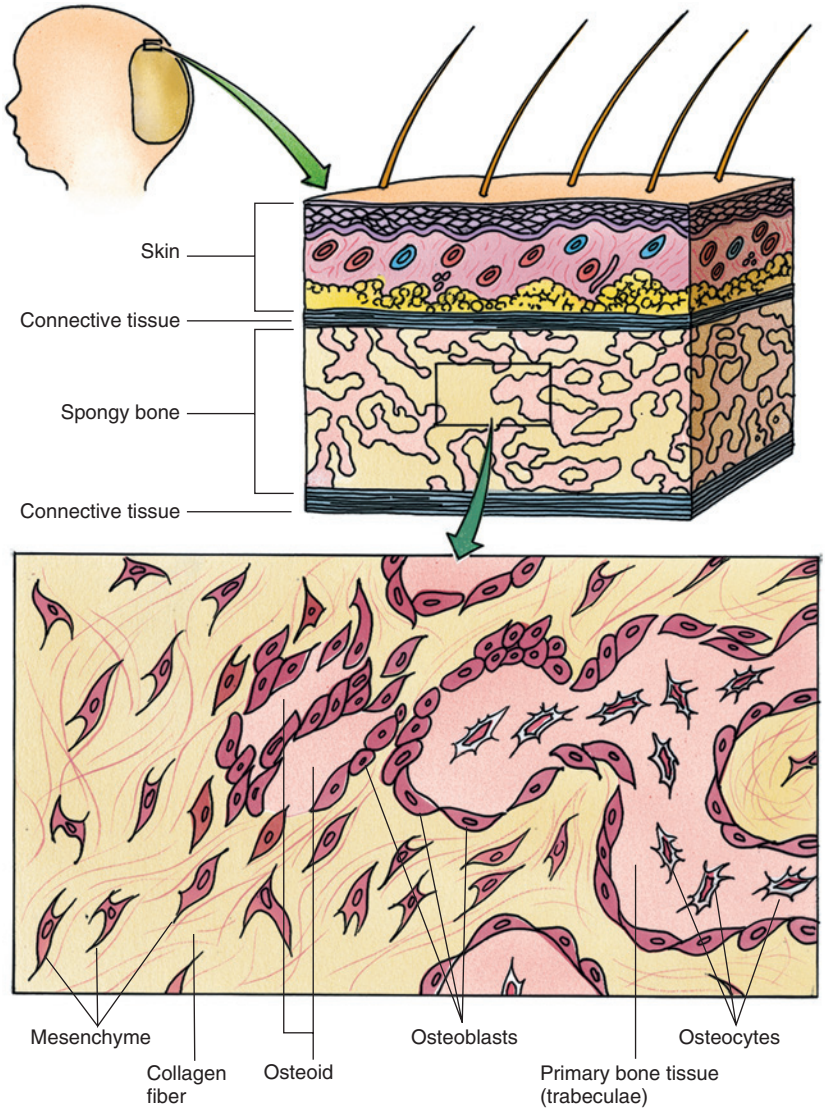


Figure 7–13 Diagram of intramembranous bone formation.

As the sponge-like network of trabeculae is established, the vascular connective tissue in their interstices is transformed into bone marrow. The addition of trabeculae to the periphery increases the size of the forming bone. Larger bones, such as the occipital bone of the base of the skull, have several ossification centers, which fuse with one another to form a single bone. The fontanelles ("soft spots") on the frontal and parietal bones of a newborn infant represent ossification centers that are not fused prenatally.

Regions of the mesenchymal tissues that remain uncalcified differentiate into the periosteum and endosteum of developing bone. Moreover, the spongy bone deep to the periosteum and the periosteal layer of the dura mater of flat bones are transformed into compact bone, forming the **inner** and **outer tables** with the intervening diploë.

CLINICAL CORRELATIONS

It has been shown that mesenchymal cells of mouse embryos that are **osterix null**, that is, they do not express osterix, cannot differentiate into osteoblasts and, as a consequence, cannot form bone.

Endochondral Bone Formation

Endochondral bone formation requires the presence of a cartilage template.

Figure 7–14 Light micrograph of intramembranous ossification (×132). Trabeculae of bone are being formed by osteoblasts lining their surface *(arrows)*. Observe osteocytes (Os) trapped in lacunae *(arrowheads)*. Primitive osteons are beginning to form.

Most of the long and short bones of the body develop by **endochondral bone formation**. This type of bone formation occurs in two steps: (1) a miniature hyaline cartilage model is formed, and (2) the cartilage model continues to grow and serves as a structural scaffold for bone development and is eventually resorbed and replaced by bone. Table 7–3 summarizes the events in endochondral bone formation, and Figure 7–15 illustrates the process.

Events in Endochondral Bone Formation (Primary Center of Ossification)

1. In the region where bone is to grow within the embryo, a **hyaline cartilage model of that bone is developed**. This event begins in exactly the same way that hyaline cartilage at any location would be developed (discussed previously). This model grows, both appositionally and interstitially, for a period of time. Eventually, the chondrocytes in the center of the cartilage model hypertrophy, accumulate glycogen in their cytoplasm, and become vacuolated (Fig. 7–16). Hypertrophy of the chondrocytes results in the enlargement of their lacunae with a concomitant reduction in the intervening cartilage matrix septa, which become calcified.
2. Concurrently, the perichondrium at the **midriff of the diaphysis of cartilage becomes vascularized** (Fig. 7–17). When this happens, chondrogenic cells become osteoprogenitor cells that differentiate to form osteoblasts, and this portion of the overlying perichondrium becomes a periosteum.
3. The newly formed **osteoblasts secrete bone matrix**, **forming the subperiosteal bone collar** on the surface of the cartilage template by *intramembranous bone formation* (see Fig. 7–17).
4. The bone collar prevents the diffusion of nutrients to the hypertrophied chondrocytes within the core of the cartilage model, causing them to die. This process is responsible for the presence of empty, confluent lacunae forming large concavities—the future marrow cavity in the center of the cartilage model.

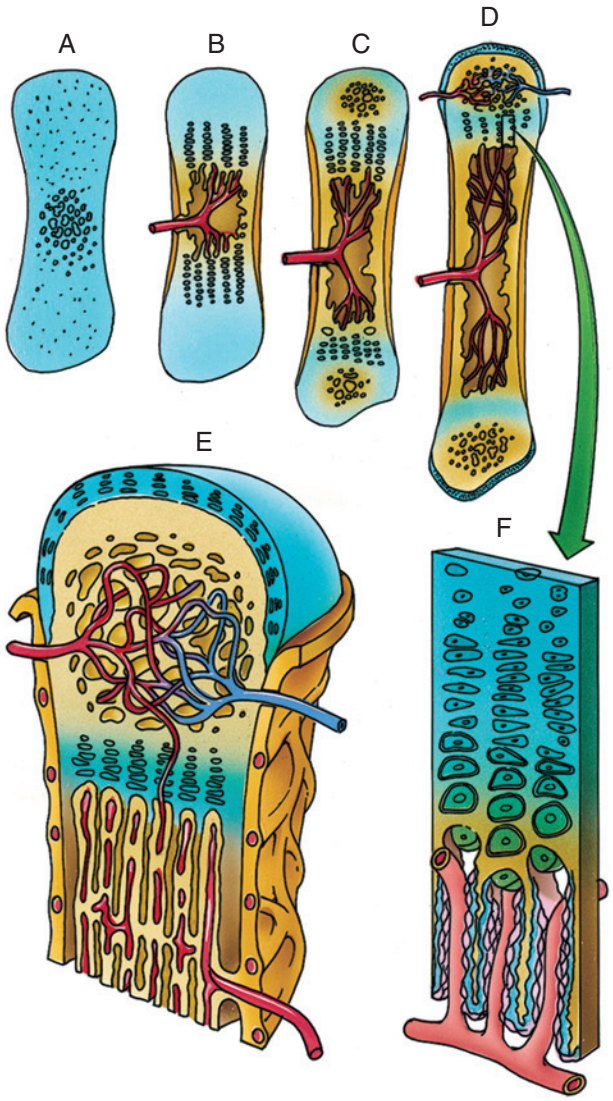


Figure 7–15 Diagram of endochondral bone formation. *Blue* represents the cartilage model upon which bone is formed replacing the cartilage.

TABLE 7–3 Events in Endochondral Bone Formation

Event	Description
Hyaline cartilage model formed	Miniature hyaline cartilage model formed in region of developing embryo where bone is to develop. Some chondrocytes mature, hypertrophy, and die. Cartilage matrix becomes calcified.
Primary Center of Ossification	
Perichondrium at the midriff of diaphysis becomes vascularized	Vascularization of perichondrium changes it to periosteum. Chondrogenic cells become osteoprogenitor cells.
Osteoblasts secrete matrix, forming subperiosteal bone collar	The subperiosteal bone collar is formed of primary bone (intramembranous bone formation).
Chondrocytes within the diaphysis core hypertrophy, die, and degenerate	Presence of periosteum and bone prevents diffusion of nutrients to chondrocytes. Their degeneration leaves lacunae, opening large spaces in cartilage.
Osteoclasts etch holes in subperiosteal bone collar, permitting entrance of osteogenic bud	Holes permit osteoprogenitor cells and capillaries to invade cartilage model, now calcified, and begin elaborating bone matrix.
Formation of calcified cartilage–calcified bone complex	Bone matrix laid down on septa of calcified cartilage forms this complex. Histologically, calcified cartilage stains blue, and calcified bone stains red.
Osteoclasts begin resorbing the calcified cartilage–calcified bone complex	Destruction of the calcified cartilage–calcified bone complex enlarges the marrow cavity.
Subperiosteal bone collar thickens, begins growing toward epiphyses	This event, over a period of time, completely replaces diaphyseal cartilage with bone.
Secondary Center of Ossification	
Ossification begins at epiphysis	Begins in same way as primary center except there is no bone collar. Osteoblasts lay down bone matrix on calcified cartilage scaffold.
Growth of bone at epiphyseal plate	Cartilaginous articular surface of bone remains. Epiphyseal plate persists—growth added at epiphyseal end of plate. Bone added at diaphyseal end of plate.
Epiphysis and diaphysis become continuous	At end of bone growth, cartilage of epiphyseal plate ceases proliferation. Bone development continues to unite the diaphysis and epiphysis.

5. Holes etched in the bone collar by osteoclasts permit a **periosteal buds** (osteogenic buds), composed of osteoprogenitor cells, hemopoietic cells, and blood vessels, to enter the concavities within the cartilage model (see Fig. 7–15).
6. Osteoprogenitor cells divide to form more osteoprogenitor cells, some of which differentiate into osteoblasts. These newly formed cells elaborate bone matrix on the surface of the calcified cartilage. The bone matrix becomes calcified to form a **calcified cartilage–calcified bone complex**. This complex can be appreciated in routinely stained histological sections because calcified cartilage stains blue, whereas calcified bone stains pink with hematoxylin and eosin (Figs. 7–18 and 7-19).
7. As the subperiosteal bone collar increases in thickness and grows in each direction from the midriff of the diaphysis toward the two epiphyses, osteoclasts begin resorbing the calcified cartilage–calcified bone complex, enlarging the marrow cavity. As this process continues, the cartilage of the diaphysis is replaced by bone, except for the **epiphyseal plates**, which are responsible for the continued lengthening of the bone for the next 18 to 20 years.

Events Occurring at Secondary Centers of Ossification

Secondary centers of ossification begin to form at the epiphysis at each end of the forming bone by a process similar to that in the diaphysis, except that a bone collar is not formed. Rather, osteoprogenitor cells invade the cartilage of the epiphysis, differentiate into osteoblasts, and begin secreting matrix on the cartilage scaffold (see Fig. 7–15). These events take place and progress much as they do in the diaphysis, and, eventually, the cartilage of the epiphysis is replaced with bone, except at the articular surface and at the epiphyseal plate. The articular surface of the bone remains cartilaginous throughout life. The process at the epiphyseal

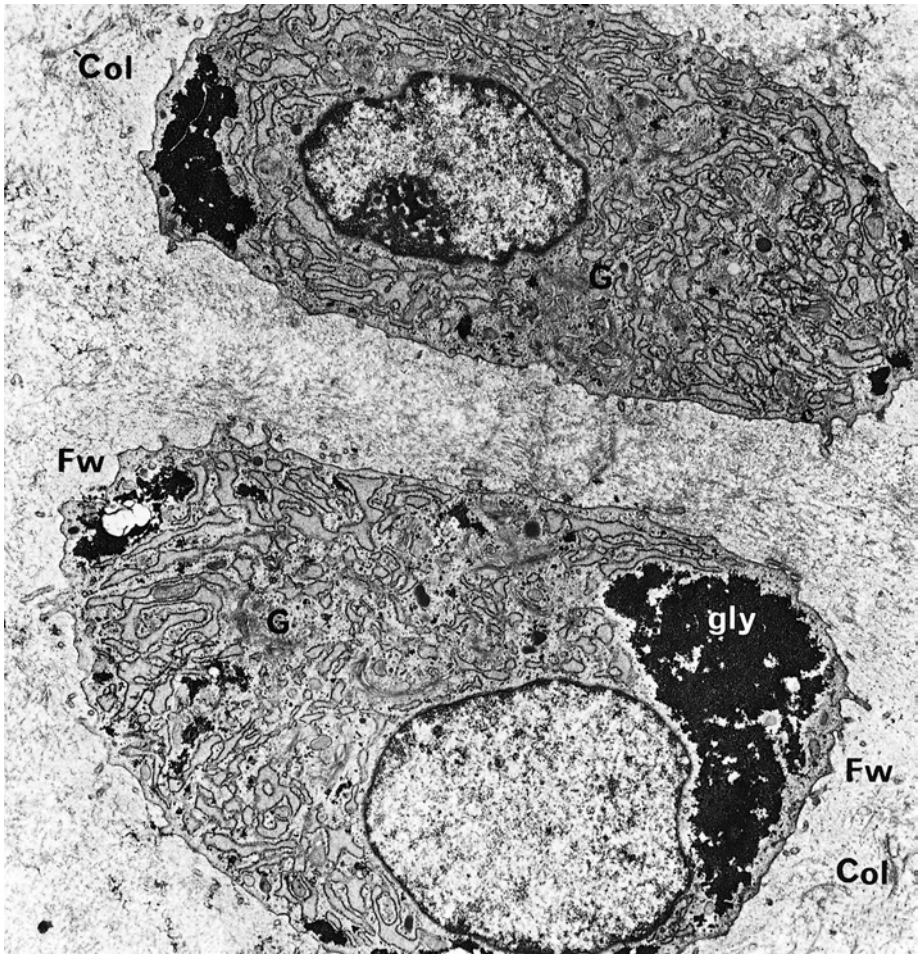


Figure 7–16 Electron micrograph of hypertrophic chondrocytes in the growing mandibular condyle (×83000). Observe the abundant rough endoplasmic reticulum and developing Golgi apparatus (G). Note also glycogen (gly) deposits in one end of the cells, a characteristic of these cells shortly before death. Col, Collagen fibers; Fw, territorial matrix. (From Marchi F, Luder HU, Leblond CP. Changes in cells' secretory organelles and extracellular matrix during endochondral ossification in the mandibular condyle of the growing rat. *Am J Anat*. 1991;190:41-73. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

plate, which controls bone length, is described in the next section.

These events form a dynamic continuum that is completed over a number of years as bone growth and development progress toward the growing epiphyses at each end of the bone (see Table 7–3). At the same time, the bone is constantly being remodeled to meet the changing forces placed on it.

Bone Growth in Length

The continued lengthening of bone depends on the epiphyseal plate.

The chondrocytes of the epiphyseal plate proliferate and participate in the process of endochondral bone formation. Proliferation occurs at the epiphyseal aspect, and replacement by bone takes place at the diaphyseal side of the plate. Histologically, the epiphyseal plate is divided into five recognizable zones. These zones, beginning at the epiphyseal side, are as follows:

- **Zone of reserve cartilage**. Chondrocytes randomly distributed throughout the matrix are mitotically active.
- **Zone of proliferation**. Chondrocytes, rapidly proliferating, form rows of isogenous cells that parallel the direction of bone growth. This proliferation rate is under the control of a signaling molecule, a paracrine hormone known as ***Indian hedgehog***, manufactured and released by the chondrocytes of this zone to act on all the chondrocytes in their

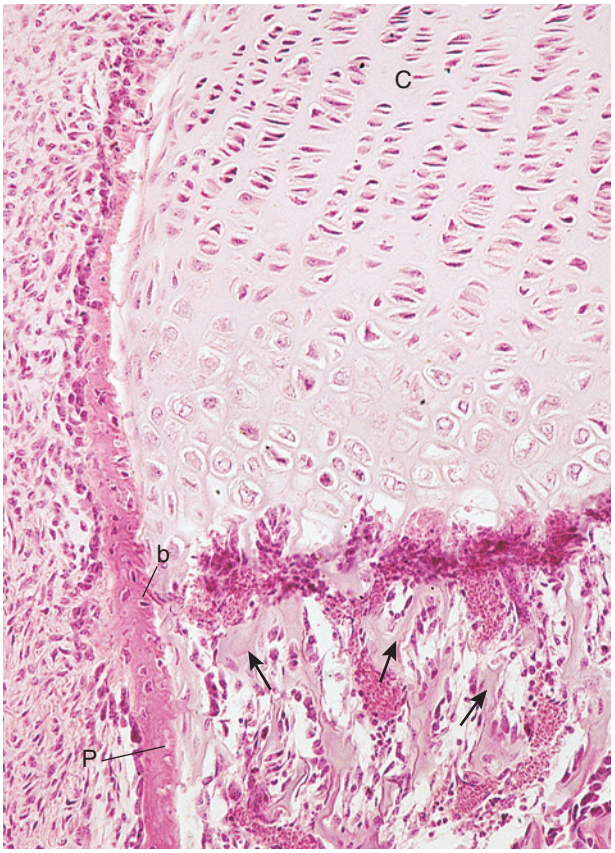


Figure 7–17 Light micrograph of endochondral bone formation (×14). The *upper* half of the photograph demonstrates cartilage (C) containing chondrocytes that mature, hypertrophy, and calcify at the interface; the *lower* half shows where calcified cartilage–bone complex *(arrows)* is being resorbed and bone (b) is being formed. P, Subperiosteal bone collar.

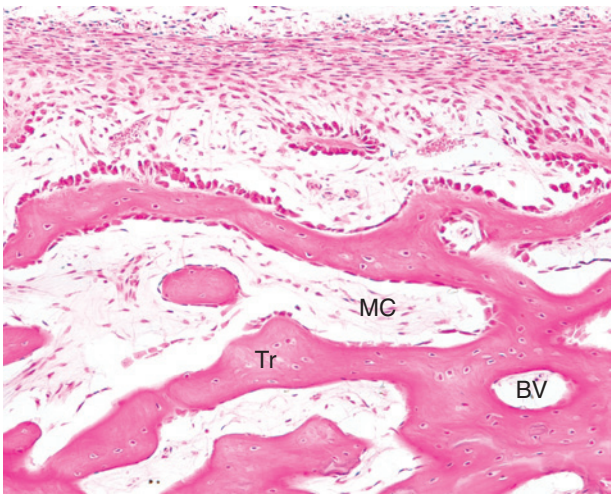


Figure 7–18 Light micrograph of endochondral bone formation. Observe a blood vessel (BV), bone-covered trabeculae of calcified cartilage (Tr), and the medullary cavity (MC) (×132).

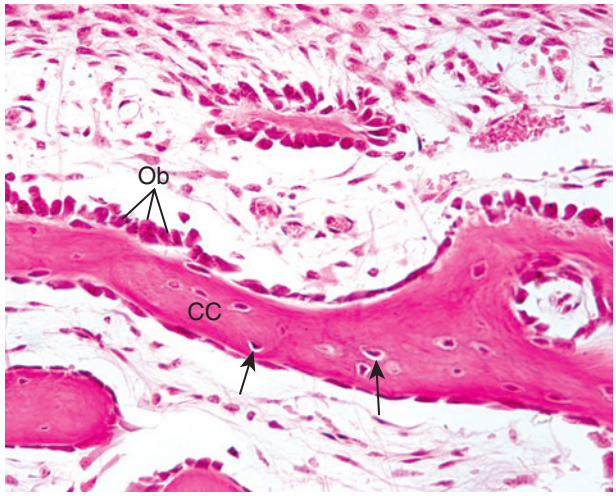


Figure 7–19 Higher magnification of endochondral bone formation. The trabeculae of calcified cartilage (CC) are covered by a thin layer bone *(darker red)* with osteocytes embedded in it *(arrows)* and with osteoblasts (Ob) lying next to the bone (×270).

immediate vicinity. This factor not only induces proliferation of the chondrocytes but also delays chondrocytic hypertrophy, thus maintaining the requisite breadth of the epiphyseal plate. The chondrocytes of this zone are also sensitive to **insulin-like growth factor 1 (IGF-1)** and to a very limited extent to IGF-2. IGF-1 is a hormone manufactured by hepatocytes in response to **growth hormone** secreted by the pituitary gland, and chondrocytes of this zone proliferate in response to being exposed to IGF-1. Although, as noted previously, osteocytes also manufacture and release IFG-1. In the case of the epiphyseal plate, it is the hepatocyte-produced IFG-1 that acts on the chondrocytes.

- **Zone of maturation and hypertrophy**. Chondrocytes mature, hypertrophy, and accumulate glycogen in their cytoplasm (see Fig. 7–16). The interterritorial matrix between their lacunae narrows with a corresponding enlargement of the chondrocytes within the lacunae. The chondrocytes of this zone eventually undergo apoptosis and die, but while enlarging, they secrete **vascular endothelial growth factor**, a cytokine that encourages the invasion of blood vessels, which bring calcium ions and specialized macrophage precursors into the area.
- **Zone of calcification**. Lacunae become confluent, hypertrophied chondrocytes die, and cartilage matrix becomes calcified using the calcium ions brought in by the blood vessels.
- **Zone of ossification**. Osteoprogenitor cells invade the area and differentiate into osteoblasts, which elaborate matrix on the surface of calcified cartilage. This is followed by calcification of the bone matrix

and the resorption of the calcified cartilage–calcified bone complex by the specialized macrophages recruited by the hypertrophied chondrocytes.

- As long as the rate of mitotic activity in the zone of proliferation equals the rate of resorption in the zone of ossification, the epiphyseal plate remains the same thickness, and the bone continues to grow longer. At about the 20th year of age, the rate of mitosis decreases in the zone of proliferation, and the zone of ossification overtakes the zones of proliferation and the zone of reserve cartilage. The cartilage of the epiphyseal plate becomes replaced by a plate of calcified cartilage–calcified bone complex, which becomes resorbed by osteoclastic activity, and the marrow cavity of the diaphysis becomes confluent with the bone marrow cavity of the epiphysis. After the epiphyseal plate is resorbed, growth in length is no longer possible.

Bone Growth in Width

The events previously described detail how lengthening of a long bone is accomplished by **endochondral bone formation** via the proliferation and interstitial growth of the chondrocytes of the epiphyseal plate, which is eventually replaced by bone. Growth of the diaphysis in girth, however, takes place by **appositional growth**. The **osteoprogenitor cells** of the osteogenic layer of the periosteum proliferate and differentiate into osteoblasts that begin elaborating bone matrix on the subperiosteal bone surface. This process occurs continuously throughout the total period of bone growth and development, so that, in a mature long bone, the shaft is built via subperiosteal intramembranous bone formation.

During bone growth and development, bone resorption is as important as bone deposition. Formation of bone on the outside of the shaft must be accompanied by osteoclastic activity internally so that the marrow space can be enlarged.

Calcification of Bone

Calcification begins when there are deposits of calcium phosphate on the collagen fibril.

Exactly how calcification occurs is still unclear, although it is known to be stimulated by certain proteoglycans and the Ca^2-binding glycoprotein **osteonectin** as well as **bone sialoprotein**. One theory, called ***heterogeneous nucleation***, is that collagen fibers in the matrix are nucleation sites for the metastable calcium and phosphate solution, and the solution begins to crystallize into the gap region of the collagen. After this region has "nucleated," calcification proceeds.

The most commonly accepted theory of calcification is based on the presence of matrix vesicles within the osteoid. Osteoblasts release these small, membrane-bounded matrix vesicles, 100 to 200 nm in diameter, which contain a high concentration of Ca^{2+} and PO_4^{3-} ions, cAMP, adenosine triphosphate (ATP), adenosine triphosphatase (ATPase), alkaline phosphatase, pyrophosphatase, calcium-binding proteins, and phosphoserine. The matrix vesicle membrane possesses numerous calcium pumps, which transport Ca^{2+} ions present in the bone matrix into the vesicle. As the concentration of calcium Ca^{2+} ions within the vesicle increases, crystallization occurs, and the growing calcium hydroxyapatite crystal pierces the membrane, bursting the matrix vesicle and releasing its contents.

Alkaline phosphatase cleaves pyrophosphate groups from the macromolecules of the matrix. The liberated pyrophosphate molecules are inhibitors of calcification, but they are cleaved by the enzyme pyrophosphatase into PO_4^{3-} ions, increasing the concentration of this ion in the microenvironment.

The calcium hydroxyapatite crystals released from the matrix vesicles act as **nidi of crystallization**. The high concentration of ions in their vicinity, along with the presence of calcification factors and calcium-binding proteins, fosters the calcification of the matrix. As crystals are deposited into the gap regions on the surface of collagen molecules, water is resorbed from the matrix.

Mineralization occurs around numerous closely spaced nidi of crystallization; as it progresses, these centers enlarge and fuse with each other. In this fashion, an increasingly large region of the matrix is dehydrated and calcified.

CLINICAL CORRELATIONS

Osteoporosis affects about 10 million chronically immobilized Americans, especially in women older than 40 years of age but mostly in postmenopausal women. Osteoporosis is related to decreasing bone mass, which becomes more serious as estrogen secretion drops appreciably after menopause. Binding of estrogen to specific receptors on osteoblasts activates the cells to manufacture and secrete bone matrix. With diminished secretion of estrogen, osteoclastic activity is greater than bone deposition, potentially reducing bone mass to the point at which the bone cannot withstand stresses and breaks easily.

For decades, estrogen replacement therapy coupled with calcium supplements and pain killers were used to alleviate or eliminate this condition. However, in 2004, it was determined that estrogen replacement therapy increases the risk for heart disease, stroke, breast cancer, and blood clots. Therefore, instead of estrogen, a new group of drugs, the bisphosphonates, has been developed that reduces the incidence of osteoporosis fractures. An early diagnostic tool, dual-energy x-ray absorptiometry, is being used as a reliable method for increasing bone density even in individuals with osteoporosis.

Bone Remodeling

In adults, bone development is balanced with bone resorption as bone is remodeled to meet stresses placed on it.

In a young person, bone development exceeds the rate of bone resorption because more haversian systems are being added than resorbed. After the desired bone growth has been attained, under normal conditions, the rate of bone formation equals the rate of bone resorption.

Growing bones largely retain the general architectural shape from the beginning of bone development in a fetus to the end of bone growth in an adult. This is accomplished by **surface remodeling**, a process involving bone deposition under certain regions of the periosteum with concomitant bone resorption under other regions of the periosteum. Similarly, bone is being deposited in certain regions of the endosteal surface, whereas it is being resorbed in other regions. The bones of the calvaria are being reshaped in a similar way to accommodate the growing brain; however, how this process is regulated is unclear.

Cortical bone and cancellous bone, however, are not remodeled in the same fashion, probably because osteoblasts and osteoprogenitor cells of cancellous bone are located within the confines of bone marrow and, therefore, they are under the direct, paracrine influence of nearby bone marrow cells. The factors produced by these bone marrow cells include IL-6, tumor necrosis factor-α (TNF-α), M-CSF, OPG, RANKL, and TGF-β. The osteoprogenitor cells and occasional osteoblasts of compact bone are located in the cellular layer of the periosteum and in the lining of haversian canals and thus are too far from the cells of bone marrow to be under their paracrine influence. Instead, these cells of compact bone respond to **systemic factors**, such as calcitonin and parathyroid hormone.

The internal structure of adult bone is continually being altered because bone must be resorbed from one area and added to another to meet changing stresses that are placed on it, such as weight changes, postural alterations, or microfractures of bone involving individual osteons. This process is known as ***internal remodeling*** and is performed by the **bone remodeling unit** whose two elements are the **cutting cone (resorption cavity)** and the **closing cone (lamellar formation)**.

In regions where compact bone is to be remodeled, osteoclasts are recruited to the area to resorb the bone, forming cone-shaped tunnels in compact bone, known as ***cutting cones*** **(resorption cavities)**. Continual osteoclastic activity increases the diameter and length of these cutting cones, which may reach as much as 1.5 mm in length and 100 μm in radius. After these tunnels reach their maximum size, they become invaded by blood vessels, osteoblasts, and osteoprogenitor cells. At this point, bone resorption ceases, and osteoblasts deposit new concentric lamellae around the blood vessels, forming new haversian systems **(closing cone)**. Not only is primary bone remodeled in this fashion, which strengthens the bone by establishing ordered collagen alignment about the haversian system, but also remodeling continues throughout life as resorption is replaced by deposition and the formation of new haversian systems. This process of bone resorption, followed by bone replacement, is known as a ***coupled system of activation***, ***resorption***, and ***formation*** **(coupling)**. The interstitial lamellae that are observed in adult bone are remnants of remodeled haversian systems.

Bone Repair

Bone repair involves both intramembranous and endochondral bone formation.

A bone fracture causes damage and destruction to the bone matrix, death to cells, tears in the periosteum and endosteum, and possible displacement of the ends of the broken bone (fragments). Blood vessels are severed near the break, and localized hemorrhaging fills in the zone of the break, resulting in blood clot formation at the site of injury. Soon the blood supply is shut down in a retrograde fashion from the injury site back to regions of anastomosing vessels, which can establish a new circulation route. As a consequence of the cessation of the circulation, there is a widening zone of injury, on either side of the original break, due to the lack of a blood supply to many haversian systems, thus causing the zone of dead and dying osteocytes to increase appreciably. Because bone marrow and the periosteum are highly vascularized, the initial injury site in either of these two areas does not grow significantly, and there is no notable increase in dead and dying cells much beyond the original injury site. Wherever the bone's haversian systems are without a blood supply, osteocytes become pyknotic, undergo lysis, and leave empty lacunae behind.

The blood clot filling the site of the fracture is invaded by small capillaries and fibroblasts from the surrounding connective tissue, forming **granulation tissue**. Approximately 48 hours after injury, osteoprogenitor cells build up because of increased mitotic activity of the osteogenic layer of the periosteum. The deepest layer of proliferating osteoprogenitor cells of the periosteum (those closest to the bone), which are in the vicinity of capillaries, differentiate into osteoblasts and begin elaborating a collar of bone, cementing it to the dead bone about the injury site. Although the capillaries are growing, their rate of proliferation is much slower than that of the osteoprogenitor cells; thus, the osteoprogenitor cells in the middle of the proliferating mass are now without a profuse capillary bed. This

results in lowered oxygen tension, and these cells become chondrogenic cells, giving rise to chondroblasts that form cartilage in the outer parts of the bony collar.

The outermost layer of the proliferating osteoprogenitor cells (those adjacent to the fibrous layer of the periosteum) have some capillaries in their vicinity; therefore, they continue to proliferate as osteoprogenitor cells. Thus, the collar under the periosteum exhibits three zones that blend together: (1) the deepest stratum consists of a layer of new bone cemented to the bone of the fragment, (2) an intermediate layer of cartilage, and (3) a proliferating osteogenic layer on the surface. In the meantime, the collars formed on the ends of each fragment fuse into a single, combined collar, known as the ***external callus***, leading to an external union of the fragments. Continued growth of the external collar is derived mainly from proliferation of osteoprogenitor cells and, to some degree, from interstitial growth of the cartilage in its intermediate zone.

The cartilage matrix adjacent to the new bone formed in the deepest region of the external collar becomes calcified and is eventually replaced with cancellous bone. Ultimately, all of the cartilage is replaced with primary bone by endochondral bone formation.

A similar event occurs in the marrow cavities as a clot forms; the clot is soon invaded by osteoprogenitor cells of the endosteum and multipotential cells of the bone marrow, forming an **internal callus** of bony trabeculae within 1 week or so subsequent to the break (Fig. 7–20).

After the fragments of bone are united by bridging with cancellous bone both on the external and the marrow surfaces, it is necessary to remodel the injury site by replacing the primary bone with secondary bone and resolving the callus.

The first bone elaborated against injured bone develops by intramembranous bone formation, and the new trabeculae become firmly cemented to the injured or dead bone. Matrices of dead bone, located in the empty spaces between newly developing bony trabeculae, are resorbed, and the spaces are filled in by new bone. Eventually, all of the dead bone is resorbed and replaced by new bone formed by the osteoblasts that invade the region. These events are concurrent, resulting in repair of the fracture with cancellous bone surrounded by a bony callus.

Through the events of remodeling, the primary bone of intramembranous bone formation is replaced with secondary bone, further reinforcing the mended fracture zone; at the same time, the callus is resorbed. It appears that the healing and remodeling processes at the fracture site are in direct response to the stresses placed on it; eventually, the repaired zone is restored to its original shape and strength. It is interesting that bone repair involves cartilage formation and both intramembranous and endochondral bone formation.

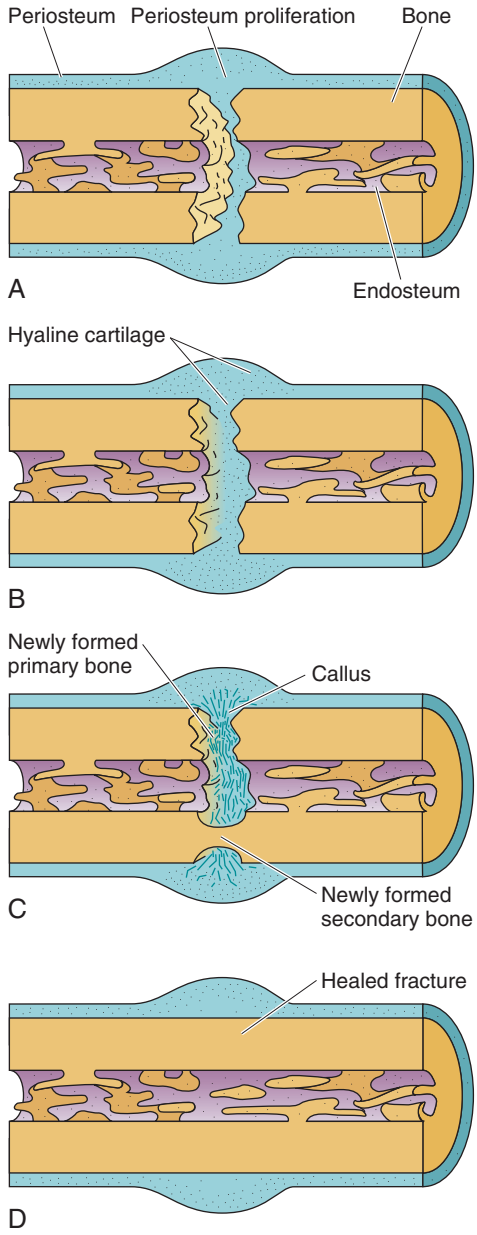


Figure 7–20 Diagram of the events in bone fracture repair.

Recapitulation of the Principal Hormones and Factors Affecting Bone

The following systemic hormones affect bone:

- PTH is released from the chief cells of the parathyroid gland when calcium blood levels fall below approximately 8.8 mg/dL (in adults). It acts in an indirect fashion by binding to PTH receptors on osteoblasts, and those cells respond by releasing

CLINICAL CORRELATIONS

1. If segments of bone are lost or damaged so severely that they have to be removed, a "**bony union**" is not possible; that is, the process of bone repair cannot occur because a bony callus does not form. In cases of this sort, a bone graft is required. Since the 1970s, bone banks have become available to supply viable bone for grafting purposes. The bone fragments are harvested and frozen to preserve their osteogenic potential and are then used as transplants by orthopedic surgeons. **Autografts** are the most successful because the transplant recipient is also the donor. **Homografts** are from different individuals of the same species and may be rejected because of an immunological response. **Heterografts**, grafts from different species, are least successful, although it has been shown that calf bone loses some of its antigenicity after being refrigerated, making it a worthy bone graft when necessary.
2. Skeletal maturation is also influenced by hormones produced in the male and female gonads. Closure of the epiphyseal plates is normally rather stable and constant and is related to sexual maturation. For example, precocious sexual maturation stunts skeletal development because the epiphyseal plates are stimulated to close too early. In other people whose sexual maturation is retarded, skeletal growth continues beyond normal because the epiphyseal plates do not close.
3. **Acromegaly** occurs in adults who produce an excess of somatotropin, causing an abnormal increase in bone deposition without normal bone resorption. This condition creates thickening of the bones, especially those of the face, in addition to disfiguring soft tissue.

factors noted previously to recruit and activate osteoclasts to resorb bone thereby increasing blood calcium levels.

- Calcitonin, released by C cells (parafollicular cells) of the thyroid gland has an opposite effect. When calcium blood levels are above approximately 10.5 mg/dL (in adults), calcitonin is released and binds directly to calcitonin receptors on osteoclasts and causes them to undergo apoptosis, thereby decreasing blood calcium levels.

The following local factors and cytokines affect bone:

- BMP-6 (also to a limited extent, BMP-2 and BMP-4) and TGF-β induce osteoprogenitor cells to differentiate into osteoblasts.
- Osteoblasts synthesize RANKL, M-CSF, alkaline phosphatase, IGF-1, and PTH receptors, all of which they place on their cell membranes.
- Osteoblasts synthesize and release osteocalcin, a signaling molecule responsible for mineralization of bone; osteonectin, a glycoprotein that assists in the binding of calcium hydroxyl apatite crystals to collagen; osteopontin, which assists in the formation of the sealing zone of osteoclasts; bone sialoprotein, which assists osteoblasts to adhere to the bone matrix; and OPG, a glycoprotein that can bind to RANKL and thus interfere with osteoclast formation.
- Osteoblasts are transformed into osteocytes under the influence of two transcription factors, Cbfa1/Runx2 and osterix, both of which appear to be dependent on BMP-2 to be expressed. This is especially evident during intramembranous bone formation.
- Osteocytes release cAMP, osteocalcin, and IGF-1 in response to tension placed on bone to facilitate the recruitment of osteoprogenitor cells.
- In response to low calcium levels in the extracellular fluid in their lacunae, osteocytes secrete sclerostin, a paracrine hormone that inhibits bone formation and encourages bone resorption, thereby elevating blood calcium levels.
- Osteoblasts secrete four signaling molecules: (1) M-CSF, which binds to a receptor on the osteoclast precursor, inducing it to proliferate and to express RANK on the osteoclast precursor cell membrane; when the signaling molecule RANKL on the osteoblast cell membrane binds to the RANK receptor on the osteoclast precursor cell membrane, the osteoclast precursor is induced to differentiate into the multinucleated osteoclast, activating it and enhancing bone resorption. (2) IL-6 facilitates the recruitment and differentiation of osteoclasts. (3) IL-1 activates osteoclast precursors to proliferate; it also has an indirect role in osteoclast stimulation. (4) Signaling molecule, OPG, a member of the TNFR family, can serve as a decoy by interacting with RANKL, thus prohibiting it from binding to the macrophage and thereby inhibiting osteoclast formation.
- Osteoclasts have receptors for osteoclast-stimulating factor, colony-stimulating factor, OPG, RANK, and calcitonin, among others.
- TNF-α, released by activated macrophages, acts in a fashion similar to IL-1.
- Interferon-γ, released by T lymphocytes, inhibits differentiation of osteoclast precursors into osteoclasts.
- TGF-β, liberated from bone matrix during bone resorption, by osteoclasts induces osteoblasts to manufacture bone matrix and enhances the process of matrix mineralization; also, it inhibits proliferation of osteoclast precursors and their differentiation into mature osteoclasts.

The following factors and cytokines affect the epiphyseal plate:

- Chondrocytes of the zone of proliferation of the epiphyseal plate release a paracrine hormone known as ***Indian hedgehog*** to act on all of the chondrocytes in the immediate vicinity inducing not only the proliferation of the chondrocytes but also delays chondrocytic hypertrophy, thus maintaining the requisite breadth of the epiphyseal plate.
- The chondrocytes of zone of proliferation are also sensitive to **IGF-1** and, to a very limited extent, to insulin-like growth factor-2 (IGF-2). IGF-1 is a hormone manufactured by hepatocytes in response to **growth hormone** secreted by the pituitary gland, and chondrocytes of this zone proliferate in response to being exposed to IGF-1. Although osteocytes also manufacture and release IFG-1, in the case of the epiphyseal plate, it is the hepatocyte-produced IFG-1 that acts on the chondrocytes.
- The chondrocytes of the zone of maturation and hypertrophy eventually undergo apoptosis and die, but, before they die, they secrete vascular endothelial growth factor, a cytokine that encourages the invasion of blood vessels, which bring calcium ions and specialized macrophage precursors into the area.

Nutritional Effects

Normal bone growth is sensitive and dependent on several nutritional factors. Unless a person's intake of protein, minerals, and vitamins is sufficient, the amino acids essential for collagen synthesis by osteoblasts are lacking, and collagen formation is reduced. Insufficient intake of calcium or phosphorus leads to poorly calcified bone, which is subject to fracture. A deficiency of vitamin D prevents calcium absorption from the intestines, causing **osteomalacia** in adults and **rickets** in children. Vitamins A and C are also necessary for proper skeletal development (Table 7–4). The consequence of excess or insufficient vitamin A availability results in a decreased height of the affected individual. Vitamin C is required for collagen production; therefore, a deficiency of vitamin C results in the condition known as *scurvy*.

CLINICAL CORRELATIONS

Rickets is a disease in children who are deficient in vitamin D. Without vitamin D, the intestinal mucosa cannot absorb calcium even though there may be adequate dietary intake. This results in disturbances in ossification of the epiphyseal cartilages and disorientation of the cells at the metaphysis, giving rise to poorly calcified bone matrix. Children with rickets display deformed bones, particularly in the legs, simply because the bones cannot bear their weight.

Osteomalacia, or adult rickets, results from prolonged deficiency of vitamin D. When this occurs, the newly formed bone in the process of remodeling does not calcify properly. This condition may become severe during pregnancy because the fetus requires calcium, which must be supplied by the mother.

Scurvy is a condition resulting from a deficiency of vitamin C. One effect is deficient collagen production, causing a reduction in formation of bone matrix and bone development. Healing is also delayed.

TABLE 7–4 Vitamins and Their Effects on Skeletal Development

Vitamin	Effects on Skeletal Development
Vitamin A deficiency	Inhibits proper bone formation as coordination of osteoblast and osteoclast activities fails. Failure of resorption and remodeling of cranial vault to accommodate the brain with serious damage to the central nervous system.
Hypervitaminosis A	Erosion of cartilage columns without increases of cells in proliferation zone. Epiphyseal plates may become obliterated, ceasing growth prematurely.
Vitamin C deficiency	Mesenchymal tissue affected as connective tissue is unable to produce and maintain extracellular matrix. Deficient production of collagen and bone matrix results in retarded growth and delayed healing. Scurvy.
Vitamin D deficiency	Ossification of epiphyseal cartilages disturbed. Cells become disordered at metaphysis, leading to poorly calcified bones, which become deformed by weight bearing. In children—rickets. In adults—osteomalacia.

Joints

Bones articulate or come into close proximity with one another at joints, which are classified according to the degree of movement available between the bones of the joint. Those that are closely bound together with only a minimum of movement between them are called ***synarthroses***; joints in which the bones are free to articulate over a fairly wide range of motion are classified as ***diarthroses***.

There are three types of **synarthrosis joints** according to the tissue making up the union:

1. **Synostosis**. There is little if any movement, and joint-uniting tissue is bone (e.g., skull bones in adults).
2. **Synchondrosis.** There is little movement, and joint-uniting tissue is hyaline cartilage (e.g., joint of first rib and sternum).

3. **Syndesmosis**. There is little movement, and bones are joined by dense connective tissue (e.g., pubic symphysis).

Most of the joints of the extremities are **diarthroses** (Fig. 7–21). The bones making up these joints are covered by persistent **hyaline cartilage**, or **articular cartilage**. Usually, ligaments maintain the contact between the bones of the joint, which is sealed by the **joint capsule**. The **capsule** is composed of an outer **fibrous layer** of dense connective tissue, which is continuous with the periosteum of the bones, and an inner cellular **synovial layer**, which covers all nonarticular surfaces. Some prefer to call this a ***synovial membrane***.

Two kinds of cells are located in the synovial layer: **Type A cells** are macrophages displaying a well-developed Golgi apparatus and many lysosomes but only a small amount of RER. These phagocytic cells are responsible for removing debris from the joint space. **Type B cells** resemble fibroblasts, exhibiting a well-developed RER; these cells are thought to secrete the **synovial fluid**.

Synovial fluid contains a high concentration of **hyaluronic acid** and the glycoprotein **lubricin** combined with filtrate of plasma. In addition to supplying nutrients and oxygen to the chondrocytes of the articular cartilage, this fluid has a high content of hyaluronic acid and lubricin that permits it to function as a lubricant for the joint. Moreover, macrophages in the synovial fluid act to phagocytose debris in the joint space.

PATHOLOGICAL CONSIDERATIONS

See Figures 7–21 through 7–25.

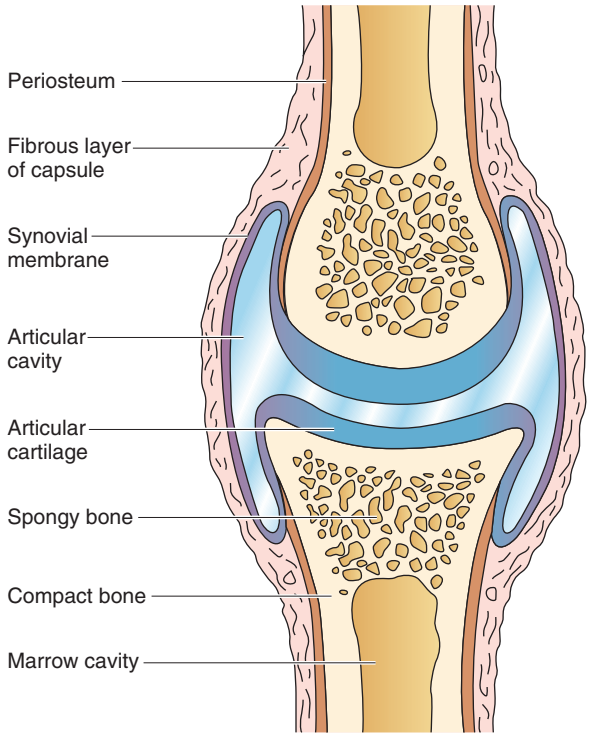


Figure 7–21 Illustrated anatomy of a diarthrodial joint.

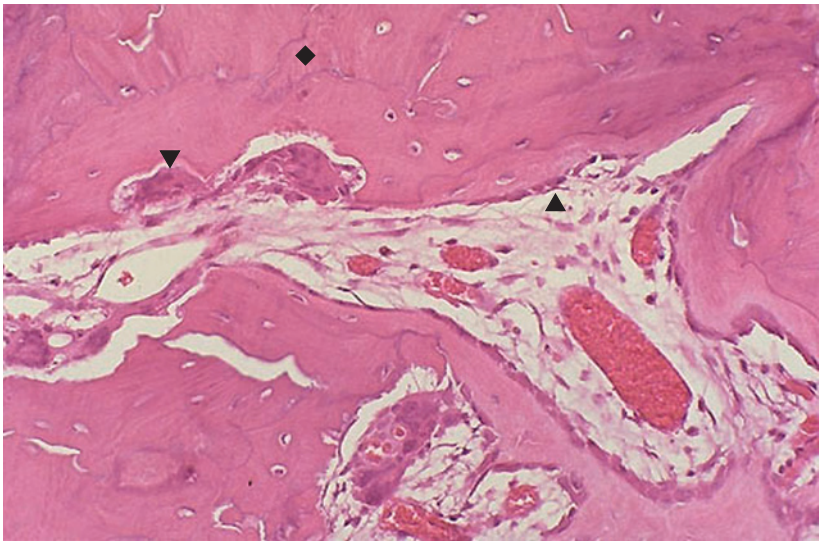


Figure 7–22 Photomicrograph of Paget disease of bone. Note that there is an active osteoclastic *(down arrowhead)* and osteoblastic *(up arrowhead)* activity, leading to a disorganized microscopic appearance of the lamellar pattern of the bone. Note also the irregularly arranged cement line *(diamond)*. (From Klatt EC. *Robbins and Cotran Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:446, with permission.)

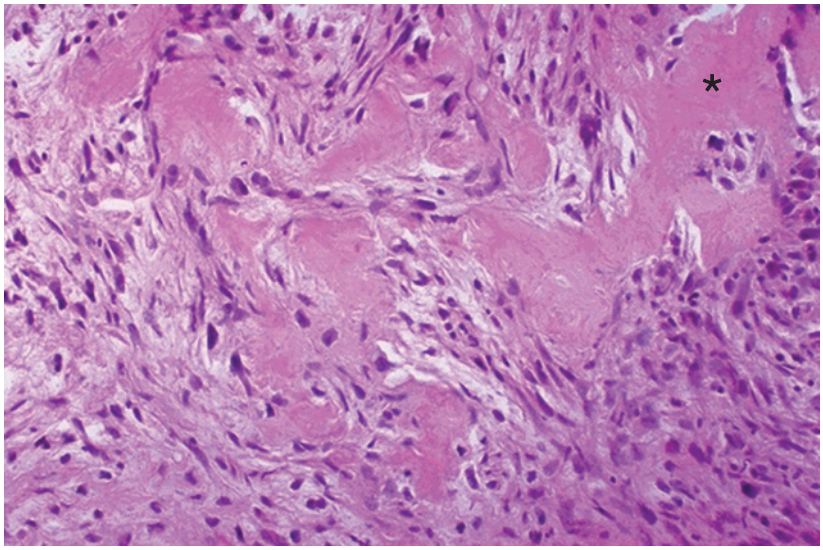


Figure 7–23 Photomicrograph of osteosarcoma. Note that the neoplastic spindle-shaped cells are manufacturing a pinkish osteoid (*asterisk*), a characteristic feature of osteosarcoma. (From Klatt EC. *Robbins and Cotran Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:453, with permission.)

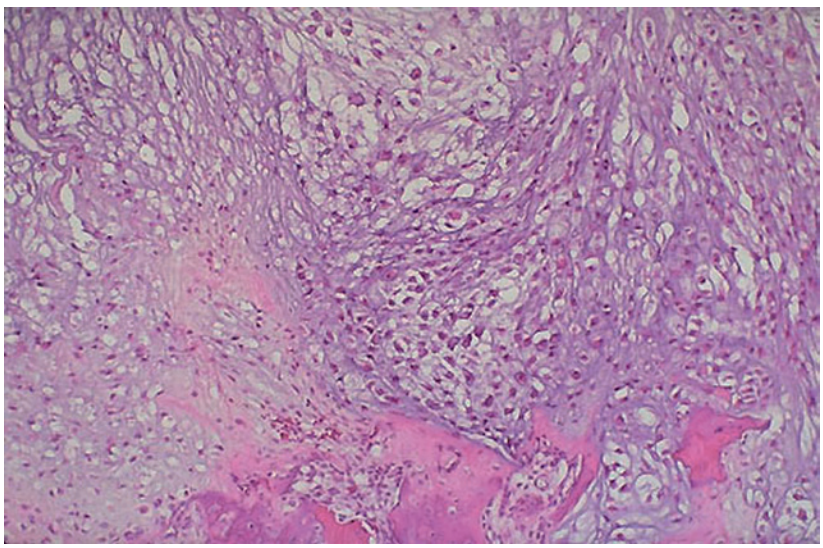


Figure 7–24 Photomicrograph of chondrosarcoma. Note that the chondrocytes of the cartilage are haphazardly arranged and that the bone at the bottom of the photomicrograph is being invaded and destroyed by the malignant cells. (From Klatt EC. *Robbins and Cotran Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:456, with permission.)

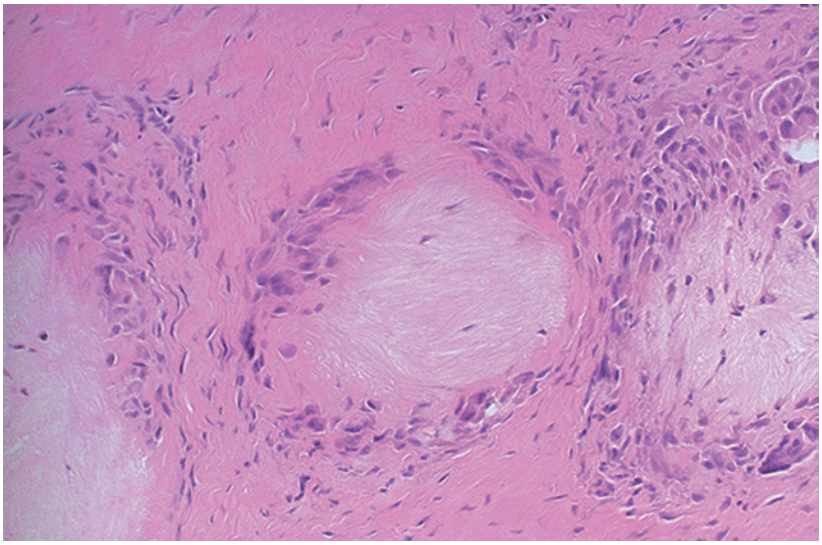
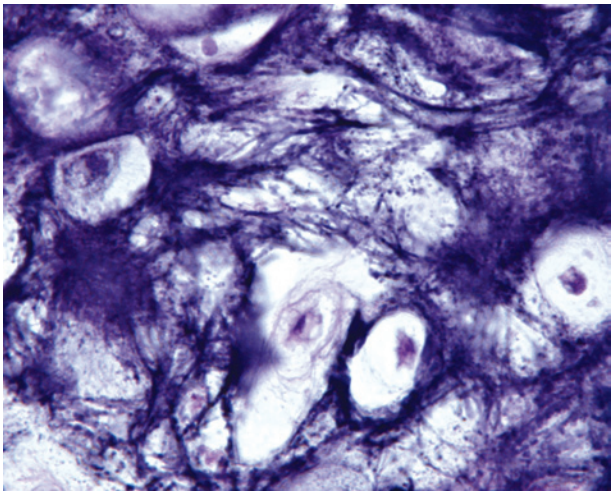
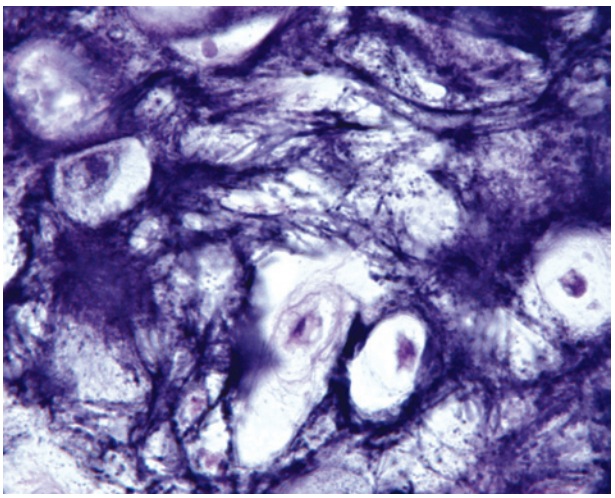


Figure 7–25 Photomicrograph of gouty arthritis of a joint. Note that the pale areas in the center of the photomicrograph are regions of urate crystal deposition. The urate crystals elicit an inflammatory response, as evidenced by the chronic inflammatory cells surrounding the crystal deposits. (From Klatt EC. *Robbins and Cotran Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:464, with permission.)

REVIEW QUESTIONS

1. A very slim 82-year-old female patient is brought into the emergency department with severe back pain that radiates to the sides of the lower thoracic region. She has been losing height for the past few years, and she has developed a "dowager's hump." Her possible diagnosis is
   a. osteopetrosis.
   b. Paget disease.
   c. osteomalacia.
   d. osteoma.
   e. osteoporosis.
2. A short-statured patient inquires about the genetic implication of her condition and would like to know the reason for it. The physician knows that the patient has achondroplasia. Which of the following would be the correct reason for the condition?
   a. Lack of calcification of the epiphyseal plate
   b. Lack of matrix formation in the epiphyseal plate
   c. Excess calcification of the epiphyseal plate
   d. Excess chondrocyte formation in the epiphyseal plate
   e. Reduction in chondrocyte formation in the epiphyseal plate
3. The enzyme lipoprotein lipase is located on the endothelial lining of blood vessels. This enzyme
   a. functions to synthesize chylomicrons.
   b. functions to synthesize VLDL (very-low-density lipoproteins).
   c. is manufactured by fibroblasts.
   d. is manufactured by adipocytes.
   e. is manufactured by macrophages.
4. Macrophages are cells that
   a. endocytose lipids.
   b. are derived from lymphocytes.
   c. are derived from monocytes.
   d. synthesize and release interferon gamma (IFN-γ).
   e. phagocytose dust cells.
5. A patient who has been diagnosed with chronic inflammatory response will have an abundance of which of the following cells?
   a. Macrophages
   b. Neutrophils
   c. Basophils
   d. Eosinophils
   e. Lymphocytes
6. 

   A patient presents with a nodule within his oral cavity/oral pharynx. The photomicrograph was from the normal area of his biopsy. The structure housing the nodule was the
   a. uvula.
   b. hard palate.
   c. soft palate.
   d. epiglottis.
   e. palatine tonsil.
7. 

   The predominant fiber types in this light micrograph are elastic fibers and
   a. type I collagen.
   b. type II collagen.
   c. type II collagen.
   d. type IV collagen.
   e. type V collagen.

8. A patient who has been diagnosed with an acute inflammatory response will have an abundance of which of the following cells?
   a. Macrophages
   b. Neutrophils
   c. Basophils
   d. Eosinophils
   e. Lymphocytes
9. A patient is seen by his physician, who determines that he has a defective gene that codes for an inactive form of the hormone leptin. This patient
   a. drinks too much fluid.
   b. does not drink enough fluids.
   c. has very little appetite.
   d. has a voracious appetite.
   e. has bouts of uncontrollable vomiting.
10. Which of the following occurs during endochondral bone formation?
    a. The periosteum is transformed into a perichondrium.
    b. Chondrocytes hypertrophy and die.
    c. Interstitial growth enlarges the subperiosteal bone collar.
    d. Calcified cartilage becomes decalcified.
    e. Calcified bone is replaced by cartilage.

ANSWERS

1. E. Osteoporosis is an age-related decrease in bone density in which both the calcium content and the mass of the bone are decreased. The patient has had several of her vertebrae collapsed that causes an alteration in the normal curvature of the spine, resulting in a hump in the area. Osteopetrosis, a familial disease, results in an increase, rather than a decrease, in bone density. Paget disease is an abnormality of the bony skeleton in that the bones become thicker but much less calcified than normal bone. Osteomalacia is the adult form of rickets and is a disease of bone due to vitamin C deficiency. Osteoma is a small bony tumor that may appear almost anywhere in the skeleton.
2. E. Achondroplasia is due to a point mutation (arginine for glycine) in fibroblast growth factor receptor 3 (FGFR3). When FGF binds to its receptor, the proliferation of chondrocytes in the zone of proliferation and hypertrophy of the epiphyseal plate is inhibited, and the epiphyseal plate does not increase in thickness, and the bone does not grow in length. The mutated form of FGFR3 is always turned on and, therefore, the epiphyseal plate does not increase in thickness, and the person's long bones do not increase in length, resulting in dwarfism. Calcification and matrix formation appear to be normal in patients with achondroplasia.
3. D. Lipoprotein lipase, an enzyme produced and released by fat cells, enters the lumen of blood vessels; adheres to the endothelial lining; and catalyzes the degradation of chylomicrons, triglycerides, and VLDL to free fatty acids and glycerol. Chylomicrons are formed in the mucosa of the small intestines, and VLDL is manufactured by hepatocytes of the liver.
4. C. When monocytes leave the bloodstream and enter the extracellular matrix of connective tissue spaces, they become known as *macrophages*. Some macrophages enter specific regions of the body and acquire a specific name, such as the dust cells of the respiratory system. These cells phagocytose foreign particulate matter as well as cellular debris resulting from dead or destroyed cells. Macrophages are activated by IFN-γ, and they manufacture TNF-α (tumor necrosis factor alpha).
5. E. A patient who has chronic inflammation will display a great abundance of lymphocytes at the site of the inflammation. Although macrophages and eosinophils will also be attracted to the site, they do not appear in such huge numbers as do lymphocytes. Neutrophils abound in a site of acute inflammation. Basophils are also increased to a certain extent.
6. D. The tissue is elastic cartilage, and only the epiglottis in this list possesses elastic cartilage in its core.
7. B. The tissue is elastic cartilage, and the two major fiber types embedded in its matrix are elastic fibers and type II collagen fibers.
8. B. A patient who has acute inflammation will display a great abundance of neutrophils at the site of the inflammation. Although macrophages and eosinophils will also be attracted to the site, they do not appear in such huge numbers as do neutrophils. Lymphocytes abound in a site of chronic inflammation. Basophils are also increased to a certain extent.
9. D. Leptin is the hormone that controls the appetite center of the hypothalamus. Individuals who do not produce leptin or produce an inactive form of the hormone have a voracious appetite and gain weight in an uncontrollable fashion. Leptin does not control the thirst center of the brain and does not induce vomiting.
10. B. Chondrocytes of the cartilage template undergo proliferation followed by hypertrophy and cell death. As the subperiosteal bone collar is formed, the perichondrium is transformed into a periosteum. The subperiosteal bone collar is calcified and, therefore, it is unable to grow via interstitial growth. Instead, it becomes thicker due to appositional growth. The calcified cartilage does not become decalcified; instead, it becomes replaced by bone.

This page intentionally left blank

Muscle

8

Although many cells of multicellular organisms have limited contractile abilities, it is the capability of muscle cells, which are specialized for contraction, that permits animals to move. Organisms harness the contraction of muscle cells and the arrangement of the extracellular components of muscle to permit locomotion, constriction, pumping, and other propulsive movements.

Cells of muscle are elongated and are called ***striated*** or ***smooth*** muscle, depending on the respective presence or absence of a regularly repeated arrangement of myofibrillar contractile proteins, the myofilaments. **Striated** muscle cells display characteristic alternations of light and dark cross-bands, which are absent in smooth muscle (Fig. 8–1). There are two types of striated muscle: **skeletal muscle**, accounting for most of the voluntary muscle mass of the body and approximately 40% of the total body weight, and involuntary **cardiac muscle**, limited almost exclusively to the heart.

Smooth muscle is located in the walls of blood vessels and the viscera as well as in the dermis of the skin.

Unique terms are often used to describe the components of muscle cells. Thus, muscle cell membrane is referred to as ***sarcolemma***; the cytoplasm, as ***sarcoplasm***; the smooth endoplasmic reticulum (SER), as ***sarcoplasmic reticulum***; and, occasionally, the mitochondria, as ***sarcosomes***. Because they are much longer than they are wide, muscle cells frequently are called ***muscle fibers***; unlike collagen and elastic fibers, however, they are living entities.

All three muscle types are derived from mesoderm. Cardiac muscle originates in splanchnopleuric mesoderm, most smooth muscle is derived from splanchnic and somatic mesoderm, and most skeletal muscles originate from somatic mesoderm.

SKELETAL MUSCLE

Skeletal muscle is composed of long, cylindrical, multinucleated cells that undergo voluntary contraction to facilitate movement of the body or its parts.

During embryonic development, several hundred **myoblasts**, precursors of skeletal muscle fibers, line up end to end, fusing with one another to form long multinucleated cells known as ***myotubes***. These newly formed myotubes manufacture cytoplasmic constituents as well as the contractile elements of muscle, called ***myofibrils***. Myofibrils are composed of specific arrays of **myofilaments**, the proteins responsible for the contractile capability of the cell.

Muscle fibers are arranged parallel to one another, with their intervening intercellular spaces housing parallel arrays of **continuous capillaries**. Each skeletal muscle fiber is long, cylindrical, multinucleated, and striated. The diameters of the fibers vary, ranging from 10 to 100 µm, although hypertrophied fibers may exceed the latter figure. The relative strength of a muscle fiber directly depends on its diameter, whereas the strength of the entire muscle is a function of the number and thickness of its component fibers.

Skeletal muscle is pink to red because of its rich vascular supply as well as the presence of **myoglobin pigments**, oxygen-transporting proteins that resemble, but are smaller than, hemoglobin. Depending on the fiber diameter, quantity of myoglobin, number of mitochondria, extensiveness of the sarcoplasmic reticulum, concentration of various enzymes, and rate of contraction, the muscle fiber may be classified as **red**, **white**, or **intermediate** (Table 8–1).

Usually, a gross anatomical muscle (e.g., biceps) contains all three types of muscle fibers (red, white, and intermediate) in relatively constant proportions that are characteristic of that particular muscle. In chickens, for instance, thigh muscles are predominantly red, and breast muscles are predominantly white. The innervation of the muscle fiber appears to be the factor that determines fiber type. If the innervation is experimentally switched, the fiber accommodates itself to the new nerve supply.

Connective Tissue Investments

The connective tissue investments of skeletal muscle are the epimysium, perimysium, and endomysium.

The entire muscle is surrounded by **epimysium**, a dense irregular collagenous connective tissue. **Perimysium**, a less dense collagenous connective tissue derived from epimysium, surrounds bundles **(fascicles)** of muscle fibers. **Endomysium**, composed of reticular fibers and an **external lamina** (basal lamina), surrounds each muscle cell (Fig. 8–2).

Because these connective tissue elements are interconnected, contractile forces exerted by individual muscle cells are transferred to them. Tendons and aponeuroses, which connect muscle to bone and to other tissues, are continuous with the connective tissue encasements of muscle and, therefore, act in harnessing the contractile forces for motion.

SUMMARY

Muscle, one of the four basic tissues, specializes in contraction. There are three types of muscles—**skeletal**, **cardiac**, and **smooth**—that differ from each other in location, structure, function, and the type of innervation that they possess. All muscle cells are elongated, and, to perform their function, they become shorter. All muscle cells contain **actin** and **myosin filaments** that slide past each other during **muscle contraction**, resulting in a decrease in the length of the cell. Skeletal muscles compose about 40% of the total body weight, they are innervated by the **somatic nervous system**, and they are responsible for voluntary movement of the body. Cardiac muscle is limited in location to the **heart**, functions in **propelling blood** throughout the body, and is controlled by the **autonomic nervous system**. Usually, **smooth muscle** forms sheets of cells, is located in the **walls of blood vessels**, the **walls of hollow viscera**, as well as in the **dermis** of the skin, and the **iris** and **ciliary body** of the eyeball. Smooth muscle functions in peristalsis, blood vessel accommodation, elevation of hair follicles, and accommodation of the lens of the eye and is controlled by the **autonomic nervous system**.

KEY WORDS

Actin filaments; autonomic nervous system; blood vessels; cardiac muscle; ciliary body; dermis; heart; hollow viscera; iris; muscle contraction; myosin filaments; skeletal muscle; smooth muscle; somatic nervous system

Light Microscopy

Light microscopy of skeletal muscle fibers displays long, cylindrical, multinucleated cells whose numerous nuclei are peripherally located.

Skeletal muscle fibers are multinucleated cells, with their numerous nuclei peripherally located just beneath the cell membrane (Fig. 8–3). Each cell is surrounded by endomysium, whose fine reticular fibers intermingle with those of neighboring muscle cells. Small **satellite cells (myogenic stem cells)**, which have a single nucleus and act as regenerative cells, are located in shallow depressions on the muscle cell's surface, sharing the muscle fiber's external lamina. The chromatin network of the satellite cell nucleus is denser and coarser than that of the muscle fiber.

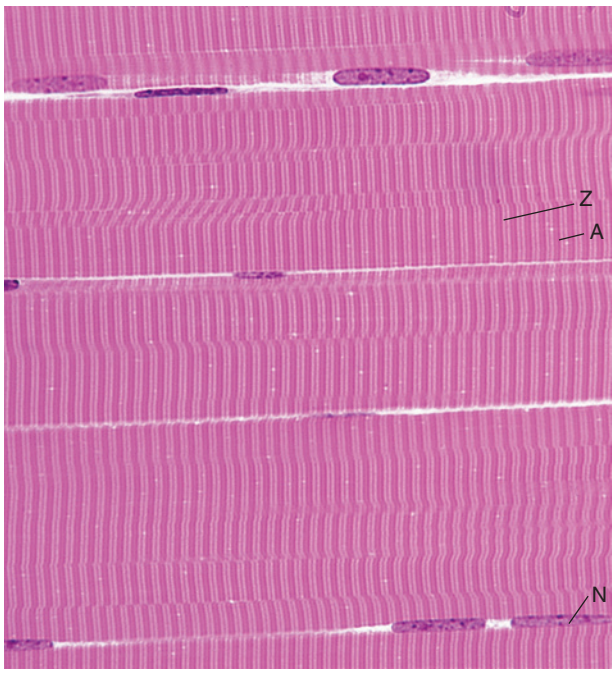


Figure 8–1 Photomicrograph of a longitudinal section of skeletal muscle. Note the peripherally located nuclei as well as the very fine connective tissue elements between individual muscle fibers (×540).

Much of the skeletal muscle cell sarcoplasm is composed of longitudinal arrays of cylindrical **myofibrils**, each 1 to 2 μm in diameter (Fig. 8–4). They extend the entire length of the cell and are aligned precisely with their neighbors. This strictly ordered parallel arrangement of the myofibrils is responsible for the cross-striations of light and dark banding that are characteristic of skeletal muscle viewed in longitudinal section (see Fig. 8–1).

The dark bands are known as ***A bands*** (anisotropic with polarized light), and the light bands are known as ***I bands*** (isotropic with polarized light). The center of each A band is occupied by a pale area, the **H band**, which is bisected by a thin **M line (M bridge)**. Each I band is bisected by a thin dark line, the **Z disk (Z line)**. The region of the myofibril between two successive Z disks, known as a ***sarcomere***, is 2.5 μm in length and is considered to be the contractile unit of skeletal muscle fibers (Fig. 8–5; see also Fig. 8–4).

During muscle contraction, the various transverse bands behave characteristically. The I band becomes narrower, the H band and M line are extinguished, and the Z disks move closer together (approaching the interface between the A and I bands), but the width of the A bands remains unaltered.

Fine Structure of Skeletal Muscle Fibers

Electron microscopy has helped reveal the functional and morphological significance of skeletal muscle cross-striations and other structural components.

TABLE 8–1 Types of Skeletal Muscle Fibers*

Characteristics	Red Muscle Fibers	White Muscle Fibers
Vascularization	Rich vascular supply	Poorer vascular supply
Innervation	Smaller nerve fibers	Larger nerve fibers
Fiber diameter	Smaller	Larger
Contraction	Slow but repetitive; not easily fatigued; weaker contraction	Fast but easily fatigued; stronger contraction
Sarcoplasmic reticulum	Not extensive	Extensive
Mitochondria	Numerous	Few
Myoglobin	Rich	Poor
Enzymes	Rich in oxidative enzymes; poor in adenosine triphosphatase	Poor in oxidative enzymes; rich in phosphorylases and adenosine triphosphatase

*Intermediate muscle fibers have characteristics between red and white fibers.

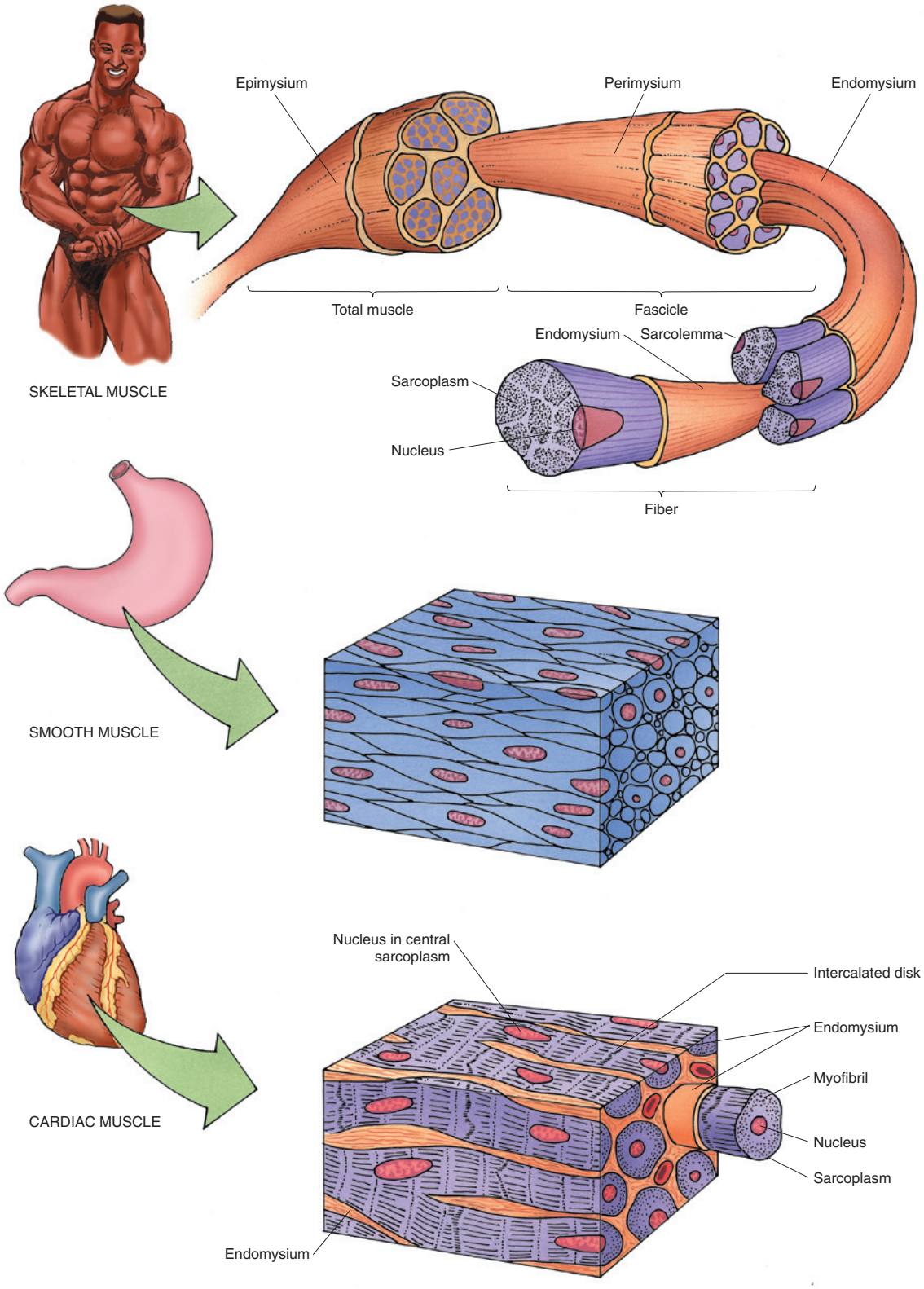


Figure 8–2 Diagram of the three types of muscle. *Top,* Skeletal muscle. *Center,* Smooth muscle. *Bottom,* Cardiac muscle.

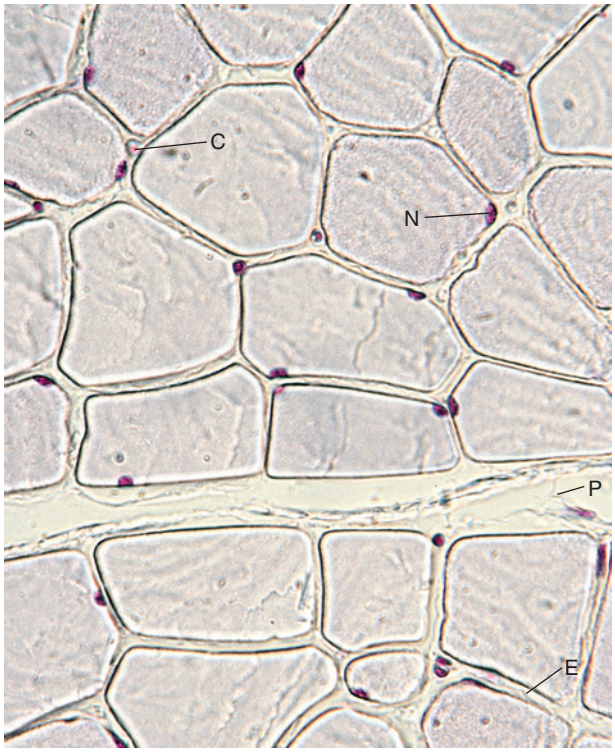


Figure 8–3 Photomicrograph of a cross-section of skeletal muscle. Note the peripheral location of the nuclei as well as the capillary (C) located in the slender connective tissue elements of the endomysium (E). Also observe the perimysium (P) that envelops bundles of muscle fibers (×540).

T Tubules and Sarcoplasmic Reticulum

T tubules and sarcoplasmic reticulum are essential components involved in skeletal muscle contraction.

The fine structure of the sarcolemma is similar to that of other cell membranes. As indicated in Chapter 2, one of the functions of the cell membrane is to maintain a potential difference between its cytoplasmic and extracellular surfaces. This potential difference is known as the ***resting membrane potential***. Some cells, such as neurons and muscle cells, are capable of altering their resting membrane potential to effect certain changes, such as blocking or expediting the transmission of a signal by neurons or reducing the length of the cell, as in muscle contraction. Cells are capable of making this potential difference greater than in the resting potential, known as ***hyperpolarization***, or less than the resting potential, known as ***depolarization***.

A distinguishing feature of the skeletal muscle membrane is that it is continued within the skeletal muscle fiber as numerous long, tubular invaginations resembling tunnels that intertwine among the myofibrils, known as ***T tubules (transverse tubules)*** (see Fig. 8–5).

T tubules pass transversely across the muscle cell and, in mammalian skeletal muscle cells, lie specifically in the plane of the junction of the A and I bands. These tubules branch and anastomose but usually remain in a single plane; hence, each sarcomere possesses two sets of T tubules, one at each interface of the A and I bands. Thus, T tubules extend deep into the interior of the muscle cell and facilitate the conduction of waves of depolarization along the sarcolemma (Figs. 8–6 and 8–7).

Associated with this system of T tubules is the **sarcoplasmic reticulum**, which is maintained in close register with the A and I bands as well as with the T tubules. The sarcoplasmic reticulum, which stores **intracellular calcium**, forms a meshwork around each myofibril and displays dilated **terminal cisternae** at each A-I junction. Thus, two of these cisternae are always in close apposition to a T tubule, forming a **triad** in which a T tubule is flanked by two terminal cisternae. This arrangement permits a wave of depolarization to spread, almost instantaneously, from the surface of the sarcolemma throughout the cell, reaching the terminal cisternae, which have **voltage-gated calcium release channels (junctional feet)** in their membrane.

The sarcoplasmic reticulum regulates muscle contraction through controlled sequestering (resulting in muscle relaxation) and release (resulting in muscle contraction) of calcium ions (Ca^{2+}) within the sarcoplasm. The trigger for the calcium ion release is the wave of depolarization transmitted by T tubules, which causes opening of the calcium release channels of the terminal cisternae, resulting in the release of calcium ions into the cytosol in the vicinity of the myofibrils.

Myofibrils are held in precise register with one another by the intermediate filaments **desmin** in the postnatal individual (and **vimentin** and some desmin in the embryo) as well as by the following desmin-associated complex of molecules. Desmin filaments bind to **plectin**, which secures these intermediate filaments to each other and to the periphery of the Z disks of neighboring myofibrils. Another protein, **αβ-crystallin**, a chaperone-like molecule, binds to desmin molecules at their union with the Z disk, ensuring that the force of muscle contraction does not damage the integrity of the desmin molecules. Desmin also has been noted to bind the Z disks to mitochondria, to the nucleus of the skeletal muscle cell, and to the actin filaments of the muscle fiber's cytoskeleton. Therefore, in some fashion that is not as yet understood, desmin filaments are responsible for maintaining the architectural integrity of the muscle fiber.

The peripheral-most bundles of myofibrils located just adjacent to the sarcolemma are tethered by desmin filaments to specific locations on the cytoplasmic aspect

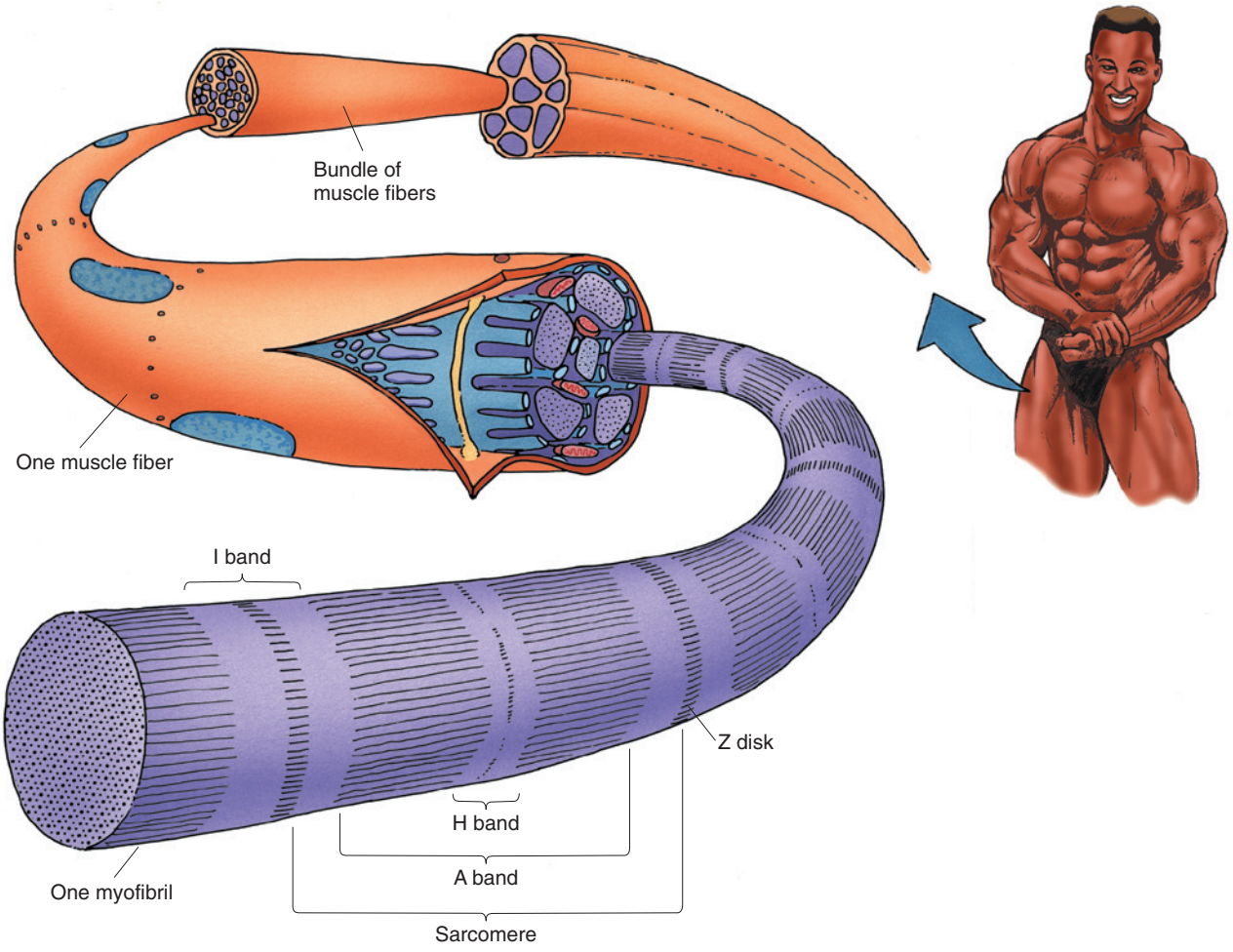


Figure 8–4 Diagram of the organization of myofibrils and sarcomeres within a skeletal muscle cell. Note that the entire gross muscle is surrounded by a thick connective tissue investment, known as the *epimysium*, which provides finer connective tissue elements (the perimysium) that surround bundles of skeletal muscle fibers. Individual muscle cells are surrounded by still finer connective tissue elements, the endomysium. Individual skeletal muscle fibers possess a sarcolemma that has tubular invaginations, the T tubules that course through the sarcoplasm, and are flanked by terminal cisternae of the sarcoplasmic reticulum. The contractile elements of the skeletal muscle fiber are organized into discrete cylindrical units, known as *myofibrils*. Each myofibril is composed of thousands of sarcomeres.

of the sarcolemma, known as ***costameres***. These costameres are rich in an intricate group of sarcolemmal integral and associated proteins known as ***dystrophin-associated glycoprotein complex***. This complex is composed of **dystrophin**, **dystroglycan complex**, **sarcoglycan complex**, **dystrobrevin**, **syntrophin**, and a few other proteins of lesser importance.

Dystrophin is a very long, slim protein whose one end is attached to the actin filaments of the cytoskeleton just adjacent to the sarcolemma, whereas the other end is attached to dystrobrevins, to the β subunit of the dystroglycan molecule, and to syntrophins. Dystrophin protects the sarcolemma from injury during muscle contraction.

- **Dystroglycan complex** is a glycoprotein composed of an extracellular **alpha subunit** and a transmembrane **beta subunit**. The intracellular moiety of the beta subunit binds to dystrophin whereas the extracellular moiety binds to its alpha subunit. In turn, the alpha subunit binds to **laminin** of the **external lamina**.
- **Sarcoglycan complex** is composed of five transmembrane proteins (α, β, γ, δ, and ε) that bind to both the alpha and beta subunits of the dystroglycan,

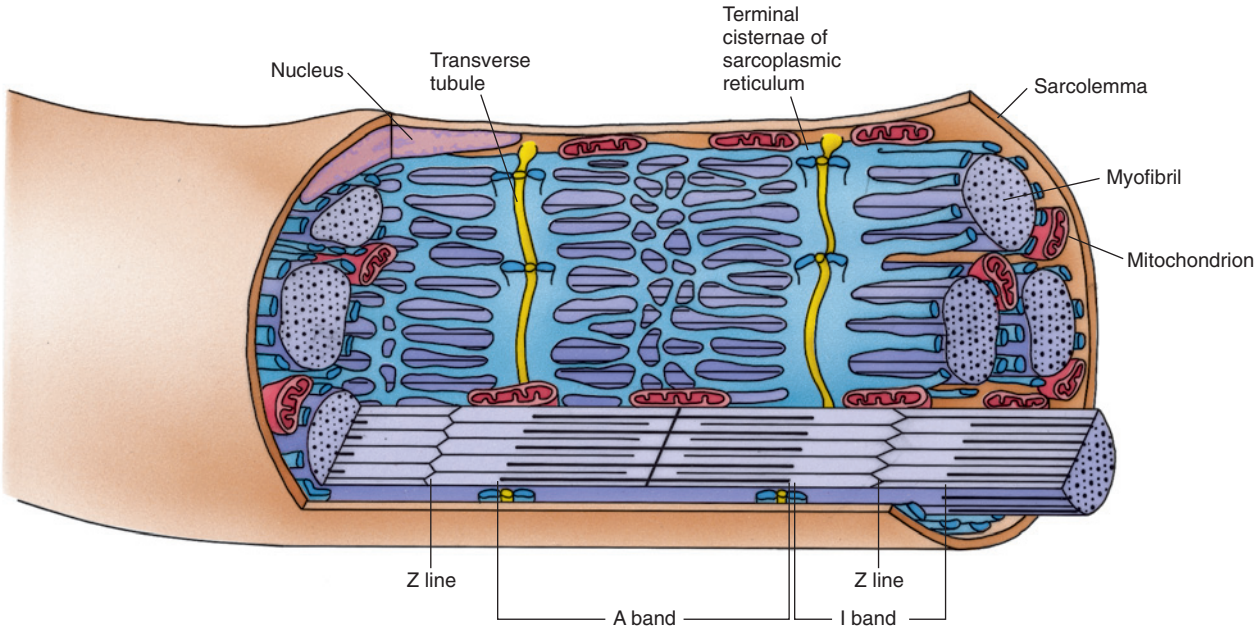


Figure 8–5 Diagram of the organization of triads and sarcomeres of skeletal muscle. Note that, in skeletal muscle, the triad is always located at the junction of the A and I bands, permitting the quick release of calcium ions from the terminal cisternae of the sarcoplasmic reticulum just in the region where the interaction of the thick and thin filaments can produce efficient sarcomere shortening. Observe the presence of mitochondria around the periphery of the myofibrils.

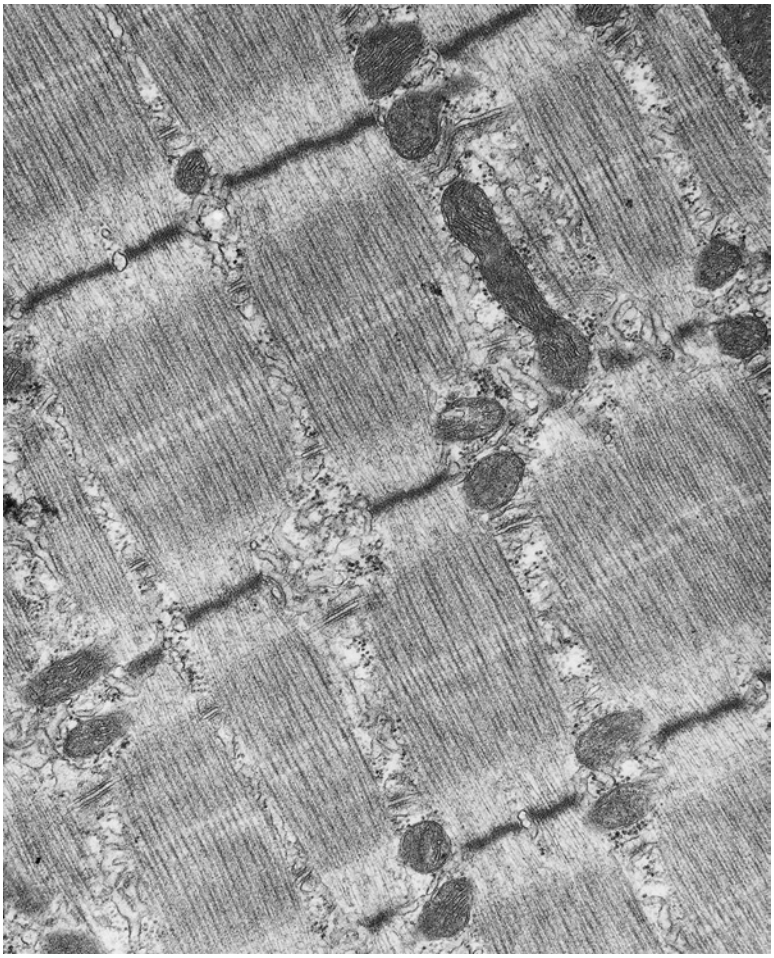


Figure 8–6 Electron micrograph of longitudinal section of rat skeletal muscle (×19330). (Courtesy Dr. J. Strum.)

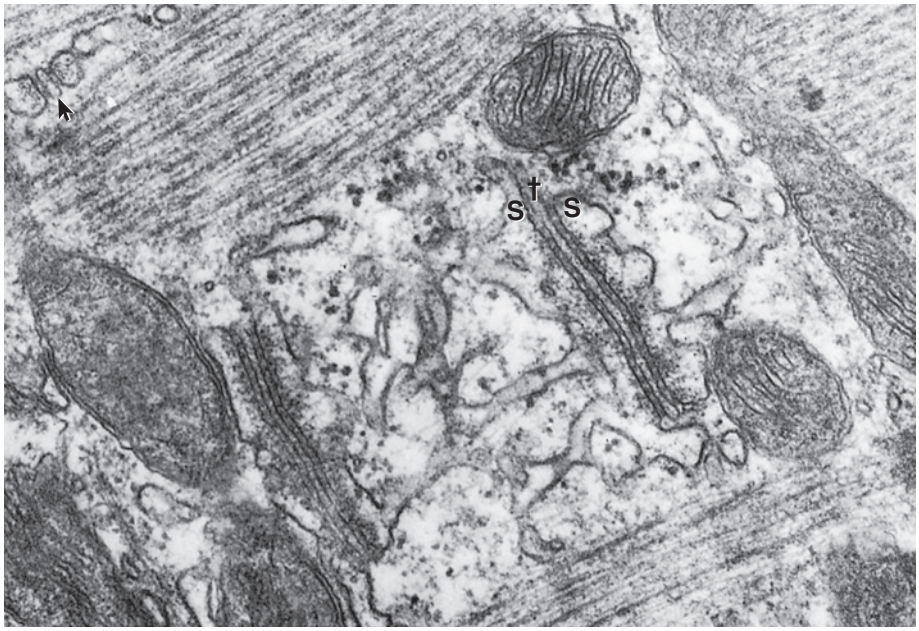


Figure 8–7 Electron micrograph of triads and sarcoplasmic reticulum in skeletal muscle (×57847). The *arrow* represents a cross-section of T tubule flanked by terminal cisternae. s, Terminal cisternae of the sarcoplasmic reticulum; t, T tubule. (From Leeson TS, Leeson CR, Papparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

thereby reinforcing the skeletal muscle cell membrane.

- **Dystrobrevin** is a small, rod-shaped intracellular protein that binds to both dystrophin and to syntrophins, thereby providing support to dystrophin.
- **Syntrophin** binds to dystrobrevin and to dystrophin, providing further support to the costameres. Additionally, syntrophin forms bonds with the enzyme **nitric oxide synthase**, and during muscle contraction, it facilitates the formation of nitric oxide, a vasodilator that increases the diameter of regional blood vessels, thereby increasing blood flow to the area.

Deep to the sarcolemma and interspersed between and among myofibrils are numerous elongated mitochondria with many highly interdigitating cristae. The mitochondria may either parallel the longitudinal axis of the myofibril or wrap around the myofibril. Moreover, numerous mitochondria are located just deep to the sarcoplasm.

Structural Organization of Myofibrils

Myofibrils are composed of interdigitating thick and thin myofilaments.

Electron microscopy reveals the same banding as noted by light microscopy but also demonstrates the presence of parallel, interdigitating, rod-like **thick filaments (thick myofilaments)** and **thin filaments (thin myofilaments)**. The **thick filaments** (15 nm in diameter and 1.5 μm long) are composed of **myosin II** and associated proteins, whereas the **thin filaments** (7 nm in diameter and 1 μm long) are composed primarily of **F-actin** and associated proteins. The detailed structures of the thick and thin filaments are presented in the following paragraphs.

The **plus ends** of thin filaments originate at the Z disk and project toward the center of the two adjacent sarcomeres, thus pointing in opposite directions. Hence, a single sarcomere has two groups of parallel arrays of thin filaments, each attached to one Z disk, with all of the filaments in each group pointing toward the middle of the sarcomere (Fig. 8–8). Thick filaments also form parallel arrays, interdigitating with the thin filaments in a specific fashion.

In a relaxed skeletal muscle fiber, the thick filaments do not extend the entire length of the sarcomere, and the thin filaments projecting from the two Z disks of the sarcomere do not meet in the midline. Therefore, there are regions of each sarcomere, on either side of each Z disk, where only thin filaments are present. These adjacent portions of two successive sarcomeres correspond to the I band seen by light microscopy; for instance, the region of each sarcomere that encompasses the entire length of the thick filaments is the A band, and the zone in the middle of the A band, which is devoid of thin

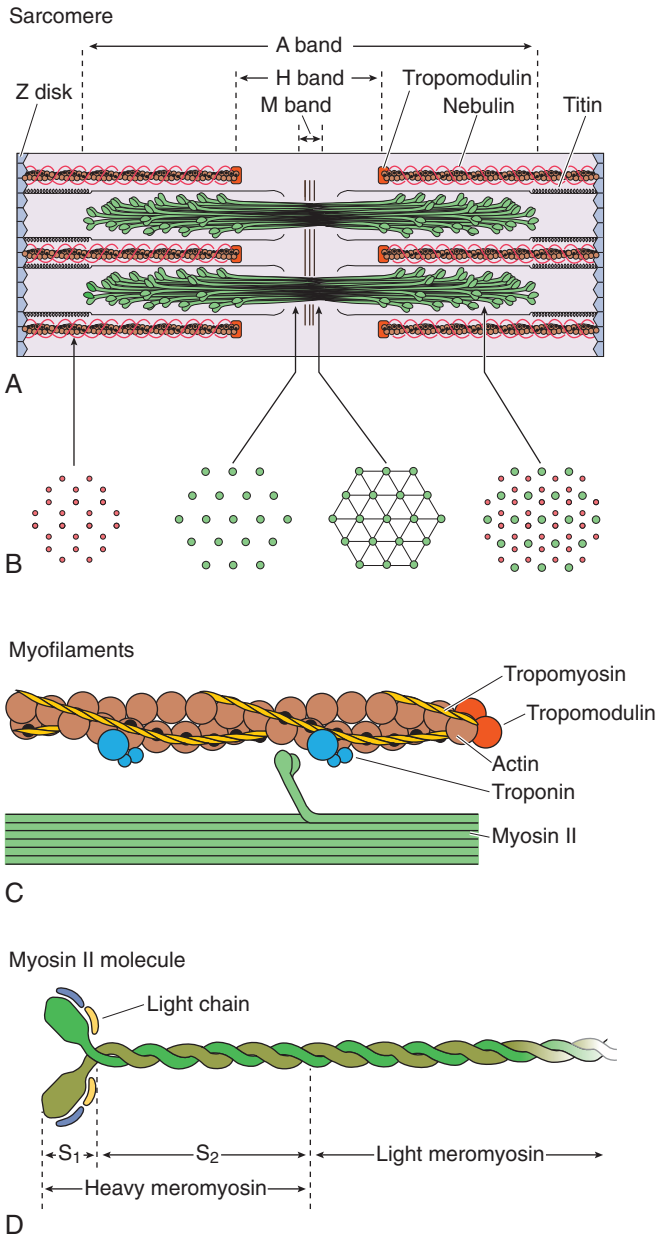


Figure 8–8 Diagram of a sarcomere and its components. **A,** Sarcomere. The myosin molecules are arranged in an antiparallel fashion so that their heads are projecting from each end of the thick filament, and each thick filament is anchored in position by four titin molecules that extend from the Z disk to the center of the thick filament at the M line. Additionally, each thin filament is fixed in place by nebulin molecules that extend from the Z disk to the distal end of the thin filament. **B,** Cross-sectional profiles of sarcomere at indicated regions. Each thick filament is surrounded equidistally by six thin filaments, so that there are always two thin filaments between neighboring thick filaments. **C,** Thick and thin filaments. Each thin filament is composed of two chains of F actins, where each F actin is composed of numerous G actin molecules assembled head to toe. Each groove of a thin filament is occupied by linear proteins, tropomyosin, that are positioned in such a fashion that they block the myosin binding site of each G actin molecule. Additionally, the tripartite molecule, troponin, is associated with each tropomyosin molecule. When the troponin C moiety of troponin binds calcium, the conformational change in the troponin molecule pushes the tropomyosin deeper into the groove, unmasking the myosin binding site of the G actin and permitting muscle contraction to occur. **D,** Myosin molecule. Each myosin molecule is composed of two light chains and two heavy chains. The heavy chains can be cleaved by trypsin into light and heavy meromyosin, and each heavy meromyosin can be cleaved by papain into S1 and S2 fragments.

filaments, is the H band. As noted earlier, the H band is bisected by the M line, which consists of **myomesin**, **C protein**, **creatine kinase**, and other as yet poorly characterized proteins that interconnect thick filaments to maintain their specific lattice arrangement.

During contraction, individual thick and thin filaments do not shorten; instead, the two Z disks are brought closer together as the thin filaments slide past the thick filaments (Huxley's **sliding filament theory**). Thus, when contraction occurs, the motion of the thin filaments toward the center of the sarcomere creates a greater overlap between the two groups of filaments, effectively reducing the widths of the I and H bands without influencing the width of the A band.

The arrangement of the thick and thin filaments bears a specific and constant relationship. In mammalian skeletal muscle, each thick filament is surrounded equidistantly by six thin filaments. Cross-sections through the region of overlapping thin and thick filaments display a hexagonal pattern, with thin filaments for the apices of each hexagon, the center of which is occupied by a thick filament (Fig. 8–9; see also

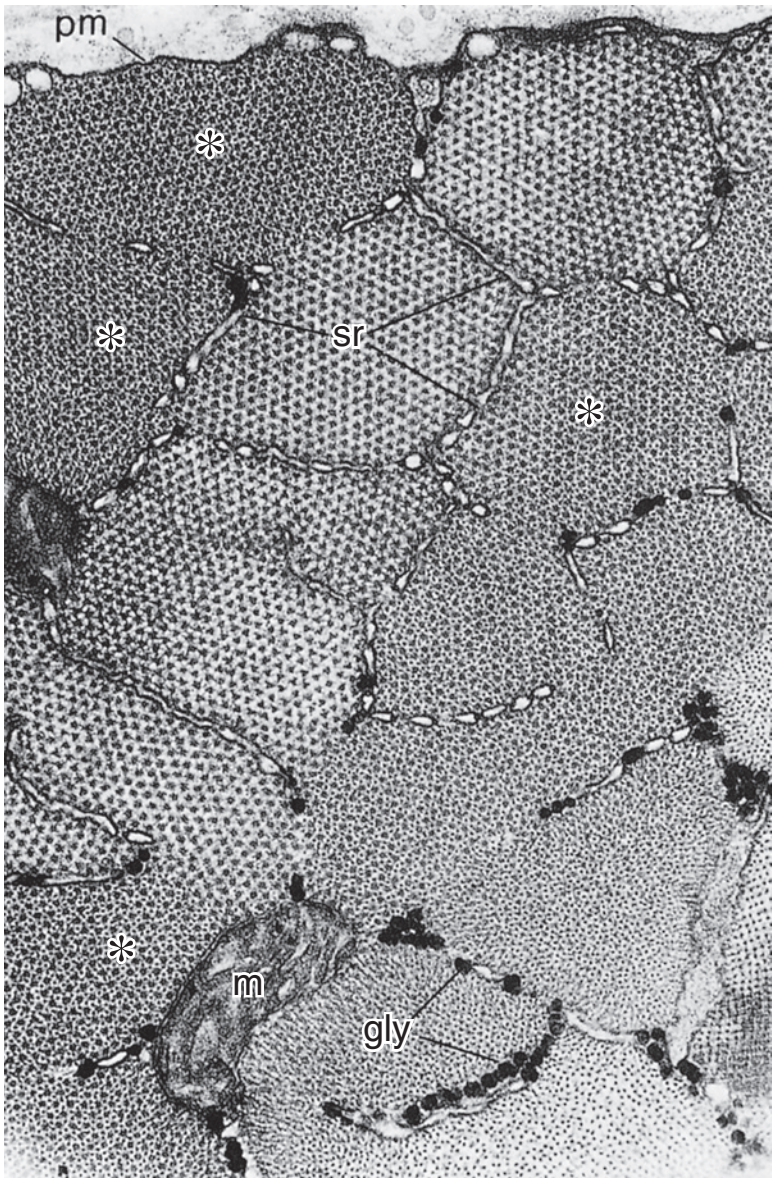


Figure 8–9 Cross-section of skeletal muscle fiber. *Asterisks* represent thick and thin filaments. gly, Glycogen; m, mitochondria; pm, plasma membrane. (Courtesy Dr. C. Peracchia; from Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

TABLE 8–2 Proteins Associated with Skeletal Muscle

Protein	Molecular Weight (kD)	Subunits and Their Molecular Weight	Function
Myosin II	510	2 heavy chains, 222 kD each; 2 pairs light chains, 18 kD and 22 kD	Major protein of thick filament; its interaction with actin hydrolyzes ATP and produces contraction
Myomesin	185	None	Cross-links thick filaments that are next to each other at M line
Titin	2500	None	Forms an elastic lattice that anchors thick filaments to Z disks
C protein	140	None	Binds to thick filaments at the M line
G actin	42	None	Polymerizes to form thin filaments of F actin; interaction of G actin with myosin II assists in hydrolyzing ATP, resulting in contraction
Tropomyosin	64	2 chains, 32 kD each	Occupies grooves of the thin filaments
Troponin	78	TnC, 18 kD	Binds calcium
		TnT, 30 kD	Binds to tropomyosin
		TnI, 30 kD	Binds to actin, thus inhibiting actin-myosin interaction
α-Actinin	190	2 units, each 95 kD	Anchors plus ends of thin filaments to Z disk
Nebulin	600	None	Z disk protein that may assist α-actinin anchor thin filaments to Z disk
Cap Z			Forms part of the Z disk and caps the plus end of the thin filament
Tropomodulin	43 kD		Caps the minus end of the thin filament

ATP, Adenosine triphosphate.

Fig. 8–8). Thick filaments are separated from each other by a distance of 40 to 50 nm, whereas the distance between thick and thin filaments is 15 to 20 nm only.

The structural organization of myofibrils is maintained largely by five proteins:

- Titin
- α-actinin
- Cap Z
- Nebulin
- Tropomodulin

Thick filaments are positioned precisely within the sarcomere with the assistance of **titin**: a large, linear, elastic protein. Two titin molecules extend from each half of a thick filament to the adjacent Z disk; thus, four titin molecules anchor each thick filament between the two Z disks of each sarcomere.

Thin filaments are held in register by the rod-shaped protein **α-actinin**, a component of the Z disk that can bind thin filaments in parallel arrays as well as by an additional component protein of the Z disk, known as ***Cap Z***, that also prevents the addition or subtraction of G actin molecules to or from the thin filament, thus assisting in the maintenance of its precise length. In addition, two molecules of **nebulin** (a long, nonelastic protein) are wrapped around the entire length of each thin filament, further anchoring it in the Z disk and ensuring the maintenance of the specific array of the thin filaments. Moreover, nebulin acts as a "ruler" ensuring the precise length of the thin filament. It is assisted in this function by the protein **tropomodulin**, a cap on the minus end of the thin filament that, similarly to Cap Z, prevents the addition or the deletion of G actin molecules to or from the thin filament (see Fig. 8–8).

Table 8–2 presents the proteins that constitute myofilaments and that keep them correctly positioned within myofibrils.

Thick Filaments

Thick filaments are composed of myosin II molecules aligned end to end.

Every thick filament consists of 200 to 300 myosin II molecules. Each **myosin II** molecule (150 nm long; 2 to 3 nm in diameter) is composed of two identical **heavy chains** and two pairs of **light chains**.

The heavy chains resemble two golf clubs, whose rod-like polypeptide chains are wrapped around each other in an α-helix. The heavy chains can be cleaved by trypsin into

- **Light meromyosin**. Rod-like tail composed of most of the two rod-like polypeptide chains wrapped around each other

- **Heavy meromyosin**. Two globular heads with the attendant short proximal portions of the two rod-like polypeptide chains wrapped around each other

The light meromyosin functions in the proper assembly of the molecules into the bipolar thick filament. Heavy meromyosin is cleaved by papain into two globular (**S_1**) moieties and a short, helical, rod-like segment (**S_2**) (see Fig. 8–8). The S_1 subfragment binds **adenosine triphosphate (ATP)** and functions in the formation of cross-bridges between the thick and thin myofilaments.

Light chains (not to be confused with light meromyosin) are of two types, and one of each type is associated with each S_1 subfragment of the myosin II molecule. For each heavy chain, therefore, there are two light chains; thus, a ***myosin II molecule is composed of two heavy chains and four light chains***.

Myosin II molecules are closely packed in a specific fashion in the thick filament. They are lined up in a parallel but staggered manner, spaced at regular intervals, lying arranged head to tail, so that the middle of each thick filament is composed solely of tail regions, whereas the two ends of the thick filament consist of both heads and tails. The spatial orientation of the myosin II molecules permits the heavy meromyosin portion to project from the thick filament at a 60-degree angle relative to neighboring heavy meromyosin, so that head regions are always in register with thin filaments.

Each myosin II molecule appears to have two flexible regions, one at the junction of the heavy meromyosin with the light meromyosin and the other at the junction of the S_1 and S_2 subfragments. The flexible region between the heavy and light meromyosins permits each myosin II molecule to contact the thin filament, forming a cross-bridge between itself and the actin filament. As discussed later, the flexible region between the S_1 and S_2 subfragments enables the myosin II molecule to drag the thin filament, incrementally, toward the middle of the sarcomere.

Thin Filaments

Thin filaments are composed of two chains of F-actin filaments wrapped around each other in association with tropomyosin and troponin.

The major component of each thin filament is **F-actin**, a polymer of globular **G-actin** units. Although G-actin molecules are globular, they all polymerize in the same spatial orientation, imparting a distinct polarity to the filament. The **plus end** of each filament is bound to the Z disk by α-actinin; the **minus end** extends toward the center of the sarcomere. Both plus and minus ends of the actin filament are capped; the plus end (at the Z disk) is capped by cap Z, whereas the minus end (near the center of the sarcomere) is capped by tropomodulin. These two caps greatly impede the addition or the deletion of G actin monomers to or from the F actin filament, maintaining its constant length.

Each G-actin molecule comprising the F actin chain possesses an **active site**, where the head region (S_1 subfragment) of myosin II binds. Two chains of F-actin are wound around each other in a tight helix (36-nm periodicity) like two strands of pearls (see Fig. 8–8).

Running along the length of the F-actin double-stranded helix are two shallow grooves. Pencil-shaped **tropomyosin molecules**, about 40 nm long, polymerize to form head-to-tail filaments that occupy the shallow grooves of the double-stranded actin helix. Bound tropomyosin masks the active sites on the actin molecules by partially overlapping them.

Approximately 25 to 30 nm from the beginning of each tropomyosin molecule is a single **troponin molecule**, composed of three globular polypeptides: TnT, TnC, and TnI. The **TnT** subunit binds the entire troponin molecule to tropomyosin; **TnC** has a great affinity for calcium; and **TnI** binds to actin, preventing the interaction between actin and myosin II. Binding of calcium by **TnC** induces a conformational shift in tropomyosin, exposing the previously blocked active sites on the actin filament so that myosin II molecules can flex, forming the cross-bridges, and so that the S_1 moieties (myosin heads) can bind to the active site on the actin molecule (see the next section).

Muscle Contraction and Relaxation

Muscle contraction obeys the all-or-none law and is followed by muscle relaxation.

Contraction effectively reduces the resting length of the muscle fiber by an amount that is equal to the sum of all shortenings that occur in all sarcomeres of that particular muscle cell. The process of contraction, usually triggered by **neural impulses**, obeys the **all-or-none law**, in that a single muscle fiber either contracts or does not as a result of stimulation. The stimulus is transferred at the **neuromuscular junction (synapse)**. During muscle contraction, the thin filaments slide past the thick filaments, as proposed by Huxley's **sliding filament theory**.

The strength of contraction of a gross anatomical muscle, such as the biceps, is a function of the number of muscle cells that undergo contraction. Based on past experience, the brain determines how many muscle cells have to contract to lift a particular object. For example, if a glass of water is to be picked up, much fewer muscle fibers will be directed to contract than if a 5-gallon container filled with water is to be picked up.

The following sequence of events leads to contraction in skeletal muscle after depolarization of the skeletal muscle membrane along the skeletal muscle cell membrane (see later section on impulse transmission at

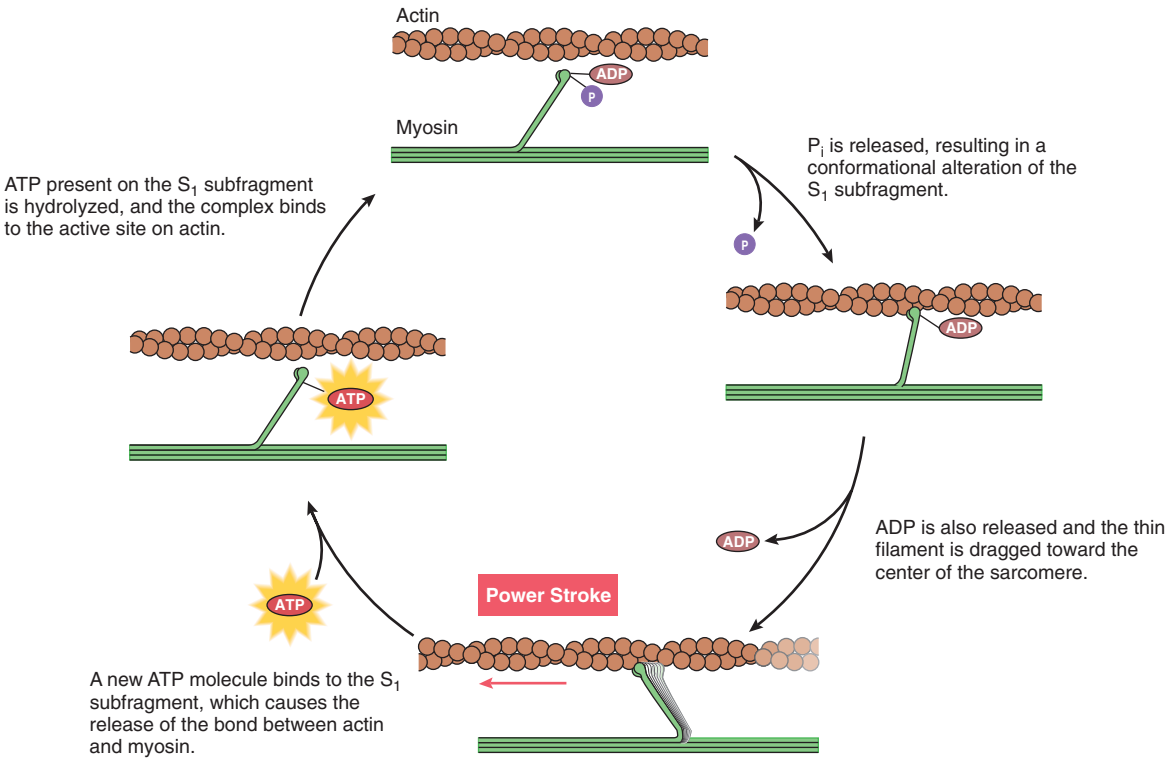


Figure 8–10 Diagram of the role of adenosine triphosphate (ATP) in muscle contraction. ADP, Adenosine diphosphate; P and P_i, inorganic phosphate; S_1 subfragment, fragment of myosin. (Modified from Alberts B, Bray D, Lewis J, et al. *Molecular Biology of the Cell*. New York: Garland Publishing; 1994.)

the neuromuscular junction to see how this depolarization occurs):

1. An impulse, generated along the sarcolemma, is transmitted into the interior of the fiber via the T tubules. The membrane of the T tubules possesses **voltage-sensitive dihydropyridine (DHP) receptors** whose conformation is altered. Because these DHP receptors are in contact with the **Ca^{2+}-release channels (ryanodine receptors)** of the terminal cisternae of the sarcoplasmic reticulum, the altered conformation in the DHP receptors causes the Ca^{2+}-release channels to open (see Fig. 8–5).
2. Calcium ions leave the terminal cisternae through the open **calcium release channels**, enter the cytosol, and bind to the TnC subunit of troponin, altering its conformation.
3. Conformational change in troponin shifts the position of tropomyosin deeper into the groove of the thin filament, unmasking the active site (myosin binding site) on the actin molecule.
4. ATP present on the S_1 subfragment of myosin II is hydrolyzed, but both adenosine diphosphate (ADP) and inorganic phosphate (P_i) remain attached to the S_1 subfragment, which twists so that the complex can bind to the active site on actin (Fig. 8–10).
5. Inorganic phosphate is released, resulting not only in a greater bond strength between the actin and myosin II, but also in a conformational alteration of the S_1 subfragment.
6. ADP is also released from the S_1 subfragment of myosin II, and the thin filament is dragged toward the center of the sarcomere ("power stroke"). Each power stroke moves the thin filament for a distance of approximately 5 nm toward the center of the sarcomere.
7. A new ATP molecule binds to the S_1 subfragment, causing the release of the bond between actin and myosin II.

The attachment and release cycles must be repeated about 200 times for contraction to be completed. Each attachment and release cycle requires ATP for the conversion of chemical energy into motion.

As long as the cytosolic calcium concentration is high enough, actin filaments remain in the active state, and

CLINICAL CORRELATIONS

Shortly after the death of an animal or a human being, the joints become immoveable. This stiffening of the joints is referred to as ***rigor mortis***, and depending on the ambient temperature, it may last as long as 3 days. Because the dead cells are unable to manufacture ATP, the dissociation of the thick and thin filaments cannot occur, and the myosin heads remain bound to the active site of the actin molecule until the muscle begins to decompose. The time of death may be estimated by the state of rigor mortis, when correlated with a record of the ambient temperature fluctuations. It is interesting to note that the facial muscles are the first to undergo rigor mortis and that the maximum rigor occurs 12 to 24 hours after death.

contraction cycles continue. Once the stimulating impulses cease, however, muscle relaxation occurs, involving a reversal of the steps that led to contraction.

First, **calcium pumps** in the membrane of the sarcoplasmic reticulum actively drive Ca^{2+} ions back into the terminal cisternae, where the ions are bound by the protein **calsequestrin**. The reduced levels of Ca^{2+} in the cytosol cause TnC to lose its bound Ca^{2+} and to return to its original conformation, permitting tropomyosin to resume its original location in the groove of the thin filament and mask the active site on the actin molecule, thus preventing the interaction of actin and myosin II.

Energy Sources for Muscle Contraction

Energy sources for muscle contraction are the phosphagen energy system, glycolysis, and aerobic energy system.

Because the process of muscle contraction requires a great deal of energy, skeletal muscle cells maintain a high concentration of the energy-rich compounds ATP and **creatine phosphate** (or **phosphocreatine**). Because both ATP and creatine phosphate contain high-energy phosphate bonds, they constitute the **phosphagen energy system** and can provide enough energy for about a total of 9 seconds of maximum muscle activity (3 seconds for ATP and 6 seconds for creatine phosphate).

Additional energy can be derived from the breakdown of glycogen to glucose and the consequent anaerobic metabolism of glucose **(glycolysis)**, which results in the formation and buildup of lactic acid. This is known as the ***glycogen–lactic acid system***. This system provides about 90 to 100 seconds of energy at almost maximal muscle activity.

The third system, known as the ***aerobic energy system***, uses the normal diet for the manufacture of ATP. The aerobic system does not support maximal muscle activity, but it can sustain normal muscle activity indefinitely if the dietary intake is maintained and the nutrients persist.

ATP is manufactured via oxidative phosphorylation within the abundant mitochondria of muscle cells during periods of inactivity or low activity. Lipid droplets and glycogen, which abound in the sarcoplasm, also are readily converted into energy sources. The three metabolic systems of skeletal muscle are harnessed to supply the energy requirement of the muscle according to their activity modalities. During bursts of muscle contraction, the ADP that is generated is rephosphorylated by two means: (1) **glycolysis**, leading to accumulation of lactic acid, and (2) transfer of high-energy phosphate from creatine phosphate (phosphagen system) catalyzed by **phosphocreatine kinase**. During prolonged but normal muscle activity, however, the aerobic system of energy production is employed.

CLINICAL CORRELATIONS

The nodal regulator **peroxisome proliferator-activated receptor γ coactivator 1α (PCG-1α)** is a well-known regulator not only of mitochondrial self-replication and energy utilization but also of the activity of a number of transcription factors. Recently, it has been shown that PCG-1α expression is increased in skeletal muscle after exercise. Interestingly, exercise is also a mood enhancer, and it relieves stress-related depression. Studies have demonstrated that PCG-1α expression elevates levels of **kynurenine aminotransferase** within skeletal muscle cells, and this enzyme converts **kynurenine** into its acidic form, **kynurenic acid**. When kynurenine is released from skeletal muscle cells, it enters the bloodstream and is able to penetrate the blood–brain barrier. It has been reported that elevated levels of kynurenine in the brain has been associated with depression; however, kynurenic acid cannot cross the blood–brain barrier; consequently, after exercise, there is a decrease in kynurenine levels in the brain. Interestingly, diabetes and obesity also cause depression, and both diabetic and obese mice display reduced levels of PCG-1α in skeletal muscle cells, suggesting that this nodal regulator may have an important function in alleviating depression.

Myotendinous Junctions

The connective tissue elements of the muscle fiber are continuous with the tendon to which the muscle is attached. At the myotendinous junctions, the cells become tapered and highly fluted. Collagen fibers of the tendon penetrate deep into these infoldings and probably become continuous with the reticular fibers of the endomysium. Within the cell, the myofilaments are

anchored to the internal aspect of the sarcolemma so that the force of contraction is transmitted to the collagen fibers of the tendon.

Innervation of Skeletal Muscle

Skeletal muscle cells and the single motoneuron that innervates them constitute a motor unit.

Each skeletal muscle receives at least two types of nerve fibers: **motor** and **sensory**. The motor nerve functions in eliciting contraction, whereas the sensory fibers pass to muscle spindles and Golgi tendon organs (see section on muscle spindles and Golgi tendon organs). Additionally, autonomic fibers supply the vascular elements of skeletal muscle. The specificity of motor innervation is a function of the muscle innervated. If the muscle acts fastidiously, as do some muscles of the eye, a single motoneuron may be responsible for as few as 5 to 10 skeletal muscle fibers, whereas a muscle located in the abdominal wall may have as many as 1000 muscle fibers under the control of a single motoneuron. Each motoneuron and the muscle fibers it controls form a **motor unit**. The muscle fibers of a *single* motor unit contract in unison and follow the **all-or-none law** of muscle contraction.

Impulse Transmission at the Neuromuscular Junction

Impulse transmission from the motoneuron to the skeletal muscle fiber occurs at the neuromuscular junction.

Motor fibers are **myelinated axons** of **α-motoneurons** that pass in the connective tissue of the muscle. The axon arborizes, eventually losing its myelin sheath (but not its Schwann cells). The terminal of each arborized twig becomes dilated and overlies the cell membrane of individual muscle fibers. Each of these muscle–nerve junctions, known as a ***neuromuscular junction*** **(myoneural junction)**, is composed of an axon terminal, a synaptic cleft, and the modified muscle cell membrane (known specifically as the ***postsynaptic membrane*** or ***motor end plate***) (Figs. 8–11 to 8–13).

The **sarcolemma at the postsynaptic membrane** is modified, forming a trough-like structure, known as the ***primary synaptic cleft***, occupied by the **axon terminal**. Opening into the primary synaptic clefts are numerous tubular invaginations known as ***junctional folds*** **(secondary synaptic clefts)**, a further modification of the sarcolemma. Both the primary synaptic cleft and the junctional folds are lined by a basal lamina–like **external lamina**, manufactured by the skeletal muscle cell. The sarcoplasm in the vicinity of the secondary synaptic cleft is rich in glycogen, nuclei, ribosomes, and mitochondria.

The **axon terminal** is covered by Schwann cells on its entire surface except on its **presynaptic membrane**, the surface facing the postsynaptic membrane. The axon terminal houses mitochondria, SER, and as many as 300,000 **synaptic vesicles** (each 40 to 50 nm in diameter) containing the neurotransmitter **acetylcholine**. The function of the neuromuscular junction is to transmit a stimulus from the nerve fiber to the muscle cell.

Stimulus transmission across a synaptic cleft involves the following sequence of events (Fig. 8–14):

1. A stimulus, traveling along the axon, depolarizes the membrane of the axon terminal, thus opening the **voltage-gated calcium channels**, located in the vicinity of linearly arranged structures known as ***dense bars***.
2. The influx of calcium ions into the axon terminal results in the fusion of about 120 synaptic vesicles per nerve impulse with the axon terminal's membrane **(presynaptic membrane)** and subsequent release of acetylcholine (along with proteoglycans and ATP) into the primary synaptic cleft. Fusion occurs along specific regions of the presynaptic membrane, known as ***active sites***, adjoining the dense bars.
3. The neurotransmitter acetylcholine (ligand) is liberated in large quantities, known as ***quanta*** (equal to 10,000 to 20,000 molecules), from the nerve terminal.
4. Acetylcholine then diffuses across the synaptic cleft and binds to postsynaptic **acetylcholine receptors** in the muscle cell membrane. These receptors, located in the vicinity of the presynaptic active sites, are **transmitter-gated sodium ion channels**, which open in response to the binding of acetylcholine. The resulting ion influx leads to **depolarization** of the muscle cell membrane and creation of an **action potential** (see Chapter 9).
5. The impulse generated spreads quickly throughout the muscle fiber via the system of T tubules (see previous section on muscle contraction and relaxation), initiating muscle contraction.

To prevent a single stimulus from eliciting multiple responses, **acetylcholinesterase**, an enzyme located in the external lamina lining the primary and secondary synaptic clefts, degrades acetylcholine into acetate and choline, thus permitting the reestablishment of the **resting potential**. Degradation is so rapid that all of the released acetylcholine is cleaved within a few hundred milliseconds.

Choline is transported back into the axon terminal by a **sodium-choline symport protein** powered by the sodium concentration gradient. Within the axon terminal, the acetylcholine is synthesized from activated acetate (produced in mitochondria) and the recycled choline, a reaction catalyzed by **choline acetyl transferase**. The newly formed acetylcholine is transported, through the use of an antiport system powered

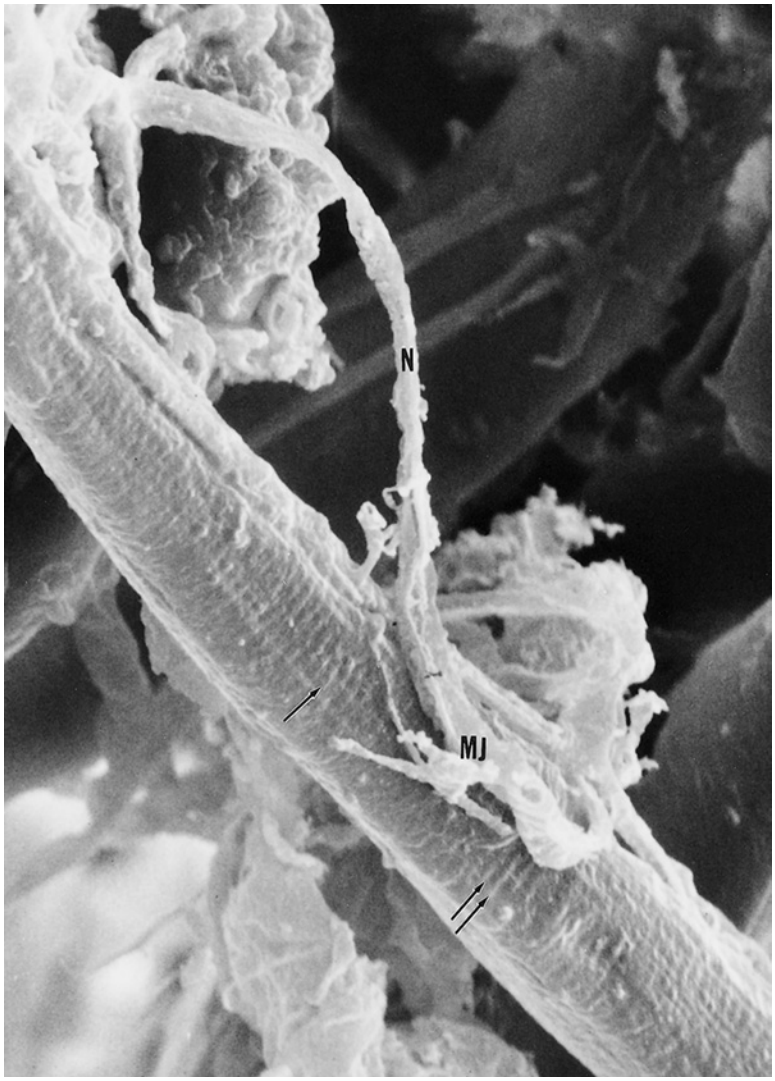


Figure 8–11 Scanning electron micrograph of a neuromuscular junction from the tongue of a cat (×2315). *Arrows* indicate striations. MJ, Neuromuscular junction; N, nerve fiber. (Courtesy Dr. L. Litke.)

by a proton concentration gradient, into newly formed synaptic vesicles.

In addition to recycling of choline, the synaptic vesicle membrane is recycled to conserve the surface area of the presynaptic membrane. This membrane recycling is accomplished by the formation of **clathrin-coated endocytotic vesicles**, which become the newly formed synaptic vesicles.

Muscle Spindles and Golgi Tendon Organs

Muscle spindles and Golgi tendon organs are sensory receptors that monitor muscle contraction.

The neural control of muscle function requires not only the capability of inducing or inhibiting muscle contraction but also the ability to monitor the status of the muscle and its tendon during muscle activity. This monitoring is performed by two types of sensory receptors:

- **Muscle spindles**, which provide feedback about the changes in muscle length as well as the rate of alteration in muscle length
- **Golgi tendon organs**, which monitor the tension as well as the rate at which the tension is being produced during movement

Information from these two sensory structures is generally processed at unconscious levels, within the

Figure 8–12 Electron micrograph of a mouse neuromuscular junction. (From Feczko D, Klueber KM. Cytoarchitecture of muscle in a genetic model of murine diabetes. *Am J Anat*. 1988;182:224-240. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

CLINICAL CORRELATIONS

Botulism is usually caused by ingestion of improperly preserved canned foods. The toxin, produced by the microbe *Clostridium botulinum*, interferes with the release of acetylcholine, with resultant muscle paralysis and, without treatment, death.

Myasthenia gravis is an autoimmune disease in which autoantibodies attach to acetylcholine receptors, blocking their availability to acetylcholine. Receptors thus inactivated are endocytosed and replaced by new receptors, which are also inactivated by the autoantibodies. Thus, the number of locations for the initiation of muscle depolarization is reduced, and the skeletal muscles (including the diaphragm) weaken gradually. Certain **neurotoxins**, such as the bungarotoxin of some poisonous snakes, also bind to acetylcholine receptors, causing paralysis and eventual death due to respiratory compromise.

Botulinum Toxin Type A **(Botox Cosmetic)** is an inhibitor of acetylcholine release by motor fibers that cause skeletal muscle contraction. This toxin, produced by *Clostridium botulinum,* when injected into particular muscles, specifically inhibits the contraction of that muscle. For cosmetic purposes, the procerus and corrugator muscles are usually injected with Botox, thus diminishing the frown lines that the contraction of those facial muscles otherwise produce and, by eradicating the "wrinkles," make the face appear smoother and younger looking. It is interesting to note that, in 2012, almost 6 million Botox injections were performed in the United States for cosmetic purposes. The effect of the injections lasts for less than 3 months, and many patients repeat the procedure two to three times per year. There appear to be no serious side effects, although, if injected into the wrong muscles, ptosis (drooping) of the eyelids could persist for several months. Occasionally, individuals may have headaches, cold-like symptoms, nausea, as well as muscle weakness, pain, and inflammation in the area of the injection for as long as 4 months.

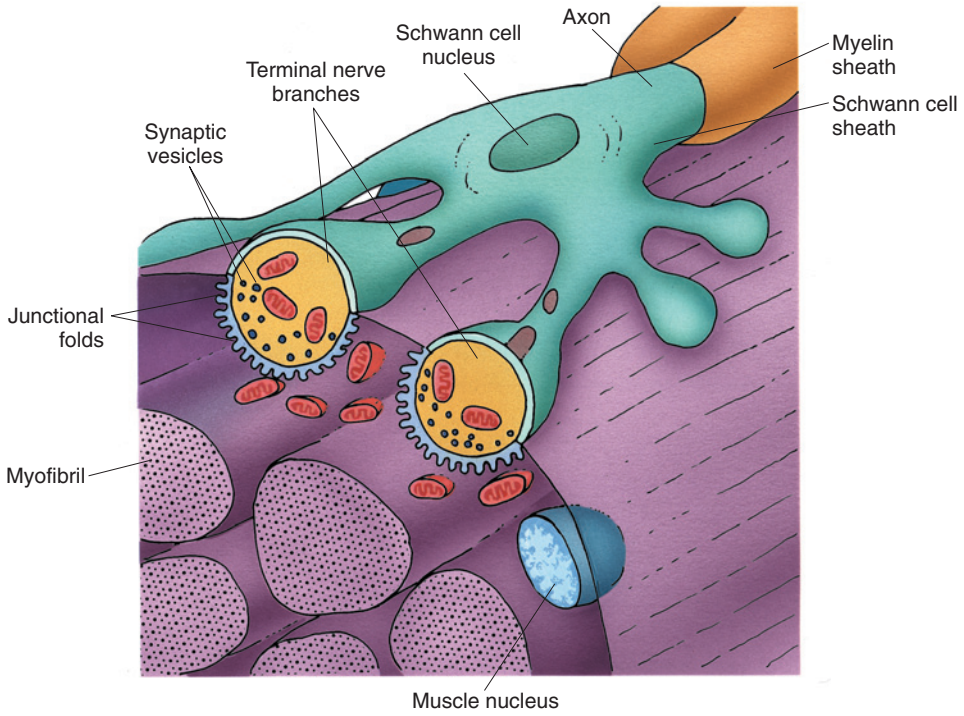


Figure 8–13 Schematic diagram of the neuromuscular junction. Note that the myelin sheath *(yellow)* stops as the axon arborizes over the skeletal muscle fiber, but the Schwann cell sheath *(green)* continues to insulate the nerve fiber. The terminal nerve branches expand to form axon terminals that overlie the motor end plates of individual muscle fibers.

spinal cord. The information also reaches the cerebellum and even the cerebral cortex, however, so a person may sense muscle position.

Muscle Spindles

Muscle spindles continuously monitor the length and the changes in length of the muscle.

When muscle is stretched, it normally undergoes reflex contraction, or **stretch reflex**. This proprioceptive response is initiated by the **muscle spindle**, an encapsulated sensory receptor located among, and in parallel with, the muscle cells (Fig. 8–15). Each muscle spindle is composed of 8 to 10 elongated, narrow, very small, modified muscle cells called ***intrafusal fibers***, surrounded by the fluid-containing **periaxial space**, which, in turn, is enclosed by the capsule. The connective tissue elements of the capsule are continuous with the collagen fibers of the perimysium and endomysium. The skeletal muscle fibers surrounding the muscle spindle are unremarkable and are called ***extrafusal fibers***.

Intrafusal fibers are of two types: **nuclear bag fibers** and the more numerous, thinner **nuclear chain fibers**. Furthermore, there are two categories of nuclear bag fibers: **static** and **dynamic**. The nuclei of both types of fibers occupy the centers of the cells; their myofibrils are located on either side of the nuclear region, limiting contraction to the polar regions of these spindle-shaped cells. The central regions of the intrafusal fibers do not contract. The nuclei are aggregated in the nuclear bag fibers, whereas they are aligned in a single row in nuclear chain fibers.

Within a specific muscle spindle, a single, myelinated, large, sensory nerve fiber **(group Ia)** wraps spirally around the nuclear regions of each of the three types of intrafusal fibers, forming the **primary sensory endings** (also known as ***dynamic*** and ***Ia*** sensory endings). Additionally, **secondary sensory nerve endings** (also known as ***static*** and ***II*** sensory nerve endings) are formed by **group II** nerve fibers, which wrap around every nuclear chain fiber as well as around the static nuclear bag fibers.

The contractile regions of the intrafusal fibers receive two types of γ-motoneurons. Dynamic nuclear bag fibers are innervated by a **dynamic γ-motoneuron**, whereas all nuclear chain fibers as well as all of the static nuclear bag fibers are innervated by a **static γ-motoneuron**.

The extrafusal fibers receive their normal nerve fibers, which are the large, rapidly conducting axons of **α-efferent neurons (motoneurons)**.

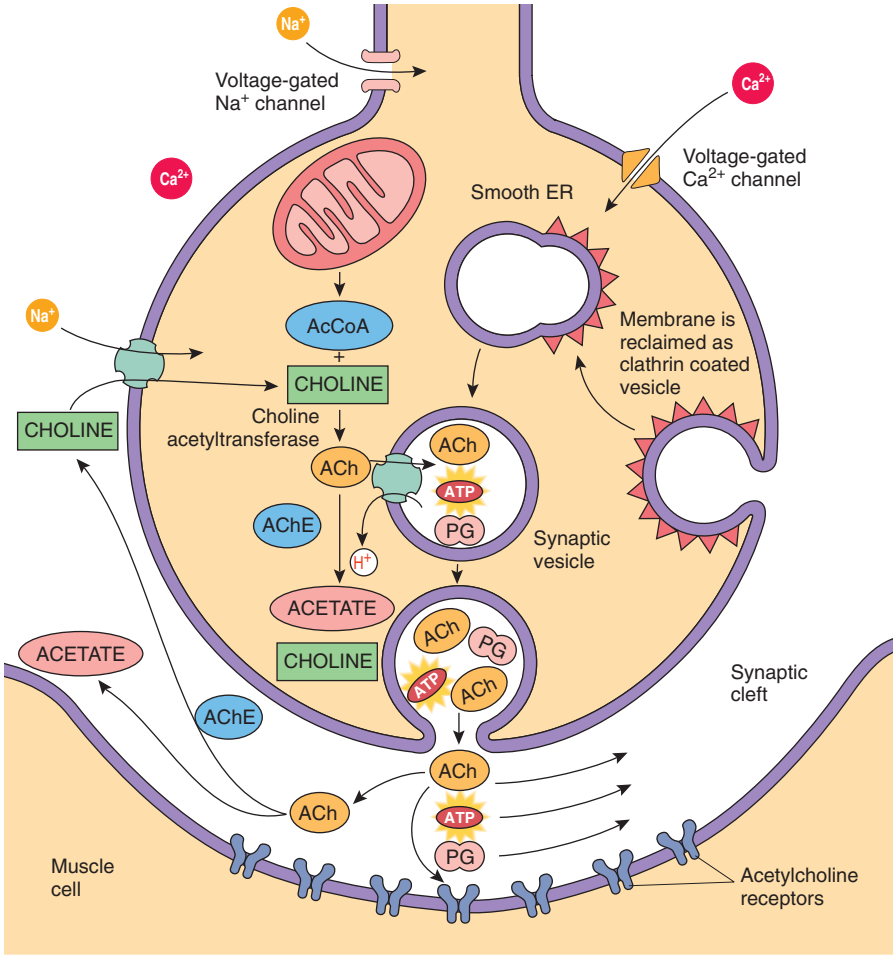
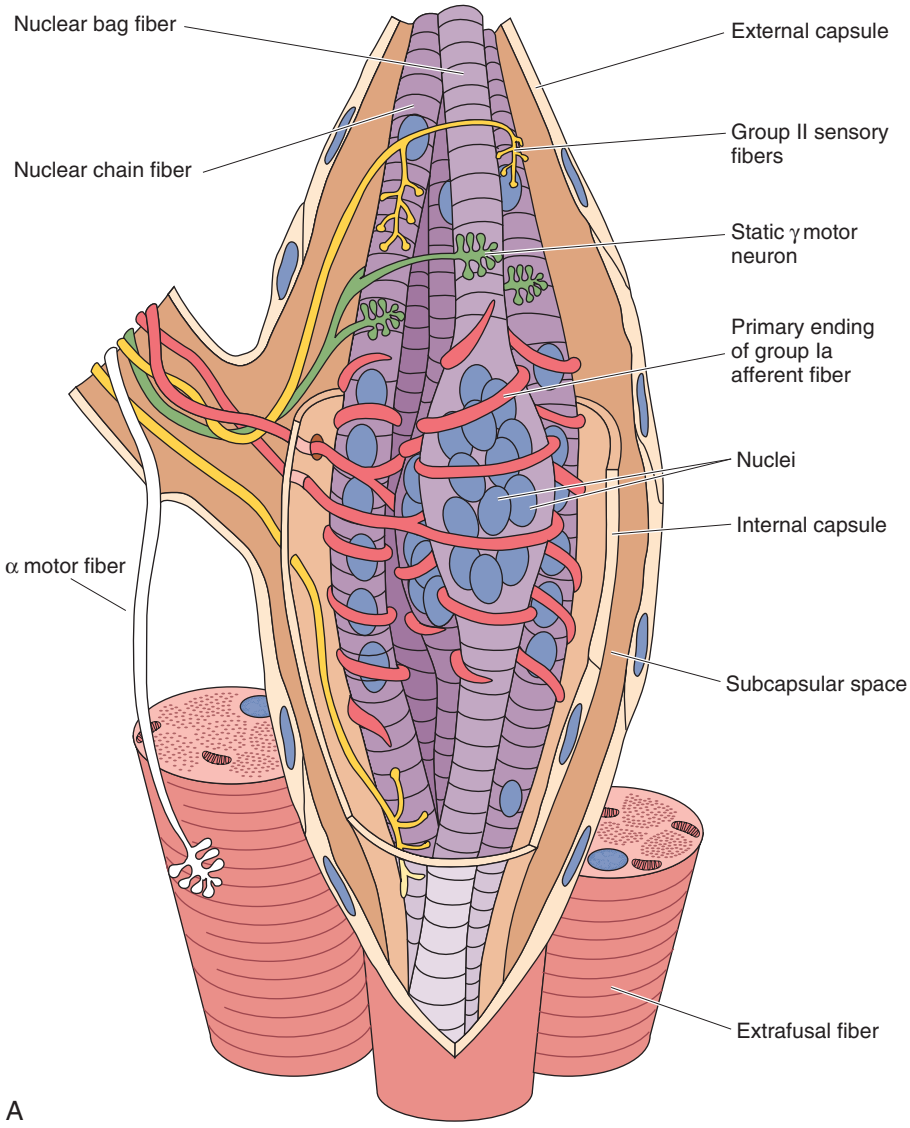


Figure 8–14 Schematic diagram of the events occurring at the neuromuscular junction during the release of acetylcholine. AcCoA, Acetyl CoA; Ach, acetylcholine; AchE, acetylcholinesterase; ATP, adenosine triphosphate; Ca^{2+}, calcium ion; H^+, hydrogen ion; Na^+, sodium ion; PG, proteoglycan. (Modified from Katzung BG. *Basic and Clinical Pharmacology*. 4th ed. East Norwalk, Conn: Appleton & Lange; 1989.)

As a muscle is stretched, the intrafusal muscle fibers of its muscle spindle are also stretched, causing the primary (group Ia, dynamic) and secondary (group II, static) sensory nerve fibers to initiate an action potential; with increased stretching, these nerve fibers accelerate their rate of firing. Both group Ia and group II fibers respond to a stretching of the muscle at a *constant rate* **(static response)**. Only group Ia fibers, however, respond to a *change in the rate* **(phasic response)** at which stretching occurs, thus furnishing information concerning both the rapidity of movement and unanticipated stretching of the muscle.

Firing of the γ-motoneurons causes the polar regions of the intrafusal fibers to contract. When this occurs, the noncontractile regions of the intrafusal fibers are stretched from both directions, resulting in activation of the primary and secondary sensory nerve endings. Modulation of γ-motoneuron activity sensitizes the muscle spindle so that it can react even to a small degree of muscle stretching, as follows:

- Firing of dynamic γ-motoneurons primes the dynamic nerve endings but not the static nerve endings (because their firing does not cause contraction of the static nuclear bag fibers)
- Firing of static γ-motoneurons increases the continuous, steady response of both group Ia and group II sensory fibers (because both fibers form sensory nerve endings on static nuclear bag and all nuclear chain intrafusal fibers); however, the dynamic sensory fiber response decreases (because static



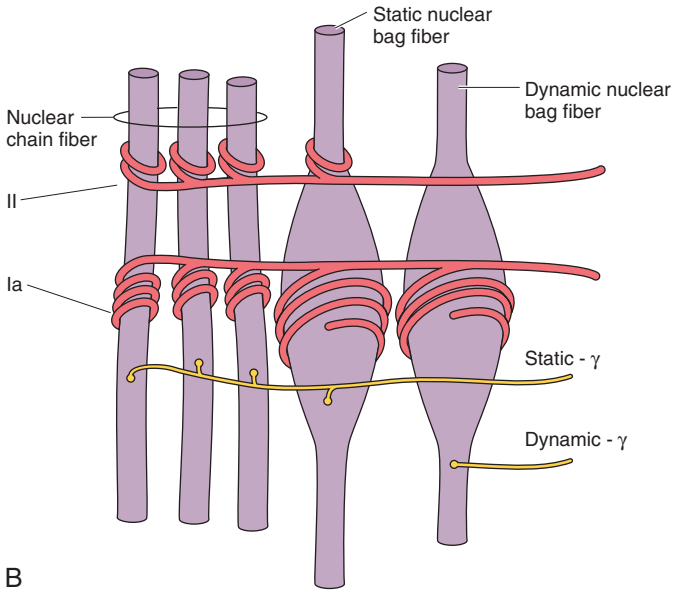


Figure 8–15 **A,** Schematic diagram of a muscle spindle. **B,** The various fiber types of the muscle spindle and their innervation are presented in a spread-out fashion. Ia, Group Ia sensory fiber; II, group II sensory fiber. (**A,** Modified from Krstic RV. *Die Gewebe des Menschen und der Saugertiere*. Berlin: Springer-Verlag; 1978. **B,** Modified from Hulliger M. The mammalian muscle spindle and its central control. *Rev Physiol Biochem Pharmacol*. 1984;101:1-110.)

γ-motoneurons do not innervate dynamic nuclear bag fibers)

Thus, modulation of the γ-motoneuron activity gives the nervous system the ability to adjust the sensitivity of the muscle spindle.

CLINICAL CORRELATIONS

The **simple reflex arc**, such as the knee jerk, is an example of the function of muscle spindles. Tapping on the patellar tendon results in a sudden stretching of the muscle (and of the muscle spindles). The primary and secondary nerve endings are stimulated, relaying the stimulus to the **α-motoneurons of the spinal cord**, **resulting in muscle contraction**. Therefore, when a muscle is overstretched either too much or for too long a period of time, the muscle spindle reacts by stimulating muscle contraction to oppose the stretching.

Golgi Tendon Organs (Neurotendinous Spindles)

Golgi tendon organs (neurotendinous spindles) monitor the intensity of muscle contraction.

Golgi tendon organs, also called ***neurotendinous spindles***, are cylindrical structures about 1 mm in length and 0.1 mm in diameter. They are located at the juncture of a muscle with its tendon and are positioned in series with the muscle fibers. Golgi tendon organs are composed of **wavy collagen fibers** and the nonmyelinated continuation of a single **type Ib axon** that ramifies as free nerve endings in the interstices between the collagen fibers. When the muscle contracts, it places tensile forces on the collagen fibers, straightening them, with a consequent compression and firing of the entwined nerve endings. The rate of firing is directly related to the amount of tension placed on the tendon.

When a muscle undergoes strenuous contraction, it may generate a great amount of force. To protect the muscle, bone, and tendon, **Golgi tendon organs** provide an inhibitory feedback to the **α-efferent neurons (motoneurons)** of the muscle, resulting in relaxation of the contracting tendon's muscle. Thus, the Golgi tendon organs monitor the force of muscle contraction, whereas muscle spindles monitor the stretching of the muscle in which they are located. These two sensory organs act in concert to integrate spinal reflex systems.

CLINICAL CORRELATIONS

The ability of a person to touch his or her nose in absolute darkness is due to the integrative activities of muscle spindles and, possibly, to Golgi tendon organs. These structures provide not only feedback about the amount of tension placed on the muscle and tendon, but also input to the cerebellum and cerebral cortex supplying information about the body's position in three-dimensional space, and this ability is referred to as *proprioception*.

CARDIAC MUSCLE

Cardiac muscle is nonvoluntary striated muscle limited to the heart and the proximal portions of the pulmonary veins.

Cardiac muscle (heart muscle), another form of striated muscle, is found only in the heart and in pulmonary veins where they join the heart. Cardiac muscle is derived from a strictly defined mass of splanchnic mesenchyme, the **myoepicardial mantle**, whose cells give rise to the epicardium and **myocardium**.

The adult myocardium consists of an anastomosing network of branching cardiac muscle cells arranged in layers **(laminae)**. Laminae are separated from one another by slender connective tissue sheets that convey blood vessels, nerves, and the conducting system of the heart. Capillaries, derived from these branches, invade the intercellular connective tissue, forming a rich, dense network of capillary beds surrounding every cardiac muscle cell, explaining the ability of these cells to use aerobic respiration for almost 90% of their energy supply.

Cardiac muscle differs from skeletal and smooth muscles in that it possesses an **inherent rhythmicity** as well as the ability to **contract spontaneously**. A system of modified cardiac muscle cells has been adapted to ensure the coordination of its contractile actions. This specialized system as well as the associated autonomic nerve supply is discussed in Chapter 11.

Cardiac Muscle Cells

Although the resting lengths of individual cardiac muscle cells vary, on average they are 15 μm in diameter and 80 μm long. Each cell possesses a single, large, oval, centrally placed nucleus, although two nuclei are occasionally present (Figs. 8–16 to 8–18).

Intercalated Disks

Cardiac muscle cells form highly specialized end-to-end junctions, referred to as ***intercalated disks*** (Figs. 8–19 to 8–21; see Fig. 8–16). The cell membranes involved in these junctions approximate each other, so that, in most areas, they are separated by a space of less than 15 to 20 nm.

Intercalated disks have **transverse portions**, where fasciae adherentes and desmosomes abound, as well as **lateral portions** rich in gap junctions (see Figs. 8–19 and 8–21). On the cytoplasmic aspect of the sarcolemma of intercalated disks, **thin myofilaments** attach to

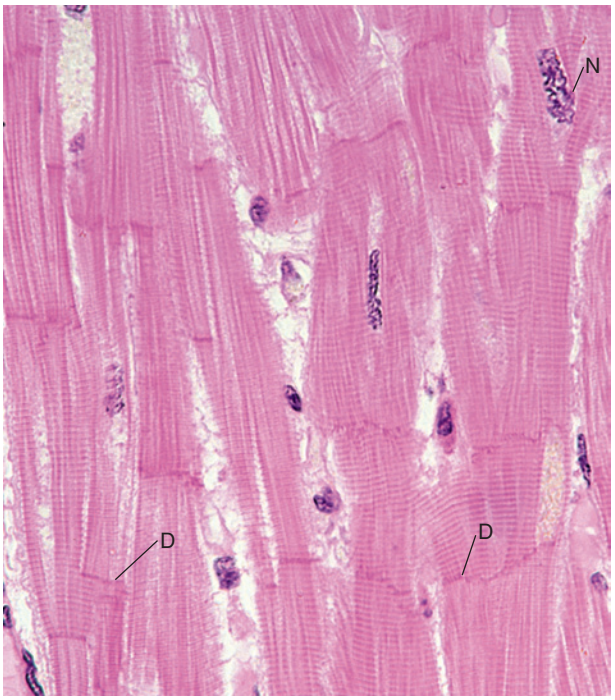


Figure 8–16 Photomicrograph of cardiac muscle in longitudinal section. Note the nucleus (N) and the presence of intercalated disks (D), regions where the cardiac muscle cells form desmosomes, fasciae adherents, and gap junctions with each other (×540).

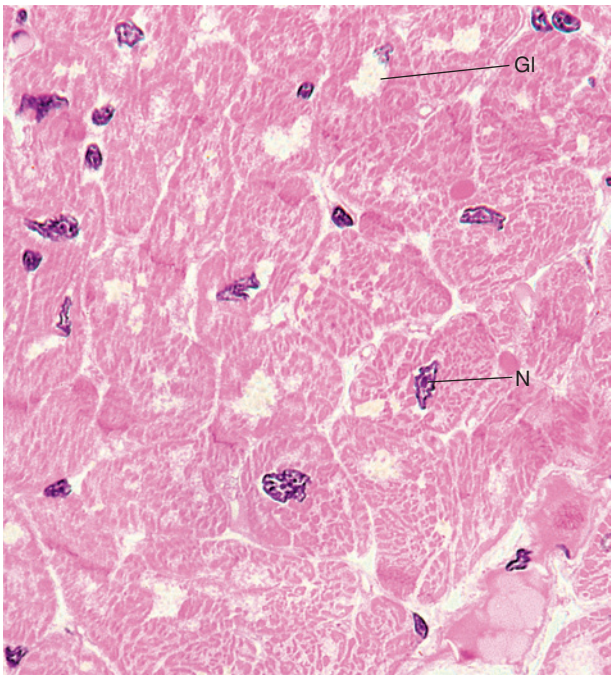


Figure 8–17 Photomicrograph of cardiac muscle in cross-section. The nucleus (N) is centrally located and, at each pole of the nucleus, the glycogen deposits (Gl) have been extracted during the histological preparation (×540).

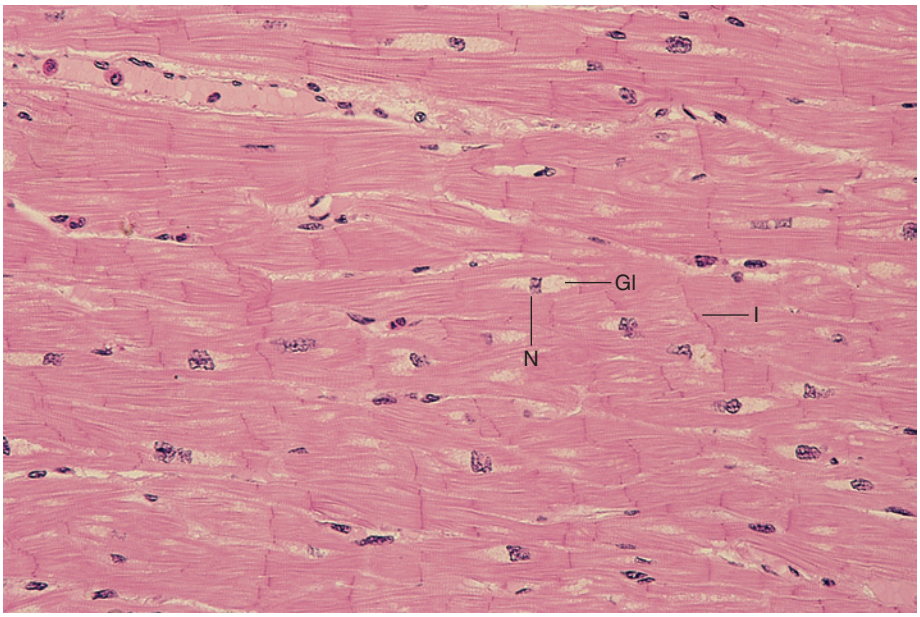


Figure 8–18 Cardiac muscle cells in longitudinal section displaying their characteristic branching patterns and glycogen deposits (Gl). The branching of the cardiac muscle fibers, the central location of the nuclei (N), and the presence of intercalated disks (I) are identifying characteristics of cardiac muscle (×270).

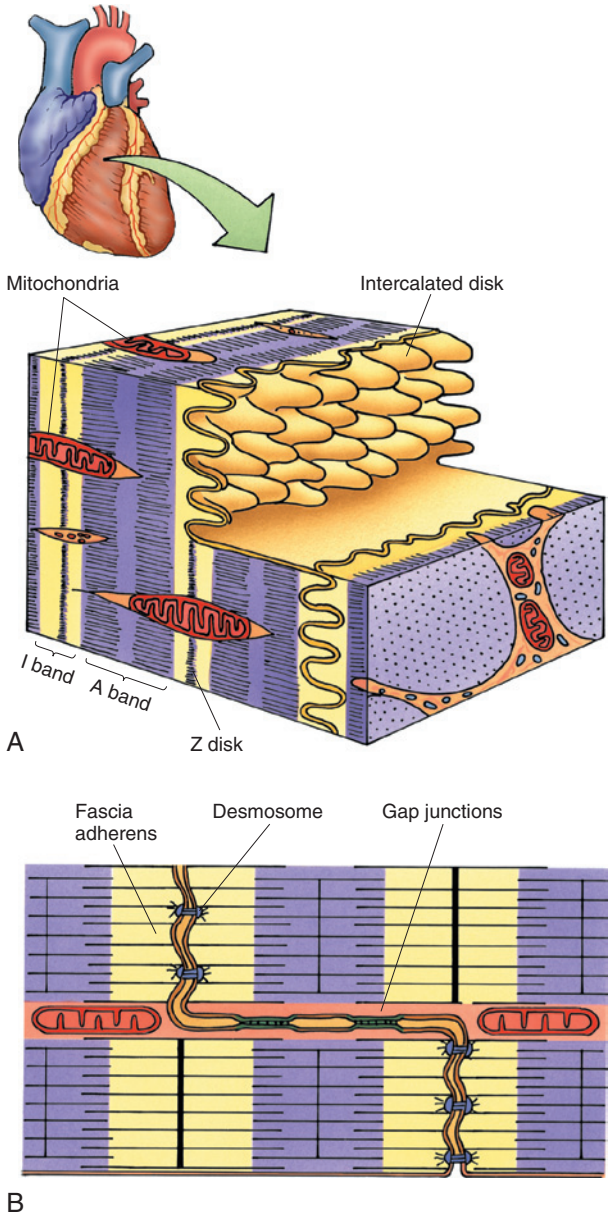


Figure 8–19 Schematic diagram of cardiac muscle. **A,** Three-dimensional view of the intercalated disk. **B,** Two-dimensional view of the intercalated disk with a display of adhering and communicating junctions. The transverse portions of the intercalated disk acts as a Z plate, and thin filaments are embedded in them.

the fasciae adherentes, which are thus analogous to Z disks. Gap junctions, which function in permitting rapid flow of information from one cell to the next, also form in regions where cells lying side by side come in close contact with each other. Because of the presence of gap junctions, cardiac muscle cells are contracting in synchrony with each other, forming a **functional syncytium**.

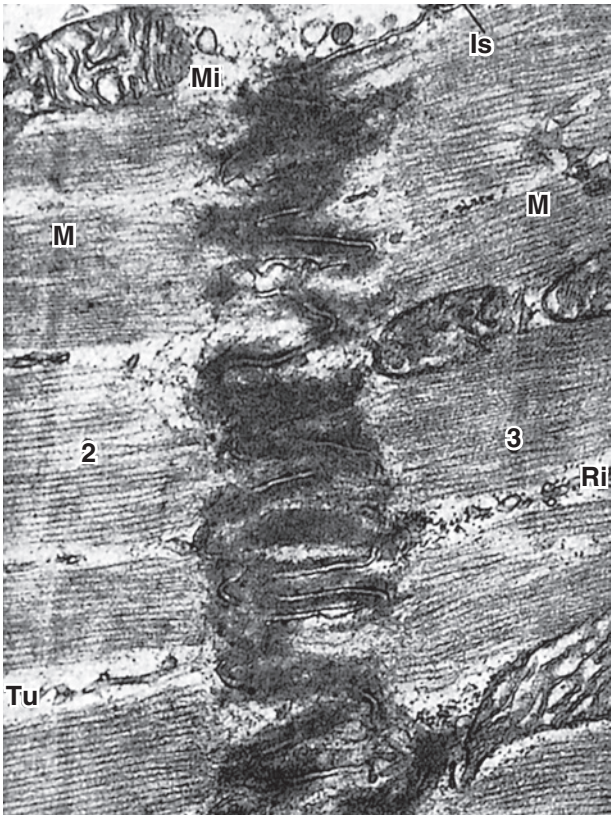


Figure 8–20 Electron micrograph of an intercalated disk from a steer heart (×29622). Is, Intercellular space; M, M-line; Mi, mitochondrion; Ri, ribosomes; Tu, sarcoplasmic reticulum. Numbers 2 and 3 denote the two cells, one on either side of the intercalated disk. (From Rhodin JAG. *An Atlas of Ultrastructure*. Philadelphia: WB Saunders; 1963.)

Organelles

The extracellular fluid is the primary calcium source for cardiac muscle contraction.

The bandings of cardiac muscle fibers are identical with those of skeletal muscle, including alternating I and A bands. Each sarcomere possesses the same substructure as its skeletal muscle counterpart; therefore, the mode and mechanism of contraction are virtually identical in the two striated muscles. However, in skeletal muscle, the **nebulin molecules** extend along the entire length of the thin filament all the way to the H zone, whereas, in cardiac muscle, the nebulin extends only about 20% of the length of the thin filament, far short of the H zone. Several major differences should be noted, however; they are in the sarcoplasmic reticulum, the arrangement of T tubules, the Ca^{2+} supply of cardiac muscle, the ion channels of the plasmalemma, and the duration of the action potential.

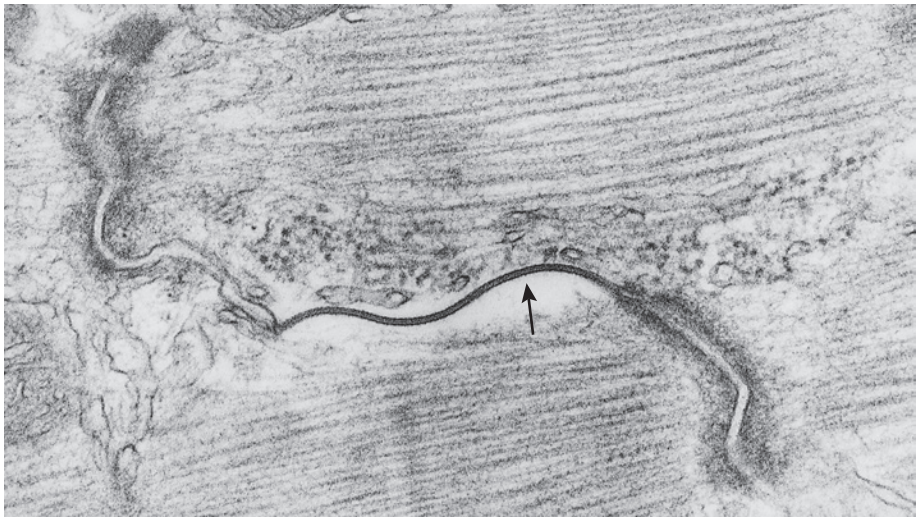


Figure 8–21 Intercalated disk from the atrium of a mouse heart (×57810). The *arrow* points to gap junctions. (From Forbes MS, Sperelakis N. Intercalated disks of mammalian heart: a review of structure and function. *Tissue Cell*. 1985;17:605.)

The sarcoplasmic reticulum of cardiac muscle does not form terminal cisternae and is not nearly as extensive as in skeletal muscle; instead, small terminals of sarcoplasmic reticulum that possess **ryanodine receptors** are referred to as ***corbular sarcoplasmic reticulum***. These structures approximate the T tubules but do not normally form a triad, as in skeletal muscle; rather, the association is usually limited to two partners, resulting in a **dyad**. Unlike in skeletal muscle, where the triads are located at the A-I interfaces, the dyads in cardiac muscle cells are located in the vicinity of the Z line. The T tubules of cardiac muscle cells are almost two-and-one-half times the diameter of those in skeletal muscle and, unlike in skeletal muscle fibers, they are lined by an **external lamina**.

Because the sarcoplasmic reticulum is relatively sparse, it cannot store enough calcium to accomplish a forceful contraction; therefore, additional sources of calcium are available. Because the T tubules open into the extracellular space and have a relatively large bore, extracellular calcium flows through the T tubules and enters the cardiac muscle cells at the time of depolarization. Moreover, the negatively charged external lamina coating of the T tubule stores calcium for instantaneous release. An additional method whereby calcium can enter the cardiac muscle cells is through the large calcium-sodium channels described below.

Skeletal muscle cell action potential is achieved by an abundance of **fast sodium channels**, which open and close within a few ten-thousandths of a second, leading to the generation of very rapid action potentials. In addition to fast sodium channels, cardiac muscle cell membranes possess **calcium-sodium channels (slow sodium channels)**. Although these channels are slow to open initially, they remain open for a considerable period of time (several tenths of a second). During this time, a remarkable number of sodium and calcium ions enter the cardiac muscle cell cytoplasm, thus increasing the calcium ion concentration supplied by the T tubule and the sarcoplasmic reticulum. An additional difference between the ion movements in skeletal and cardiac muscle cells is that potassium ions can leave the skeletal muscle cells extremely quickly, thus reestablishing the resting membrane potential; however, in cardiac muscle cells, the egress of potassium ions is impeded, thus contributing to the protracted action potential. When contraction is over, the calcium ions are returned to the sarcoplasmic reticulum. This process is controlled by **phospholamban**, an integral protein in the sarcoplasmic reticulum of cardiac muscle that is the principal target for phosphorylation by cycle adenosine monophosphate **(cAMP)-dependent protein kinase**. When phospholamban is *not phosphorylated*, it inhibits the **calcium pump** of the sarcoplasmic reticulum, and *calcium ions cannot return into the sarcoplasmic reticulum*. However, when *phospholamban is phosphorylated* by cAMP-dependent protein kinase, its conformation alters and permits the **calcium pumps** of the sarcoplasmic reticulum *to open*, and calcium can leave the cytosol and enter the sarcoplasmic reticulum, causing *relaxation of the cardiac muscle cell*. Therefore, phospholamban regulates relaxation of cardiac muscle, and, in that fashion, it is responsible for regulating **diastole**.

There are additional differences between cardiac and skeletal muscle cells. Almost half of the volume of the

cardiac muscle cell is occupied by mitochondria, attesting to its great energy consumption. During basal heart rate, triglycerides form approximately 60% of the energy supply of the heart, and only about 40% is derived from glycogen. Because the oxygen requirement of cardiac muscle cells is high, they contain an abundant supply of the oxygen-binding molecule myoglobin.

Muscle cells of the atria are somewhat smaller than those of the ventricles. These cells also house granules (especially in the right atrium) containing **atrial natriuretic peptide** and the ventricles house granules containing **B-type natriuretic peptide**, substances that function to lower blood pressure (Fig. 8–22). These peptides act by decreasing the capabilities of renal tubules to resorb (conserve) sodium and water and also by interfering with vasoconstriction, thus further decreasing blood pressure.

CLINICAL CORRELATIONS

1. During **cardiac hypertrophy**, the number of myocardial fibers is not increased; instead, the cardiac muscle cells become longer and larger in diameter. Damage to the heart does not result in regeneration of muscle tissue; instead, the dead muscle cells are replaced by fibrous connective tissue.
2. Lack of Ca^{2+} in the extracellular compartment results in cessation of cardiac muscle contraction within 1 minute, whereas skeletal muscle fibers can continue to contract for several hours.
3. Although a small amount of energy production may be achieved by anaerobic metabolism (up to 10% during hypoxia), totally anaerobic conditions cannot sustain ventricular contraction.

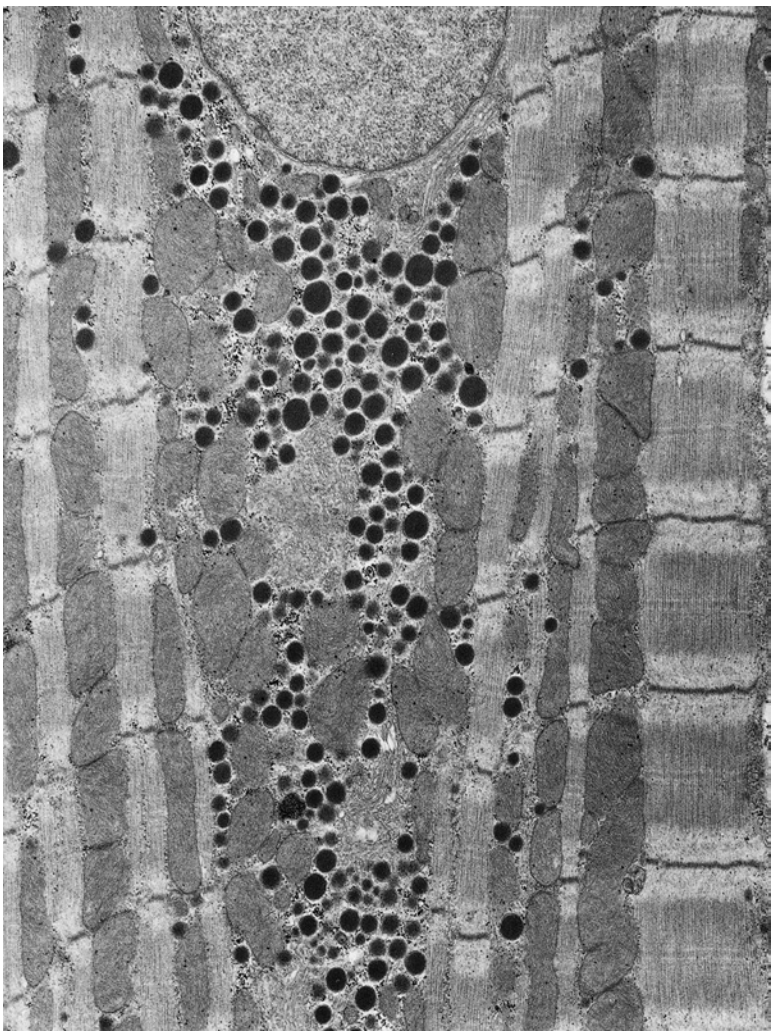


Figure 8–22 Electron micrograph of a rat atrial muscle cell (×14174). Observe the secretory granules containing atrial natriuretic peptide. (Courtesy Dr. Stephen C. Pang.)

SMOOTH MUSCLE

The cells of the third type of muscle exhibit no striations; therefore, they are referred to as ***smooth muscle***. Additionally, smooth muscle cells do not possess a system of T tubules. Smooth muscle is found in the walls of hollow viscera (e.g., the gastrointestinal tract, some of the reproductive tract, and the urinary tract), walls of blood vessels, larger ducts of compound glands, respiratory passages, and small bundles within the dermis of skin. Smooth muscle is not under voluntary control; it is regulated by the autonomic nervous system, hormones (such as bradykinins), and local physiological conditions. Hence, smooth muscle is also referred to as ***involuntary muscle***.

There are two types of smooth muscle:

- Cells of **multiunit smooth muscle** can contract independently of one another, because each muscle cell has its own nerve supply.
- Cell membranes of **unitary (single-unit, visceral) smooth muscle** form **gap junctions** with those of contiguous smooth muscle cells, and nerve fibers form synapses with only a few of the muscle fibers. Thus, cells of unitary smooth muscle cannot contract independently of one another.

In addition to its contractile functions, some smooth muscle is capable of exogenous **protein synthesis**. Among the substances manufactured by smooth muscle cells for extracellular utilization are collagen, elastin, glycosaminoglycans, proteoglycans, and growth factors.

Light Microscopy of Smooth Muscle Fibers

Light microscopy reveals that smooth muscle fibers are short, spindle-shaped cells with a centrally placed nucleus.

Smooth muscle fibers are **fusiform**, elongated cells whose average length is about 0.2 mm with a diameter of 5 to 6 µm. The cells taper at either end, and the expanded central portion contains an oval nucleus housing two or more nucleoli (Figs. 8–23 and 8–24; see Fig. 8–2). During muscle shortening, the nucleus assumes a characteristic corkscrew appearance, as a result of the method of smooth muscle contraction (Fig. 8–25).

Each smooth muscle cell is surrounded by an **external lamina**, which invariably separates the sarcolemmae of contiguous muscle cells from each other (Fig. 8–26). Embedded in the external lamina are numerous **reticular fibers**, which appear to envelop individual smooth muscle cells and function in harnessing the force of contraction.

With hematoxylin and eosin stain, the cytoplasm of smooth muscle fibers appears unremarkable; however, iron hematoxylin stain demonstrates the presence of **dense bodies** adhering to the cytoplasmic aspect of the cell membrane. In addition to dense bodies, thin, longitudinal striations may be evident in the sarcoplasm of smooth muscle cells, representing clumped associations of **myofilaments**.

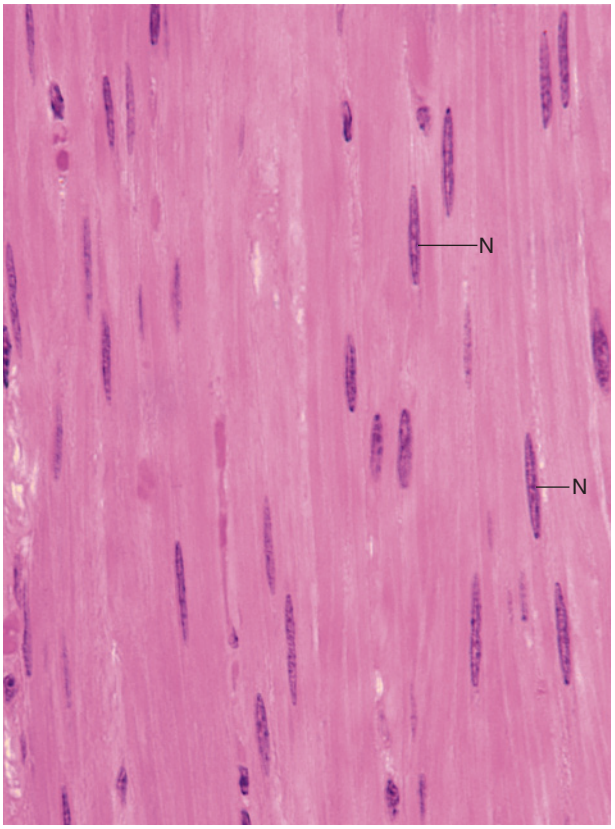


Figure 8–23 Photomicrograph of smooth muscle in longitudinal section. Note that the nuclei (N) are located in the midline of the cell but off center, so that they are closer to one lateral cell membrane than to the other. Observe that the nuclei are not corkscrew-shaped, indicating that the muscle is not undergoing contraction (×540).

Smooth muscle cells usually form sheets of various thicknesses, although they may also occur as individual cells. When they form sheets, the cells are arranged so that they form a continuous network in which their tapered portions fit almost precisely into existing spaces between the expanded regions of neighboring smooth muscle cells (see Fig. 8–2). In cross-section, outlines of various diameters may be noted, some containing nuclei, some not (see Fig. 8–24). Cross-sections without nuclei represent the tapered ends of smooth muscle cells as they interdigitate with the other smooth muscle fibers.

Sheets of smooth muscle cells are frequently arranged in two layers perpendicular to each other, as in the digestive and urinary systems. This arrangement permits waves of peristalsis to be established.

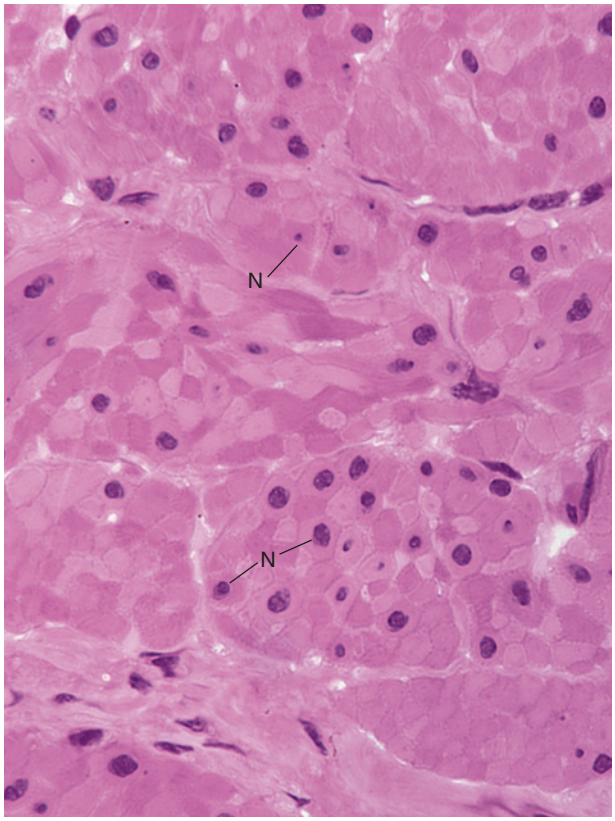


Figure 8–24 Photomicrograph of smooth muscle in cross section. Observe that the nuclei (N) are of various diameters, indicating that they are spindle-shaped and that they have been sectioned at various regions along their length. Also, knowing that the nucleus of the cell is located at its center and that the cell is much longer than the nucleus, it is reasonable to expect that there will be many smooth muscle cells in the field that do not display their nuclei, because they have been sectioned along regions of the cell that are away from the center (×540).

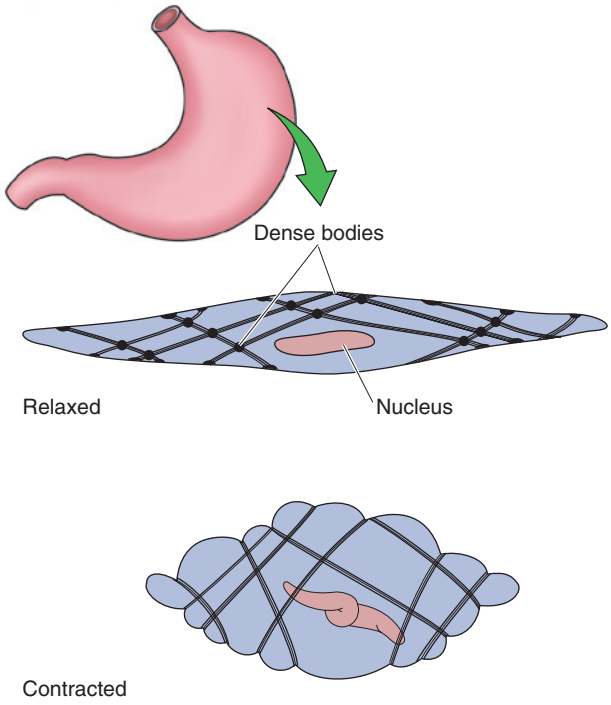


Figure 8–25 Schematic diagram of a relaxed smooth muscle cell and a contracted smooth muscle cell. Note that, in a contracted smooth muscle cell, the nucleus appears to be corkscrew-shaped.

Fine Structure of Smooth Muscle

The perinuclear cytoplasm of smooth muscle cells, especially the regions adjacent to the two poles of the nucleus, contains numerous mitochondria, Golgi apparatus, rough endoplasmic reticulum, SER, and inclusions such as glycogen (see Fig. 8–26). Additionally, an extensive array of interweaving thin filaments (7 nm) and thick filaments (15 nm) is also present. The **thin filaments** are composed of **actin** with its associated **caldesmon**, a protein that blocks, in association with **tropomyosin**, the active site of G-actin, and **calponin**, a protein that inhibits myosin ATPase (note that calponin assumes the role of **troponin** in smooth muscle thin filaments). The **thick filaments** are composed of the same **myosin II** as present in skeletal muscle.

Myofilaments of smooth muscle are not arranged in the paracrystalline fashion of striated muscle, and the organization of the thick filaments is not the same. Instead, the myosin II molecules are lined up so that the **heavy meromyosin heads** (S_1) project from the thick filaments throughout the length of the filament, with the two ends lacking heavy meromyosin. The middle of the filament, unlike that of striated muscle, also possesses heavy meromyosin, thus resulting in the availability of a larger surface area for the interaction of actin with myosin II and permitting **contractions of long duration**. Unlike in skeletal muscle, instead of 6 thin filaments, approximately 15 thin filaments surround each thick filament. When smooth muscle is in its relaxed state, the myosin II molecules are unable to contact the thin filaments because their light meromyosin moieties are folded over and are bound to their own heavy meromyosin component, effectively masking that region of the heavy meromyosin that would bind to the **active site** of the actin molecules.

The all-or-none law for striated muscle contraction does not apply to smooth muscle. The entire cell, or only a portion of the cell, may contract at a given instant even though the method of contraction probably follows the sliding filament theory of contraction.

The contractile forces are harnessed, intracellularly, by an additional system of intermediate filaments, which

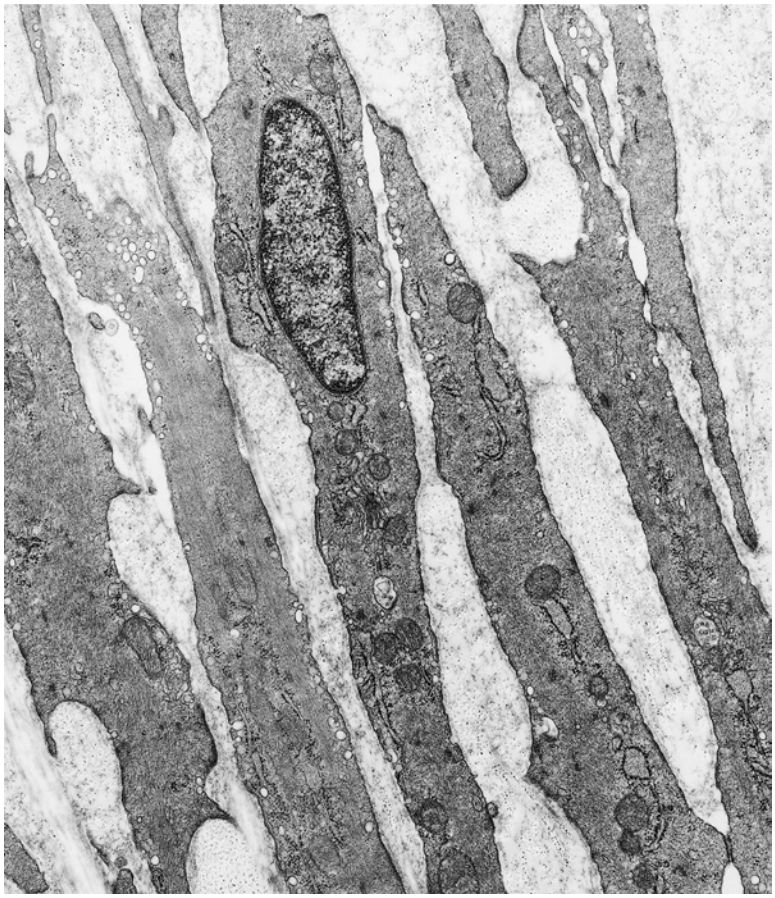


Figure 8–26 Electron micrograph of smooth muscle cells. (Courtesy Dr. J. Strum.)

consist of **vimentin** and **desmin** in unitary smooth muscle (visceral smooth muscle) and **desmin** (only) in multiunit smooth muscle (nonvisceral smooth muscle). These intermediate filaments as well as thin filaments insert into **dense bodies**, formed of **α-actinin** and other Z disk–associated proteins. Dense bodies may be located in the cytoplasm or associated with the cytoplasmic aspect of the smooth muscle sarcolemma. They are believed to resemble Z disks in function and, in three dimensions, may even be more extensive than formerly assumed in that they form interconnected branching networks that extend throughout the cytoplasm. The force of contraction is relayed, through the association of myofilaments with dense bodies, to the intermediate filaments, which act to twist and shorten the cell along its longitudinal axis.

Associated with the cell membrane domains are structures known as ***caveolae*** that act, among other functions, as T tubules of skeletal and cardiac muscle, in regulating the cytosolic free calcium ion concentration.

Control of Smooth Muscle Contraction

Although the regulation of contraction in smooth muscle depends on Ca^{2+}, the control mechanism differs from that encountered in striated muscle because smooth muscle thin filaments are devoid of troponin. Additionally, myosin II molecules of relaxed smooth muscle cells assume a different configuration, in that their actin binding site is masked by their light meromyosin moiety (Fig. 8–27), and their light chains are different from those of striated muscle.

Contraction of smooth muscle fibers proceeds as follows:

1. Calcium ions, released from the sarcoplasmic reticulum as well as entering the cell at plasma membrane caveolae, bind to **calmodulin** (a regulatory protein ubiquitous in living organisms), thereby altering its conformation. The Ca^{2+}-calmodulin complex binds to **caldesmon** causing it and its associated **tropomyosin molecule** to expose the active site of G-actin and then activates **myosin light chain kinase**.

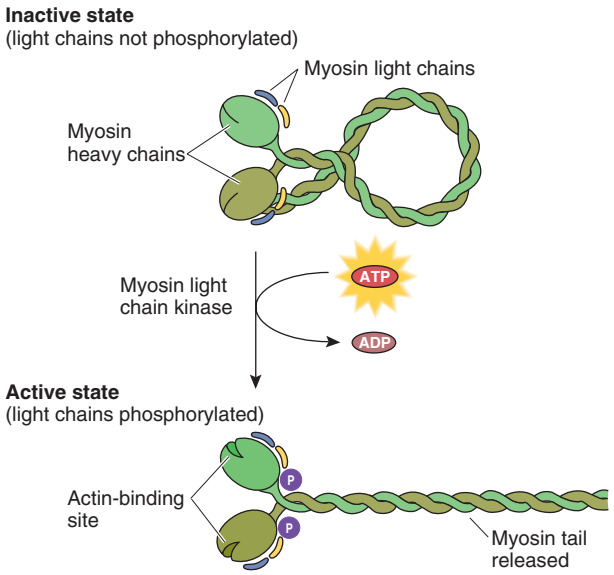


Figure 8–27 Schematic diagram of activation of a myosin molecule of smooth muscle. ADP, Adenosine diphosphate; ATP, adenosine triphosphate; P, myosin light chain bound phosphate. (Modified from Alberts B, Bray D, Lewis J, et al. *Molecular Biology of the Cell*. New York: Garland Publishing; 1994. Reprinted with permission from Taylor & Francis, Inc./Routledge, Inc.)

2. The presence of calcium ions is also responsible for the phosphorylation of **calponin**, which changes its conformation and is no longer able to function in its capacity to inhibit muscle contraction.
3. Myosin light chain kinase phosphorylates one of the **myosin light chains**, known as the **regulatory chain**, permitting the unfolding of the light meromyosin moiety to form the typical golf club–shaped myosin II molecule (see Fig. 8–27), thereby permitting myosin II molecules to associate and form a thick filament.
4. The phosphorylated light chain permits the interaction between actin and the S_1 subfragment of myosin II that results in contraction.
5. Because both phosphorylation and the attachment-detachment of the myosin cross-bridges occur slowly, the process of smooth muscle contraction takes longer than skeletal or cardiac muscle contraction. It is interesting that ATP hydrolysis also occurs much more slowly, and the myosin heads remain attached to the thin filaments for a longer time in smooth muscle than in striated muscles. Thus, smooth muscle contraction not only is *prolonged* but also requires *less energy*.
6. Decrease in the sarcoplasmic calcium level results in the dissociation of the **calmodulin–calcium complex**, causing inactivation of myosin light chain kinase. The subsequent **dephosphorylation of myosin light chain**, catalyzed by the enzyme **myosin phosphatase**, brings about **masking** of the myosin's actin binding site and the subsequent **relaxation** of the muscle.

Innervation of Smooth Muscle

Neuromuscular junctions in smooth muscle are not as specifically organized as those in skeletal muscle. The synapses may vary from 15 to 100 nm in width. The neural component of the synapse is the **en passant** type, which occurs as axonal swellings that contain **synaptic vesicles**, housing either **norepinephrine** for sympathetic innervation or **acetylcholine** for parasympathetic innervation.

In certain cases, every smooth muscle cell receives individual innervation, as in the iris and the vas deferens. As indicated previously, smooth muscle innervated in this fashion is referred to as ***multiunit***.

Other smooth muscle cells, such as those of the gastrointestinal tract and uterus, do not possess individual innervation; rather, only a few muscle cells are equipped with neuromuscular junctions. As indicated previously, impulse transmission in these muscles, referred to as ***unitary*** (***single-unit*** or ***visceral smooth muscles***), occurs via **nexus** (gap junctions) formed between neighboring smooth muscle cells. Visceral smooth muscle may also be regulated by humoral or microenvironmental factors, such as oxytocin in the uterus or stretching of the muscle fibers in the intestines.

Still other smooth muscles of the body are of an **intermediate** type, in which a certain percentage (30% to 60%) of the cells receives individual innervation.

Table 8–3 summarizes the similarities and differences among skeletal, cardiac, and smooth muscles.

REGENERATION OF MUSCLE

- Although **skeletal muscle** cells do not have the capability of mitotic activity, the tissue can regenerate because of the presence of satellite cells (myogenic stem cells). In the adult skeletal muscle cell, approximately 95% of the nuclei evident by light microscopy belong to the muscle cell, whereas about 5% of the nuclei belong to satellite cells. There are two types of satellite cells, those that are not in the process of forming muscle cells, said to be in the **quiescent state**, and those that are in the process of cell division and forming muscle cells, said to be in the **activated state**. It has been shown that satellite cells express the homeobox gene **Pax7** and a number of myogenic regulatory factors, especially **MyoD**, **Myf5**, and **myogenin**. Most satellite cells are in the quiescent state until they are exposed to a number of local factors, which cause them to become activated. When the skeletal muscle cell is injured by

TABLE 8–3 Comparison of the Three Types of Muscle

Feature	Skeletal Muscle	Cardiac Muscle	Smooth Muscle
Sarcomeres and myofibrils	Yes	Yes	No
Nuclei	Multinucleated; peripherally located	One (or two); centrally located	One; centrally located
Sarcoplasmic reticulum	Well developed with terminal cisterns	Poorly defined; some small terminals	Some smooth endoplasmic reticulum
T tubules	Yes; small, involved in triad formation	Yes; large, involved in dyad formation	No
Cell junctions	No	Intercalated disks	Nexus (gap junctions)
Contraction	Voluntary; all-or-none	Involuntary; rhythmic and spontaneous	Involuntary; slow and forceful; not all-or-none
Calcium control	Calsequestrin in terminal cisternae	Calcium from extracellular sources and the sarcoplasmic reticulum	Calcium from extracellular sources (via caveolae) and the sarcoplasmic/endoplasmic reticulum
Calcium binding	Troponin C	Troponin C	Calmodulin
Regeneration	Yes, via satellite cells	Very limited, perhaps 1% per year	Yes
Mitosis	No	No	Yes
Nerve fibers	Somatic motor	Autonomic	Autonomic
Connective tissue	Epimysium, perimysium, and endomysium	Connective tissue sheaths and endomysium	Connective tissue sheaths and endomysium
Distinctive features	Long; cylinder-shaped; many peripheral nuclei	Branched cells; intercalated disks; one or two nuclei	Fusiform cells with no striations; single nucleus

physical or chemical insults or due to disease process, the satellite cells become activated, and they proliferate. Most of the newly formed cells are destined to repair the damage, but a small percentage of the newly formed cells replenish the satellite cell population. In response to muscle cell injury, both Pax7 and MyoD become expressed in satellite cells, causing them to leave the quiescent and enter the active stage and undergo proliferation; these proliferating cells become known as ***myoblasts (myogenic precursor cells)***. The protein myogenic factor Myf5 accumulates in the myoblast cytoplasm and triggers these cells to embark on the sequence of actions, leading to myogenesis. Therefore, the myoblasts line up end to end and, under the influence of myogenin, fuse to form **myotubes** and synthesize the organelles and myofilaments that result in the genesis of a mature skeletal muscle cell.

- Under certain other conditions, such as "muscle building," activated satellite cells may fuse with existing muscle cells, thus increasing muscle mass during skeletal muscle **hypertrophy**. Skeletal muscle cells regulate their number and their size by the secretion of a member of the TGF-β superfamily of extracellular signaling molecules, **myostatin (growth differentiation factor 8)**. Certain mutant mice, whose skeletal muscle fibers cannot produce myostatin, have enormous muscles that not only have many more cells, but also whose muscle cells are much larger than those of normal mice.
- **Cardiac muscle** is capable of a very limited degree of regeneration as was demonstrated in individuals who were exposed to atmospheric ^{14}C prior to termination of above-ground nuclear testing. In these individuals, a very small percent of their cardiac muscle cells had ^{14}C, indicating that these cells were formed postnatally. In fact, approximately 1% of their cardiac muscle cells were regenerated per year. However, this amount of regeneration is not enough to repair major damage to the heart. Instead, following damage, such as a myocardial infarct, **fibroblasts** invade the damaged region, undergo cell division, and form fibrous connective tissue (scar tissue) to repair the damage.
- **Smooth muscle cells** retain their mitotic capability to form more smooth muscle cells. This ability is especially evident in the pregnant uterus, where the muscular wall becomes thicker both by hypertrophy of individual cells and by hyperplasia derived from mitotic activity of the smooth muscle cells. Small defects, subsequent to injury, may result in the formation of new smooth muscle cells. These new cells may be derived via mitotic activity of existing smooth muscle cells, as in the gastrointestinal and urinary

tracts, or from differentiation of relatively undifferentiated **pericytes** accompanying some blood vessels.

MYOEPITHELIAL CELLS AND MYOFIBROBLASTS

Certain cells associated with glandular secretory units possess contractile capabilities. These **myoepithelial cells** are modified to assist in the delivery of the secretory products into the ducts of the gland. Myoepithelial cells are flattened and possess long processes that wrap around the glandular units (see Chapter 5, Figs. 5–24 and 5–25). Myoepithelial cells contain both actin and myosin as well as intermediate filaments and peripheral and cytoplasmic densities necessary to harness the interactions between actin and myosin. Mechanisms and control of contraction in myoepithelial cells resemble, but are not identical to, those in smooth muscle.

In lactating mammary glands, myoepithelial cells contract upon the release of **oxytocin**; in the lacrimal gland, they contract because of the action of **acetylcholine**.

Myofibroblasts resemble fibroblasts but have abundant actin and myosin. They can contract and are especially evident during wound contraction as they bring the edges of a wound closer to each other.

PATHOLOGICAL CONSIDERATIONS

See Figures 8–28 through 8–30.

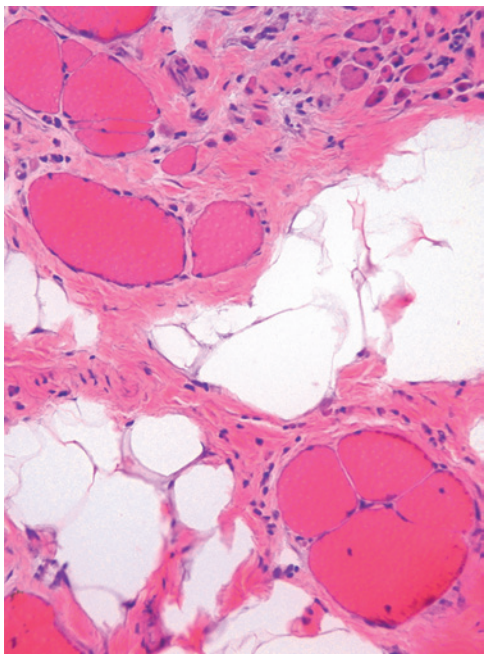


Figure 8–28 Photomicrograph of a 9-year-old male child with Duchenne muscular dystrophy. Observe that the muscle fibers are of various diameters with greatly enlarged fibrotic endomysia. Additionally, many of the muscle cells have been replaced by fatty infiltrates. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:1243.)

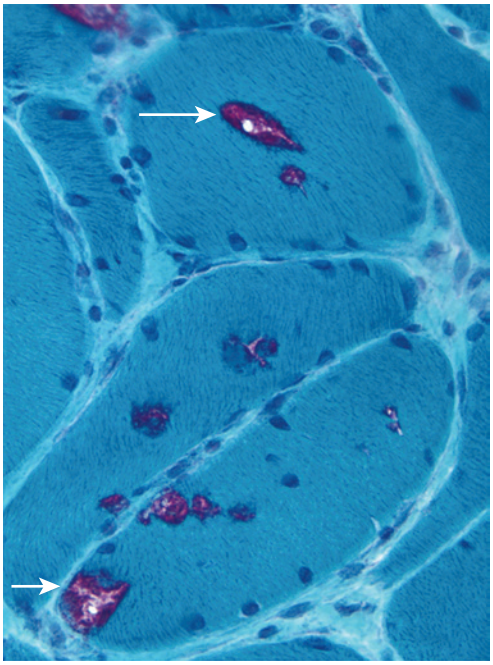


Figure 8–29 Photomicrograph displaying a condition known as *inclusion body myositis*, a poorly understood disease that may be a process of inflammation or degenerative condition with associated inflammation. Note the presence of "rimmed" vacuoles, cytoplasmic inclusions with reddish granular rimming *(arrows)*. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:1240.)

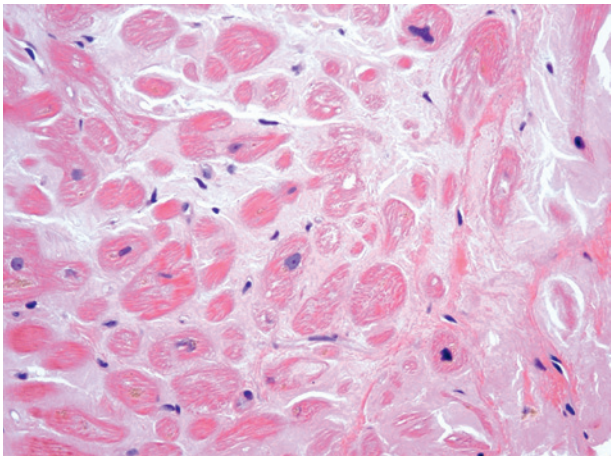


Figure 8–30 Photomicrograph of a heart muscle displaying cardiac amyloidosis. This disease is characterized by the presence of amyloid protein deposition in the extracellular spaces surrounding cardiac muscle cells. Note the amorphous pink amyloid deposited around the cardiac muscle cells. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:573.)

REVIEW QUESTIONS

1. An entire skeletal muscle is invested by a connective tissue element known as
   a. endomysium.
   b. basement membrane.
   c. external lamina.
   d. epimysium.
   e. perimysium.
2. Which of the following occurs during skeletal muscle contraction?
   a. A band elongates.
   b. H band disappears.
   c. I band becomes wider.
   d. Z disks disappear.
   e. A band becomes narrower.
3. Which of the following is true concerning human skeletal muscle? It has
   a. dyads.
   b. triads.
   c. caveolae.
   d. a centrally positioned nucleus.
   e. intercalated disks.
4. The sarcoplasmic reticulum of skeletal muscle
   a. sequesters Na^+ ions.
   b. possesses voltage-gated K^+ channels.
   c. is depolarized by T tubules.
   d. is responsible for acetylcholine synthesis.
   e. is responsible for synthesis of actin and myosin.
5. Myofibrils are held in register with each other by structures known as
   a. desmin.
   b. dystrophin.
   c. keratin.
   d. lamins.
   e. neurofilaments.
6. Thick filaments are held in register with one another by
   a. titin.
   b. nebulin.
   c. tropomyosin.
   d. troponin.
   e. tropomodulin.
7. Each myosin II molecule of the heavy chain can be digested into smaller constituents, one of which binds ATP. Which is that constituent?
   a. Light meromyosin
   b. S1 subfragment
   c. S2 subfragment
   d. Papain
   e. Trypsin
8. In early May, a pathologist is called to a crime scene in Baltimore, Maryland, to examine a cadaver. She notes that the body is in maximal rigor mortis and determines that the person has been dead for
   a. 2 hours.
   b. 6 hours.
   c. 18 hours.
   d. 2 days.
   e. 1 week.
9. A medical student and her nurse boyfriend present in the emergency department of a hospital complaining about nausea, vomiting, diarrhea, stomach cramps, double vision, and drooping eyelids. Their symptoms began less than 24 hours after dinner at home, and they both remembered that the can of corn that they consumed with dinner was damaged. The doctor should suspect
   a. acid reflux.
   b. myocardial infarct.
   c. stroke.
   d. botulism.
   e. duodenal ulcer.
10. A patient is diagnosed with the autoimmune disease, myasthenia gravis. This condition is due to the patient's inability to
    a. release acetylcholine.
    b. release norepinephrine.
    c. make norepinephrine receptors.
    d. bind norepinephrine by norepinephrine receptors.
    e. bind acetylcholine by acetylcholine receptors.

ANSWERS

1. D. A thick dense irregular collagenous connective tissue element, the epimysium, surrounds the entire skeletal muscle and forms tendons at its origin and insertion. Endomysium surrounds individual muscle fibers, and a basal lamina-like material (the external lamina) is interposed between the flimsy connective tissue endomysium and the sarcolemma. The perimysium is also a dense irregular collagenous connective tissue that surrounds bundles of skeletal muscle fibers, where a number of such bundles constitute the entire skeletal muscle.
2. B. During skeletal muscle contraction, the thin filaments slide toward the center of the sarcomere and, thus, extinguish the H band, the region in the middle of the sarcomere where, in the relaxed muscle, there is no overlap of the thick and thin filaments. The A band does not change its width, whereas the I band becomes narrower. The Z disks remain visible.
3. B. Skeletal muscle has two triads per sarcomere, each located at the A-I junction. Each triad is composed of a T tubule flanked on either side by the dilated terminal cisternae of the sarcoplasmic reticulum. Dyads and intercalated disks are present in cardiac not skeletal muscle. Nuclei of skeletal muscle are positioned peripherally, whereas those of smooth and cardiac muscle are centrally located.
4. C. The sarcoplasmic reticulum, a modified smooth ER, when depolarized by the T tubule in its proximity, releases its sequestered Ca^+ ions via its voltage-gated Ca^+ to channels to effect contraction of the cell. The sarcoplasmic reticulum does not have voltage-gated K^+ channels or sequester Na^+ ions. Acetylcholine is synthesized by the motoneuron that innervates the skeletal muscle, and synthesis of actin and myosin occurs on the rough endoplasmic reticulum not on the sarcoplasmic reticulum.
5. A. Myofibrils are held in register with each other by the intermediate filaments, desmin and vimentin, which secure the periphery of the Z disks of neighboring myofibrils to each other. Dystrophin, a transmembrane protein, binds to actin molecules, securing the peripheral myofibrils to the cytoplasmic aspect of the sarcolemma. Keratins are intermediate filaments of epithelial cells, and neurofilaments are intermediate filaments of the cells of the nervous system. Lamins are intermediate filaments that form the architectural support of the inner nuclear membranes.
6. A. Each thick filament is held in register with other thick filaments by two pairs of large elastic proteins known as *titin*. Each pair of titin molecules extends from the Z disk to the center of the thick filament at the M line coming close to but not contacting the titin molecules from the other Z disk of the same sarcomere. Nebulin wraps around the thin filament and secures it in place. Tropomyosin and troponin are proteins associated with the thin filament and assist in masking and unmasking the myosin binding sites of each actin molecule. Tropomodulin is a protein that caps the minus end of the actin filament, preventing the addition or deletion of G actin molecules from that end of the thin filament.
7. B. The enzyme trypsin cleaves the golf club–shaped myosin II molecule into a heavy meromyosin, the head of the golf club, and the narrow handle of the golf club, the light meromyosin. The enzyme papain cleaves the heavy meromyosin into an S1 subfragment that binds ATP and an S2 subfragment that attaches the heavy meromyosin to the light meromyosin.
8. C. Rigor mortis occurs because the muscles consume all of their available ATP and cannot produce any more ATP to permit the myosin heads (S1 subfragment) from releasing their bond with the active site of the actin molecules. It takes approximately 2 to 4 hours for rigor mortis to set in. Maximal rigor mortis occurs between 12 and 24 hours, and decomposition of muscles will loosen rigor mortis in about 3 days.
9. D. *Clostridium botulinum*, the microorganism that causes botulism, produces a toxin that inhibits the release of acetylcholine from motoneurons. The toxin, as well as the microbe, can be acquired from canned foods that become contaminated if the can is damaged or if the canning process is defective. Because both patients shared the same can of corn and both presented with the same symptoms, it is safe to assume that all of the other possibilities may be ruled out.
10. E. Autoantibodies bind to acetylcholine receptors, which disable them from binding acetylcholine. The inactivated receptors are endocytosed and replaced by newly formed acetylcholine receptors that are also attacked by the autoantibodies. The release of acetylcholine is not affected by this condition, and norepinephrine release or its ability to bind to norepinephrine receptors is not affected by myasthenia gravis.

This page intentionally left blank

Nervous Tissue

9

Nervous tissue, comprising perhaps as many as a trillion neurons with multitudes of interconnections, forms the complex system of neuronal communication within the body. Certain neurons have **receptors**, elaborated on their terminals, that are specialized for receiving different types of stimuli (e.g., mechanical, chemical, thermal) and transducing them into nerve impulses that may eventually be conducted to nerve centers. These impulses are then transferred to other neurons for processing and transmission to higher centers for perceiving sensations or for initiating motor responses.

To accomplish these functions, the nervous system is organized anatomically into the **central nervous system (CNS)**, which comprises the brain and spinal cord, and the **peripheral nervous system (PNS)**. The PNS, located outside of the CNS, includes cranial nerves, emanating from the brain; spinal nerves, emanating from the spinal cord; and their associated ganglia.

Functionally, the PNS is divided into a **sensory (afferent) component**, which receives and transmits impulses to the CNS for processing, and a **motor (efferent) component**, which originates in the CNS and transmits impulses to effector organs throughout the body. The motor component is further subdivided as follows:

1. In the **somatic system**, impulses originating in the CNS are transmitted directly, via a single neuron, to skeletal muscles.
2. In the **autonomic system**, in contrast, impulses from the CNS first are transmitted to an autonomic **ganglion** via one neuron; a second neuron originating in the autonomic ganglion then transmits the impulses to smooth muscles, cardiac muscles, or glands.

In addition to neurons, nervous tissue contains numerous other cells, collectively called ***neuroglial cells***, which do not receive or transmit impulses; instead, these cells support and assist neurons in various ways.

DEVELOPMENT OF NERVOUS TISSUE

The nervous system develops from the ectoderm of the embryo in response to signaling molecules from the notochord.

As the notochord develops early in embryonic life, it releases signaling molecules that induce the overlying ectoderm to form **neuroepithelium**, which thickens and forms the **neural plate**. As the margins of this plate continue to thicken, the plate buckles, forming a **neural groove** whose edges continue to grow toward each other until they come together, forming the **neural tube**. The rostral (anterior) end of this structure develops into the brain; the remaining (caudal) portion of the neural tube develops into the spinal cord. Additionally, the neural tube gives rise to neurons, neuroglia, ependyma, and the choroid plexus.

A small mass of cells at the lateral margins of the neural plate, which does not become incorporated into the neural tube, forms the **neural crest cells**. This group of cells begins to migrate away from the developing neural tube early in development. Once they reach their destinations, these cells eventually form many structures, including the following:

- Most of the sensory components of the PNS
- Sensory neurons of cranial and spinal sensory ganglia (dorsal root ganglia)
- Autonomic ganglia and the postganglionic autonomic neurons originating in them
- Much of the mesenchyme of the anterior head and neck
- Melanocytes of the skin and oral mucosa
- Odontoblasts (cells responsible for production of dentin)
- Chromaffin cells of the adrenal medulla
- Cells of the arachnoid and pia mater
- Satellite cells of peripheral ganglia
- Schwann cells

CELLS OF THE NERVOUS SYSTEM

Cells of the nervous system are classified into two categories: neurons and neuroglia.

The cells of the nervous system may be subdivided into two categories.

Neurons are responsible for the receptive, integrative, and motor functions of the nervous system. **Neuroglial cells** are responsible for supporting, protecting, and assisting neurons in neural transmission.

Neurons

The cells responsible for the reception and transmission of nerve impulses to and from the CNS are the **neurons**. Ranging in diameter from 5 to 150 μm, neurons are among both the smallest and the largest cells in the body.

SUMMARY

The **nervous system** is separated into two physically and functionally interconnected components, namely the **central nervous system (CNS)**, that is, the **brain** and **spinal cord** and the **peripheral nervous system (PNS)**, that is, **ganglia** and **peripheral nerves**. The basic functional unit of the nervous system are cells known as ***neurons***, and these are supported in various manner by cells known as ***neuroglia***. Functionally, the nervous system is separated into two major categories, the **sensory (afferent) component** that receives information and transmits it to the CNS for processing and the **motor (efferent) component** that transmits responses to the sensory information. The motor component also has two divisions, the **somatic nervous system** where the response is directed to skeletal muscles and the **autonomic nervous system** where the response is directed to smooth muscles, cardiac muscles, or glands. Neurons have processes known as ***dendrites*** which deliver information to the cell body of the neuron and a single **axon** that transmits information from the neuron's cell body, known as ***soma***, to target cells, be they other neurons, various types of muscle cells, and/or secretory cells of glands. The information is transmitted to and from neurons by chemical signals released at specialized intercellular junctions known as **synapses**. Axons can be very long (100 or more cm in length) and frequently are wrapped by **myelin sheaths** that increase the velocity of impulse transmission.

KEY WORDS

Autonomic nervous system; axon; central nervous system; dendrites; ganglia; motor component of the nervous system; myelin sheath; neuroglia; neurons; peripheral nerves; peripheral nervous system; sensory component of the nervous system; soma; somatic nervous system; synapse

CLINICAL CORRELATIONS

Abnormal organogenesis of the CNS results in various types of congenital malformations. **Spina bifida** is a defective closure of the spinal column. In severe cases, the spinal cord and meninges may protrude through the unfused areas. **Spina bifida anterior** is a defective closure of the vertebrae. Severe cases may be associated with defective development of the viscera of the thorax and abdomen.

Anencephaly is failure of the developmental anterior neuropore to close with a poorly formed brain and absence of the cranial vault. It is usually not compatible with life.

Epilepsy may result from abnormal migration of cortical cells, which disrupts normal interneuronal functioning.

Hirschsprung disease, also known as ***congenital megacolon***, is caused by failure of the neural crest cells to invade the wall of the gut. The wall lacks **Auerbach plexus**, a portion of the parasympathetic system innervating the distal end of the colon. Absence of the plexus leads to dilatation and hypertrophy of the colon.

Phenylketonuria (PKU) is a hereditary condition where the newborn child's liver is unable to manufacture the enzyme **phenylalanine hydroxylase** and, therefore, cannot metabolize the essential amino acid phenylalanine. Unless the child is provided with a phenylalanine-free diet, the baby will have mental retardation, seizures, and other intellectual problems. In most developed countries, all newborns are tested for PKU, and, if present, the mother is placed on a special diet during the period of breastfeeding. It is recommended that the affected individual adhere to a phenylalanine-free diet for life.

Structure and Function of Neurons

Neurons are composed of a cell body, dendrites, and an axon.

Most neurons are composed of three distinct parts: a **cell body**, **multiple dendrites**, and a **single axon**. The **cell body** of a neuron, also known as the ***perikaryon*** or ***soma***, is the central portion of the cell where the nucleus and perinuclear cytoplasm are contained. Generally, neurons in the CNS are polygonal (Fig. 9–1), with concave surfaces between the many cell processes, whereas neurons in the dorsal root ganglion (a sensory ganglion of the PNS) have a round cell body from which only one process exits (Fig. 9–2). Cell bodies exhibit different sizes and shapes that are characteristic for their type and location. These different morphologies are described below in discussions of the various regions of the nervous system.

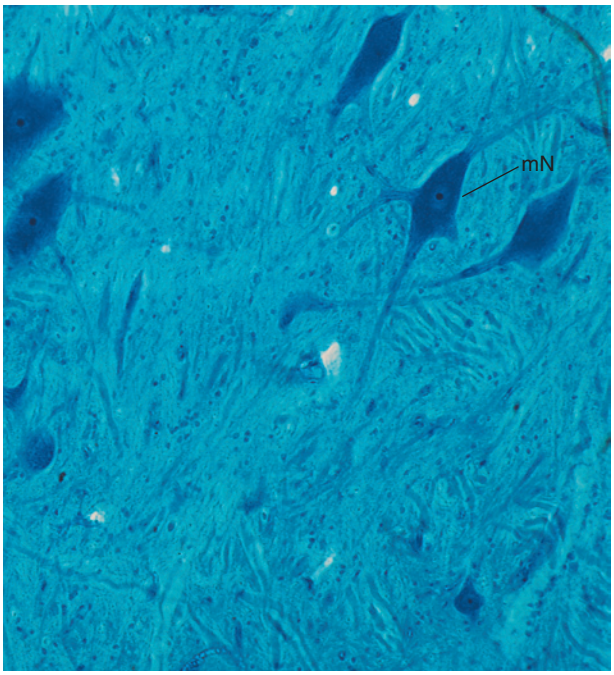


Figure 9–1 Light micrograph of the gray matter of the spinal cord (×270). Observe the multipolar neuron (mN) cell bodies and their processes.

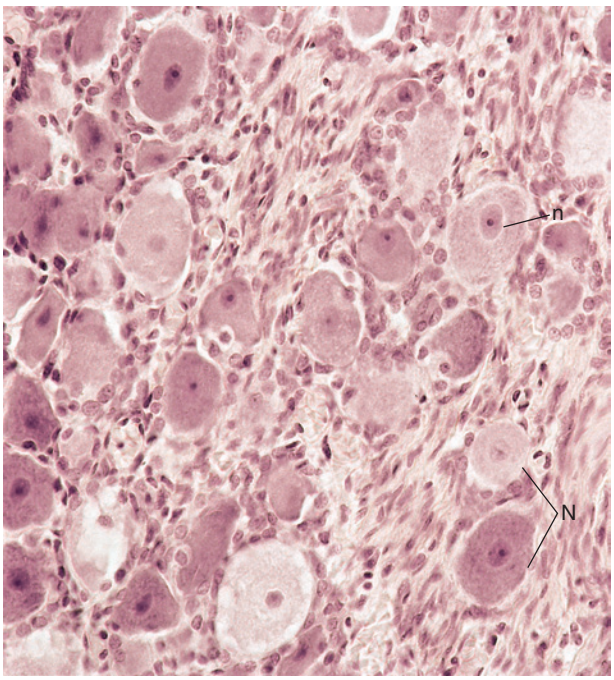


Figure 9–2 Light micrograph of a sensory ganglion (×270). Observe the large neuronal cell bodies (N) with singular nucleoli (n).

Projecting from the cell body are the **dendrites**, processes specialized for receiving stimuli from sensory cells, axons, and other neurons (Fig. 9–3). Often the dendrites are multibranched. They are arborized so that they can receive multiple stimuli from many other neurons simultaneously. The nerve impulses received by the dendrites are then transmitted toward the soma.

Each neuron possesses a single **axon**, a process of varying diameter and up to 100 cm in length. The axon conducts impulses away from the soma to other neurons, muscles, or glands, but it may also receive stimuli from other neurons, which may modify its behavior. Similar to dendrites, the axon arborizes, and each branch usually has terminal dilatations, known as ***axon terminals***, at or near its end. These axon terminals, also known as ***end bulbs (terminal boutons)***, approach other cells to form a **synapse**, a submicroscopic gap between the axon and the plasma membrane of the target cell where impulses can be transmitted between cells.

Neurons can be classified according to their shape and the arrangement of their processes (Fig. 9–4). The prevalence and typical locations of the various neuron types are described in the next section.

Neuronal Cell Body (Soma, Perikaryon)

The cell body is the region of the neuron containing the large pale-staining nucleus and perinuclear cytoplasm.

The cell body is the most conspicuous region of the neuron, but the largest volume of the neuron's cytoplasm is located in the processes originating from the cell body. The **nucleus** is large, usually spherical to ovoid, and centrally located. It contains finely dispersed chromatin, indicative of a rich synthetic activity, although smaller neurons may present some condensed, inactive heterochromatin. A well-defined nucleolus is also common.

The **cytoplasm** of the cell body has abundant rough endoplasmic reticulum (RER) with many cisternae in parallel arrays, a characteristic especially prominent in large motor neurons. Polyribosomes are also scattered throughout the cytoplasm. When these stacked RER cisternae and polyribosomes are stained with basic dyes, they appear as clumps of basophilic material called ***Nissl bodies***, which are visible with the light microscope. RER is also present in the dendritic region of the neuron, but only as scattered short or branching cisternae. RER is absent at the **axon hillock**, the region on the cell body where the axon arises; however, smooth endoplasmic reticulum (SER) is present in the axon.

Although Nissl bodies in each type of neuron have a characteristic size, shape, and form, no specific, repeating pattern has been observed. Generally, small neurons display small granular Nissl bodies, but not all large neurons display larger Nissl bodies. These differences may be related to changing physiological and pathological conditions within a specific neuron.

Most neurons have abundant SER throughout the cell body; this reticulum extends into the dendrites and the axon, forming **hypolemmal cisternae** directly beneath the plasmalemma. These hypolemmal cisternae are continuous with the RER in the cell body and weave between the Nissl bodies on their way into the dendrites and axon. Although it is unclear how they function, it is known that hypolemmal cisternae sequester calcium and contain protein. These cisternae may serve as a conduit for the distribution of protein throughout the cell. Some authors suggested that transport and synaptic vesicles bud from these cisternae, but much of this issue is still unclear.

A prominent juxtanuclear **Golgi complex** is present in the neuronal cell bodies, composed of several closely associated cisternae exhibiting a dilated periphery, characteristic of protein-secreting cells. The Golgi complex is also responsible for the packaging of neurotransmitter substances or the enzymes essential for their production in the axon.

Numerous **mitochondria** are scattered throughout the cytoplasm of the soma, dendrites, and axon, but they are most abundant at the axon terminals. Generally, the mitochondria in neurons are more slender than those in other cells, and, occasionally, their cristae are oriented longitudinally rather than transversely. It has been demonstrated that neuronal mitochondria are in constant motion along microtubules in the cytoplasm.

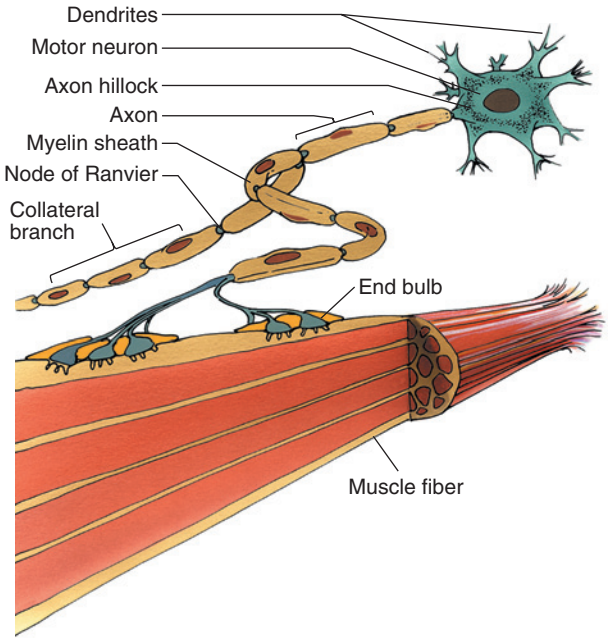
Most adult neurons display only one **centriole** associated with a basal body of a primary cilium; it possesses the 9 + 0 arrangement of microtubules (see Chapter 5 on primary cilia).

Inclusions

Inclusions located in neuronal cell bodies encompass nonliving substances such as melanin and lipofuscin pigments as well as lipid droplets.

Dark brown to black **melanin granules** are found in neurons in certain regions of the CNS (e.g., mostly in the substantia nigra and locus ceruleus, with lesser amounts in the dorsal motor nucleus of the vagus and the spinal cord) and in the sympathetic ganglia of the PNS. The function of these granules in these various locations is unknown. However, dihydroxyphenylalanine, or methyldopa, the precursor of this pigment, is also the precursor of the neurotransmitters dopamine and noradrenaline. It has been suggested, therefore, that melanin may accumulate as a by-product of the synthesis of these neurotransmitters.

Lipofuscin, an irregularly shaped, yellowish brown pigment granule, is more prevalent in the neuronal cytoplasm of older adults and is thought to be the remnant of lysosomal enzymatic activity. Lipofuscin



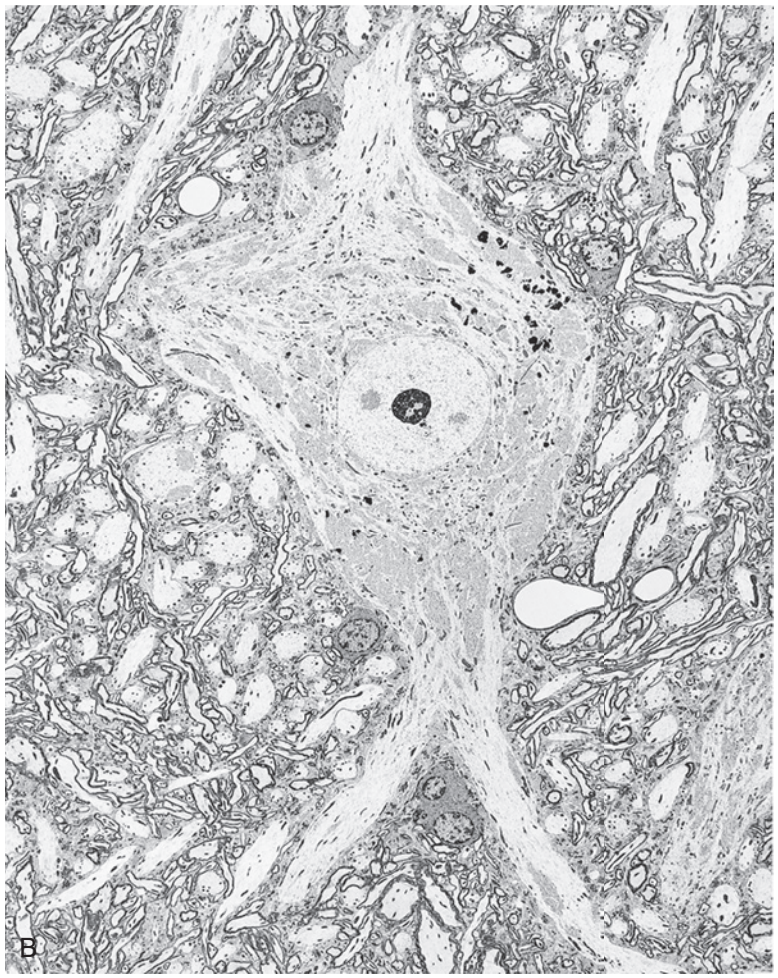


Figure 9–3 Motor neuron. **A,** Diagram of a typical motor neuron. **B,** Electron micrograph of a ventral horn neuron with several of its dendrites (×1300). (From Ling EA, Wen CY, Shieh JY, et al. Neuroglial response to neuron injury: a study using intraneural injection of *Ricinus communis* agglutinin-60. *J Anat.* 1989;164:201-213. Reprinted with the permission of Cambridge University Press.)

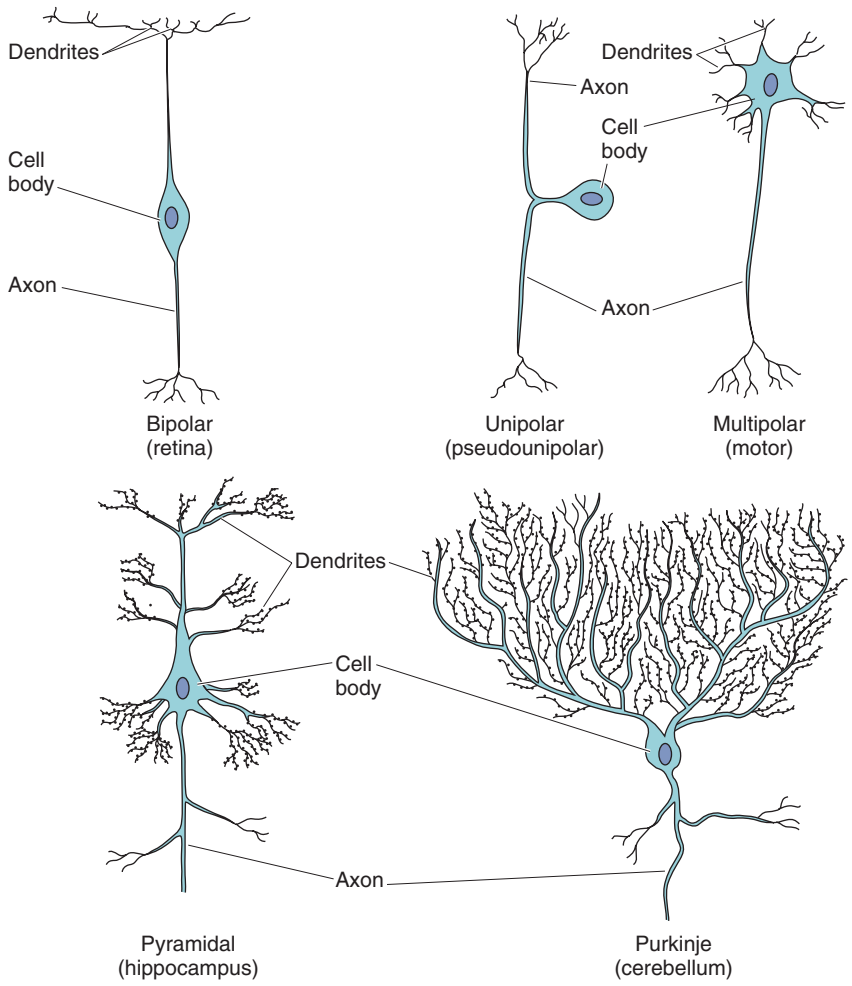


Figure 9–4 Diagram of the various types of neurons.

granules increase with advancing age and may even crowd the organelles and nucleus to one side in the cell, possibly affecting cellular function. It is interesting that certain cells (e.g., Purkinje cells of the cerebellar cortex) do not accumulate lipofuscin. Iron-containing pigments also may be observed in certain neurons of the CNS and may accumulate with age.

Lipid droplets sometimes are observed in the neuronal cytoplasm and may be either the result of faulty metabolism or energy reserves.

Secretory granules are observed in neurosecretory cells; many of them contain signaling molecules.

Cytoskeletal Components

When prepared by silver impregnation for visualization with light microscopy, the neuronal cytoskeleton exhibits **neurofibrils** (up to 2 µm in diameter) coursing through the cytoplasm of the soma and extending into the processes. Electron microscopic studies reveal three different filamentous structures: **microtubules** (24 nm in diameter), **neurofilaments** (intermediate filaments 10 nm in diameter), and **microfilaments** (6 nm in diameter). The neurofibrils observed with light microscopy possibly represent clumped bundles of neurofilaments, a suggestion supported by the fact that neurofilaments are stained by silver nitrate. Microfilaments (actin filaments) are associated with the plasma membrane. The microtubules in neurons are identical to those in other cells, except that the **microtubule-associated protein MAP-2** is found in the cytoplasm of the cell body and dendrite, whereas **MAP-3** is present in the axon only.

Dendrites

Dendrites receive stimuli from other nerve cells.

Dendrites are elaborations of the receptive plasma membrane of the neuron. In some neurons, however, the cell body and the proximal end of the axon may also

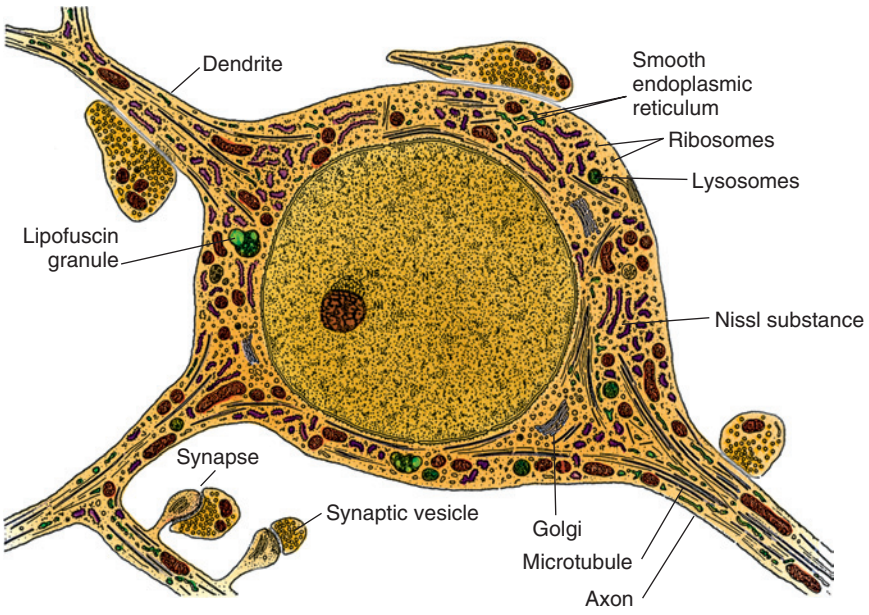


Figure 9–5 Diagram of the ultrastructure of a neuronal cell body. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

serve in a receptive capacity. Most neurons possess multiple dendrites, each of which arises from the cell body, usually as a single, short trunk that ramifies several times into smaller and smaller branches, tapering at the ends like branches of a tree. The specific dendrite branching pattern is characteristic of each particular type of neuron. The base of the dendrite arises from the cell body and contains the usual complement of organelles, except for Golgi complexes (Fig. 9–5). Farther away from the base, toward the distal end of the dendrite, many of the organelles become sparse or are absent.

In the dendrites of most neurons, neurofilaments are reduced to small bundles or single filaments, which may be cross-linked to microtubules. Mitochondria, however, are abundant in dendrites. The branching of dendrites, which results in numerous synaptic terminals, permits a neuron to receive and integrate multiple, perhaps, as in Purkinje cells of the cerebellum, for instance, even hundreds of thousands of impulses. **Spines** located on the surfaces of some dendrites permit them to form synapses with other neurons. These spines diminish with age and with poor nutrition, and they may exhibit structural changes in persons with trisomy 13 and trisomy 21 (Down syndrome) and other anomalous conditions. Dendrites sometimes contain vesicles and transmit impulses to other dendrites.

Axons

Axons transmit impulses to other neurons or effector cells, namely, cells of muscle and glands.

The **axon** arises from the cell body at the axon hillock as a single thin process extending longer distances from the cell body than do the dendrites. In some instances, axons of motor neurons may be 1 m or even more in length. Axon thickness is directly related to conduction velocity, so that velocity increases as axon diameter increases. Although axon thickness varies, it is constant for a particular type of neuron. Some axons possess **collateral branches**, which arise at right angles from the axonal trunk (see Fig. 9–3A). As the axon terminates, it may ramify, forming many small branches **(terminal arbor)**.

The **axon hillock**, a pyramid-shaped region of the soma, is devoid of ribosomes and is usually located on the opposite side of the soma from the dendrites. The portion of the axon from its origin at the axon hillock to the beginning of the myelin sheath is called the ***initial segment***. Deep to the **axolemma** (plasmalemma of the axon) of the initial segment, when viewed with the electron microscope, thin, electron-dense layer is visible whose function is not known, though it resembles the layer located at the nodes of Ranvier (see section on astrocytes). This area of the neuron lacks RER and ribosomes but houses abundant microtubules and neurofilaments that are believed to facilitate the regulation of the axon's diameter. In some neurons, the number of neurofilaments may increase threefold in the initial segment, whereas the number of microtubules increases only slightly. It is in this initial segment, also referred to as the ***spike trigger zone***, where excitatory and inhibitory impulses are

summed to determine whether propagation of an action potential is to occur (see section on generation and conduction of nerve impulses).

The axoplasm (the cytoplasm within the axon) contains short profiles of SER and remarkably long, thin mitochondria and many microtubules; however, it lacks RER and polyribosomes. Thus, the axon relies on the soma for its maintenance. Microtubules are grouped in small bundles at the origin of the axon and in the initial segment; distally, however, they become arranged as uniformly spaced, single microtubules interspersed with neurofilaments.

The plasmalemma of certain neuroglial cells forms a **myelin sheath** around some axons in both the CNS and the PNS, referred to as ***myelinated axons*** (Figs. 9–6 and 9–7). The process of myelination is described in detail in the section on Schwann cells. Axons lacking myelin sheaths are called ***unmyelinated axons*** (Fig. 9–8). Nerve impulses are conducted much faster along myelinated axons than along unmyelinated axons. In the fresh state, the myelin sheath imparts a white, glistening appearance to the axon. The presence of myelin permits the subdivision of the CNS into **white matter** and **gray matter**.

In addition to impulse conduction, an important function of the axon is **axonal transport** of materials between the soma and the axon terminals. In **anterograde transport**, the direction of transport is from the cell body to the axon terminal; in **retrograde transport**, the direction of transport is from the axon terminal to the cell body. Axonal transport is as crucial to **trophic relationships** within the axon as it is between neurons and muscles or glands. If these relationships are interrupted, the target cells atrophy.

Axonal transport occurs at three velocities: fast, intermediate, and slow. The most rapid transport (up to 400 mm/day) takes place in anterograde transport of organelles, which move more rapidly in the cytosol. In retrograde transport, the fastest speed is less than half that observed in anterograde transport, with the slowest being only about 0.2 mm/day. Axonal transport speeds between these two extremes are considered intermediate.

Anterograde transport is used in the translocation of organelles and vesicles as well as of macromolecules, such as actin, myosin, and clathrin, and some of the enzymes necessary for neurotransmitter synthesis at the axon terminals. Items returned to the cell body from the axon in **retrograde transport** include protein building blocks of neurofilaments, subunits of microtubules, soluble enzymes, and materials taken up by endocytosis (e.g., viruses and toxins). Additionally, small molecules and proteins destined for degradation are transported to endolysosomes of the soma.

Axonal transport not only distributes materials for nerve conduction and neurotransmitter synthesis, but

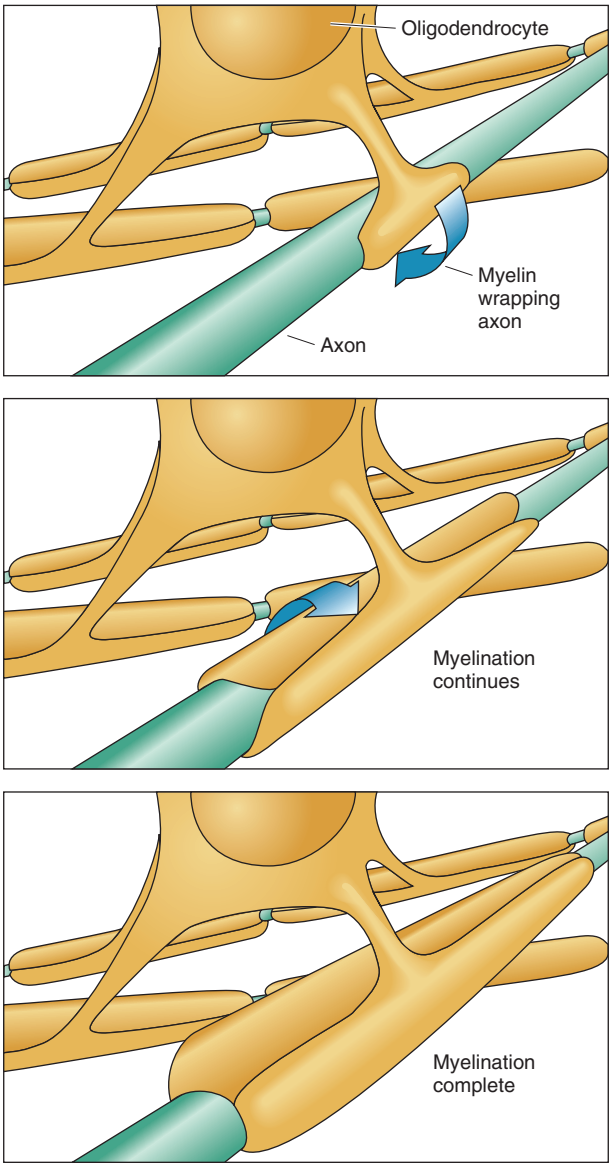


Figure 9–6 Schematic diagram of the process of myelination in the central nervous system. Unlike the Schwann cell of the peripheral nervous system, each oligodendroglion is capable of myelinating several axons.

CLINICAL CORRELATIONS

Retrograde axonal transport is used by certain viruses (e.g., herpes simplex and rabies virus) to spread from one neuron to the next in a chain of neurons. It is also the method whereby toxins (e.g., tetanus) are transported from the periphery into the CNS.

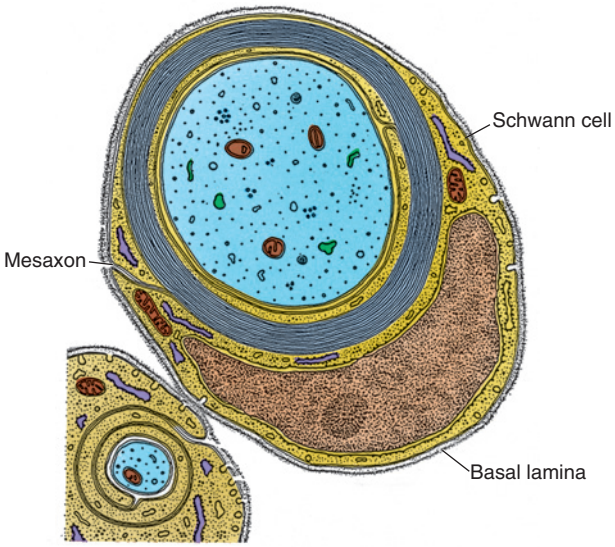


Figure 9–7 Diagram of the fine structure of a myelinated nerve fiber and its Schwann cell. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

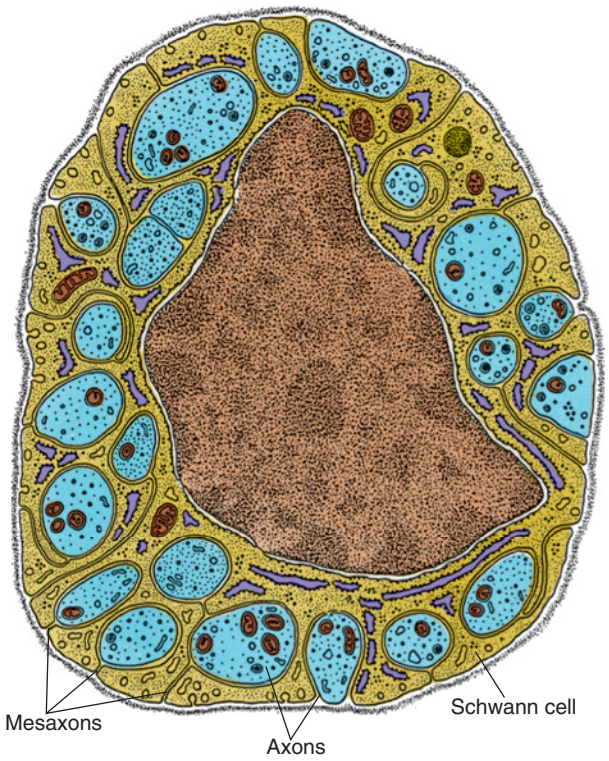


Figure 9–8 Diagram of the fine structure of an unmyelinated nerve fiber. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

also serves to provide and ensure general maintenance of the axon cytoskeleton.

Since the 1970s, much has been learned about the nature and functioning of the neuron through study of the mechanism of axonal retrograde transport with the use of the enzyme **horseradish peroxidase**. In fact, it has become one of the most used techniques in the study of retrograde transport. When this enzyme is injected into the axon terminal, it can be detected later by histochemical techniques that mark its pathway to the cell body. In studying anterograde axonal transport, researchers inject radiolabeled amino acids into the cell body and then later determine the radioactivity at the axon terminals using autoradiography.

Microtubules are important to fast anterograde transport because they exhibit a polarity, with their plus ends directed toward the axon terminal. **Tubulin dimers**, reaching the axoplasm via anterograde transport, are assembled onto the microtubules at their plus ends and depolymerized at their minus ends. The mechanism for anterograde transport involves **kinesin**, a microtubule-associated protein, because one end attaches to a vesicle and the other end interacts in a cyclical fashion with a microtubule, thus permitting the kinesin to transport the vesicle at a speed of about 3 mm/sec. **Dynein**, another microtubule-associated protein, is responsible for moving vesicles along the microtubules in retrograde transport.

CLINICAL CORRELATIONS

Although **neurological tumors** account for about 50% of the intracranial lesions, tumors of neurons of the CNS are rare. Most intracranial tumors originate from neuroglial cells (e.g., **benign oligodendrogliomas** and fatal **malignant astrocytomas**). Tumors that arise from cells of connective tissue associated with nervous tissue (e.g., **benign fibroma** or **malignant sarcoma**) are connective tissue tumors and are not related to the nervous system. Tumors of neurons in the PNS may be extremely malignant (e.g., **neuroblastoma** in the suprarenal gland, which attacks mostly infants and young children).

Classification of Neurons

Neurons are classified morphologically into three major types according to their shape and the arrangement of their processes.

The major types of neurons are as follows (see Fig. 9–4):

- **Bipolar neurons** possess two processes emanating from the soma, a single dendrite and a single axon. Bipolar neurons are located in the vestibular and cochlear ganglia and in the olfactory epithelium of the nasal cavity.

- **Unipolar neurons** (also known as ***pseudounipolar neurons***) possess only one process emanating from the cell body, but this process branches later into a peripheral branch and a central branch. The central branch enters the CNS, and the peripheral branch proceeds to its destination in the body. Each of the branches is morphologically axonal and can propagate nerve impulses, although the very distal aspect of the peripheral branch arborizes and displays small dendritic ends, indicating its receptor function. Unipolar neurons develop from embryonic bipolar neurons whose processes migrate around the cell body during development and eventually fuse into a single process, which, as indicated above, bifurcate into two processes. During impulse transmission, the impulse passes from the end of the peripheral process to the central process without involving the cell body. Unipolar neurons are present in the dorsal root ganglia and in some of the cranial nerve ganglia.
- **Multipolar neurons**, the most common neuron type, possess various arrangements of multiple dendrites emanating from the soma as well as a single axon. Multipolar neurons are present throughout the nervous system, and most of them are motoneurons (in older terminology, they were called *motor neurons*). Some multipolar neurons are named according to the morphology of their somata (e.g., pyramidal cells) or after the scientist who first described them (e.g., Purkinje cells).

Neurons also are classified into three general groups according to their function:

- **Sensory (afferent) neurons** receive sensory input at their dendritic terminals and conduct impulses to the CNS for processing. Those located in the periphery of the body monitor changes in the environment, and those within the body monitor the internal environment.
- **Motoneurons (efferent)** originate in the CNS and conduct their impulses to muscles, glands, and other neurons.
- **Interneurons (intercalated neurons)**, located completely in the CNS, function as interconnectors or integrators that establish networks of neuronal circuits between sensory and motoneurons and other interneurons. With evolution, the number of neurons in the human nervous system has grown enormously, but the greatest increase has involved the interneurons, which are responsible for the complex functioning of the body.

Neuroglial Cells

Neuroglial cells function not only in the physical and metabolic support of neurons but also in a regulatory capacity.

Cells whose function is the metabolic and mechanical support and protection of neurons collectively form the **neuroglia** (Fig. 9–9); they also have a role in the regulation of neuronal propagation of impulses. There may be as many as 10 times more neuroglial cells than neurons in the nervous system. Neuroglial cells undergo mitosis, whereas neurons possess a more limited capability of cell division. Although neuroglial cells form gap junctions with other neuroglial cells, they do not react to or propagate nerve impulses, although they assist neurons in the performance of their neural transmission by

- Keeping a check on synapses
- Regulating the flow of **cerebrospinal fluid (CSF)** through the substance of the brain
- Scavenging neurotransmitters released by the axon terminals of neurons
- Releasing **gliotransmitter** substances such as glutamate into the region of the synapse that may regulate processes at the synapse

Neuroglial cells that reside exclusively in the CNS include astrocytes, oligodendrocytes, microglia (microglial cells), and ependymal cells. Schwann cells, although located in the PNS, are also considered to be neuroglial cells.

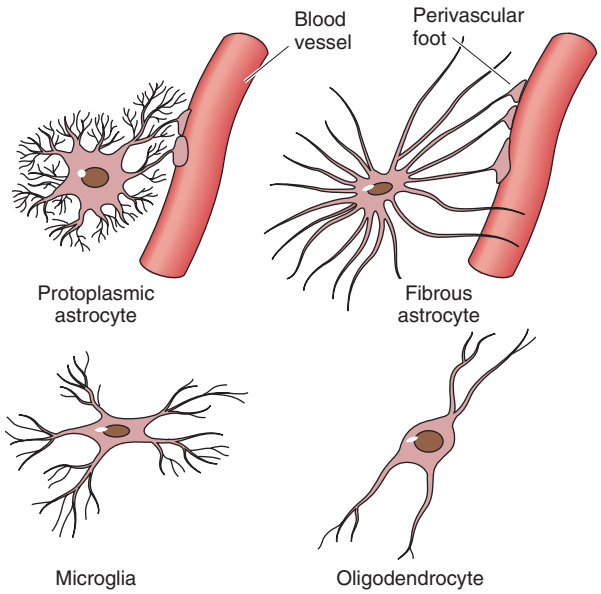


Figure 9–9 Diagram of the various types of neuroglial cells.

Astrocytes

Astrocytes provide structural and metabolic support to neurons and act as scavengers of ions and neurotransmitters released into the extracellular space.

Astrocytes are the largest of the neuroglial cells and exist as two distinct types: (1) protoplasmic astrocytes in the gray matter of the CNS and (2) fibrous astrocytes present mainly in the white matter of the CNS. It is difficult to distinguish the two types of astrocytes in light

micrographs. Some researchers have suggested that they may be the same cells functioning in different environments. Electron micrographs display distinct cytoplasmic bundles of 8- to 11-nm intermediate filaments composed of **glial fibrillar acidic protein**, which is unique to astrocytes.

Protoplasmic astrocytes are stellate-shaped cells displaying abundant cytoplasm, a large nucleus, and many short branching processes (Fig. 9–10). The tips of some processes end as **pedicels (vascular feet)** that come into contact with blood vessels. Other astrocytes lie adjacent to blood vessels with their cell bodies apposed to the vessel wall. Still other protoplasmic astrocytes near the brain or surface of the spinal cord exhibit pedicel-tipped processes that contact the pia mater, forming the **pia-glial membrane**. Protoplasmic astrocytes also function in regulating the flow of CSF through the substance of the brain (see the section on

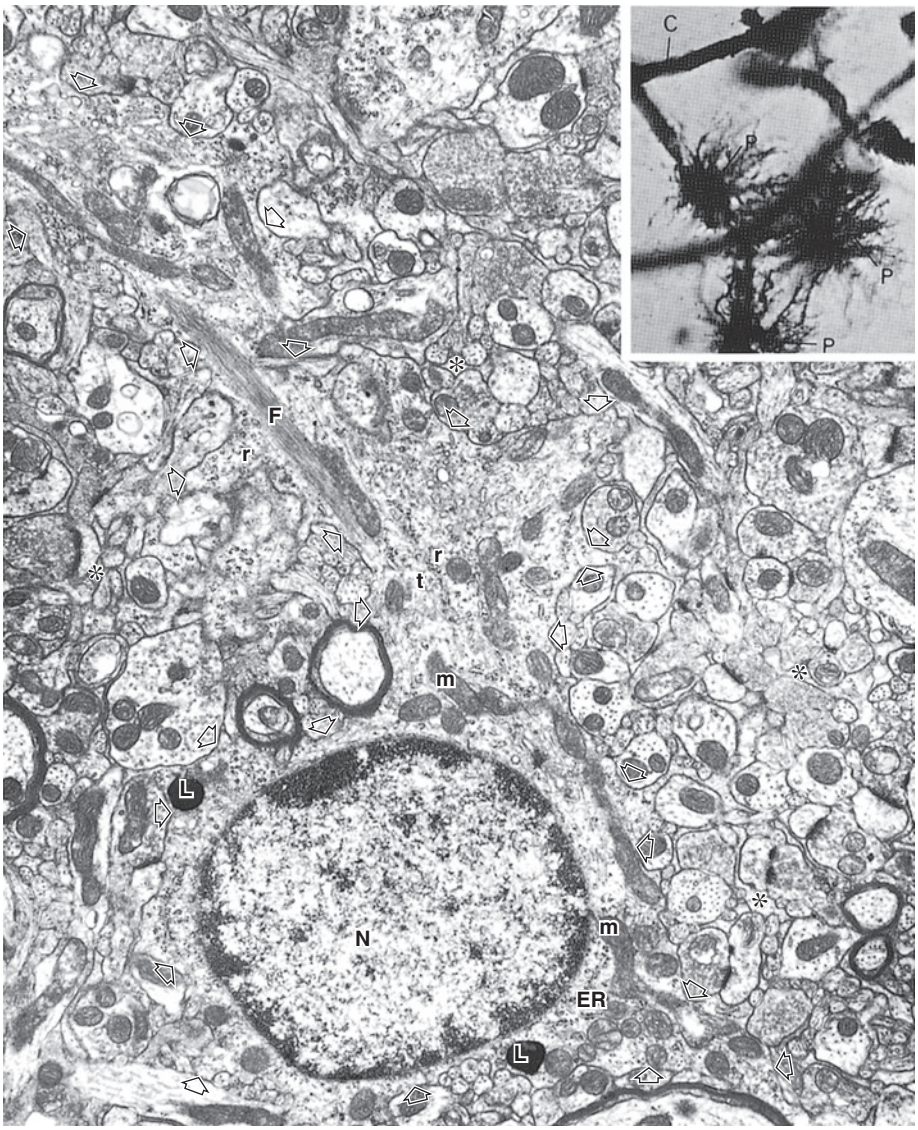


Figure 9–10 Electron micrograph of protoplasmic astrocyte (×11400). Observe the nucleus (N), filaments (F), mitochondria (m), microtubules (t), free ribosomes (r), and granular reticulum (ER). Two lysosomes (L) are also identified in the processes of the neuroglia. Note the irregular cell boundary, indicated by *arrowheads*. *Asterisks* indicate processes of other neuroglial cells of the neuropil. (From Peters A, Palay SL, Webster HF. *The Fine Structure of the Nervous System*. Philadelphia: WB Saunders; 1976.) **Inset**, Light micrograph of three highly branched protoplasmic astrocytes (P) surrounding capillaries (C). (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

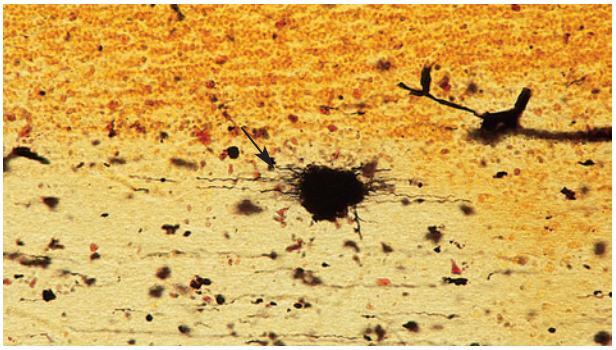


Figure 9–11 Light micrograph of a fibrous astrocyte *(arrow)* in the human cerebellum (×132).

CSF). Some smaller protoplasmic astrocytes located adjacent to neuronal cell bodies are a form of satellite cells.

Fibrous astrocytes possess a euchromatic cytoplasm containing only a few organelles, free ribosomes, and glycogen (Fig. 9–11). The processes of these cells are long and mostly unbranched. These processes are closely associated with the pia mater and blood vessels but are separated from these structures by their own basal lamina.

Astrocytes function in removing ions, neurotransmitters, and remnants of neuronal metabolism, such as potassium ions (K^+), glutamate, and γ-aminobutyric acid (GABA), accumulated in the microenvironment of the neurons, especially at the nodes of Ranvier, where they provide a cover for the axon. These cells also contribute to energy metabolism within the cerebral cortex by releasing glucose from their stored glycogen when induced by the neurotransmitters norepinephrine and vasoactive intestinal peptide (VIP). Astrocytes located at the periphery of the CNS form a continuous layer over the blood vessels and may assist in maintaining the **blood–brain barrier**. Astrocytes are also recruited to damaged areas of the CNS, where they form cellular scar tissue **(glial scar)**.

Oligodendrocytes

Oligodendrocytes function in electrical insulation and in myelin production in the CNS.

Oligodendrocytes resemble astrocytes but are smaller and contain fewer processes with sparse branching. The darkest-staining neuroglial cells, oligodendrocytes are located in both the gray and the white matter of the CNS. Their dense cytoplasm contains a relatively small nucleus, abundant RER, many free ribosomes and mitochondria, and a conspicuous Golgi complex (Fig. 9–12). Microtubules also are present, especially in the perinuclear zone and in the processes.

Interfascicular oligodendrocytes, located in rows beside bundles of axons, are responsible for manufacturing and maintaining **myelin** about the axons of the CNS, serving to insulate them (see Fig. 9–6). In producing myelin, oligodendrocytes function similarly to the Schwann cells of the PNS, except that a single oligodendrocyte may form a number of processes, each of which wraps a small region **(internode)** of as many as 50 axons with segments of myelin, whereas a single Schwann cell wraps a small region **(internode)** of only one axon with myelin. Schwann cells also differ from interfascicular oligodendrocytes in the following ways: Schwann cells possess a basal lamina and retain some cytoplasm within the intracellular domains of the myelin lamellae, and connective tissue invests the myelin sheaths and their surrounding Schwann cells. During active myelin synthesis, interfascicular oligodendrocytes have a very high metabolic rate because they can produce as much as 300% of their weight in myelin synthesis on a daily basis. Subsequent to completion of myelinization of all of the internodes under their control, these cells must maintain responsibility over the metabolic fate of the myelin that they produced.

Satellite oligodendrocytes are closely applied to cell bodies of large neurons and are present only in gray matter. Their function is not understood completely, but they appear to monitor the extracellular fluid around neuronal cell bodies, and, according to some investigators, they may act in a reserve capacity, and, if the need arises, they may migrate into the white matter to replenish interfascicular oligodendrocytes.

CLINICAL CORRELATIONS

Progressive multifocal leukoencephalopathy is a terminal but rare viral disease caused by a **polyoma virus (JC virus)** that attacks oligodendrocytes and causes demyelinization of axons especially in the occipital and parietal lobes of the brain. Although JC virus is present in almost 50% of the adult population in the United States, it is benign until the patient becomes immunosuppressed and immunodeficient.

Microglial Cells

Microglia are members of the mononuclear phagocyte system.

Scattered throughout the CNS, **microglial cells** are small, dark-staining cells that faintly resemble oligodendrocytes. These cells exhibit scant cytoplasm, an oval to triangular nucleus, and irregular short processes. Spines also adorn the cell body and processes. These cells function as phagocytes in clearing debris and damaged structures in the CNS. Microglial cells also protect the nervous system from viruses, microorganisms and tumor formation. When activated by the presence of pathogens or damaged neurons in their vicinity, they secrete interferon-γ which prompts other microglia to

Figure 9–12 Electron micrograph of an oligodendrocyte (×2925). Note the nucleus (N), endoplasmic reticulum (ER), Golgi apparatus (G), and mitochondria (m). Processes of fibrous astrocytes (As) contact the oligodendrocyte. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

become activated. Microglia also release signaling molecules to recruit T lymphocytes and then present epitopes to these T lymphocytes, thereby acting as antigen presenting cells. Recent studies have demonstrated that microglia also react to the **complement-associated proteins C1q** and **C3** by attacking and destroying synapses that present this protein. This is especially true in the developing brain where neurons form a large number of synapses, many of which are unnecessary and are marked by C1q and/or C3.

Unlike the other neuroglial cells, which are derived embryologically from the neural tube, microglial cells originate in the bone marrow and are part of the mononuclear phagocytic cell population.

CLINICAL CORRELATIONS

1. Large populations of microglial cells are present in the brains of patients with AIDS and HIV-1. Although HIV-1 does not attack neurons, it does attack microglial cells, which then secrete neurotoxic cytokines.
2. The complement-associated protein C1q appears to build up at synapses as the individual ages, which results in activation of microglia and subsequent destruction of synapses. It is possible that, after enough synapses belonging to a specific neuron are destroyed, the neuron itself may undergo degeneration.

Ependymal Cells

Ependymal cells (ependymocytes) are low columnar to cuboidal epithelial cells lining the ventricles of the brain and central canal of the spinal cord. They are derived from embryonic neuroepithelium of the developing nervous system. Their cytoplasm contains abundant mitochondria and bundles of intermediate filaments. In some regions, these cells are ciliated, a feature that facilitates the movement of CSF. In an embryo, processes emanating from the cell body reach the surface of the brain, but, in an adult, the processes are reduced, ending on nearby cells.

Where the neural tissue is thin, ependymal cells form an **internal limiting membrane** lining the ventricle and an **external limiting membrane** beneath the pia, both formed by thin-fused pedicels. Modifications of some of the ependymal cells in the ventricles of the brain participate in the formation of the **choroid plexus**, which is responsible for secreting and maintaining the chemical composition of the CSF.

Tanycytes, specialized ependymal cells, extend processes into the hypothalamus, where they terminate near blood vessels and neurosecretory cells. It is believed that tanycytes transport CSF to these neurosecretory cells and, possibly under control from the anterior lobe of the pituitary, may respond to changes in hormone levels in the CSF by discharging secretory products into capillaries of the median eminence.

Schwann Cells

Schwann cells form both myelinated and unmyelinated coverings over axons of the peripheral nervous system.

Unlike other neuroglial cells, **Schwann cells** are located in the PNS, where they envelop axons. They can form either myelinated or unmyelinated coverings over axons. Axons that have myelin wrapped around them are referred to as ***myelinated nerves***.

Schwann cells are flattened cells whose cytoplasm contains a flattened nucleus, a small Golgi apparatus, and a few mitochondria. Electron microscopy has revealed that myelin is the plasmalemma of the Schwann cell organized into a sheath that is wrapped several times around the axon. Interruptions occur in the myelin sheath at regular intervals along the length of the axon, exposing the axon; these interruptions are called ***nodes of Ranvier*** (Fig. 9–13). Each node indicates an interface between the myelin sheaths of two adjacent Schwann cells located along the axon. Nodes of Ranvier are very rich in **voltage-gated Na^+ ion channels**, a feature that permits an impulse transmission known as ***saltatory conduction***, but internodes have few, if any, of these channels (see section on generation and conduction of nerve impulses).

The outer portion of Schwann cells is covered by a basal lamina that dips into the nodes of Ranvier, covering the overlapped areas of the myelin sheath lamellae of adjacent Schwann cells. Thus, each Schwann cell is covered by a basal lamina, as is the axon at the node of Ranvier. After nerve injury, the regenerating nerve is guided by the basal lamina to its location.

Areas of the axon covered by concentric lamellae of myelin and the single Schwann cell that produced that section of the myelin sheath are called ***internodal segments***, which range in length from 200 to 1000 µm. Light microscopy has revealed several cone-shaped, oblique clefts in the myelin sheath of each internodal segment called ***clefts (incisures)*** **of Schmidt-Lanterman**. These clefts, viewed with the electron microscope, are demonstrated to be Schwann cell cytoplasm trapped within the lamellae of myelin.

As the membrane spirals around the axon, it produces a series of alternating wide, dense lines with narrower, less dense lines occurring at 12-nm intervals. The wider line (3 nm in width) is known as the ***major dense line***. It represents the fused cytoplasmic surfaces of the Schwann cell plasma membrane. The narrower **intraperiod line** represents the apposing outer leaflets of the Schwann cell plasma membrane. High-resolution electron microscopy has revealed small gaps within the intraperiod line between spiraled layers of the myelin sheath called ***intraperiod gaps***. These gaps are thought to provide access for small molecules to reach the axon. The region of the intraperiod line that is in intimate contact with the axon is known as the ***internal mesaxon***, whereas its outermost aspect, which is in contact with the body of the Schwann cell, is the **external mesaxon** (Fig. 9–14; see Fig. 9–7).

The mechanism of **myelination**, that is, the process whereby the Schwann cell located in the PNS (or oligodendrocyte located in the CNS) concentrically wraps its membrane around the axon to form the myelin sheath, is unclear. It is believed to begin when a Schwann cell envelops an axon and somehow wraps its membrane around the axon. The wrapping may continue for more than 50 turns. During this process, the cytoplasm is squeezed back into the body of the Schwann cell, bringing the cytoplasmic surfaces of the membranes in contact with each other, thus forming the major dense line that spirals through the myelin sheath. A single Schwann cell can myelinate only one internode of a single axon (and in the PNS only), whereas oligodendrocytes can myelinate an internode of as many as 50 axons (and in the CNS only).

Nerves are not myelinated simultaneously during development. Indeed, the onset and completion of myelination vary considerably in different areas of the nervous system. This variation appears to be correlated with function. For example, motor nerves are nearly completely myelinated at birth, whereas sensory roots are not myelinated for several months thereafter. Some CNS nerve tracts and commissural axons are not fully myelinated until several years after birth.

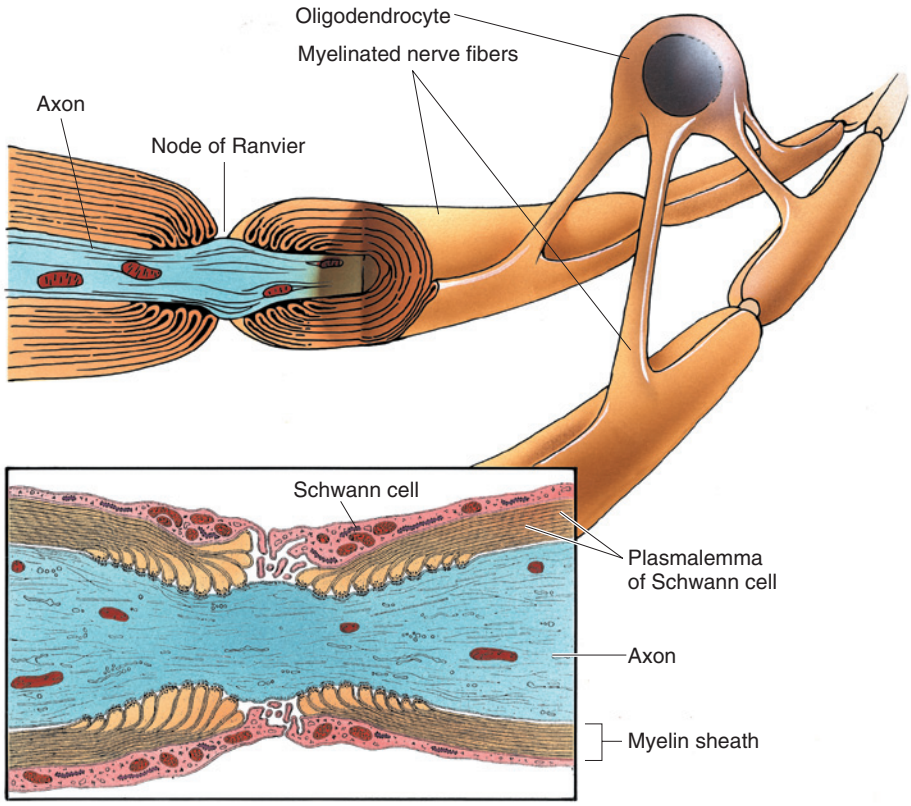


Figure 9–13 Diagrammatic representation of the myelin structure at the nodes of Ranvier of axons in the central nervous system and peripheral nervous system *(inset)*.

Some axons in the PNS are not wrapped with the many layers of myelin typical of myelinated axons. These unmyelinated axons are surrounded by a single layer of Schwann cell plasma membrane and cytoplasm of the Schwann cell (see Fig. 9–8). Although a single Schwann cell can myelinate one axon only, several unmyelinated axons may be enveloped by a single Schwann cell.

GENERATION AND CONDUCTION OF NERVE IMPULSES*

Nerve impulses are generated in the spike trigger zone of the neuron and are conducted along the axon to the axon terminal.

*Although negatively charged proteins within the cytoplasm of the neuron do not cross the cell membrane, they do affect the behavior of the various charged species. However, their role in the generation and conduction of nerve impulses is not described here. The interested reader is referred to textbooks of physiology or neuroscience for an in-depth explanation of these phenomena.

Nerve impulses are electrical signals that are most readily generated at an area of the axon hillock that is exceptionally rich in voltage-gated sodium channels. This region of the axon hillock is known as the ***spike trigger zone*** of a neuron and, as the result of **membrane depolarization**, impulses are conducted along the axon to the axon terminal. Transmission of impulses from the terminals of one neuron to another neuron, a muscle cell, or a gland occurs at synapses (see section on synapses and the transmission of the nerve impulse).

Neurons and other cells are electrically **polarized** with a **resting potential** of about −70 mV (the cytoplasm adjacent the neuronal cell membrane is *less positive* than the extracellular fluid adjacent the neuronal extracellular aspect of the cell membrane) across the plasma membrane, although in smaller muscle cells and small nerve fibers, this differential may be as low as −40 to −60 mV. This potential arises because of the difference between ion concentrations inside and outside of the cell. In mammalian cells, the concentration of potassium (K^+) ions is much higher inside of the cell than outside of the cell, whereas the concentration of sodium

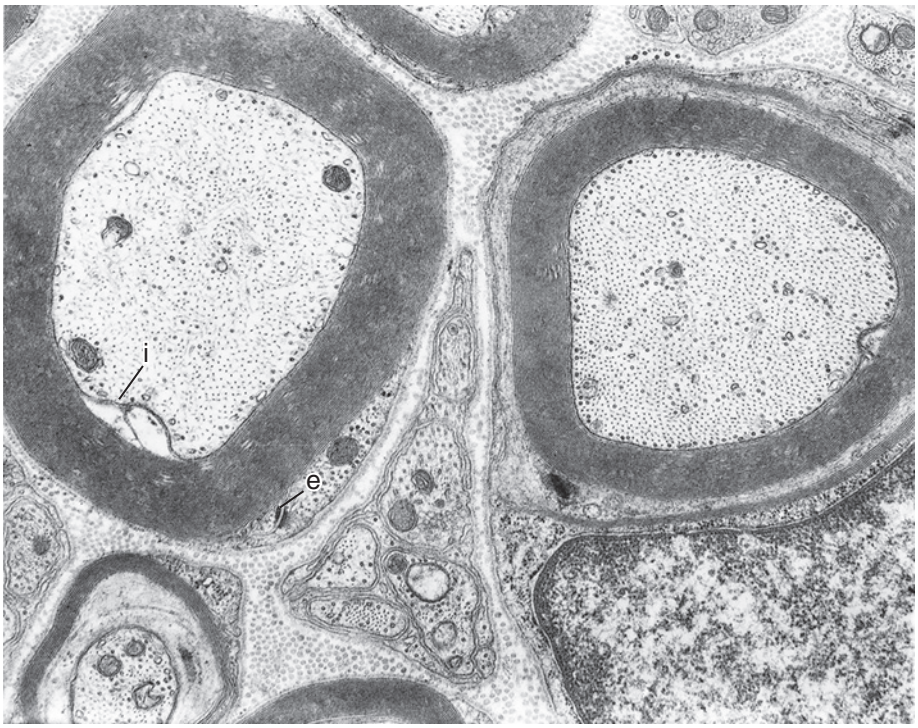


Figure 9–14 Electron micrograph of a myelinated peripheral nerve. Note the internal (i) and external (e) mesaxons as well as the Schwann cell cytoplasm and nucleus. (From Jennes L, Traurig HH, Conn PM. *Atlas of the Human Brain*. Philadelphia: Lippincott-Raven; 1995.)

CLINICAL CORRELATIONS

Multiple sclerosis, a relatively common disease affecting myelin, is 1.5 times more common in females than in males. It usually occurs between 15 and 45 years of age, and its principal pathological feature is demyelination in the CNS (optic nerve, cerebellum, and white matter of the cerebrum, spinal cord, and cranial and spinal nerves). The disease is distinguished by episodes of random, multifocal inflammation, edema, and subsequent demyelination of axons in the CNS, followed by periods of remission that may last for several months to decades. Each episode may further jeopardize the patient's vitality. Any single episode of demyelination may cause deterioration or malignancy of the affected nerves and may lead to death in a matter of months. Because this demyelination is thought to result from an autoimmune disease with inflammatory features (which may manifest as a possible aftermath of an infectious agent), immunosuppression with corticosteroids is the most common therapy for multiple sclerosis, although the anti-inflammatory activity of the therapy is thought to be most beneficial. Recent studies have demonstrated that physical, social, and intellectual activity is able to help protect the individual from certain neurological conditions such as demyelination. It appears that certain cells of the immune system in these individuals release small membrane-bound vesicles, **exosomes**, into the bloodstream and lymphatic fluid which then ferry them throughout the body. These exosomes contain various proteins, enzymes, signaling molecules, as well as micro-ribonucleic acids (miRNAs). Reports of research performed on rodents demonstrated that some of these exosomes were able to trigger oligodendrocytes of the brain to repair the damaged myelin and reverse some of the damage caused by multiple sclerosis. Whether this is possible to replicate in humans is not as yet known.

Radiation therapy can lead to demyelination of the brain or spinal cord when these structures are in the radiation field during therapy. Toxic agents, such as those used in **chemotherapy** for cancer, may also lead to demyelination, resulting in neurological problems.

Guillain-Barré syndrome is a immune disorder that produces inflammation and rapid demyelination within the peripheral nerves and the motor nerves arising from the ventral roots. This disease is associated with recent gastrointestinal infection, especially with *Campylobacter jejuni*. Interestingly, some of the *Campylobacter* lipopolysaccharide contains ganglioside-like epitopes that resemble some of the lipids present in myelin which then elicits an autoimmune response resulting in axonal demyelination. A symptom of this disease is muscle weakness in the extremities reaching a high point within just a few weeks followed by a more serious condition of demyelination of the nerves serving the diaphragm, making breathing difficult at first and impossible eventually. Early recognition of the condition followed by physical therapy, respiratory therapy, and autoimmune globulin treatments results in possibly complete reversal of the condition.

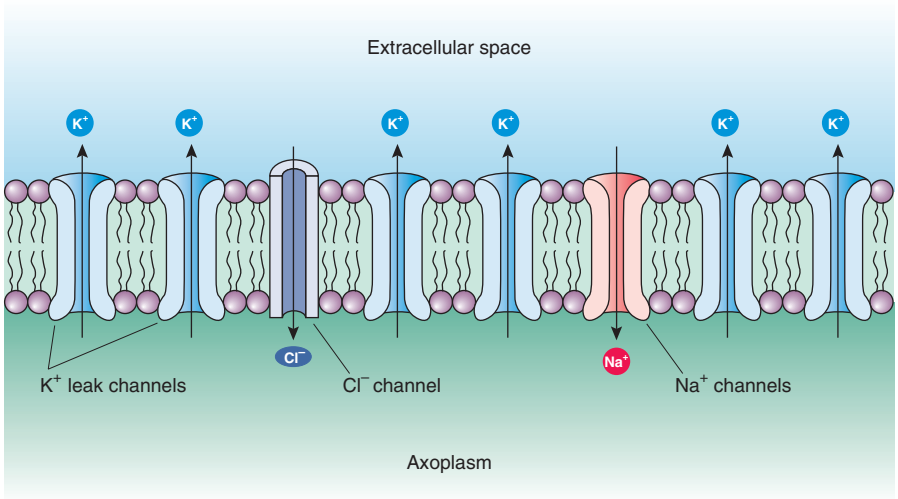


Figure 9–15 Schematic diagram of the establishment of the resting potential in a typical neuron. Observe that the potassium ion (K^+) leak channels outnumber the sodium ion (Na^+) and calcium ion (Cl^-) channels; consequently, more K^+ can leave the cell than Na^+ or Cl^- can enter. Because there are more positive ions outside than inside of the cell, the outside is more positive than the inside, establishing a potential difference across the membrane. Ion channels and ion pumps not directly responsible for the establishment of resting membrane potential are not shown.

ions (Na^+) and chloride ions (Cl^-) is much higher outside of the cells than inside of the cell.

K^+ ion leak channels in the plasmalemma permit a relatively free flow of K^+ ions out of a cell down its concentration gradient (Fig. 9–15). Although the K^+ ion leak channel allows Na^+ ions to enter the cell, the ratio of potassium to sodium is 100 : 1, so that many more K^+ ions leave the cell than Na^+ ions enter; thus, a small net positive charge accumulates on the outside of the plasma membrane. Although maintenance of the resting potential depends primarily on K^+ ion leak channels, **Na^+-K^+ ion pumps** in the plasma membrane assist by actively pumping Na^+ ions out of the cell and K^+ ions into the cell. For every three sodium ions pumped out, two potassium ions enter the cell, also making a minor contribution to the potential difference between the two sides of the membrane.

In most cells, the potential across the plasma membrane is generally constant. In neurons and muscle cells, however, the membrane potential can undergo controlled changes, making these cells capable of conducting an electrical signal, as follows:

1. Stimulation of a neuron causes opening of **voltage-gated Na^+ ion channels** in a small region of the membrane, leading to an influx of Na^+ ions into the cell at that site (Fig. 9–16). Eventually, the overabundance of Na^+ ions inside of the cell causes a **reversal of the resting potential** (i.e., the cytoplasmic aspect of the plasma membrane becomes positive relative to its extracytoplasmic aspect), and the membrane is said to be **depolarized**.
2. As a result, the Na^+ ion channels become inactivated for 1 to 2 msec, a condition known as the ***refractory period***. This is a time during which the Na^+ ion channels are inactive; that is, they cannot open or close, and Na^+ ions cannot traverse them. The presence of the refractory period is due to the specialized construction of the voltage-gated Na^+ ion channels. These channels have two gates, an extracytoplasmic gate **(activation gate)** that opens as a result of the depolarization of the cell membrane and remains open as long as the membrane is depolarized. However, an intracytoplasmic gate **(inactivation gate)** closes within a few ten-thousandths of a second after the opening of the activation gate. Therefore, even though the activation gate remains open, Na^+ ions can no longer enter or leave the cell through these channels.
3. During the refractory period, **voltage-gated K^+ ion channels** open (*note that these are different from the K^+ ion leak channels described previously*), permitting an efflux of K^+ ions into the extracellular fluid that eventually restores the resting membrane potential; however, there may be a brief period of **hyperpolarization**.
4. Once the resting potential is restored, the voltage-gated K^+ ion channels close, and the refractory period is ended with the closing of the activation gate and

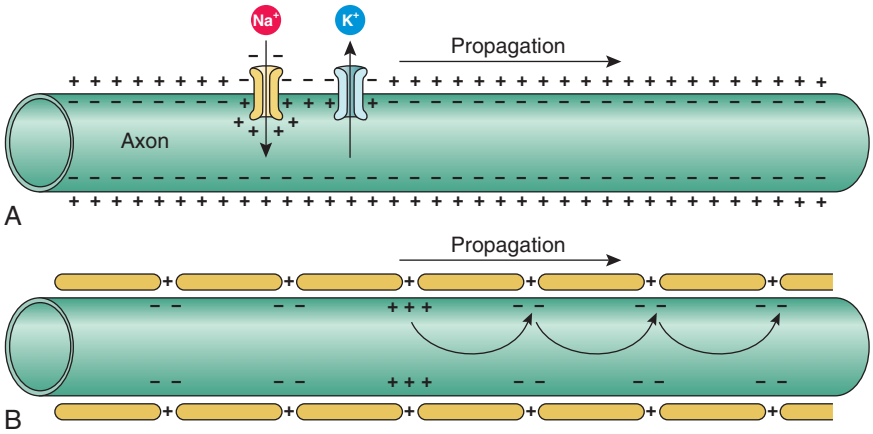


Figure 9–16 Schematic diagram of the propagation of the action potential in an unmyelinated (**A**) and a myelinated (**B**) axon (see text).

the opening of the inactivation gate of the voltage-gated Na^+ ion channel.

The cycle of membrane depolarization, hyperpolarization, and return to the resting membrane potential is called the **action potential**, an all-or-none response that can occur at rates of 1000 times/sec. The membrane depolarization that occurs with the opening of voltage-gated Na^+ ion channels at one point on an axon spreads passively for a short distance and triggers the opening of adjacent channels, resulting in the generation of another action potential. In this manner, the **wave of depolarization**, or **impulse**, is conducted along the axon. In vivo, an impulse is conducted in only one direction, from the site of initial depolarization to the axon terminal, known as **orthodromic spread**. The inactivation of the Na^+ ion channels during the refractory periods prevents retrograde propagation, known as **antidromic spread**, of the depolarization wave. In an **unmyelinated axon**, the impulse travels slowly because it involves sodium channels that are adjacent to each other. In myelinated fiber, the impulse travels much faster because it jumps, known as ***saltatory conduction***, from one node of Ranvier to the adjacent node of Ranvier, without having to involve the membrane of the internodes. As stated above, the nodes of Ranvier are richly supplied by **voltage-gated Na^+ ion channels**, whereas the internodes possess very few, if any, of these channels.

Synapses and the Transmission of the Nerve Impulse

Synapses are the sites of impulse transmission between the presynaptic and postsynaptic cells.

Synapses are the sites where nerve impulses are transmitted from a presynaptic cell (a neuron) to a postsynaptic cell (another neuron, muscle cell, or cell of a gland). Synapses thus permit neurons to communicate with each other and with effector cells (muscles and glands). Impulse transmission at synapses can occur electrically or chemically.

Although **electrical synapses** are uncommon in mammals, they are present in the brain stem, retina, and cerebral cortex. Electrical synapses are usually represented by gap junctions that permit free movement of ions from one cell to another. When this ion movement occurs between neurons, there is a flow of current. Impulse transmission is much faster across electrical synapses than across chemical synapses.

Chemical synapses are the most common mode of communication between two nerve cells. The **presynaptic membrane** releases one or more **neurotransmitters** into the **synaptic cleft**, a small (20- to 30-nm) gap, located between the presynaptic membrane of the first cell and the **postsynaptic membrane** of the second cell (Fig. 9–17). The neurotransmitter diffuses across the synaptic cleft to **gated ion-channel receptors** on the postsynaptic membrane. Binding of the

CLINICAL CORRELATIONS

The terminals of the peripheral processes of sensory neurons that are designed to transmit pain sensations possess a very specific type of Na^+ channels, known as $Na_V1.7$. When the terminus of one of these nerve fibers is stimulated, the $Na_V1.7$ channels open, permit the movement of Na^+ ions into the cell and thereby initiate the propagation of a nerve impulse. The discovery of these channels provided an explosion of research to find drugs and anesthetics directed specifically toward these channels to provide pain relief and anesthesia without affecting all other sodium channels of the region.

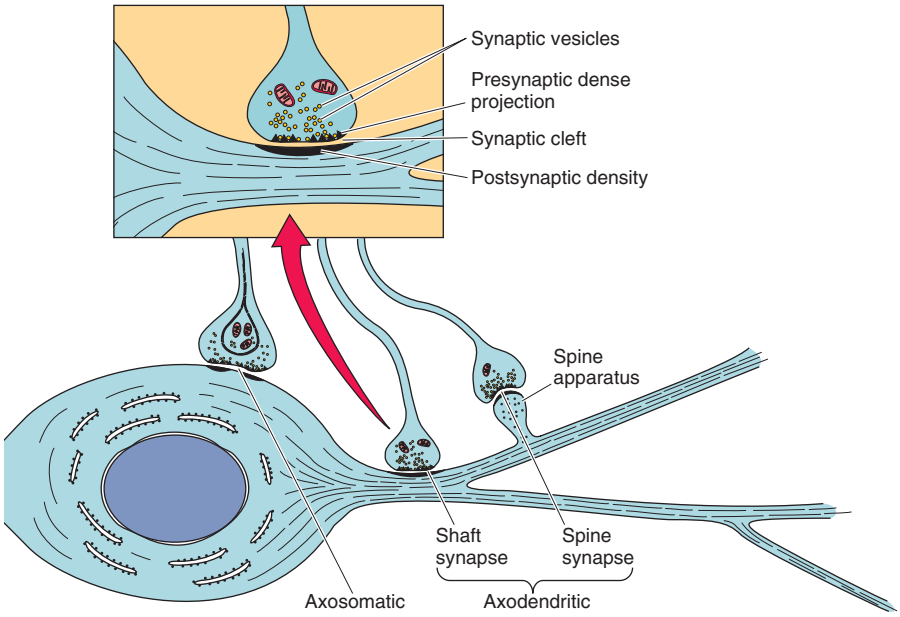


Figure 9–17 Schematic diagram of the various types of synapses.

neurotransmitter to these receptors initiates the opening of ion channels, which permits the passage of certain ions, altering the permeability of the postsynaptic membrane and reversing its membrane potential. *Neurotransmitters do not accomplish the reaction events at the postsynaptic membrane; they only activate the response*.

When the stimulus at a synapse results in depolarization of the postsynaptic membrane to a threshold value that initiates an action potential, it is called an ***excitatory postsynaptic potential***. A stimulus at the synapse that results in maintaining a membrane potential or increasing it to a state known as ***hyperpolarization*** is called an ***inhibitory postsynaptic potential***.

Various types of synaptic contacts between neurons have been observed. The following synapses are the most common (Fig. 9–18; see Fig. 9–17):

- **Axodendritic synapse** (between an axon and a dendrite)
- **Axosomatic synapse** (between an axon and a soma)
- **Axoaxonic synapse** (between two axons)
- **Dendrodendritic synapse** (between two dendrites)

Synaptic Morphology

Terminals of axons vary according to the type of synaptic contact. Often, the axon forms a bulbous expansion at its terminal end called ***bouton terminal***. Other forms of synaptic contacts in axons are derived from swellings along the axon called ***boutons en passage***, where each bouton may serve as a synaptic site.

The cytoplasm at the **presynaptic membrane** contains mitochondria, a few elements of SER, and an abundance of synaptic vesicles assembled around the presynaptic membrane (Fig. 9–19). **Synaptic vesicles** are spherical structures (40 to 60 nm in diameter) filled with neurotransmitter substance that usually was manufactured and packaged near the axon terminal. Peptide neurotransmitters, however, are manufactured and packaged in the cell body and are transported to the axon terminal via anterograde transport. Enzymes located in the axoplasm protect neurotransmitters from degradation.

Also located on the cytoplasmic aspect of the presynaptic membrane are cone-shaped densities that project from the membrane into the cytoplasm; they appear to be associated with many of the synaptic vesicles, forming the **active site** of the synapse. Those synaptic vesicles associated with the active site are released at stimulation. **Cell adhesion molecules** are known to play an additional role in this location as signaling molecules at both the presynaptic and postsynaptic aspects of the synapse. Other synaptic vesicles, forming a reserve pool, adhere to actin microfilaments at a slight distance from the active site.

Synapsin-I, a small protein that forms a complex with the vesicle surface, appears to assist in the clustering of synaptic vesicles held in reserve. When synapsin-I is phosphorylated, these synaptic vesicles become free to move to the active site in preparation for release of the neurotransmitter; dephosphorylation of synapsin-I reverses the process.

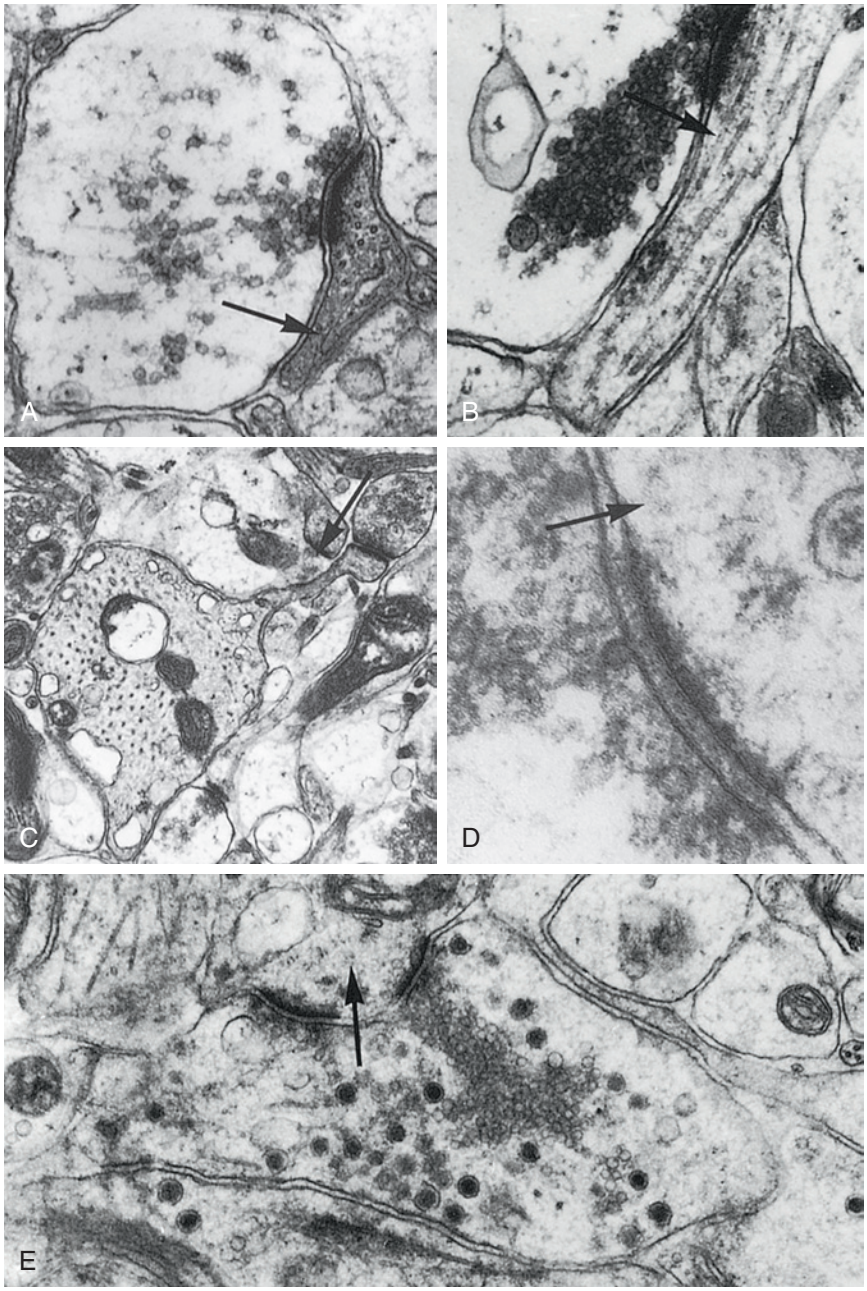


Figure 9–18 Electron micrographs of synapses. The *arrow* indicates transmission direction. **A,** Axodendritic synapse. Presynaptic vesicles are located to the left (×37,600). **B,** Axodendritic synapse. Note neurotubules in dendrite (×43,420). **C,** Dendrite in cross section. Note the synapse (×18,800). **D,** Axodendritic synapse. Note presynaptic vesicle fusing with the axolemma (×76,000). **E,** Axon terminal with clear synaptic vesicles and dense-cored vesicles (×31000). (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

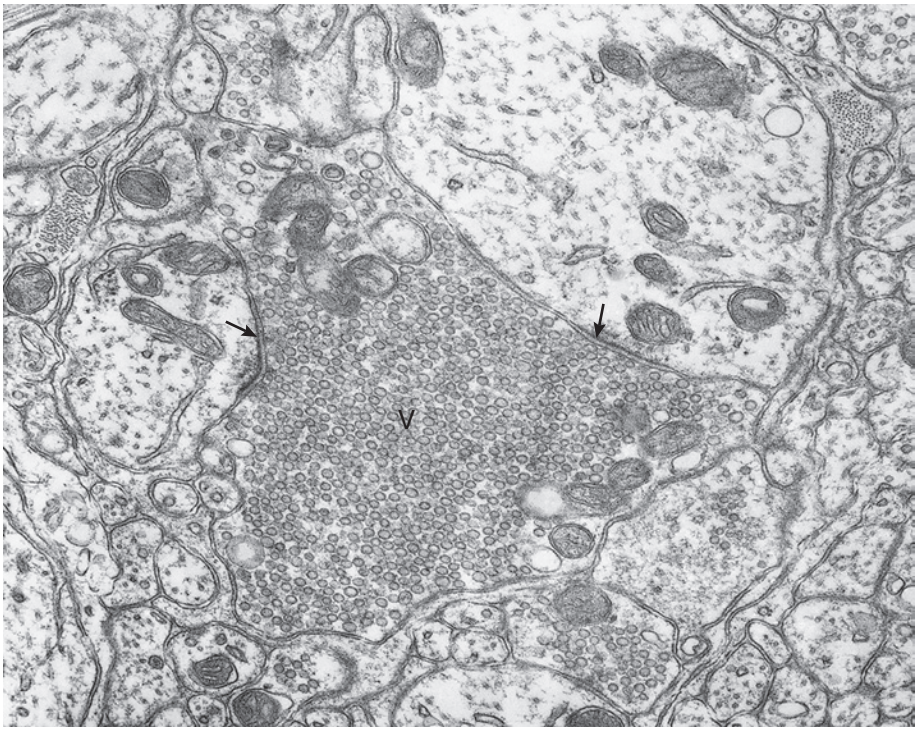


Figure 9–19 Electron micrograph of an axodendritic synapse. Observe the numerous synaptic vesicles (v) within the axon terminal synapsing with dendrites and the synaptic clefts at these sites *(arrows)*. (From Jennes L, Traurig HH, Conn PM: Atlas of the Human Brain. Philadelphia, Lippincott-Raven, 1995.)

Synapsin-II and another small protein **(rab3a)** control association of the vesicles with actin microfilaments. Docking of the synaptic vesicles with the presynaptic membrane is under control of two additional synaptic vesicle proteins: **synaptotagmin** and **synaptophysin**. When an action potential reaches the presynaptic membrane, it initiates opening of the **voltage-gated calcium ion (Ca^{2+}) channels**, permitting Ca^{2+} to enter. This Ca^{2+} ion influx causes synaptic vesicles, under the influence of **SNARE (SNAP receptor)** proteins (including **synaptobrevin**, **syntaxin**, and **soluble *N*-ethylmaleimide–sensitive–fusion protein attachment protein-25 [SNAP-25]**) to fuse with the presynaptic membrane, releasing their stored neurotransmitter material into the synaptic cleft via exocytosis.

Excess membrane is recaptured via **clathrin-mediated endocytosis**. Recycling of synaptic vesicles involves interactions between synaptotagmin and **vesicle coat protein AP-2**. The endocytic vesicle fuses with the SER, where new membrane is continuously recycled.

The **postsynaptic membrane**, a thickened portion of the plasma membrane of the postsynaptic cell, contains **neurotransmitter receptors**. Coupling of the neurotransmitter with its receptors initiates **depolarization** (an excitatory response) or **hyperpolarization** (an inhibitory response) of the postsynaptic membrane. Neuroglia have been shown to increase synaptogenesis, synaptic efficacy, and action-potential firing.

CLINICAL CORRELATIONS

The microorganism ***Clostridium botulinum*** produces a protease known as **neurotoxin B** that specifically cleaves the synaptic **vesicle fusion proteins** synaptobrevin, syntaxin, and SNAP-25. The toxin is lethal in very small quantities and can be ingested in food from damaged cans or from food that was handled improperly. Neurotoxin B selectively blocks synaptic vesicle fusion with the presynaptic membrane, thus preventing the exocytosis of the neurotransmitter acetylcholine without affecting any other aspect of nerve function. The absence of acetylcholine release at the neuromuscular junction results in flaccid paralysis of skeletal muscles. If this condition is not recognized early and antitoxins are not administered, the affected individual will succumb to respiratory failure.

The relative thicknesses and densities of the presynaptic and postsynaptic membranes, coupled with the width of the synaptic cleft, generally correlate with the nature of the response. A thick postganglionic density and a 30-nm synaptic cleft constitutes an **asymmetric synapse**, which is usually the site of **excitatory responses**. A thin postsynaptic density and a 20-nm synaptic cleft constitutes a **symmetric synapse**, which is usually the site of **inhibitory responses**.

Neurotransmitters and Neuromodulators

Neurotransmitters and neuromodulators are signaling molecules that are released at the presynaptic membranes and activate receptors on postsynaptic membranes.

Cells of the nervous system communicate mostly by the release of signaling molecules. The released molecules contact receptor molecules protruding from the plasmalemma of the target cell, eliciting a response from the target cell. These signaling molecules were called ***neurotransmitters***. However, such molecules may act on two types of receptors: (1) those directly associated with ion channels and (2) those associated with G proteins or receptor kinases, which activate a second messenger. Therefore, signaling molecules that act as "first messenger systems" (i.e., act on receptors directly associated with ion channels) are now referred to as ***neurotransmitters***. Signaling molecules that invoke the "second messenger system" are referred to as ***neuromodulators*** or ***neurohormones***. Because neurotransmitters act directly, the entire process is fast, lasting usually less than 1 msec. Events using neuromodulators are much slower and may last as long as a few minutes.

There are perhaps 100 known neurotransmitters (and neuromodulators) represented by the following four groups:

- Small-molecule transmitters
- Neuropeptides
- Gases
- Other

Small-molecule transmitters are of three major types:

- Acetylcholine (the only one in this group that is not an amino acid derivative)
- Amino acids: glutamate, aspartate, glycine, and GABA
- Biogenic amines: (monoamines) serotonin and the three catecholamines: dopamine, **norepinephrine** (noradrenaline), and epinephrine (adrenaline)

Neuropeptides, many of which are neuromodulators, form a large group. They include the following:

- Opioid peptides: enkephalins and endorphins
- Gastrointestinal peptides: produced by cells of the diffuse neuroendocrine system: substance P, neurotensin, and VIP
- Hypothalamic-releasing hormones: thyrotropin-releasing hormone and somatostatin
- Hormones: stored in and released from the neurohypophysis (antidiuretic hormone and oxytocin)

Certain **gases** act as neuromodulators. They are

- Nitric oxide (NO)
- Carbon monoxide (CO)

The **other category** of neurotransmitters include

- Anandamide, 2-Arachidonylglycerol, and virodhamine, all of which bind to cannabinoid receptors
- Adenosine triphosphate, which binds to both P2X and P2Y receptors
- Adenosine, which binds to P1 receptors (adenosine receptors)

The most common neurotransmitters are listed in Table 9–1.

CLINICAL CORRELATIONS

Huntington chorea is a hereditary condition with an onset at about the third or fourth decade of life. It begins as involuntary jerking movements that progress to severe distortions, dementia, and motor dysfunction. The condition is thought to be related to loss of cells producing **GABA**, an inhibitory neurotransmitter. Without it, outbursts are uncontrolled. The dementia associated with this disease is thought to be related to subsequent loss of acetylcholine-secreting cells.

Parkinson disease, a crippling disease related to the absence of **dopamine** in certain regions of the brain, is characterized by muscular rigidity, constant tremor, bradykinesia (slow movement), and, finally, a mask-like face and difficult voluntary movement. Because dopamine cannot cross the blood–brain barrier, therapy is administered as L-dopa (levodopa), which relieves the motor abnormalities temporarily , although the neurons in the affected area continue to die. Efforts to transplant fetal adrenal gland tissue into persons with this disease have provided only transient relief. The therapeutic grafting of genetically modified cells capable of secreting dopamine will perhaps be able establish synaptic connections to cells in the corpus striatum of the brain where dopamine it is needed.

Several principles appear to describe the functioning of neurotransmitters. First, a specific neurotransmitter may elicit different actions under varied circumstances. Second, the nature of the postsynaptic receptors determines the effect of a neurotransmitter on postsynaptic cells. Synaptic communication commonly involves multiple neurotransmitters. Additionally, there is mounting evidence for **volume transmission** as a method of

TABLE 9–1 Common Neurotransmitters and Functions Elicited by Their Receptor

Neurotransmitter	Compound Group	Function
Acetylcholine	Small molecule transmitter; not derived from amino acids	Myoneural junctions, all parasympathetic synapses, and preganglionic sympathetic synapses
Epinephrine	Small molecule transmitter; biogenic amine; catecholamine	Postganglionic sympathetic synapses (except for eccrine sweat glands); does not have psychoactive function in the brain
Norepinephrine	Small molecule transmitter; biogenic amine; catecholamine	Postganglionic sympathetic synapses (except for eccrine sweat glands); has a psychoactive function in the brain
Glutamic acid	Small molecule transmitter; amino acid	Presynaptic sensory and cortex: most common excitatory neurotransmitter of CNS
γ-Aminobutyric acid (GABA)	Small molecule transmitter; amino acid	Most common inhibitory neurotransmitter of CNS
Dopamine	Small molecule transmitter; biogenic amine; catecholamine	Basal ganglia of CNS; inhibitory or excitatory, depending on receptor
Serotonin	Small molecule transmitter; biogenic amine	Inhibits pain; mood control; sleep
Glycine	Small molecule transmitter; amino acid	Brain stem and spinal cord; inhibitory
Endorphins	Neuropeptide; opioid peptide	Analgesic; inhibit pain transmission?
Enkephalins	Neuropeptide; opioid peptide	Analgesic; inhibit pain transmission?
Adenosine triphosphate	Other	P2X receptors: induce target cell to release other neurotransmitters P2Y receptors: modify gene expression

CNS, Central nervous system.

communication between brain cells. According to this concept, chemical and electrical "neurotransmitters," believed to exist in the intercellular fluid–filled spaces between brain cells, activate groups or fields of cells that contain appropriate receptors rather than activating individual cells. Whereas synaptic communication is fast-acting, volume transmission is thought to be slow and may be related to such conditions as autonomic function, alertness, awareness, changes in brain patterns during sleep, sensitivity to pain, and moods.

PERIPHERAL NERVOUS SYSTEM

The peripheral nervous system includes the peripheral nerves and nerve cell bodies located outside of the CNS.

Peripheral Nerves

Peripheral nerves are bundles of nerve fibers (axons), located outside of the CNS, surrounded by several investments of connective tissue sheaths (Figs. 9–20 through 9–22). These bundles **(fascicles)** may be observed with the unaided eye; those that are myelinated appear white because of the presence of myelin. Usually, each bundle of nerve fibers, regardless of size, has both sensory and motor components, and are considered to be **mixed nerves**. There are, however some nerves that are purely **sensory** and some nerves that are purely **motor**.

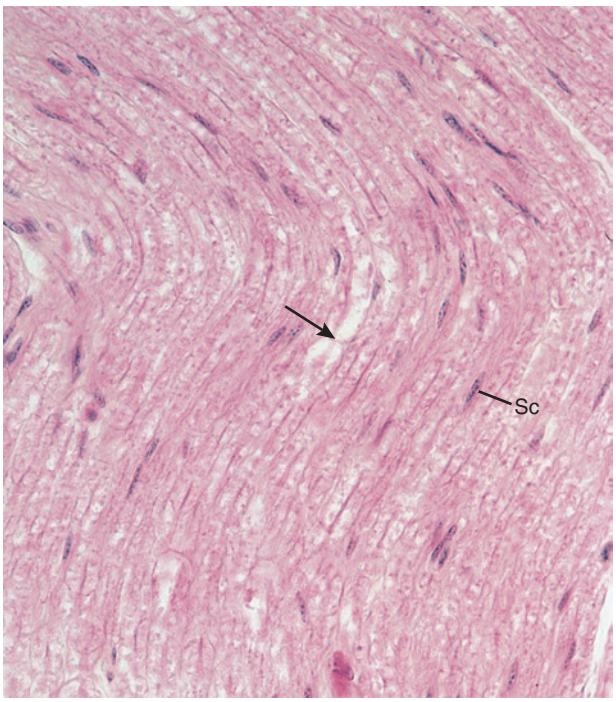


Figure 9–20 Light micrograph of a longitudinal section of a peripheral nerve (×270). Myelin and nodes of Ranvier *(arrow)* as well as the lightly stained oval nuclei of Schwann cells (Sc) may be observed.

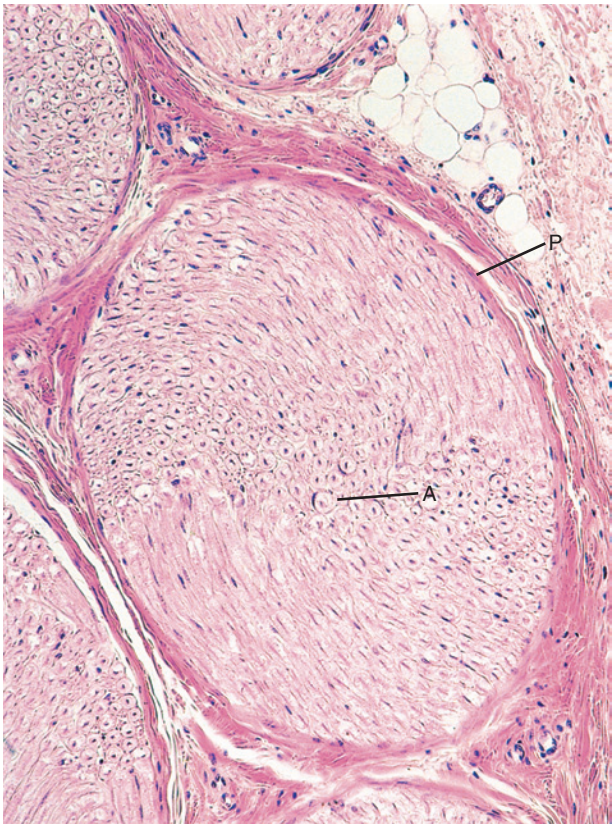


Figure 9–21 Light micrograph of a cross section of a peripheral nerve (×132). Observe the axons (A) and the perineurium (P) surrounding the fascicle.

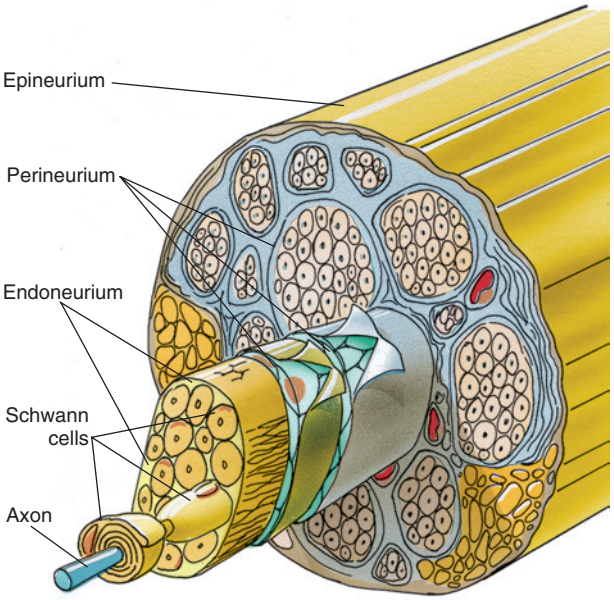


Figure 9–22 Diagram of the structure of a nerve bundle.

Connective Tissue Investments

Connective tissue investments of peripheral nerves include the epineurium, perineurium, and endoneurium.

Epineurium is the outermost layer of the three connective tissue investments covering nerves (see Fig. 9–22). The epineurium is composed of dense, irregular, collagenous connective tissue containing some thick elastic fibers that completely ensheaths the nerve. Collagen fibers within the sheath are aligned and oriented to prevent damage by overstretching of the nerve bundle. The epineurium is thickest where it is continuous with the dura covering the CNS at the spinal cord or brain, where the spinal or cranial nerves originate, respectively. The epineurium becomes progressively thinner as the nerves branch into smaller nerve components, eventually disappearing.

Perineurium, the middle layer of connective tissue investments, covers each bundle of nerve fibers (fascicle) within the nerve. The perineurium is composed of dense connective tissue but is thinner than epineurium. Its inner surface is lined by several layers of **epithelioid cells** joined to one another by **zonulae occludentes** and surrounded by a basal lamina that isolates the neural environment, and in that fashion facilitates the creation of the **blood–nerve barrier**. Between the layers of epithelioid cells are sparse collagen fibers oriented longitudinally and intertwined with a few elastic fibers. The thickness of the perineurium is progressively reduced to a sheet of flattened cells as the fascicle diminishes in diameter.

Endoneurium, the innermost layer of the three connective tissue investments of a nerve, surrounds individual nerve fibers **(axons)** and is composed of a loose connective tissue composed of a thin layer of reticular fibers (produced by the underlying Schwann cells), scattered fibroblasts, fixed macrophages, capillaries, and perivascular mast cells bathed in extracellular fluid. The endoneurium is in contact with the basal lamina of the Schwann cells. Thus, the endoneurium is housed in a compartment completely isolated from the perineurium and Schwann cells, an important factor in regulation of the microenvironment of the nerve fiber. Near the distal terminus of the axon, the endoneurium is reduced to a scant amount of reticular fibers surrounding the basal lamina of the Schwann cells of the axon.

Functional Classification of Nerves

Functionally, nerve fibers are classified as either sensory (afferent) or motor (efferent).

Nerve fibers are segregated functionally into sensory **(afferent)** fibers and motor **(efferent)** fibers. Sensory nerve fibers carry sensory input from the cutaneous areas of the body and from the viscera back to the CNS for processing. Motor nerve fibers originate in the CNS and carry motor impulses to the effector organs. The sensory roots and motor roots of the spinal nerve unite to form **mixed peripheral nerves**, the **spinal nerves**, which carry both sensory and motor fibers.

Conduction Velocity

The **conduction velocity** of peripheral nerve fibers depends on the extent of their myelination. In myelinated nerves, it is only at the nodes of Ranvier that ions can cross the axonal plasma membrane, initiating depolarization for two reasons:

1. Voltage-gated Na^+ channels of the axon plasmalemma are clustered mostly at the nodes of Ranvier.
2. The myelin sheath covering the internodes prevents the outward movement of the excess Na^+ in the axoplasm associated with the action potential.

Therefore, the excess positive ions can diffuse only through the axoplasm to the next node, triggering depolarization there. As indicated previously, the action potential "jumps" from node to node, a process called ***saltatory conduction*** (see Fig. 9–16*B*).

As noted previously, unmyelinated nerve fibers lack a myelin sheath and, therefore, lack nodes of Ranvier. These nerve fibers are surrounded by a single layer of Schwann cell plasma membrane and cytoplasm, which provides little insulation. Moreover, voltage-gated Na^+ channels are distributed along the entire length of the axon plasma membrane. Therefore, impulse propagation in unmyelinated fibers occurs by **continuous conduction**, which is slower and requires more energy than the saltatory conduction occurring in myelinated fibers.

As demonstrated in Table 9–2, peripheral nerve fibers are classified into three major groups according to their conduction velocity. In thin unmyelinated fibers, the conduction velocity ranges from about 0.5 to 2 m/sec, whereas, in heavily myelinated fibers, it ranges from 15 to 120 m/sec.

The sensory component of the PNS is presented in various chapters related to function.

SOMATIC MOTOR AND AUTONOMIC NERVOUS SYSTEMS

Functionally, the motor component is divided into the somatic and autonomic nervous systems.

The motor component of the nervous system is divided functionally into the somatic nervous system and the autonomic nervous system. The **somatic nervous system** provides motor impulses to the skeletal muscles, whereas the **autonomic nervous system** provides motor impulses to the smooth muscles of the viscera, cardiac muscle of the heart, and secretory cells of the exocrine and endocrine glands, thus helping maintain homeostasis.

Motor Component of the Somatic Nervous System

Motor innervation to skeletal muscles is provided by somatic nerves.

Skeletal muscles receive motor nerve impulses conducted to them by spinal and selected cranial nerves of the somatic nervous system. The cell bodies of these nerve fibers originate in the CNS. The cranial nerves containing **somatic efferent components** are cranial nerves III, IV, VI, and XII (excluding those nerves supplying muscles of branchiomeric rather than mesodermal origin). Most of the 31 pairs of spinal nerves contain somatic efferent components to skeletal muscles.

Cell bodies of neurons of the somatic nervous system originate in motor nuclei of the cranial nerves embedded within the brain or motor nuclei embedded in the ventral horn of the spinal cord. These neurons are multipolar, and their axons leave the brain or spinal cord and travel to the skeletal muscle via the cranial nerves or spinal nerves (Fig. 9–23). They synapse with the skeletal muscle at the motor end plate (see Chapter 8).

TABLE 9–2 Classification of Peripheral Nerve Fibers

Fiber Group	Diameter (μm)	Conduction Velocity (m/sec)	Function
Type A fibers—heavily myelinated	1–20	15–120	High-velocity fibers: acute pain, temperature, touch, pressure, proprioception, somatic efferent fibers
Type B fibers—less heavily myelinated	1–3	3–15	Moderate-velocity fibers: visceral afferents, preganglionic autonomics
Type C fibers—unmyelinated	0.5–1.5	0.5–2	Slow-velocity fibers: postganglionic autonomics, chronic pain

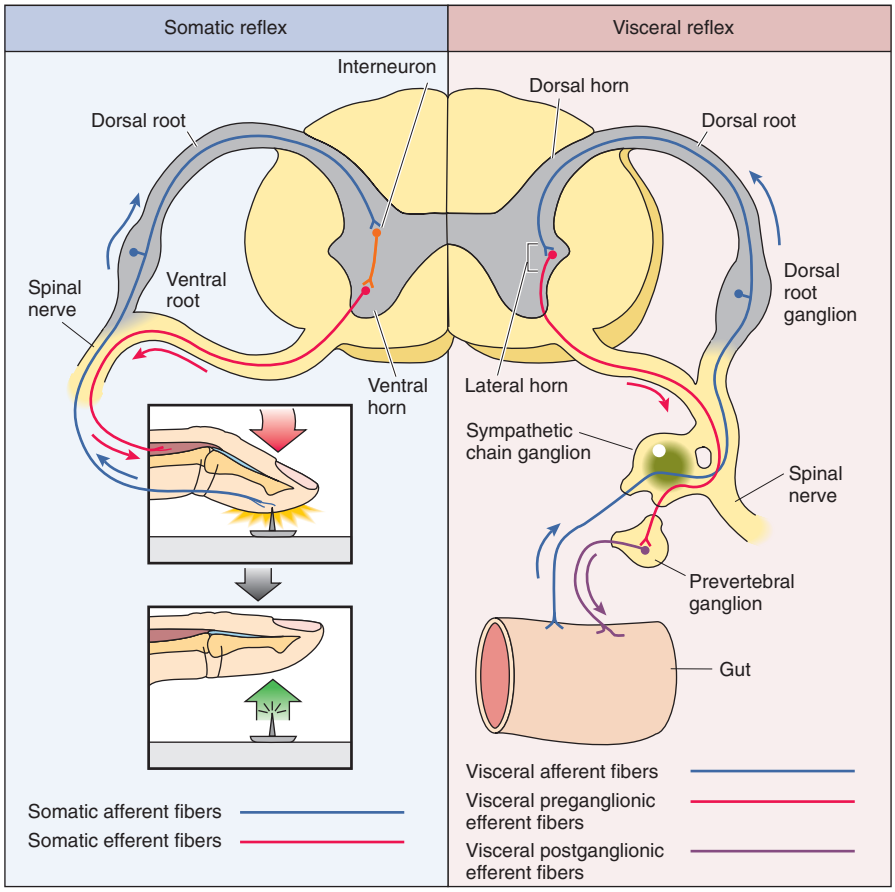


Figure 9–23 Diagram comparing somatic and visceral reflexes.

Autonomic Nervous System

Autonomic nerves provide motor innervation to smooth muscle and cardiac muscle and supply secretomotor innervation to glands.

The **autonomic (involuntary, visceral) nervous system** is generally defined as a motor system; although agreement on this point is not universal, it is regarded as a motor system in this discussion. The autonomic nervous system controls the viscera of the body by supplying the **general visceral efferent (visceral motor)** component to smooth muscle, cardiac muscle, and secretory cells of glands.

In contrast to the somatic system, in which one neuron, originating in the CNS, acts directly on the effector organ, the autonomic nervous system possesses two neurons between the CNS and the effector organ.

Cell bodies of the first neurons in the chain lie in the CNS and their axons are usually myelinated. These **preganglionic fibers (axons)** enter an **autonomic ganglion** located outside of the CNS where they synapse on multipolar cell bodies of **postganglionic neurons**. Postganglionic fibers, which are usually unmyelinated, although they are always enveloped by Schwann cells, exit the ganglion to terminate on the **effector organ** (smooth muscle, cardiac muscle, or secretory cells of gland).

In contrast to the somatic system, the autonomic system has postganglionic synapses that branch out, and the neurotransmitter diffuses out for some distance to the effector cells, thus contributing to more prolonged and widespread effects than in the somatic system. Smooth muscle cells stimulated by the neurotransmitter activate adjacent smooth muscle cells to contract by relaying the information via gap junctions.

The autonomic nervous system is subdivided into three functionally different divisions (Fig. 9–24):

- The **sympathetic nervous system** generally prepares the body for action by increasing respiration, blood pressure, heart rate, and blood flow to the skeletal muscles, dilating pupils of the eye, and generally slowing down visceral function.
- The **parasympathetic nervous system** tends to be functionally antagonistic to the sympathetic system,

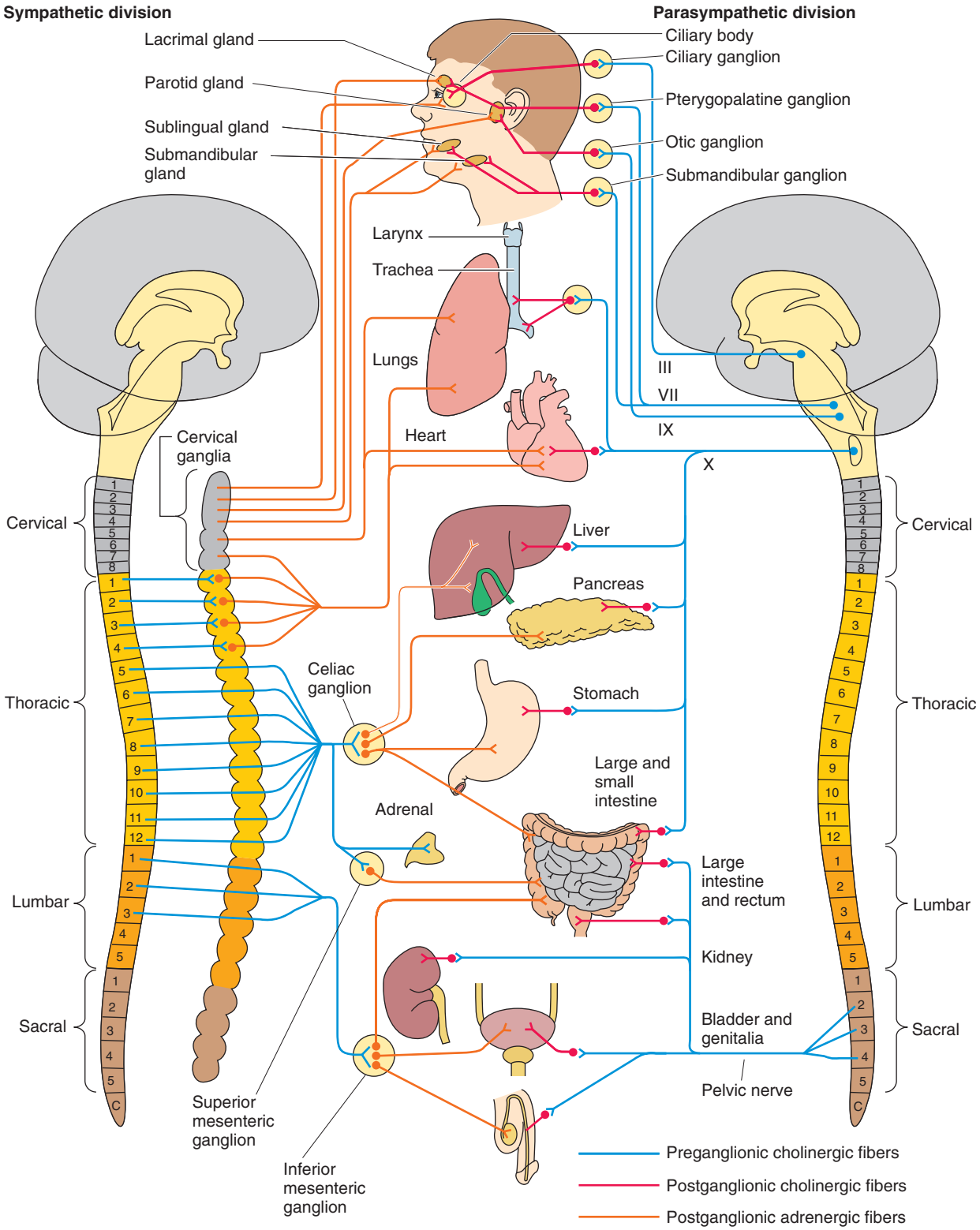


Figure 9–24 Schematic diagram of the autonomic nervous system. *Left,* Sympathetic division. *Right,* Parasympathetic division.

in that it decreases respiration, blood pressure, and heart rate; reduces blood flow to skeletal muscles; constricts the pupils; and generally increases the actions and functions of the visceral system.
  - Thus, the parasympathetic nervous system brings about homeostasis, whereas the sympathetic nervous system prepares the body for "flight or fight or freeze (faint)" (see the next section on the sympathetic nervous system). The sympathetic nervous system is broadly considered to function in **vasoconstriction**, whereas the parasympathetic nervous system is broadly considered to be **secretomotor** in function. Because the visceral components of the body receive innervation from both divisions of the autonomic nervous system, these two systems are balanced in health.
- The **enteric nervous system** is localized strictly to the gastrointestinal tract; although it can act independently in controlling the digestive process, the sympathetic and parasympathetic components of the autonomic nervous system can and do modulate its function.

Acetylcholine is the neurotransmitter at all synapses between preganglionic and postganglionic fibers and between parasympathetic postganglionic endings and effector organs. **Norepinephrine** and **epinephrine** are the neurotransmitters at synapses between postganglionic sympathetic fibers and effector organs. However, sweat glands and arrector pili muscles that elevate the hair follicles of skin are the exceptions to this rule because the postganglionic sympathetic fibers to these structures release **acetylcholine** rather than the expected epinephrine or norepinephrine.

Generally, preganglionic fibers of the sympathetic system are short, but postganglionic fibers are long. In contrast, preganglionic fibers of the parasympathetic system are long, whereas postganglionic fibers are short.

Sympathetic Nervous System

The effect of the sympathetic nervous system is to prepare the body for "flight or fight or faint."

The sympathetic nervous system originates in the spinal cord from segments of the thoracic spinal cord and upper lumbar spinal cord (T1 to L2). Thus, the sympathetic nervous system is sometimes called the ***thoracolumbar outflow*** (see Fig. 9–24). Cell bodies of preganglionic neurons are small, spindle-shaped cells that originate in the lateral horn of the spinal cord; their axons exit the cord via the ventral roots to join the spinal nerve. After a short distance, the fibers leave the peripheral nerve, via white rami communicantes, to enter one of the paravertebral chain ganglia.

Typically, the preganglionic neuron either synapses on a cell body of one of the multipolar postganglionic neurons residing in the ganglion associated with that spinal cord segment or ascends or descends in the sympathetic trunk to synapse on a cell in another of the chain ganglia. Certain preganglionic fibers do not synapse in the chain ganglia, however, and instead pass through to enter the abdominal cavity as splanchnic nerves. Here, they seek collateral ganglia located along the abdominal aorta for synapsing on cell bodies of postganglionic fibers residing there.

Axons of postganglionic neurons housed in the chain ganglia exit the ganglia, via gray rami communicantes, to reenter the peripheral nerve for distribution to effector organs in the periphery (i.e., sweat glands, blood vessels, dilator pupillae muscles, cardiac muscle, bronchial tree, salivary glands, and arrector muscles of hair).

Axons of postganglionic neurons housed in the collateral ganglia exit the ganglia and accompany the myriad blood vessels to the viscera, where they synapse on the effector organs (i.e., blood vessels and the smooth muscles and glands of the viscera).

Essentially, the sympathetic nervous system prepares the body to fight an attacker or flee from an attacker, or in the case of a catastrophic event, causes the individual to the individual freeze (or faint) to simulate death and thereby hope to stop the attack.

Parasympathetic Nervous System

The effect of the parasympathetic nervous system is to prepare the body to "rest or digest."

The **parasympathetic nervous system** originates in the brain and the sacral segments of the spinal cord (S2 to S4); thus, the parasympathetic system is called the ***craniosacral outflow*** (see Fig. 9–24).

Cell bodies of **preganglionic parasympathetic neurons** originating in the brain lie in the **visceromotor nuclei** of the four cranial nerves that carry visceral motor components (cranial nerves III, VII, IX, and X).

Axons of **preganglionic parasympathetic fibers** of cranial nerves III, VII, and IX seek **parasympathetic (terminal) ganglia** located outside the brain case, where they synapse on cell bodies of **postganglionic parasympathetic neurons** housed in the ganglia. Axons of these nerves are usually delivered by cranial nerve V to the effector organs they serve, including salivary glands and mucous glands, whereas cranial nerve III delivers postganglionic parasympathetic fibers to the ciliary muscle and the sphincter pupillae muscles of the eye.

Axons of **preganglionic parasympathetic fibers** in cranial nerve X travel to the thorax and abdomen before synapsing in the terminal ganglia within the respective viscera.

Axons of **postganglionic parasympathetic nerves** synapse on the glands, smooth muscles, and cardiac muscle.

Cell bodies of **preganglionic parasympathetic nerves** originating in segments of the sacral spinal cord are located in the lateral segment of the ventral horn and leave via the ventral root with the sacral nerves. From here, the axons project to the enteric nervous system, specifically to ganglia located in plexuses in the walls of the gastrointestinal tract.

Axons of other **postganglionic neurons** synapse on the effector organs in the viscera of the lower abdominal wall and the pelvis.

Enteric Nervous System

The function of the enteric nervous system is to control the digestive processes of the gastrointestinal tract.

The enteric nervous system has two components, those that belong only to the **enteric nervous system**, the intrinsic components, and those that belong to the sympathetic and parasympathetic nervous systems, the extrinsic components.

The **intrinsic components** are derived from neural crest material and are located completely within the wall of the alimentary canal. The neurons of the enteric nervous system project to two sets of ganglia, known as **Meissner submucosal plexus** and **Auerbach myenteric plexus**. It has been estimated that the total number of neurons intrinsic to the enteric nervous system is equal to or greater than the number of neurons located in the entire spinal cord.

- **Auerbach myenteric plexus** functions in the localized aspects of the digestive tract, such as eliciting secretions from the glands of the mucosa and contractions of the smooth muscle cells of the muscularis mucosae. This plexus is located between the inner circular and outer longitudinal layers of the muscularis externa of the digestive tract.
- **Meissner submucosal plexus** functions in the overall process of digestion, namely peristalsis, that is the movement of the food along the length of the alimentary canal. This plexus is located at the junction of the submucosa with the inner circular layer of the muscularis externa.

There is a constant communication between Auerbach and Meissner plexuses to make sure that the process of digestion progresses in the proper fashion.

The **extrinsic components** of the enteric nervous system are composed of the **sympathetic nerve fibers** derived from the splanchnic nerves and the **parasympathetic nerve fibers** carried by cranial nerve X (the vagus nerve) that impinge on and modulate the activities of the intrinsic components of the enteric nervous system. The former decreases and the latter increases the rate of peristalsis. The process of digestion is described in Chapters 17 and 18.

GANGLIA

Ganglia are aggregations of cell bodies of neurons located outside of the CNS. There are two types of ganglia: **sensory** and **autonomic**.

Sensory Ganglia

Sensory ganglia house cell bodies of sensory neurons.

Sensory ganglia are associated with cranial nerves V, VII, IX, and X and with each of the spinal nerves originating from the spinal cord. A sensory ganglion of a cranial nerve appears as a swelling of the nerve either inside the cranial vault or at its exit. Ganglia are usually identified with specific names that relate to the nerves. Sensory ganglia of the spinal nerves are called **dorsal root ganglia** and they house unipolar (pseudounipolar) cell bodies of the sensory nerves enveloped by cuboidal **capsule cells**. These capsule cells are then surrounded by a connective tissue capsule composed of **satellite cells** and collagen. The endoneurium of each axon becomes continuous with the connective tissue surrounding the ganglia. Peripheral processes of the neurons possess specialized receptors at their terminals to transduce various types of stimuli from the internal and external environments. Central processes pass from the ganglion to the brain within the cranial nerves or to the spinal cord within the spinal nerves, where they terminate on other neurons for processing.

Autonomic Ganglia

Autonomic ganglia house cell bodies of postganglionic autonomic nerves.

As discussed previously, by definition, nerve cell bodies of **autonomic ganglia** are motor in function because they cause smooth or cardiac muscle contraction or glandular secretion. In the sympathetic system, **preganglionic sympathetic fibers** synapse on postganglionic sympathetic cell bodies in the sympathetic ganglia located in either the **sympathetic chain ganglia**, adjacent to the spinal cord, or the **collateral ganglia**, along the abdominal aorta in the abdomen. **Postganglionic sympathetic nerves** originating in these ganglia are then distributed, for the most part, by peripheral nerves that they join after exiting the ganglia. They then terminate in the effector organs that they innervate.

In the parasympathetic system, **preganglionic parasympathetic fibers** originate in one of two places: in certain cranial nerves or, as previously described, in certain segments of the sacral spinal cord. These fibers synapse on postganglionic cell bodies (Fig. 9–25) located

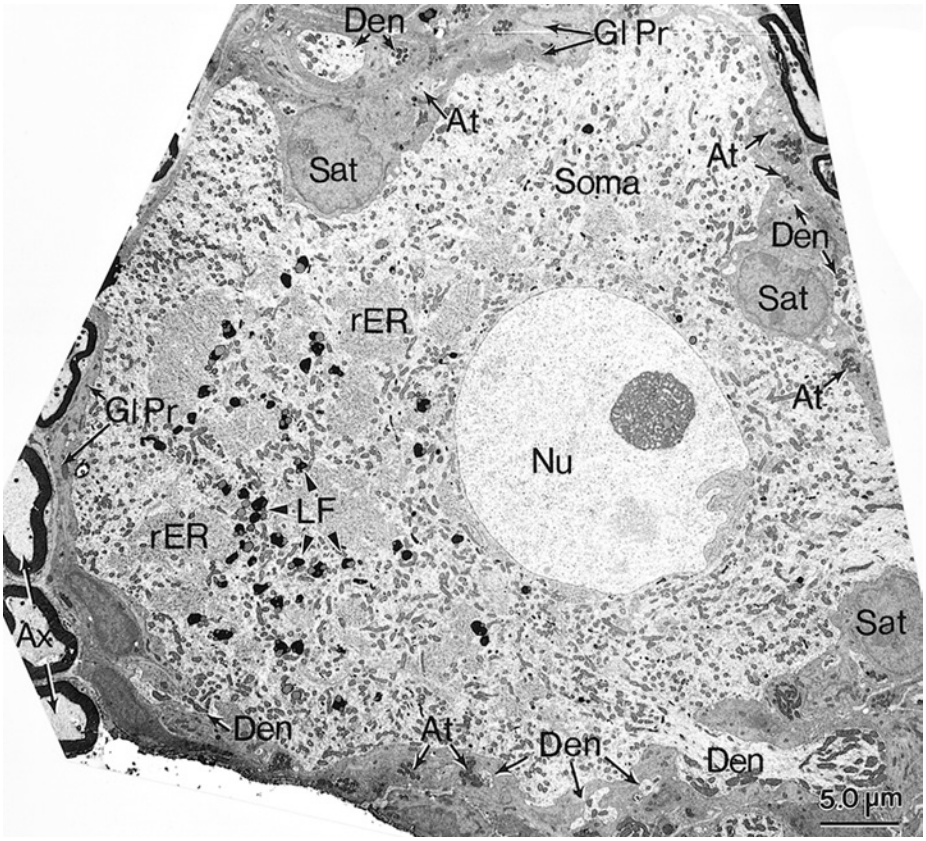


Figure 9–25 Electron micrograph of the ciliary ganglion. At, Axon terminal; Ax, axon; Den, dendrite; GIPr, gastric inhibitory peptide receptor; LF, lipofuscin granules; Nu, nucleus; rER, rough endoplasmic reticulum; Sat, satellite cells. (From May PJ, Warren S. Ultrastructure of the macaque ciliary ganglion. *J Neurocytol*. 1993;22:1073-1095.)

in **terminal ganglia**. Preganglionic parasympathetic fibers originating in the nuclei (term for a cluster of nerve cell bodies located in the CNS) of the cranial nerves conducting parasympathetic fibers synapse in one of the four **terminal ganglia** located in the head (except those of cranial nerve X). Terminal ganglia associated with cranial nerve X and for preganglionic fibers arising from the sacral spinal cord are located in the walls of the viscera.

Postganglionic parasympathetic nerves originating in the terminal ganglia within the head exit the ganglia and usually join the trigeminal nerve (V) to be distributed to the effector organs. Those postganglionic parasympathetic nerves originating in the ganglia located in the walls of the viscera pass directly to the effector organs located within the viscera.

CENTRAL NERVOUS SYSTEM

The **central nervous system**, composed of the brain and the spinal cord, consists of white matter and gray matter without intervening connective tissue elements; therefore, the CNS has the consistency of a semi-firm gel.

White matter is composed mostly of myelinated nerve fibers along with some unmyelinated fibers and neuroglial cells; its white color results from the abundance of myelin surrounding the axons. **Gray matter** consists of aggregations of neuronal cell bodies, dendrites, and unmyelinated portions of axons as well as neuroglial cells; the absence of myelin causes these regions to appear gray in live tissue.

Axons, dendrites, and neuroglial processes form a tangled network of neural tissue called the ***neuropil*** (Fig. 9–26). In certain regions, aggregations of neuron cell bodies embedded in white matter are called ***nuclei***, whereas their counterparts in the PNS are called *ganglia*.

Gray matter in the brain is located at the periphery **(cortex)** of the cerebrum and cerebellum and forms the deeper basal ganglia, whereas the white matter lies deep to the cortex and surrounds the basal ganglia (it

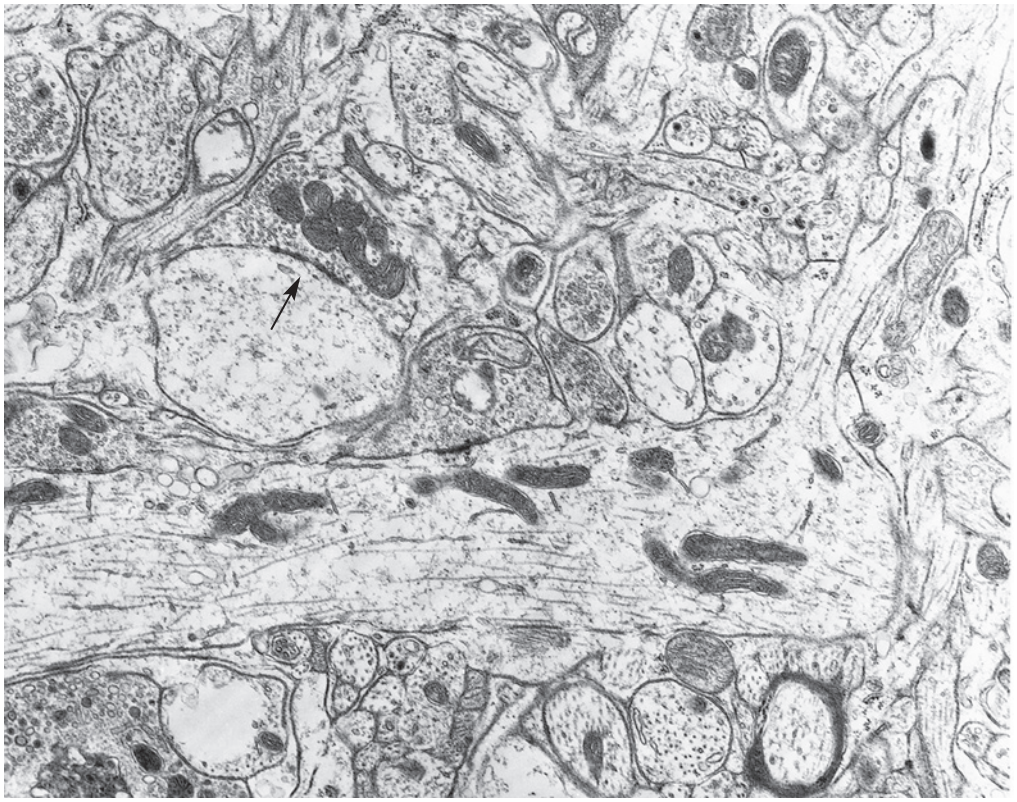


Figure 9–26 Electron micrograph of axodendritic synapses. (From Jennes L, Traurig HH, Conn PM. *Atlas of the Human Brain*. Philadelphia: Lippincott-Raven; 1995.)

should be noted that the correct term for the **basal ganglia** is ***basal nuclei***). The reverse is true in the spinal cord; white matter is located in the periphery of the spinal cord, whereas gray matter lies deep in the spinal cord, where it forms an H shape in cross-section. A small **central canal**, lined by **ependymal cells** and representing the lumen of the original neural tube, lies in the center of the crossbar of the H. The upper vertical bars of the H represent the **dorsal horns** of the spinal cord, which receive central processes of the sensory neurons whose cell bodies lie in the **dorsal root ganglion**. Cell bodies of interneurons are also located in the dorsal horns. Cell bodies of **interneurons (internuncial neurons** or **intercalated neurons)** originate in the CNS and are entirely confined there, where they form networks of communication for integration between sensory and motor neurons. Interneurons constitute the vast majority of the neurons of the body. The lower vertical bars of the H represent the **ventral horns** of the spinal cord, which house cell bodies of large multipolar motor neurons whose axons exit the spinal cord via the ventral roots.

Meninges

The three connective tissue coverings of the brain and spinal cord are the **meninges**. The outermost layer of the meninges is the **dura mater**, the intermediate layer is the **arachnoid**, and the innermost or intimate layer of the meninges is the **pia mater** (Fig. 9–27).

Dura Mater

Dura mater, the outermost layer of the meninges, is composed of a dense collagenous connective tissue.

The **dura mater** covering the brain is a dense, collagenous connective tissue composed of two layers that are closely apposed in the adult. **Periosteal dura mater**, the outer layer, is composed of osteoprogenitor cells, fibroblasts, and organized bundles of collagen fibers that are loosely attached to the inner surface of the skull, except at the sutures and base of the skull, where the attachment is firm. As the name implies, periosteal dura mater serves as the periosteum of the inner surface of the skull, and as such it is well vascularized.

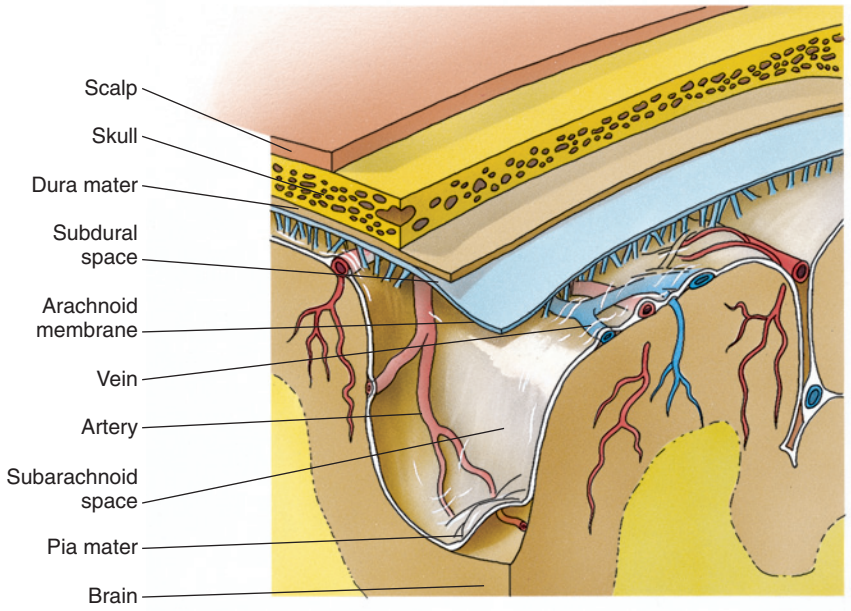


Figure 9–27 Diagram of the skull and the layers of the meninges covering the brain.

The inner layer of the dura, **meningeal dura mater**, is composed of fibroblasts displaying dark-staining cytoplasm, elongated processes, ovoid nuclei, and sheet-like layers of fine collagen fibers. This layer also contains small blood vessels.

A layer of cells internal to the meningeal dura, called the **border cell layer**, is composed of flattened fibroblasts exhibiting long processes that are occasionally attached to one another by desmosomes and gap junctions. Collagen fibers are lacking in this layer, but in their place, an extracellular, amorphous, flocculent material (believed to be composed of a proteoglycan) surrounds the fibroblasts and extends into the interface between this layer and the meningeal dura.

Spinal dura mater does not adhere to the walls of the vertebral canal; rather, it forms a continuous tube from the foramen magnum to the second segment of the sacrum and is pierced by the spinal nerves. The **epidural space**, the space between the dura and the bony walls of the vertebral canal, is filled with epidural fat and a venous plexus.

Arachnoid

The arachnoid is the intermediate layer of the meninges.

The **arachnoid** layer of the meninges is avascular, that is, it does not have a vascular supply of its own even though blood vessels course through it. This intermediate layer of the meninges consists of fibroblasts, collagen, and some elastic fibers. The fibroblasts form gap junctions and desmosomes with one another. The arachnoid is composed of two regions. The first is a flat, sheet-like membrane in contact with the dura. The second is a deeper, gossamer-like region composed of loosely arranged **arachnoid trabecular cells** (modified fibroblasts), along with a few collagen fibers, which form trabeculae that contact the underlying pia mater. These arachnoid trabeculae span the **subarachnoid space**, the space between the sheet-like portion of the arachnoid and the pia. The arachnoid trabecular cells have long processes that attach to one another via desmosomes and communicate with one another by gap junctions.

The interface between the dura and arachnoid, the **subdural space**, is considered a potential space because it appears only as the aftermath of injury resulting in subdural hemorrhage, when blood forces these two layers apart.

Blood vessels from the dura pierce the arachnoid on their way to the vascular pia mater. However, these vessels are isolated both from the arachnoid and from the subarachnoid space by a close investment of arachnoid-derived, modified fibroblasts. In certain regions, the arachnoid extends through the dura to form **arachnoid villi**, which protrude into the spaces connected to the lumina of the dural venous sinuses. These specialized regions of the arachnoid function in transporting CSF from the subarachnoid space into the venous system. In later life, the villi enlarge and become sites for calcium deposits.

The interface between the arachnoid mater and pia mater is difficult to distinguish; therefore, the two layers are often called the ***pia-arachnoid***, with both surfaces being covered by a thin layer of squamous **epithelioid cells** composed of modified fibroblasts.

Pia Mater

Pia mater, the innermost, highly vascular layer of the meninges, is in close contact with the brain.

The **pia mater** is the innermost layer of the meninges and is intimately associated with the brain tissue, following closely all of its contours. The pia mater does not quite come into contact with the neural tissue, however, because a thin layer of neuroglial processes is always interposed between them.

The pia mater is composed of a thin layer of flattened, modified fibroblasts that resemble arachnoid trabecular cells. Blood vessels, abundant in this layer, are surrounded by **pial cells** interspersed with macrophages, mast cells, and lymphocytes. Fine collagenous and elastic fibers lie between the pia and neural tissue.

The pia mater is completely separated from the underlying neural tissue by neuroglial cells. Blood vessels penetrate the neural tissues and are covered by pia mater until they form the **continuous capillaries** characteristic of the CNS. **End-feet of astrocytes**, known as ***pedicels***, rather than pia mater, cover capillaries within the neural tissue.

CLINICAL CORRELATIONS

1. **Meningiomas** are slow-growing tumors of the meninges that are usually benign and produce clinical effects by compressing the brain and increasing intracranial pressure.
2. **Meningitis** is an inflammation of the meninges resulting from bacterial or viral infection in the CSF. Viral meningitis is not as severe a condition as bacterial meningitis, which is contagious and could be a very severe condition leading to brain damage, hearing loss, learning disability, and death, if untreated. Presently, in the United States, all children 4 years of age or younger have been vaccinated for the most prevalent form of this disease. Major symptoms of bacterial meningitis include fever, headache, stiff neck, and alteration of consciousness. Diagnosis is based on spinal fluid culture to determine the bacterial species involved followed by treating with a specific antibiotic. Bacterial meningitis can be spread by the exchange of respiratory and throat secretions (while coughing and kissing).

Blood–Brain Barrier

Endothelial cells of CNS capillaries form a barrier known as the blood-brain barrier *that prevents the free passage of selective blood-borne substances into the neural tissue.*

A highly selective barrier, known as the **blood–brain barrier**, exists between specific blood-borne substances and the neural tissue of the CNS. This barrier is established by the endothelial cells lining the **continuous capillaries** that course through the CNS. These endothelial cells form fasciae occludentes with one another, retarding the paracellular route (the flow of materials between cells). Additionally, these endothelial cells have relatively few pinocytotic vesicles, and vesicular traffic is almost completely restricted to **receptor-mediated transport**.

Macromolecules injected into the vascular system cannot enter the intercellular spaces of the CNS; conversely, macromolecules injected into the intercellular spaces of the CNS cannot enter the capillary lumen. Certain substances, however, such as oxygen, water, and carbon dioxide, can easily penetrate the blood–brain barrier mostly due to the presence of **aquaporins** located in endothelial cell membrane. Other small molecules, such as lipid-soluble materials and certain drugs can also cross the blood–brain barrier. Molecules such as glucose, amino acids, certain vitamins, and nucleosides are transferred across the blood–brain barrier by specific carrier proteins, many via facilitated diffusion. Ions are also transported across the blood–brain barrier through carrier proteins via active transport. The energy requirement for this process is provided by the presence of large numbers of mitochondria within the endothelial cell cytoplasm.

Capillaries of the CNS are invested by well-defined basal laminae, which, in turn, are almost completely surrounded by the end-feet of numerous astrocytes, collectively called the ***perivascular glia limitans***. It is believed that these astrocytes help convey metabolites from blood vessels to neurons. Additionally, astrocytes remove excess K^+ and neurotransmitters from the neuron's environment, thus maintaining the neurochemical balance of the CNS extracellular milieu.

Recently, a new concept has emerged, known as the ***neurovascular unit***, consisting of the blood–brain barrier (capillary endothelium), end-feet of astrocytes contacting the blood vessels of the brain, pericytes, and neurons that adjoin the blood vessels of the brain. These components of the neurovascular unit interact with each other to control the movement of molecules, that is, whether to permit or deny them the ability to cross the blood–brain barrier.

CLINICAL CORRELATIONS

1. Because the blood–brain barrier is very selective, antibiotics, some therapeutic drugs, and certain neurotransmitters (e.g., dopamine) cannot penetrate it. Perfusion of a hypertonic solution of **mannitol** dehydrates the endothelial lining, causing these cells to shrink and thereby transiently relaxing the tight junctions of the capillary endothelial cells permitting the movement of therapeutic drugs across the blood–brain barrier. Therapeutic drugs can also be bound to antibodies developed against **transferrin receptors** in the endothelial cells of the capillaries, permitting their transport across the blood–brain barrier and into the CNS.
2. In some diseases of the CNS (e.g., stroke, infection, tumors), the integrity of the blood–brain barrier is compromised, resulting in the accumulation of toxins and extraneous metabolites in the extracellular environment.

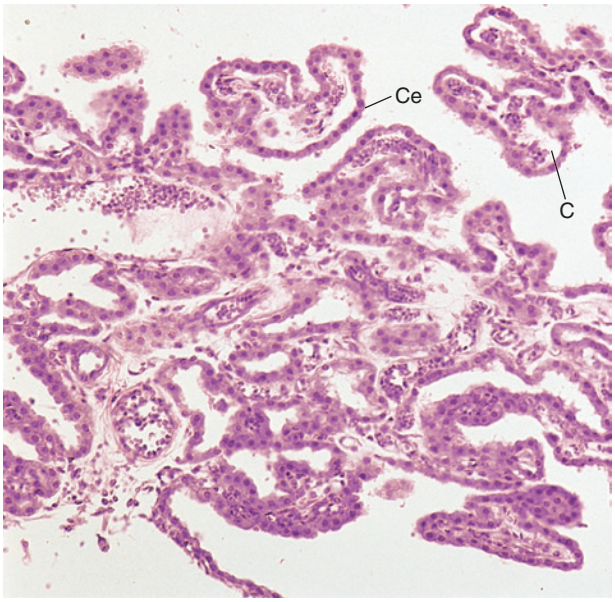


Figure 9–28 Light micrograph of the choroid plexus (×270). Observe capillaries (c) and the simple cuboidal epithelium of the choroid plexus (Ce).

Choroid Plexus

The choroid plexus, composed of folds of pia mater within the ventricles of the brain, produces the CSF.

Folds of pia mater housing an abundance of fenestrated capillaries and invested by the simple cuboidal (ependymal) lining extend into the third, fourth, and lateral ventricles of the brain, forming the **choroid plexus** (Fig. 9–28). The choroid plexus produces CSF, which fills the ventricles of the brain and central canal of the spinal cord. The CSF bathes the CNS as it circulates through the subarachnoid space. Although more than half of the CSF is produced by the choroid plexus, there is evidence that parenchyma in several other regions of the brain produce a substantial amount of CSF, which diffuses through the ependymal lining to enter the ventricles.

Cerebrospinal Fluid

CSF bathes, nourishes, and protects the brain and spinal cord.

CSF is produced by the choroid plexus at the rate of about 14 to 36 mL/hr, replacing its total volume about four to five times daily. CSF circulates through the ventricles of the brain, the subarachnoid space, the perivascular space, and the central canal of the spinal cord. CSF is low in protein but rich in sodium, potassium, and chloride ions. It is clear and has a low density. Consisting of about 90% water and ions, it may also contain a few desquamated cells and occasional lymphocytes.

CSF is important to the metabolic activity of the CNS because brain metabolites diffuse into the CSF as it passes through the subarachnoid space. It also serves as a liquid cushion for protection of the CNS. CSF is able to flow by diffusion and is reabsorbed through the thin cells of the arachnoid villi in the superior sagittal venous sinus from where the CSF is returned to the bloodstream.

Recently it was discovered that the flow of CSF through the brain tissue increases while the individual is asleep. It appears that during sleep protoplasmic astrocytes shrink in size, thereby increasing the interstitial spaces in the gray matter by at least 50%. Due to this enlargement, the flow rate and flow volume of the CSF are greatly increased though the gray matter. It is believed that this rapid flow and increased volume permit a more efficient flushing of the interstitial spaces, thus removing waste products and excess ions and neurotransmitter substances from the microenvironment of the neurons and their synapses. It is now proposed that this cleansing of the interstitial spaces of the CNS is the reason why almost all members of the animal kingdom have to sleep. Once the individual awakens, the protoplasmic astrocytes become swollen, thereby decreasing the interstitial spaces and limiting the rate and volume of CSF perfusion of the interstitial spaces of the CNS.

TABLE 9–3 Comparison Between Serum and Cerebrospinal Fluid

Constituent	Serum	Cerebrospinal Fluid
White blood cells (cells/mL)	0	0–5
Protein (g/L)	60–80	Negligible
Glucose (mmol/L)	4.0–5.5	2.1–4.0
Na^+ (mmol/L)	135–150	135–150
K^+ (mmol/L)	4.0–5.1	2.8–3.2
Cl^- (mmol/L)	100–105	115–130
Ca^{2+} (mmol/L)	2.1–2.5	1.0–1.4
Mg^{2+} (mmol/L)	0.7–1.0	0.8–1.3
pH	7.4	7.3

CLINICAL CORRELATIONS

Because CSF is constantly being produced, any decrease in absorption of the fluid by the arachnoid villi or blockage within the ventricles of the brain causes swelling in the brain tissue. This condition, called ***hydrocephalus***, leads to enlargement of the head in fetuses and neonates, impairment of mental and muscular functions, and death if left untreated.

Blood–Cerebrospinal Fluid Barrier

The chemical stability of the CSF is maintained by the **blood–CSF barrier**, which is composed of zonulae occludentes between the cells of the simple cuboidal epithelium. These tight junctions impede the movement of substances between cells, compelling the substances to take the transcellular route. The production of CSF thus depends on facilitated and active transport across the simple cuboidal epithelium, resulting in differences in composition between CSF and plasma (Table 9–3).

Cerebral Cortex

The cerebral cortex is responsible for learning, memory, sensory integration, information analysis, and initiation of motor responses.

Gray matter at the periphery of the cerebral hemispheres is folded into many **gyri** and **sulci** called the ***cerebral cortex***. This portion of the brain is responsible for learning, memory, information analysis, initiation of motor response, and integration of sensory signals.

The cerebral cortex is divided into six layers composed of neurons, which exhibit a morphology unique to the particular layer. The most superficial layer lies just deep to the pia mater; the sixth, or deepest, layer of the cortex is bordered by white matter of the cerebrum. The six layers and their components are as follows:

1. The **molecular layer** is composed mostly of nerve terminals originating in other areas of the brain, **horizontal cells**, and neuroglia.
2. The **external granular layer** contains mostly **granule** (stellate) **cells** and neuroglial cells.
3. The **external pyramidal layer** contains neuroglial cells and large **pyramidal cells**, which become increasingly larger from the external to the internal border of this layer.
4. The **internal granular layer** is a thin layer characterized by closely arranged, small **granule** (stellate) **cells**, **pyramidal cells**, and neuroglia. This layer has the greatest cell density of the cerebral cortex.
5. The **internal pyramidal layer** contains the largest **pyramidal cells** and neuroglia. This layer has the lowest cell density of the cerebral cortex.
6. The **multiform layer** consists of cells of various shapes **(Martinotti cells)**, and neuroglia.

Cerebellar Cortex

The cerebellar cortex is responsible for balance, equilibrium, muscle tone, and muscle coordination.

The layer of gray matter located in the periphery of the cerebellum is called the ***cerebellar cortex*** (Fig. 9–29). This portion of the brain is responsible for maintaining balance and equilibrium, muscle tone, and coordination of skeletal muscles. Histologically, the cerebellar cortex is divided into three layers:

1. The **molecular layer** lies directly below the pia mater and contains superficially located stellate cells, dendrites of **Purkinje cells**, basket cells, and unmyelinated axons from the granular layer.
2. The **Purkinje cell layer** contains the large, flask-shaped Purkinje cells, which are present in the cerebellum only (Fig. 9–30; see Figs. 9–4 and 9–29). Their arborized dendrites project into the molecular layer, and their myelinated axons project into the white matter. Each Purkinje cell receives hundreds of thousands of excitatory and inhibitory synapses that it must integrate to form the proper response. The Purkinje cell is the only cell of the cerebellar cortex that sends information to the outside, and it is always an **inhibitory output** using GABA as the neurotransmitter.
3. The **granular layer** (the deepest layer) consists of small granule cells and **glomeruli (cerebellar islands)**. Glomeruli are regions of the cerebellar cortex where synapses are taking place between axons entering the cerebellum and the granule cells.

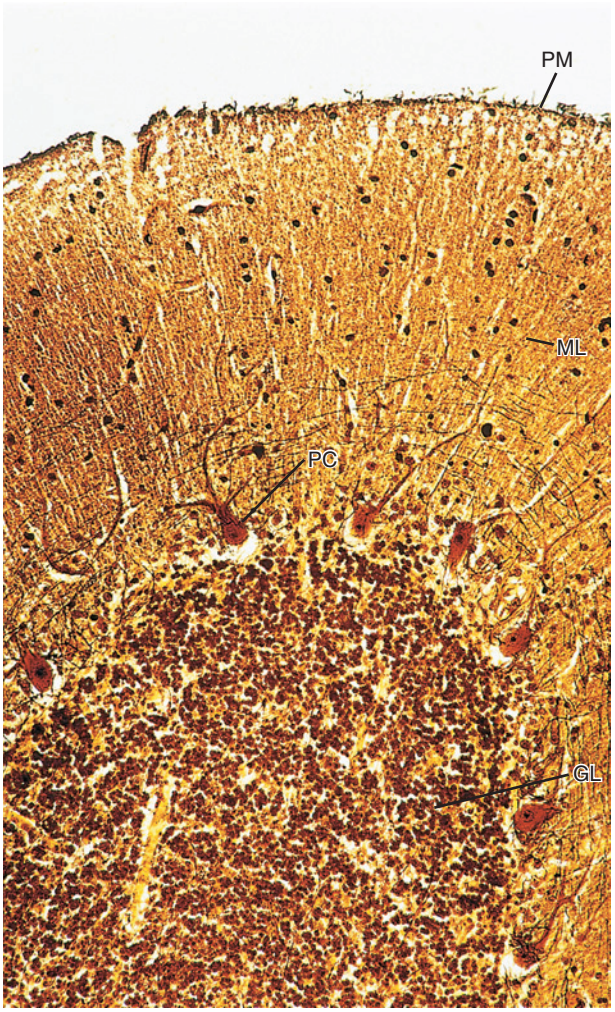


Figure 9–29 Light micrograph of the cerebellum showing its layers (×132). Pia mater (PM), molecular layer (ML), granular layer (GL). Especially note the prominent Purkinje cells (PC).

NERVE REGENERATION

Nerve cells, unlike neuroglial cells, cannot proliferate but can regenerate their axons, located in the PNS.

When a traumatic event destroys neurons, they are not replaced because usually neurons do not proliferate (although some suggest that proliferation of certain neurons may take place even within the CNS); therefore, the damage to the CNS is mostly permanent. However, if a peripheral nerve fiber is injured or transected, the neuron attempts to repair the damage, regenerate the process, and restore function by initiating a series of structural and metabolic events, collectively called the ***axon reaction***.

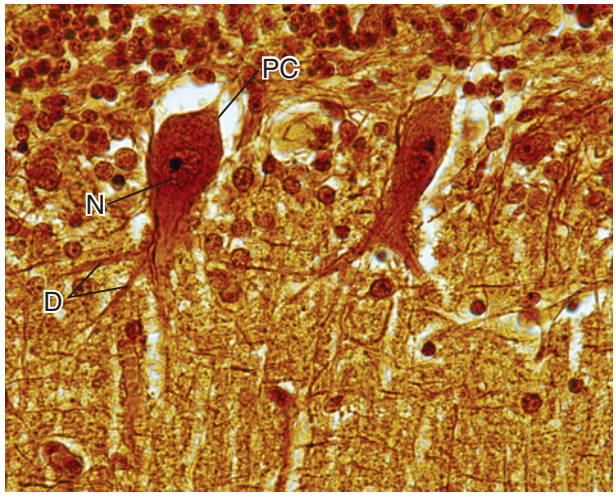


Figure 9–30 Higher magnification light micrograph of the granular layer of the cerebellum illustrating Purkinje cells. The multipolar Purkinje cells (PC) display a nucleus (N) and a dendritic tree (D). Please note that this figure is upside down compared to Figure 9–29 (×540).

Axon Reaction

The reactions to the trauma are characteristically localized in three regions of the neuron: (1) at the site of damage **(local changes)**, (2) distal to the site of damage **(anterograde changes)**, and (3) proximal to the site of damage **(retrograde changes)**. Some of the changes occur simultaneously, whereas others may occur weeks or months apart. The following description of nerve regeneration assumes that the cut ends remain near each other; otherwise, regeneration is unsuccessful (Fig. 9–31).

Local Reaction

Local reaction to injury involves repair and removal of debris by neuroglial cells.

The severed ends of the axon retract away from each other, and the cut membrane of each stump fuses to cover the open end, preventing loss of axoplasm. Each severed end begins to expand as material delivered by axoplasmic flow accumulates. Macrophages and fibroblasts infiltrate the damaged area, secrete cytokines and growth factors, and up-regulate the expression of their receptors. Macrophages invade the basal lamina and, to a certain, limited extent assisted by Schwann cells, phagocytose the debris.

Anterograde Reaction

That portion of the axon distal to an injury undergoes degeneration and is phagocytosed.

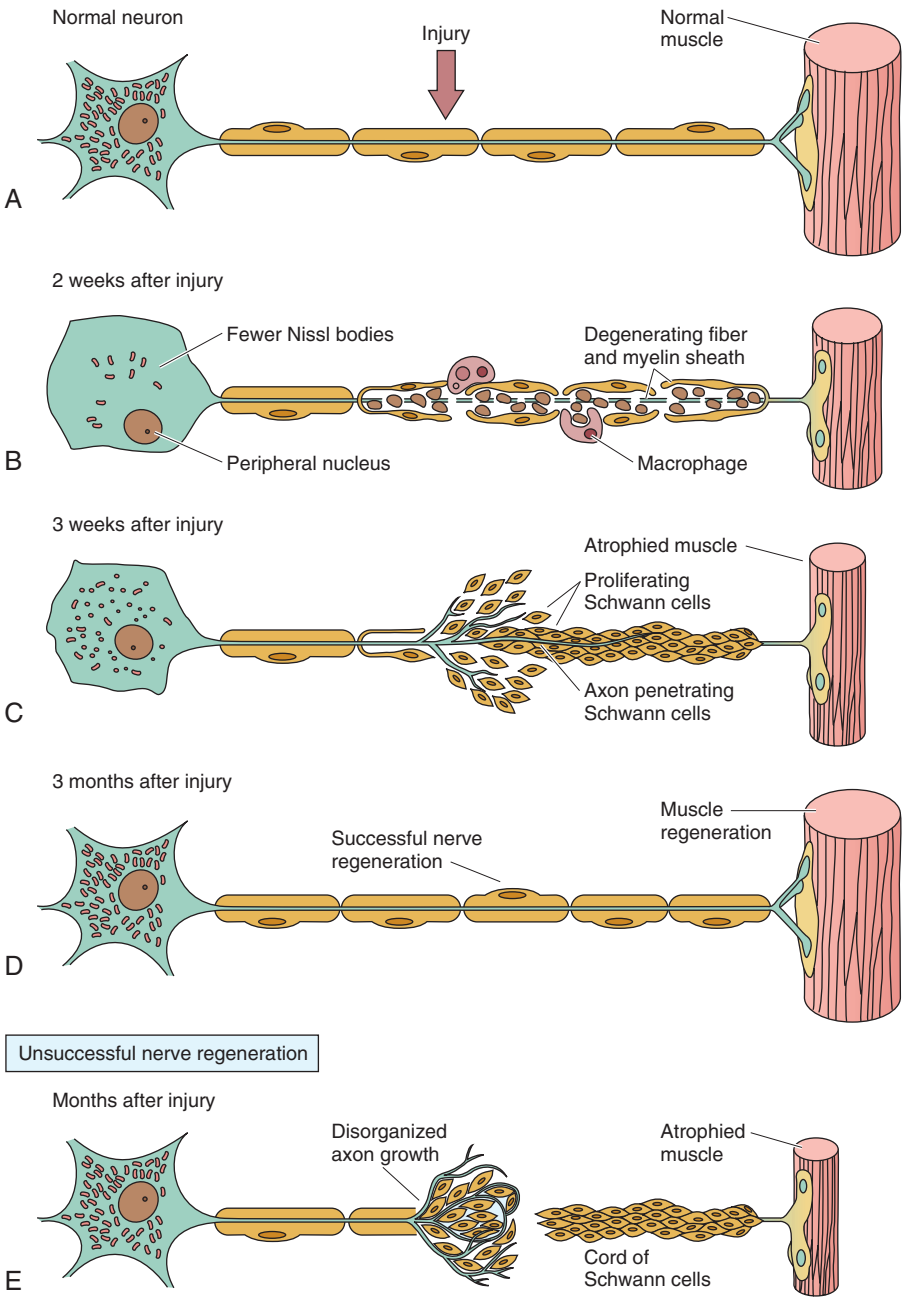


Figure 9–31 Schematic diagram of nerve regeneration.

The axon undergoes anterograde changes as follows:

1. The axon terminal becomes hypertrophied and degenerates within 1 week; as a result, contact with the postsynaptic membrane is terminated. Schwann cells proliferate and phagocytose the remnants of the axon terminal, and the newly formed Schwann cells occupy the synaptic space.
2. The distal portion of the axon undergoes **wallerian degeneration (orthograde degeneration)**, whereby distal to the lesion, the axon and the myelin disintegrate, Schwann cells dedifferentiate, and myelin synthesis is discontinued. Moreover, macrophages and, to a certain extent, Schwann cells phagocytose the disintegrated remnants.

3. Schwann cells proliferate, forming a column of Schwann cells (**Schwann tubes**) enclosed by the original basal lamina of the endoneurium.

Retrograde Reaction and Regeneration

The proximal portion of the injured axon undergoes degeneration followed by sprouting of a new axon whose growth is directed by Schwann cells.

The portion of the axon proximal to the damage undergoes the following changes:

1. The perikaryon of the damaged neuron becomes hypertrophied, its Nissl bodies disperse, and its nucleus is displaced. These events, called ***chromatolysis***, may last several months. Meanwhile, the soma is actively producing free ribosomes and synthesizing proteins and various macromolecules, including RNA. During this time, the proximal axon stump and surrounding myelin sheath degenerate as far proximally as the nearest collateral axon.
2. Several "sprouts" of axon emerge from the proximal axon stump, enter the endoneurium, and are guided by the Schwann cells to their target cell. For regeneration to occur, the Schwann cells, macrophages, and fibroblasts as well as the basal lamina must be present. These cells manufacture growth factors and cytokines and up-regulate the expression for the receptors of these signaling molecules.
3. These sprouts are guided by the Schwann cells that redifferentiate and either begin to manufacture myelin around the growing axon or, in nonmyelinated axons, form a Schwann cell sheath. The one sprout that reaches the target cell first forms a synapse, whereas the other sprouts degenerate. The process of regeneration proceeds at about 3 to 4 mm/day.

Transneuronal Degeneration

The nerve cell has a **trophic influence** on the cells it contacts. If the neuron dies, sometimes its target cells atrophy and degenerate, or other cells targeting that particular neuron also atrophy and degenerate. This process, called ***transneuronal degeneration***, may thus be anterograde or retrograde but occurs only infrequently.

Regeneration in the Central Nervous System

Regeneration in the CNS is much less likely than in the PNS because connective tissue sheaths are absent in the CNS. Injured cells within the CNS are phagocytosed by **microglia**, and the space liberated by the phagocytosis becomes occupied by proliferation of glial cells, which form a cell mass called a **glial scar**. It is believed that the glial cell masses hinder the process of repair. Thus, generally, neuronal damage within the CNS appears to be irreparable.

Although neurons do not divide readily, there is evidence that there are neural stem cells within the adult mammalian and human brain that, when provided the proper stimulus, could be activated to replace lost or injured neurons. Some of these cells have the capacity to produce glial cells and others to differentiate into neurons. It has been shown that these neural stem cells exhibit multipotential ability to differentiate into the cells of the tissue into which they were introduced.

CLINICAL CORRELATIONS

It has been shown recently that reducing additional cell damage or death within 1 hour of injury increases the survivability of neurons in the vicinity of the lesion. This information coupled with results of recent investigations concerning growth factors, applications of embryonic neural stem cells, reducing neuron growth inhibitors, applying axon grafts, and grafting axons directly into the gray matter of the spinal cord are providing promising results for the future in spinal cord therapy.

Neuronal Plasticity (Neuroplasticity, Brain Plasticity)

Neuronal plasticity is the ability of neural pathways and synapses to alter their original function adapting to various factors, such as damage, behavior, or changes in the individual's environment. Such plasticity is evident during development because the neurons that are present in excess or not making correct connections must be destroyed. However, it has been shown in adult mammals that after injury, neuronal circuits may be reestablished from the growth of neuronal processes located at some distance away from the lesion that are able to provide at least some functional recovery. Regeneration of this sort relies on growth factors called ***neurotrophins*** produced by neurons, glial cells, Schwann cells, and certain target cells. Evidence for neuronal plasticity in humans may be observed in stroke victims as well as in victims of other neurological injuries.

PATHOLOGICAL CONSIDERATIONS

See Figures 9–32 through 9–34.

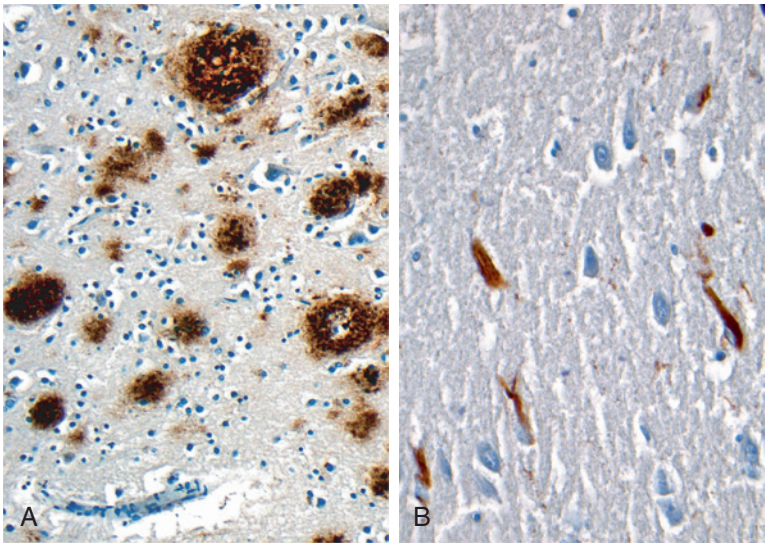


Figure 9–32 The brains of individuals with Alzheimer disease display the presence of amyloid β accumulations that may be well demonstrated by the application of histochemical staining techniques *(left photomicrograph)*. Additionally, neurofibrillary tangles, composed of cytoskeletal elements—especially microtubule-associated protein tau—are also present and can be demonstrated by immunocytochemistry *(right photomicrograph)*. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:302.)

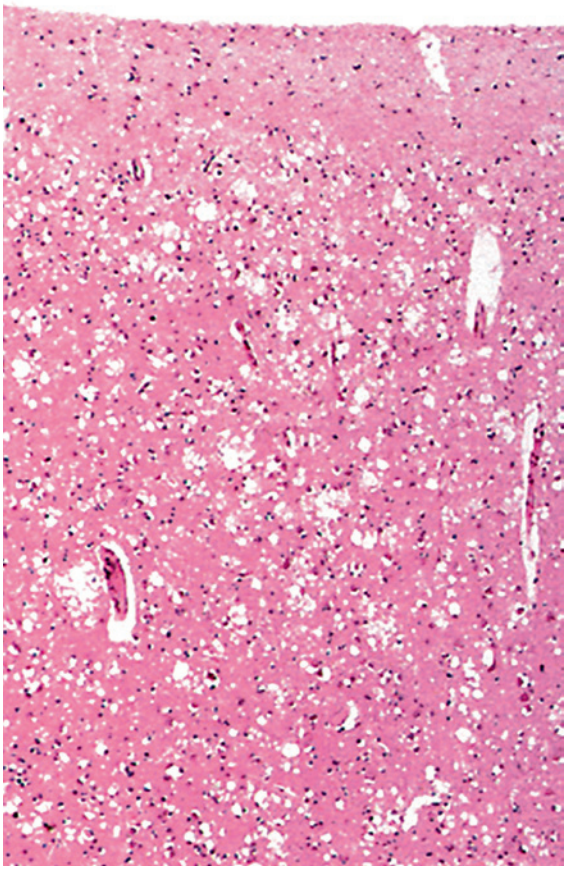


Figure 9–33 The brains of individuals with prion diseases display the presence of spongiform changes as evidenced in the sponge-like appearance of the cerebral cortex in this photomicrograph. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:304.)

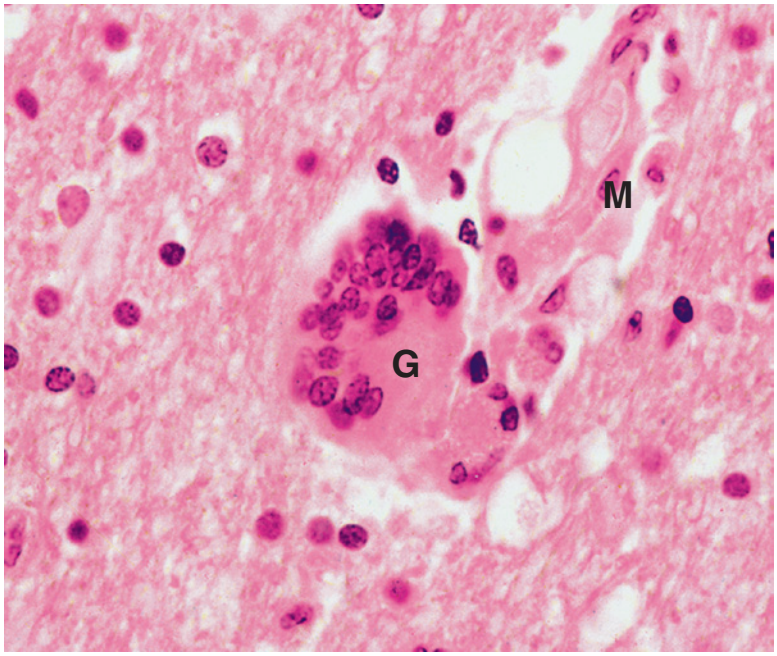
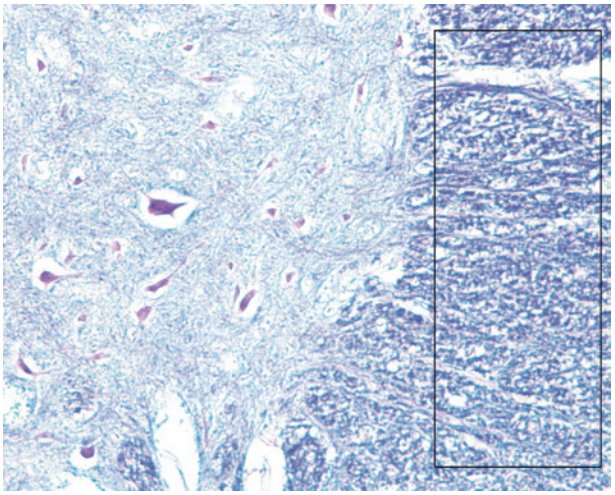
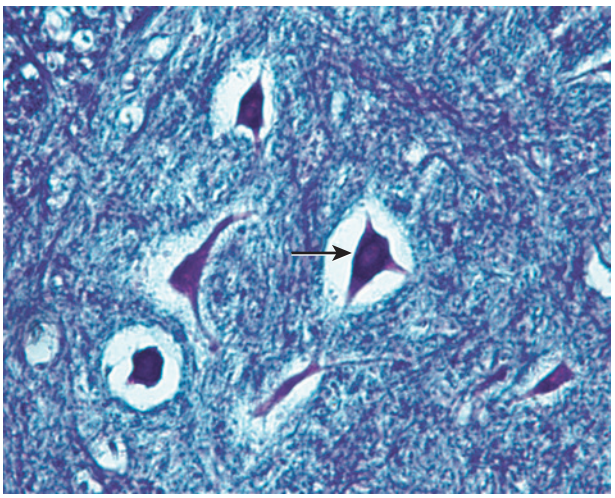


Figure 9–34 The brains of individuals with HIV-induced encephalitis display the presence of inflammation in the white matter, as evidenced by the presence of mononuclear cells (M) and multinucleated giant cells (G). (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:306.)

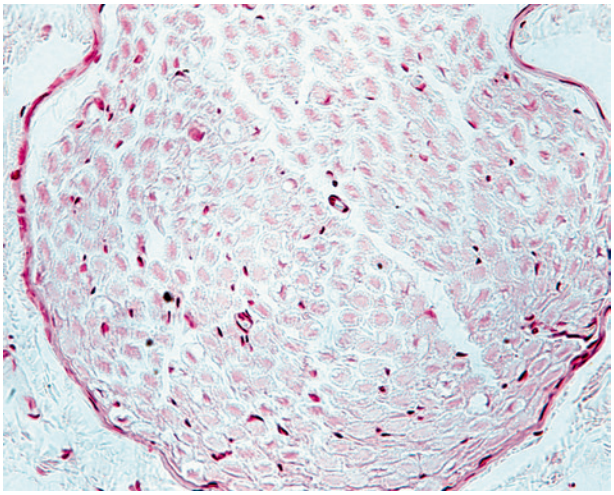
REVIEW QUESTIONS

1. The nervous system has neuron as well as associated neuroglia. Which one of the following neuroglia is derived from cells of the bone marrow?
   a. Schwann cells
   b. Astrocytes
   c. Oligodendroglia
   d. Ependymal cells
   e. Microglia
2. The neuron that is not present in a two neuron reflex arc is known as a(n)
   a. motoneuron.
   b. sensory neuron.
   c. afferent neuron.
   d. efferent neuron.
   e. interneuron.
3. Major dense lines of a myelinated axon
   a. represent the fused extracellular surface of the Schwann cell membrane.
   b. represent the fused cytoplasmic surfaces of the Schwann cell membrane.
   c. are located at the node of Ranvier.
   d. are aligned perpendicular to the intraperiod line.
   e. are absent in myelin sheaths formed by oligodendroglia on the central nervous system.
4. The maintenance of resting potential depends primarily on
   a. Na^+-K^+ pumps.
   b. Ca^+ channels.
   c. K^+ leak channels.
   d. voltage-gated K^+ channels.
   e. voltage-gated Na^+ channels.
5. When an action potential reaches the presynaptic membrane, it causes the opening of certain voltage-gated ion channels that permit the influx of specific ions that causes synaptic vesicles to fuse with the presynaptic membrane and release their neurotransmitter substances via exocytosis into the synaptic cleft. These voltage gated ion channels permit the
   a. influx of Na^+.
   b. outflow of K^+.
   c. influx of Cl^-.
   d. influx of Ca^{++}.
   e. outflow of Cl^-.
6. During embryonic development, cells derived from neural crest migrate to form which of the following?
   a. preganglionic parasympathetic neurons.
   b. preganglionic sympathetic neurons.
   c. interneurons.
   d. motoneurons of the ventral horn of the spinal cord.
   e. neurons in Auerbach plexus.
7. 

   The region within the rectangle is the
   a. cerebellar cortex.
   b. cerebellar medulla.
   c. gray matter of spinal cord.
   d. white matter of spinal cord.
   e. cerebral cortex.
8. 

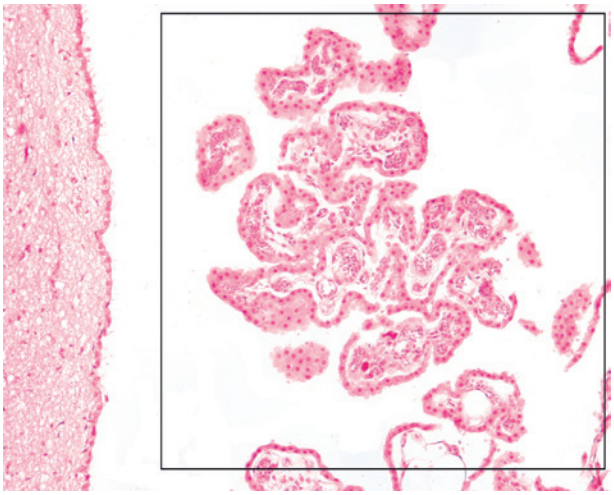
   Identify the cell at the tip of the arrow.
   a. Unipolar neuron
   b. Pseudounipolar neuron
   c. Bipolar neuron
   d. Interneuron
   e. Multipolar neuron

9.



Identify the structure in the entire field.
a. Dorsal root ganglion
b. Nerve fiber
c. Adipose tissue
d. Autonomic ganglion
e. Embryonic connective tissue

10.



The structure located in the rectangle is responsible for the
a. formation of cerebrospinal fluid.
b. elimination of cerebrospinal fluid.
c. lining of the ventricles of the brain.
d. lining of the central canal of the spinal cord.
e. formation of new neurons.

ANSWERS

1. E. Microglia are phagocytes that reside in the brain and are derived from monocyte precursors of the bone marrow. Schwann cells are present in the peripheral nervous system, where they form myelin sheaths and insulate axons. Astrocytes provide metabolic and structural support for neurons of the central nervous system. Oligodendroglia perform functions in the central nervous system that are similar to those of Schwann cells in the peripheral nervous system. Ependymal cells line the ventricles of the brain and the central canal of the spinal cord.
2. E. The three neuron reflex arc is composed of the sensory (afferent) neuron and the motor (efferent) neuron and the interneuron that is interposed between them. The interneuron is controlled by higher centers and can be "instructed" to stop the progress of the impulse relayed by the sensory neuron from reaching the motoneuron. The two neuron reflex arc, such as the patellar reflex, does not have an interneuron.
3. B. Major dense lines of myelinated axons represent the fused cytoplasmic surfaces of the Schwann cell membrane. They cannot be located at the nodes of Ranvier because there is no myelin present at the node. Major dense lines parallel the intraperiod line, and they are present in myelin sheaths formed by oligodendroglia of the central nervous system.
4. C. K^+ leak channels in the plasmalemma permit a relatively free flow of potassium ions out of the cell down its concentration gradient. Although the K^+ leak channels allow sodium ions to enter the cell, about 100 times more potassium leaves than sodium enters and its effects on the resting potential is only minimal. Na^+-K^+ pumps also have a minimal effect on the resting potential of the cell but their major contribution is to maintain the osmotic balance of the cell. Voltage-gated K^+ channels assist in reestablishing the resting potential after a depolarization event. Voltage-gated Na^+ channels are responsible for the reversal of the resting potential. Calcium ion channels permit the entry of calcium into the cell to facilitate the adherence of synaptic vesicles to the active sites of the axon end foot.
5. D. The influx of Ca^{++} causes synaptic vesicles under the influence of SNARE (SNAP receptor) proteins to fuse with the presynaptic membrane, emptying neurotransmitter into synaptic clefts via exocytosis. The other ions have no direct affect on the synaptic vesicles.
6. E. Neurons of Auerbach plexus, as all ganglia of the peripheral nervous system, originate from neural crest material. Postganglionic but not preganglionic autonomic neurons originate from neural crest material. Neither interneurons nor motoneurons of the spinal cord originate from neural crest material.
7. D. The white matter of the spinal cord is evident because the gray matter ventral horn with its large multipolar motoneurons is readily recognizable.
8. E. This neuron has three processes that are clearly evident, and a fourth process is visible; therefore, this is a multipolar neuron from the ventral horn of the gray matter of the spinal cord.
9. B. This is a cross section of a nerve fiber as is evident by the surrounding perineurium and the numerous myelinated axons. Observe that some of the axons display the nuclei of Schwann cells.
10. A. This is a photomicrograph of the choroid plexus whose function is the manufacture of cerebrospinal fluid (CSF). CSF is eliminated from the subarachnoid spaces by structures known as *arachnoid granulations*.

This page intentionally left blank

Blood and Hemopoiesis

10

Blood is a bright to dark red, viscous, slightly alkaline fluid (pH, 7.4) that accounts for approximately 7% of the total body weight. The total volume of blood of an average adult is about 5 L, and it circulates throughout the body within the confines of the circulatory system. Blood is a specialized connective tissue composed of formed elements—**red blood cells (RBCs; erythrocytes)**, **white blood cells (WBCs; leukocytes)**, and **platelets**—suspended in a fluid component (the extracellular matrix), known as ***plasma*** (Figs. 10–1 and 10–2).

Because blood circulates throughout the body, it is an ideal vehicle for the transport of materials. The primary functions of blood include conveying nutrients from the gastrointestinal system to all of the cells of the body and subsequently delivering the waste products of these cells to specific organs for elimination. Numerous other metabolites, cellular products (e.g., hormones and other signaling molecules), and electrolytes are also ferried by the bloodstream to their final destinations. Oxygen (O_2) is carried by hemoglobin within erythrocytes from the lungs for distribution to the cells of the organism, and carbon dioxide (CO_2) is conveyed both by hemoglobin and by the fluid component of plasma (as bicarbonate ion, HCO_3^-, as well as in its free form) for elimination by the lungs.

Blood also helps regulate body temperature and aids in maintaining the acid-base and osmotic balance of the body fluids. Finally, blood acts as a pathway for migration of WBCs between various connective tissue compartments of the body.

The fluid state of blood necessitates the presence of a protective mechanism, **coagulation (clotting)**, to stop its flow in case of damage to the vascular tree. The process of coagulation is mediated by platelets and blood-borne factors that transform blood from a liquid to a gel state.

When blood is removed from the body and placed in a test tube, clotting occurs unless the tube is coated by an anticoagulant such as heparin. Upon centrifugation of liquid blood, the formed elements settle to the bottom of the tube as a red precipitate (44%) covered by a thin translucent layer, the **buffy coat** (1%), and the fluid plasma remains on top as the supernatant (55%). The red precipitate is composed of RBCs, and the total RBC volume is known as the ***hematocrit***, whereas the buffy coat consists of WBCs and platelets.

The finite life span of blood cells requires their constant renewal to maintain a steady circulating population. This process of blood cell formation from established blood cell precursors is called ***hemopoiesis*** (also referred to as *hematopoiesis*).

BLOOD

Blood is composed of a fluid component (plasma) and formed elements, consisting of the various types of blood cells as well as platelets.

Light microscopic examination of circulating blood cells is performed by evenly smearing a drop of blood on a glass slide, air drying the preparation, and staining it with mixtures of dyes specifically designed to demonstrate distinctive characteristics of the cells. The current methods are derived from the technique developed in the late 19th century by Romanovsky, who used a mixture of methylene blue and eosin. Most laboratories now use either the Wright or Giemsa modifications of the original procedure, and identification of blood cells is based on the colors produced by these stains. Methylene blue stains acidic cellular components blue, and eosin stains alkaline components pink. Still other components are colored a reddish blue by binding to **azures**, substances formed when methylene blue is oxidized.

Plasma

Plasma is a yellowish fluid in which cells, platelets, organic compounds, and electrolytes are suspended and/or dissolved.

During coagulation, some of the organic and inorganic components leave the **plasma** to become integrated into the clot. The remaining fluid, which no longer has the clot-forming components dissolved or suspended in it, differs from plasma, is straw colored, and is known as ***serum***.

The major component of plasma is water, constituting about 90% of its volume. Proteins compose 9%, and inorganic salts, ions, nitrogenous compounds, nutrients, and gases constitute the remaining 1%. The types, origins, and functions of the **blood proteins** are listed in Table 10–1.

Plasma leaves the capillaries and small venules to enter the connective tissue spaces as **extracellular**

SUMMARY

Blood is a specialized connective tissue that, unlike other connective tissues of the body, is a fluid composed of **formed elements** suspended in a liquid component known as ***plasma***. The primary functions of blood are to ferry material to and from the cells of the body, assist in the regulation of body temperature and the maintenance of the acid–base balance of the body, clotting of blood to staunch the flow of blood in case of damage to the blood vessels, and migration of blood cells between regions of the body to assist in defense against invading pathogens or foreign macromolecules by the process of inflammation or immune response. The formed elements are the blood cells, namely **red blood cells (RBCs, erythrocytes)**, **white blood cells (WBCs**, **leukocytes)**, and **platelets (thrombocytes)**. There are two major categories of WBCs, those without specific granules, known as ***agranulocytes***, namely, **lymphocytes** and **monocytes**, and those that house specific granules, known as ***granulocytes***, namely **neutrophils**, **eosinophils**, and **basophils**. **Bone marrow** is responsible for the formation of blood cells **(hemopoiesis)** and their delivery into the circulatory system. Bone marrow also provides a microenvironment for much of the maturation process of **B lymphocytes** and for the initial maturation of **T lymphocytes**.

KEY WORDS

Basophils; blood; B lymphocytes; bone marrow; eosinophils; erythrocytes; formed elements; hemopoiesis; leukocytes; lymphocytes; monocytes; neutrophils; plasma; platelets; thrombocytes; T lymphocytes.

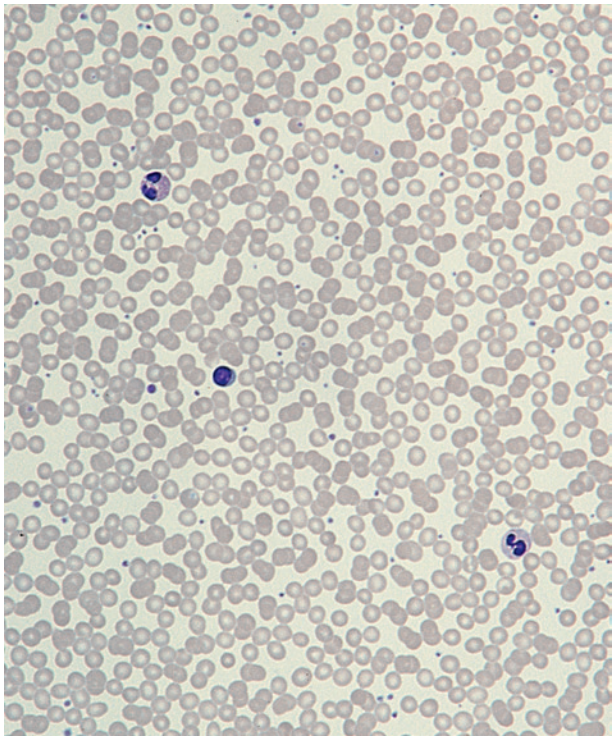


Figure 10–1 Photomicrograph of circulating blood. Note the abundance of erythrocytes as well as the three leukocytes. Also observe the presence of numerous platelets that appear as small dots interspersed among the erythrocytes (×270).

fluid, which thus has a composition of electrolytes and small molecules similar to that in plasma. The concentration of proteins in extracellular fluid is much lower than that in plasma, however, because it is difficult even for small proteins, such as albumin, to traverse the endothelial lining of a capillary or that of a venule. In fact, one of the proteins of plasma, **albumin**, is chiefly responsible for the establishment of blood's **colloid osmotic pressure**, the force that maintains normal blood volume by opposing the movement of fluid from the capillaries and venules into the interstitial spaces.

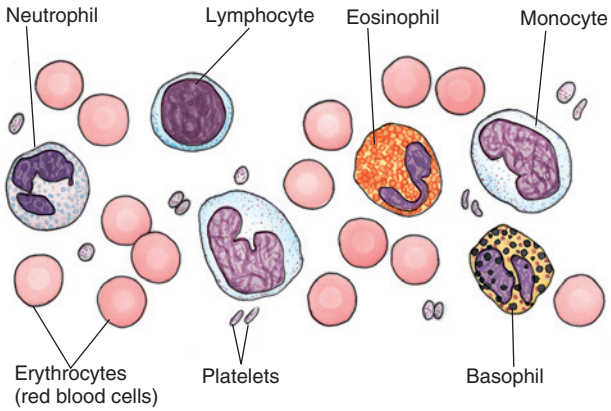


Figure 10–2 Cells and platelets of circulating blood.

TABLE 10–1 Proteins of Plasma

Protein	Size	Source	Function
Albumin	60,000–69,000 Da	Liver	Maintains colloid osmotic pressure and transports certain insoluble metabolites
Globulins α- and β-Globulins	80,000–1 × 10^6 Da	Liver	Transport metal ions, protein-bound lipids, and lipid-soluble vitamins
γ-Globulin		Plasma cells	Antibodies of immune defense
Clotting Proteins (e.g., prothrombin, fibrinogen, accelerator globulin)	Varied	Liver	Formation of fibrin threads
Complement proteins C1 through C9	Varied	Liver	Destruction of microorganisms and initiation of inflammation
Plasma Lipoproteins Chylomicrons	100–500 μm	Intestinal epithelial cells	Transport of triglycerides to liver
Very low-density lipoprotein (VLDL)	25–70 nm	Liver	Transport of triglycerides from liver to body cells
Low-density lipoprotein (LDL)	3 × 10^6 Da	Liver	Transport of cholesterol from liver to body cells

Formed Elements

Red blood cells, white blood cells, and platelets constitute the formed elements of blood.

Erythrocytes

Erythrocytes (red blood cells), the smallest and most numerous cells of blood, have no nuclei and are responsible for the transport of oxygen and carbon dioxide to and from the tissues of the body.

Each **erythrocyte (RBC)** resembles a biconcave-shaped disk that is 7.5 µm in diameter, 2.0 µm thick at its widest region, and less than 1 µm thick at its center (Figs. 10–3 and 10–4). This shape provides the cell with a large surface area relative to its volume, thus enhancing its capability for gaseous exchange. Although erythrocyte precursor cells within the bone marrow possess nuclei during development and maturation, the precursor cells or erythrocytes expel not only their nuclei but also all of their organelles prior to entering

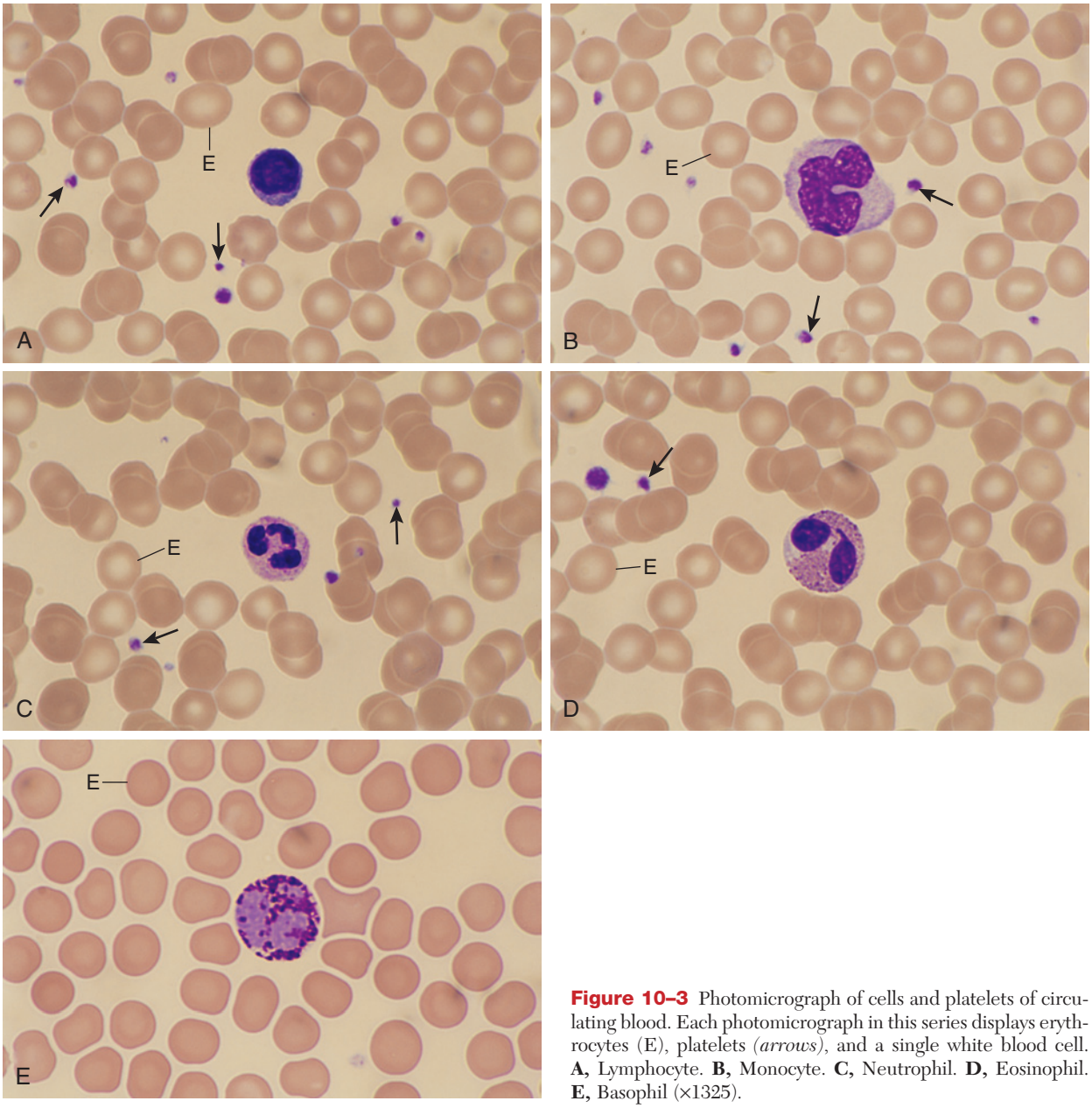


Figure 10–3 Photomicrograph of cells and platelets of circulating blood. Each photomicrograph in this series displays erythrocytes (E), platelets *(arrows)*, and a single white blood cell. **A,** Lymphocyte. **B,** Monocyte. **C,** Neutrophil. **D,** Eosinophil. **E,** Basophil (×1325).

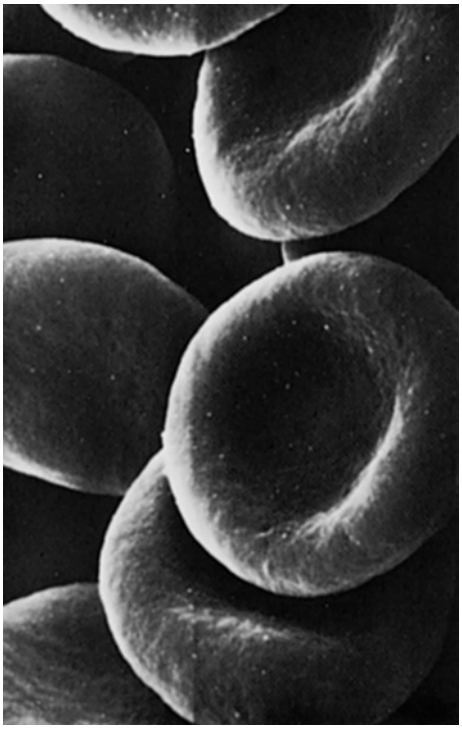


Figure 10–4 Scanning electron micrograph of circulating red blood cells displaying their biconcave disk shape (×5850). (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

the circulation. Thus, mature, circulating erythrocytes have no nuclei. When stained with Giemsa or Wright stains, erythrocytes display a salmon-pink color.

Although erythrocytes possess no organelles, they do have soluble enzymes in their cytosol. Within the erythrocyte, the enzyme **carbonic anhydrase** facilitates the formation of carbonic acid from CO_2 and water. This acid dissociates to form bicarbonate (HCO_3^-) and hydrogen (H^+). It is as bicarbonate that most of the CO_2 is ferried to the lungs for exhalation. The ability of bicarbonate to cross the erythrocyte cell membrane is mediated by the integral membrane protein **band 3**, a coupled anion transporter that exchanges intracellular bicarbonate for extracellular Cl^-; this exchange is known as the ***chloride shift***. Additional enzymes include those of the **glycolytic pathway (Embden-Meyerhoff pathway)** as well as enzymes that are responsible for the **pentose monophosphate shunt (hexose monophosphate shunt)** for the production of the high-energy molecule **reduced nicotinamide adenine dinucleotide phosphate (NADPH)**, a reducing agent. The pentose monophosphate shunt does not require the presence of oxygen and is the chief method whereby the erythrocyte produces adenosine triphosphate (ATP), necessary for its energy requirement.

Males have more erythrocytes per unit volume of blood than do females (5×10^6 vs. 4.5×10^6 per mm^3), and members of both sexes living at higher altitudes have correspondingly more RBCs than residents living at lower altitudes.

Human erythrocytes have an average life span of 120 days; when they reach that age, they display on their surface a group of oligosaccharides. RBCs bearing these sugar groups are fragile and are destroyed by macrophages of the spleen, bone marrow, and liver.

Hemoglobin

Hemoglobin is a large protein composed of four polypeptide chains, each of which is covalently bound to a heme group.

RBCs are packed with **hemoglobin**, a large tetrameric protein (68,000 Da) composed of four polypeptide chains, each of which is covalently bound to an iron-containing **heme**; this molecule is bound within a hydrophobic depression, the heme pocket, of the globin chain that protects the iron from being oxidized while permitting the binding of oxygen to it. It is hemoglobin that provides the *unstained* cell with its pale yellow color. The globin moiety of hemoglobin releases CO_2, and, in regions of high oxygen concentration, such as in the lungs, O_2 binds to the iron of each heme. When O_2 is bound to the heme, the hemoglobin molecule is in the relaxed state ([R-] Hb), the globin moieties of the molecule are less constrained and can move with respect to each other, and the O_2 can easily be released. When O_2 is released its place becomes occupied by 2,3-diphosphoglycerate, and the hemoglobin becomes known as *deoxyhemoglobin*, or *taut hemoglobin* ([T-] Hb). The number of ionic and H bonds between the globin chains of (T-) Hb is greater than that of (R-) Hb, and the movement of the globin chains with respect to each other is reduced. However, in oxygen-poor regions, as in tissues, hemoglobin releases O_2 and binds CO_2. This property of hemoglobin makes it ideal for the conveyance of respiratory gases. Hemoglobin carrying O_2 is known as ***oxyhemoglobin***, and hemoglobin carrying CO_2 is called ***carbaminohemoglobin*** (or ***carbamylhemoglobin***). Hypoxic tissues release 2,3-diphosphoglyceride, a carbohydrate that facilitates the release of oxygen from the erythrocyte. Hemoglobin also binds nitric oxide (NO), a neurotransmitter substance that causes dilation of blood vessels, permitting RBCs to release more oxygen and pick up more CO_2 within the tissues of the body.

CLINICAL CORRELATIONS

Carbon monoxide (CO) has a much greater affinity, about 250-fold, than O_2 for the heme portion of hemoglobin, and when CO binds to the iron of the heme, the hemoglobin molecule is transformed to its (R-) Hb form and increases its affinity to oxygen so that it cannot be released to the tissues even in hypoxic regions. People who are trapped in areas of poor ventilation with a running gasoline-powered engine or in a building on fire frequently succumb to CO poisoning. Many such, especially fair-skinned victims, instead of being cyanotic (with a bluish pallor) present with healthy-looking, cherry-red skin because of the color of the CO-hemoglobin complex (carbon monoxyhemoglobin) even though they are dead.

On the basis of the amino acid sequences, there are four normal, human polypeptide chains of hemoglobin, designated α, β, γ, and δ. The principal hemoglobin of the fetus, **fetal hemoglobin (HbF)**, composed of two α-chains and two γ-chains, is replaced shortly after birth by **adult hemoglobin (HbA)**. There are two types of normal HbAs, **HbA_1** ($\alpha_2\beta_2$) and the much rarer form, **HbA_2** ($\alpha_2\delta_2$). In an adult, approximately 96% of the hemoglobin is HbA_1, 2% is HbA_2, and the remaining 2% is HbF.

Erythrocyte Cell Membrane

The cell membrane of the erythrocyte and the underlying cytoskeleton are highly pliable and can withstand great shear forces.

The RBC plasma membrane, a typical lipid bilayer, is composed of about 50% protein, 40% lipids, and 10% carbohydrates. Most of the proteins are transmembrane proteins, principally **glycophorin A** (as well as lesser quantities of glycophorins B, C, and D), **ion channels** (calcium-dependent potassium channels and Na^+-K^+ adenosine triphosphatase), and the anion transporter **band 3 protein**, which transports Cl^- and HCO^-_3; it also acts as an anchoring site for **ankyrin**, band 4.1 protein, hemoglobin, and glycolytic enzymes (Fig. 10–5). Additionally, the RBC membrane also possesses the peripheral proteins band 4.1 protein, spectrin, ankyrin, and actin. **Band 4.1 protein**, acts as an anchoring site for spectrin, band 3 protein, and glycophorins. Thus, ankyrin, band 3 protein, and band 4.1 protein anchor the cytoskeleton, a hexagonal lattice composed chiefly of **spectrin tetramers**, **actin**, and **adducin**, to the cytoplasmic aspect of the RBC plasmalemma (see Chapter 2). This subplasmalemmal cytoskeleton helps maintain the biconcave disk shape of the erythrocyte.

CLINICAL CORRELATIONS

1. Several hereditary diseases result from defects in the genes encoding the hemoglobin polypeptide chains. Diseases referred to as *thalassemia* are marked by decreased synthesis of one or more hemoglobin chains. In β-thalassemia, synthesis of the β-chains is impaired. In the homozygous form of this disease, which is most prevalent among persons of Mediterranean descent, HbA is missing, and high levels of HbF persist after birth.

2. Sickle cell anemia is the result of a point mutation at a single locus of the β-chain (valine is incorporated into the sequence instead of glutamate), forming the abnormal hemoglobin HbS. When the oxygen tension is reduced (e.g., during strenuous exercise), HbS changes shape, producing abnormal-shaped (crescent-shaped) erythrocytes that are less pliant, more fragile, and more prone to hemolysis than normal cells. Sickle cell anemia is prevalent in the black population, especially in those whose ancestors lived in regions of Africa where malaria is endemic. In the United States, about 1 of 600 newborn African-American babies is stricken with this condition.

3. Individuals with sickle cell anemia are more resistant to malaria, a disease caused by a parasite than people whose hemoglobin is not mutated. It was believed that sickle-shaped erythrocytes were more resistant to the entry of the parasite *Plasmodium falciparum*, the most fatal of the five *Plasmodium* species that cause malaria. However, it has been reported recently that the erythrocytes of individuals with sickle cell anemia manufacture an increased amount of the enzyme, heme oxygenase-1. This enzyme produces CO, a gas that not only protects hemoglobin from degradation, but also limits the toxic ability of *P. falciparum*, thus providing more time for the individual's immune system to fight the parasitic invasion.

4. When plasma glucose levels are elevated, glucose molecules attach to the N-terminus of hemoglobin's beta chain, forming glycated hemoglobin, known as *hemoglobin A1c* (*HbA1c* or *A1c*). This is a nonreversible reaction, so, once attached, the hemoglobin molecules do not lose their glucose. If the plasma glucose levels remain elevated, glycated hemoglobin levels also increase; therefore, a measure of A1c levels is indicative of the blood sugar concentrations for the previous 2 to 3 months. The normal glycated hemoglobin level in adults is approximately 4% to 5.6%. Individuals with diabetes have higher A1c levels because their blood sugar concentrations are usually greater than those of individuals without diabetes. For a patient with diabetes, an A1c level below 7% is considered to be very good, indicating a good control of blood glucose levels. A1c levels higher than 7% is worrisome because the higher the level, the greater the probability of acquiring diabetes-related conditions.

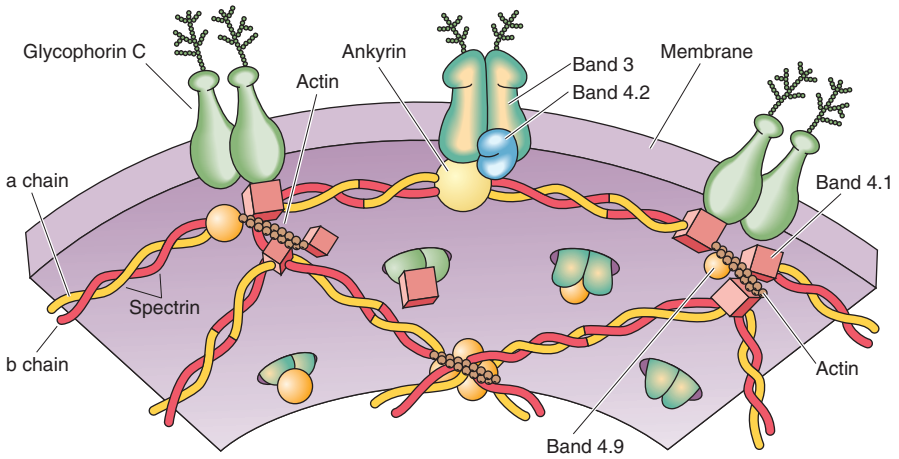


Figure 10–5 Diagram of the cytoskeleton and integral proteins of the erythrocyte plasmalemma. Spectrin forms a hexagonal lattice-work that is anchored to the erythrocyte plasma membrane by band 4.1 and band 3 proteins as well as by ankyrin.

During its 120-day life, each erythrocyte negotiates the entire circulatory system at least 100,000 times and therefore must pass through innumerable capillaries whose lumen is smaller than the cell's diameter. To navigate through such small-bore vessels, the erythrocyte undergoes deformations of its shape and becomes subject to tremendous shear forces. It is the erythrocyte cell membrane and the underlying cytoskeleton that contribute to the ability of the RBC to maintain its structural and functional integrity.

CLINICAL CORRELATIONS

1. Defects in the cytoskeletal components of erythrocytes result in various conditions marked by abnormally shaped cells. Hereditary spherocytosis, for instance, is caused by synthesis of an abnormal spectrin that exhibits defective binding to band 4.1 protein. RBCs of patients with this condition are more fragile and transport less oxygen compared with normal erythrocytes. Moreover, these spherocytes are preferentially destroyed in the spleen, leading to anemia.

2. Deficiency of glycophorin C is responsible for elliptocytic RBCs without the resultant hemolytic anemia. These cells are unstable and fragile and are less capable of deformation than normal erythrocytes.

The extracellular surface of the RBC plasmalemma has specific inherited carbohydrate chains that act as antigens and determine the blood group of an individual for the purposes of blood transfusion. The most notable of these are the **A**, **B**, and **O antigens**, which determine the four primary blood groups, **A**, **B**, **AB**, and **O** (Table 10–2). People who lack either the A or B antigen, or both, have antibodies against the missing antigen in their blood; if they undergo transfusion with blood containing the missing antigen, the donor erythrocytes are attacked by the recipient's serum antibodies and are eventually lysed.

CLINICAL CORRELATIONS

1. It is interesting to note that the carbohydrate chains of the A and B blood groups are identical to each other with the exception of their terminal sugar molecules and that people possessing the O blood group also have the same carbohydrate chains but do not have the terminal sugar molecule present in the A or B blood types (i.e., they have one fewer members in their carbohydrate group than A or B antigens). The addition of the terminal sugar group of A and B antigens each requires a specific enzyme, and neither of these two enzymes is present in individuals with O blood group. For the type A blood group, the enzyme N-acetylgalactosamine transferase adds a terminal N-acetylgalactosamine to the type O antigen. In the type B blood group, the enzyme terminal N-acetylgalactose transferase adds a terminal galactose to the type O antigen. In blood type AB, both enzymes are present and form both type A and type B. In blood type O, neither enzyme is present; therefore, neither galactose nor N-acetylgalactosamine is added to the O antigen.

2. Recent reports have demonstrated that individuals with AB blood group have a greater prevalence of impaired cognitive function as a function of age than individuals possessing other blood groups. The reason for this occurrence is not as yet understood.

TABLE 10–2 ABO Blood Group System

Blood Group	Antigens Present	Miscellaneous
A	Antigen A	
B	Antigen B	
AB	Antigens A and B	Universal acceptor
O	Neither antigen A nor B	Universal donor

The approximate distribution of the various blood types in the U.S. population is ethnicity dependent:

- Type O among Caucasians is 45%, among African Americans is 51%, among Hispanics is 57%, and among Asian Americans is 40%.
- Type A among Caucasians is 40%, among African Americans is 26%, among Hispanics is 31%, and among Asian Americans is 28%.
- Type B among Caucasians is 11%, among African Americans is 19%, among Hispanics is 10%, and among Asian Americans is 25%.
- Type AB among Caucasians is 4%, among African Americans is 4%, among Hispanics is 2%, and among Asian Americans is 7%.

Another important blood group, the **Rh** group, is so named because it was first identified in rhesus monkeys. This complex group comprises almost 50 antigens, although many are relatively rare. One of the Rh antigens, **D antigen (Rh factor)**, is so common in the human population that the erythrocytes of 85% of Americans have D antigen on their surface, and these individuals are thus said to be **Rh positive (Rh^+)**, whereas the remaining 15% of the population does not have the antigen and is said to be **Rh negative (Rh^-)**.

CLINICAL CORRELATIONS

When an Rh^- pregnant woman delivers her first Rh^+ baby, enough of the baby's blood is likely to enter her circulation to induce the formation of anti-Rh antibodies in the mother. Because the first antibody to be produced is immunoglobulin M (IgM), it is too large to be able to cross the placental barrier (see Chapter 12 on the lymphoid [immune] system). During a subsequent pregnancy with an Rh^+ fetus, the new fetus's RBCs enter the mother's bloodstream this second exposure elicits the formation of IgG antibodies, which are smaller and are able to cross the placental barrier. These antibodies attack the erythrocytes of the fetus, causing erythroblastosis fetalis, a condition that may be fatal to the newborn. Prenatal and postnatal transfusions of the fetus are necessary to prevent brain damage and death of the newborn unless the mother has been treated with anti-D globulin (RhoGAM) before or shortly after the birth of the first Rh^+ baby. The anti-D globulin complexes with D antigen, preventing the mother's immune system from recognizing it as an antigenic molecule; therefore, the mother's immune system does not produce antibodies that would otherwise attack the fetus's erythrocytes, thus preventing the occurrence of erythroblastosis fetalis.

Leukocytes

Leukocytes are white blood cells that are classified into two major categories: granulocytes and agranulocytes.

The number of **leukocytes (WBCs)** is much smaller than that of RBCs; in fact, in a healthy adult, there are only 6,500 to 10,000 WBCs per mm^3 of blood. Unlike erythrocytes, leukocytes do not function within the bloodstream but use it as a means of traveling from one region of the body to another. When leukocytes reach their destination, they leave the bloodstream by migrating between the endothelial cells of the blood vessels **(diapedesis)**, enter the connective tissue spaces, and perform their function. Within the bloodstream as well as in smears, leukocytes are round; in connective tissue, they are pleomorphic. They generally defend the body against foreign substances.

WBCs are classified into two groups:

- Granulocytes, which have specific granules in their cytoplasm
- Agranulocytes, which lack specific granules in their cytoplasm

Both granulocytes and agranulocytes possess nonspecific **(azurophilic)** granules, now known to be **lysosomes**.

There are three types of granulocytes, differentiated according to the color of their specific granules after application of Romanovsky-type stains:

- Neutrophils
- Eosinophils
- Basophils

There are two types of agranulocytes:

- Lymphocytes
- Monocytes

The differential leukocyte count and various properties of the leukocytes are detailed in Table 10–3.

Neutrophils

Neutrophils compose most of the white blood cell population; they are avid phagocytes, destroying bacteria that invade connective tissue spaces.

Neutrophils (polymorphonuclear leukocytes, polys) are granulocytes and are the most numerous of the WBCs, constituting 60% to 70% of the total leukocyte population. In blood smears, neutrophils are 9 to 12 µm in diameter and have a multilobed nucleus (see Figs. 10–2 and 10–3). The lobes, connected to each

TABLE 10–3 Leukocytes

	GRANULOCYTES			AGRANULOCYTES	
Features	**Neutrophils**	**Eosinophils**	**Basophils**	**Lymphocytes**	**Monocytes**
Number/mm^3	3500–7000	150–400	50–100	1500–2500	200–800
% of WBCs	60–70%	2–4%	<1%	20–25%	3–8%
Diameter (μm)					
(section)	8–9	9–11	7–8	7–8	10–12
(smear)	9–12	10–14	8–10	8–10	12–15
Nucleus	Three to four lobes	Two lobes (sausage shaped)	S-shaped	Round	Kidney shaped
Specific granules	0.1 μm, light pink*	1–1.5 μm, dark pink*	0.5 μm, blue/black*	None	None
Contents of specific granules	Type IV collagenase, phospholipase A_2, lactoferrin, lysozyme, phagocytin, alkaline phosphatase, vitamin B_{12}–binding protein	Aryl sulfatase, histaminase, β-glucuronidase, acid phosphatase, phospholipase, major basic protein, eosinophil cationic protein, neurotoxin, ribonuclease, cathepsin, peroxidase	Histamine, heparin, eosinophil chemotactic factor, neutrophil chemotactic factor, peroxidase, neutral proteases, chondroitin sulfate	None	None
Surface markers	Fc receptors, platelet-activating factor receptor, leukotriene B_4 receptor, leukocyte cell adhesion molecule-1	IgE receptors, eosinophil chemotactic factor receptor	IgE receptors	*T cells:* T-cell receptors, CD molecules, IL receptors *B cells:* surface immunoglobulins	Class II HLA, Fc receptors
Life span	<1 week	<2 weeks	1–2 years (in murines)	Few months to several years	Few days in blood, several months in connective tissue
Function	Phagocytosis and destruction of bacteria	Phagocytosis of antigen-antibody complex; destruction of parasites	Similar to mast cells to mediate inflammatory responses	*T cells:* cell-mediated immune response *B cells:* humorally mediated immune response	Differentiate into macrophage: phagocytosis, presentation of antigens

*Using Romanovsky-type stains (or their modifications).
CD, Cluster of differentiation; HLA, human leukocyte antigen; IgE, immunoglobulin E; IL, interleukin; WBC, white blood cell.

other by slender chromatin threads, increase in number with the age of the cell. In females, the nucleus presents a characteristic small appendage, the "drumstick," which contains the condensed, inactive second X chromosome. It is also called the ***Barr body*** or ***sex chromosome*** but is not always evident in every cell. Neutrophils are among the first cells to appear in acute bacterial infections. The neutrophil plasmalemma possesses complement receptors as well as Fc receptors for IgG.

Neutrophil Granules

Neutrophils possess specific, azurophilic, and tertiary granules.

Three types of granules are present in the cytoplasm of neutrophils:

- Small, specific granules (0.1 μm in diameter)
- Larger azurophilic granules (0.5 μm in diameter)
- Tertiary granules

Specific granules contain various enzymes and pharmacological agents that aid the neutrophil in performing its antimicrobial functions (see Table 10–3). In electron micrographs, these granules appear to be somewhat oblong (Fig. 10–6).

Azurophilic granules, as indicated previously, are lysosomes, containing acid hydrolases, myeloperoxidase (MPO), the antibacterial agent lysozyme, bactericidal permeability–increasing protein, cathepsin G (an enzyme that may contribute to the destruction and degradation of pathogens phagocytosed by these cells), elastase, and nonspecific collagenase.

Tertiary granules contain gelatinase and cathepsins as well as glycoproteins that are inserted into the plasmalemma.

Neutrophil Functions

Neutrophils phagocytose and destroy bacteria by using the contents of their various granules.

Neutrophils interact with chemotactic agents to migrate to sites invaded by microorganisms. They accomplish this by entering postcapillary venules in the region of inflammation and adhering to the various **selectin molecules** located on the luminal cell membranes of the endothelial cells of these vessels by use of their **selectin receptors**. The interaction between the neutrophil's selectin receptors and the selectins of the endothelial cells causes the neutrophils to roll slowly along the

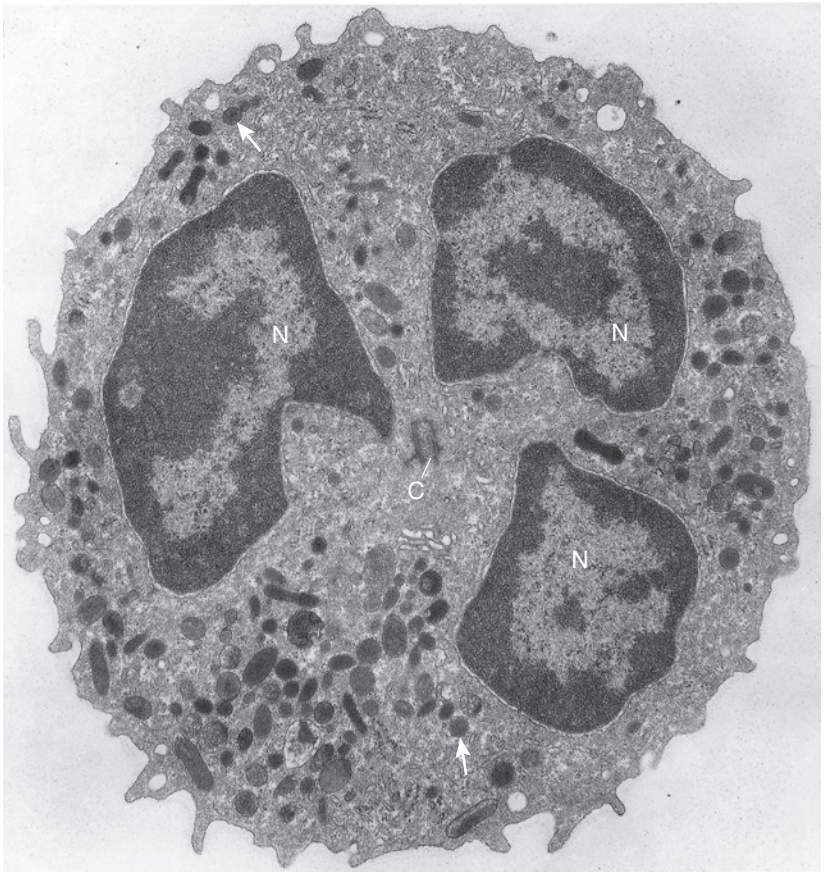


Figure 10–6 Electron micrograph of a human neutrophil. Note the three lobes of the nucleus (N), the presence of granules *(arrows)* throughout the cytoplasm, and the centrally located centriole (C). Although it appears as if there were three distinct nuclei in this image, they are lobes of the same nucleus, and the connections are merely outside of the present field of view. (From Zucker-Franklin D, et al, eds. *Atlas of Blood Cells*. Vol 1. Milan, Italy: Edi Ermes; 1981.)

vessel's endothelial lining. As the neutrophils are slowing their migrations, **interleukin-1** (IL-1) and **tumor necrosis factor (TNF)** induce the endothelial cells to express **intercellular adhesion molecule type 1 (ICAM-1)**, to which the **integrin molecules** of neutrophils avidly bind.

When binding occurs, the neutrophils stop migrating in preparation for their passage through the endothelium of the postcapillary venule to enter the connective tissue compartment. Once there, they destroy the microorganisms by phagocytosis and by the release of hydrolytic enzymes (and **respiratory burst**). In addition, by manufacturing and releasing **leukotrienes**, neutrophils assist in the initiation of the inflammatory process. The sequence of events is as follows:

1. The binding of neutrophil chemotactic factor (NCF), released by mast cells and basophils to the neutrophil's plasmalemma NCF receptors, facilitates the release of the contents of tertiary granules into the extracellular matrix.
2. Gelatinase and cathepsins degrades the basal lamina, facilitating neutrophil migration. Glycoproteins present in the tertiary granules become inserted in the cell membrane and aid the process of phagocytosis.
3. The contents of the specific granules are also released into the extracellular matrix, where they attack the invading microorganisms and aid neutrophil migration.
4. Microorganisms, phagocytosed by neutrophils, become enclosed in phagosomes (Fig. 10–7A, B). Enzymes and pharmacological agents of the azurophilic granules are usually released into the lumina of these intracellular vesicles, where they destroy the ingested microorganisms. Because of their phagocytic functions, neutrophils are also known as *microphages* to distinguish them from the larger phagocytic cells, the macrophages.
5. Bacteria are killed not only by the action of enzymes but also by the formation of reactive oxygen compounds within the phagosomes of neutrophils. These are superoxide (O_2^-), formed by the action of NADPH oxidase on O_2 in a respiratory burst; hydrogen peroxide, formed by the action of superoxide dismutase

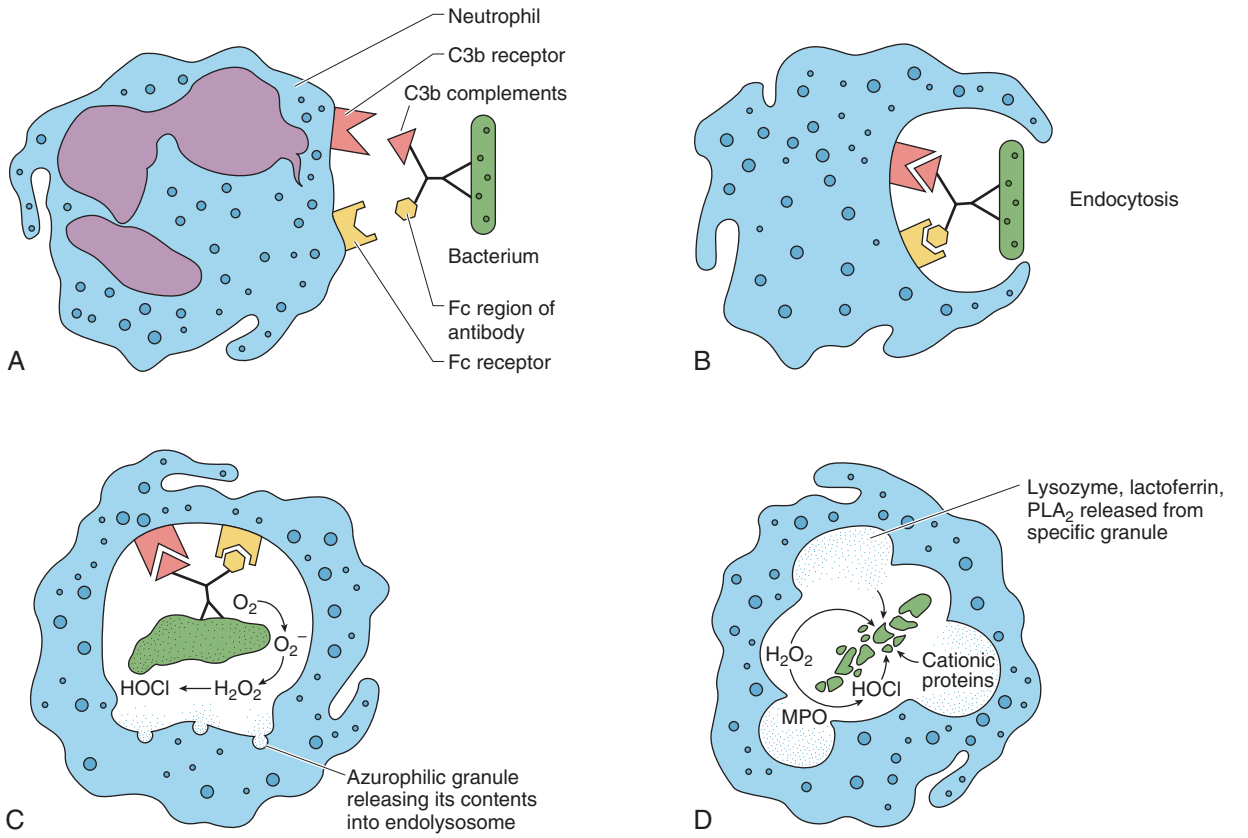


Figure 10–7 Bacterial phagocytosis and destruction by a neutrophil. These actions are dependent on the ability of the neutrophil to recognize the bacterium via the presence of complement and/or antibody attached to the microorganism. H_2O_2, Hydrogen peroxide; HOCl, hypochlorous acid; MPO, myeloperoxidase O_2^-, superoxide.

on superoxide; and hypochlorous acid, formed by the interaction of MPO (myeloperoxidase) and chloride ions with hydrogen peroxide (see Fig. 10–7 *C, D*).

6. Frequently, the contents of the azurophilic granules are released into the extracellular matrix, causing tissue damage, but usually catalase and glutathione peroxidase limit the tissue injury by degrading hydrogen peroxide.
7. Once neutrophils perform their function of killing microorganisms, they also die, resulting in the formation of pus, the accumulation of dead leukocytes, bacteria, and extracellular fluid.
8. Not only do neutrophils destroy bacteria, but also they synthesize leukotrienes from arachidonic acids in their cell membranes. These newly formed leukotrienes aid in the initiation of the inflammatory process.

CLINICAL CORRELATIONS

1. Children with hereditary deficiency of NADPH oxidase are subject to persistent bacterial infections because their neutrophils cannot form a respiratory burst response to the bacterial challenge. Their neutrophils cannot generate superoxide, hydrogen peroxide, or hypochlorous acid during phagocytosis of bacteria.

2. Individuals suffering from neutropenia, low levels of neutrophils in circulating blood, have problems fighting bacterial infections. This condition may be acute, lasting less than 3 months, or chronic, lasting more than 3 months. Neutropenia may be mild (1000 to 1500 neutrophils per mm^3 of blood), moderate (500 to 1000 neutrophils per mm^3 of blood), or severe (less than 500 neutrophils per mm^3 of blood). The causes of neutropenia may be due to decreased neutrophil production by the bone marrow or excess neutrophil destruction outside the bone marrow.

Eosinophils

Eosinophils phagocytose antigen-antibody complexes and kill parasitic invaders.

Eosinophils are granulocytes and they constitute less than 4% of the total WBC population. They are round cells in suspension and in blood smears, but they may be pleomorphic during their migration through connective tissue. Their cell membrane has receptors for IgG, IgE, and complement. Eosinophils are 10 to 14 µm in diameter (in blood smears) and have a sausage-shaped, bilobed nucleus in which the two lobes are connected by a thin chromatin strand and surrounding nuclear envelope (see Figs. 10–2 and 10–3). Electron micrographs display a small, centrally located Golgi apparatus, a limited amount of rough endoplasmic reticulum (RER), and only a few mitochondria, usually in the vicinity of the centrioles near the cytocenter. Eosinophils are produced in the bone marrow, and **interleukin-5 (IL-5)** causes proliferation of their precursors and their differentiation into mature cells; in the absence of IL-5, basophils develop instead of eosinophils (see later sections for more detailed information about hemopoiesis).

Eosinophil Granules

The specific granules of eosinophils possess an externum and an internum.

Eosinophils possess specific granules and azurophilic granules. Specific granules are oblong (1.0 to 1.5 µm in length, <1.0 µm in width) and stain deep pink with Giemsa and Wright stains. Electron micrographs demonstrate that specific granules have a crystal-like, electron-dense center, the **internum**, surrounded by a less electron-dense **externum** (Fig. 10–8). The internum contains **major basic protein**, **eosinophilic cationic protein**, and **eosinophil-derived neurotoxin**, the first two of which are highly efficacious agents in combating parasites. The externum contains the enzymes listed in Table 10–3.

The nonspecific azurophilic granules are lysosomes (0.5 µm in diameter) containing hydrolytic enzymes similar to those found in neutrophils. These function both in the destruction of parasitic worms and in the hydrolysis of antigen-antibody complexes internalized by eosinophils.

Eosinophil Functions

Eosinophils assist in the elimination of antibody-antigen complexes and in the destruction of parasitic worms.

Eosinophils are associated with the following functions:

- The binding of histamine, leukotrienes, and eosinophil chemotactic factor (released by mast cells, basophils, and neutrophils) to eosinophil plasmalemma receptors results in the migration of eosinophils to the site of allergic reaction, inflammatory reaction, or parasitic worm invasion.
- Eosinophils degranulate their major basic protein or eosinophil cationic protein on the surface of the parasitic worms, killing them by forming pores in their pellicles, thus facilitating access of agents, such as superoxides and hydrogen peroxide to the parasite cell membrane and cytoplasm.
- They release substances that inactivate the pharmacological initiators of the inflammatory response, such as histamine and leukotriene C.
- They engulf antigen-antibody complexes that pass into the eosinophil's endosomal compartment for eventual degradation.
- The ribonucleases in the azurophilic granules of eosinophils combat viral pathogens.
- They participate in the degradation of fibrin.

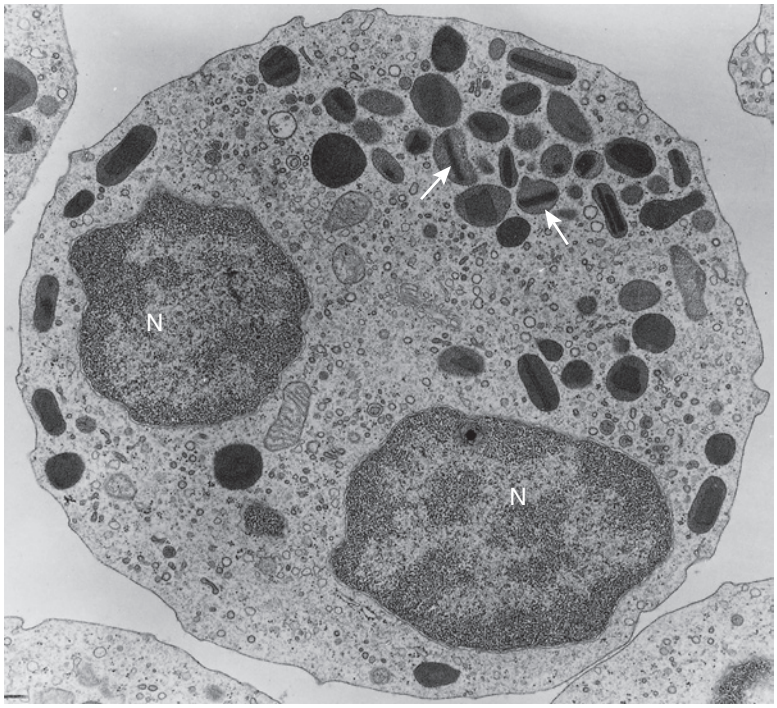


Figure 10–8 Electron micrograph of a human eosinophil. Note the electron-dense internum *(arrows)* of the eosinophilic granules and the two lobes of the nucleus (N). (From Zucker-Franklin D. Eosinophil function and disorders. *Adv Intern Med.* 1974;19:1-25.)

CLINICAL CORRELATIONS

Connective tissue cells in the vicinity of antigen-antibody complexes release the pharmacological agents histamine and IL-5, causing increased formation and release of eosinophils from the bone marrow. On the contrary, elevation of blood corticosteroid levels depresses the number of eosinophils in circulation.

Basophils

Basophils are similar to mast cells in function even though they originate from different precursors in the bone marrow.

Basophils are granulocytes, and they constitute less than 1% of the total leukocyte population. They are round cells in suspension but may be pleomorphic during migration through connective tissue. They are 8 to 10 µm in diameter (in blood smears) and have an **S**-shaped nucleus, which is commonly masked by the large specific granules present in the cytoplasm (see Figs. 10–2 and 10–3). In electron micrographs, the small Golgi apparatus, few mitochondria, extensive RER, and occasional glycogen deposits are clearly evident. Basophils have several surface receptors on their plasmalemma, including **immunoglobulin E (IgE) receptors (FcεRI)**. Basophils originate in the bone marrow, and as indicated above the formation of basophils requires the absence of IL-5.

Basophil Granules

Basophils possess specific and azurophilic granules.

The **specific granules** of basophils stain dark blue to black with Giemsa and Wright stains. They are approximately 0.5 µm in diameter and frequently press against the periphery of the cell, creating the basophil's characteristic "roughened" perimeter, as seen by light microscopy. The granules contain heparin, histamine, eosinophil chemotactic factor, neutrophil chemotactic factor, neutral proteases, chondroitin sulfate, and peroxidase (see Table 10–3). The nonspecific **azurophilic granules** are lysosomes, which contain enzymes similar to those of neutrophils.

Basophil Functions

Basophils function as initiators of the inflammatory process.

In response to the presence of some antigens in certain individuals, plasma cells manufacture and release a particular class of immunoglobulin, IgE. The Fc portions of the IgE molecules become attached to the **FcεRI** receptors of basophils and mast cells without any apparent effect. However, the next time that the same

antigens enter the body, they bind to the IgE molecules on the surface of these cells. Although mast cells and basophils appear to have similar functions, they are different cells and they originate from different precursors in the bone marrow.

Although the following sequence of steps occurs in both mast cells and basophils, the basophil is used for descriptive purposes:

1. Binding of antigens to the IgE molecules to FcεRI receptors on the surface of a basophil causes the cell to release the contents of its specific granules into the extracellular space.
2. In addition, the enzyme phospholipase A generates arachidonic acid residues from the plasma membrane, which then is fed into the cyclooxygenase or the lipoxygenase pathway to produce chemical factors that mediate the inflammatory response. The factors are platelet activating factor, leukotriene B_4, prostaglandin D_2, thromboxane A_2, leukotriene C_4, leukotriene D_4, leukotriene E_4 (formerly called *slow-reacting substance of anaphylaxis*, or *SRS-A*), adenosine, bradykinin, superoxide, TNF-α, IL-4, IL-5, IL-6, and granulocyte-monocyte colony-stimulating factor (GM-CSF).
3. The release of histamine causes vasodilation, smooth muscle contraction (in the bronchial tree), and leakiness of blood vessels.
4. Leukotrienes have similar effects, but these actions are slower and more persistent than those associated with histamine. In addition, leukotrienes activate leukocytes, causing them to migrate to the site of antigenic challenge.

CLINICAL CORRELATIONS

In certain hyperallergic individuals, a second exposure to the same allergen may result in an intense generalized response. A large number of basophils (and mast cells) degranulate, resulting in widespread vasodilation and sweeping reduction in blood volume (because of vessel leakiness). Thus, the person goes into circulatory shock. The smooth muscles of the bronchial tree constrict, causing respiratory insufficiency. The combined effect is a life-threatening condition known as *anaphylactic shock*.

Monocytes

Monocytes, the largest of the circulating blood cells, are agranulocytes; they enter the connective tissue spaces, where they are known as macrophages.

Monocytes are agranulocytes and are the largest of the circulating blood cells (12 to 15 µm in diameter in blood smears) and constitute 3% to 8% of the leukocyte population. They have a large, acentric, kidney-shaped nucleus that frequently has a "moth-eaten," soap-bubbly appearance and whose lobe-like extensions seem to overlap one another. The chromatin network is coarse but not overly dense, and typically two nucleoli are present, although they are not always evident in smears. The cytoplasm is bluish gray and has numerous azurophilic granules (lysosomes) and occasional vacuole-like spaces (see Figs. 10–2 and 10–3).

Electron micrographs display both heterochromatin and euchromatin in the nucleus as well as two nucleoli. The Golgi apparatus is usually near the indentation of the kidney-shaped nucleus. The cytoplasm contains deposits of glycogen granules, a few profiles of RER, some mitochondria, free ribosomes, and numerous lysosomes. The periphery of the cell displays microtubules, microfilaments, pinocytotic vesicles, and filopodia.

Monocytes stay in circulation for only a few days; they then migrate through the endothelium of venules and capillaries into the connective tissue, where they differentiate into **macrophages** or **dendritic cells**. Recently, it has been suggested that there are three types of monocytes (classical, intermediate, and nonclassical, depending on their membrane markers) and three types of dendritic cells (plasmacytoid, CD1c and CD141, depending on their membrane markers). Monocytes and dendritic cells and their subtypes are discussed in greater detail in Chapter 12; an introduction to the properties and functions of macrophages follows here.

Function of Macrophages

Macrophages phagocytose unwanted particular matter, produce cytokines that are required for the inflammatory and immune responses, and present epitopes to T lymphocytes.

- Macrophages are avid phagocytes and, as members of the mononuclear phagocyte system, they phagocytose and destroy dead and defunct cells (e.g., senescent erythrocytes) as well as antigens and foreign particulate matter (e.g., bacteria). The destruction occurs within the phagosomes through both enzymatic digestion and the formation of superoxide, hydrogen peroxide, and hypochlorous acid.
- Macrophages produce cytokines that activate the inflammatory response as well as the proliferation and maturation of other cells.
- Certain macrophages, as well as dendritic cells, are antigen-presenting cells that phagocytose antigens and present the most antigenic fragments of these macromolecules, known as *epitopes*, in conjunction with the integral proteins, class II human leukocyte antigen **(class II HLA)**; also known as *major histocompatibility complex antigens* [MHC II]), to immunocompetent T lymphocytes.
- In response to large foreign particulate matter, macrophages fuse with one another, forming foreign-body giant cells that are large enough to phagocytose the foreign particle.

Lymphocytes

Lymphocytes are agranulocytes and form the second largest population of white blood cells.

Lymphocytes are agranulocytes that constitute 20% to 25% of the total circulating leukocyte population. They are round cells in blood smears, but they may be pleomorphic as they migrate through connective tissue. Lymphocytes are somewhat larger than erythrocytes, 8 to 10 µm in diameter (in blood smears), and have a slightly indented, round nucleus that occupies most of the cell. The nucleus is dense, rich in heterochromatin, and somewhat acentrically located. The peripherally situated cytoplasm stains a light blue and contains a few azurophilic granules. On the basis of size, lymphocytes may be described as small (8–10 µm in diameter), medium (12 to 15 µm), or large (15 to 18 µm), although the latter two are much less numerous (see Figs. 10–2 and 10–3).

Electron micrographs of lymphocytes display a scant amount of peripheral cytoplasm housing a few mitochondria, a small Golgi apparatus, and a few profiles of RER. A small number of lysosomes, representing azurophilic granules 0.5 µm in diameter, and an abundant supply of ribosomes are also evident (Fig. 10–9).

Lymphocytes are discussed in greater detail in Chapter 12; an introduction to their properties and functions follows here.

Types of Lymphocytes

There are three types of lymphocytes: T lymphocytes, B lymphocytes, and null cells.

Lymphocytes are subdivided into three functional categories: **B lymphocytes (B cells)**, **T lymphocytes (T cells)**, and **null cells**. Although morphologically, they are indistinguishable from each other, they may be recognized immunocytochemically by the differences in their surface markers (see Table 10–3). Approximately 80% of the circulating lymphocytes are T cells, about 15% are B cells, and the remainder are null cells. Their life spans also differ widely: some T cells may live for years, whereas some B cells may die in a few months.

Functions of B and T Cells

In very general terms, B cells are responsible for the humorally mediated immune system, whereas T cells are responsible for the cellularly mediated immune system.

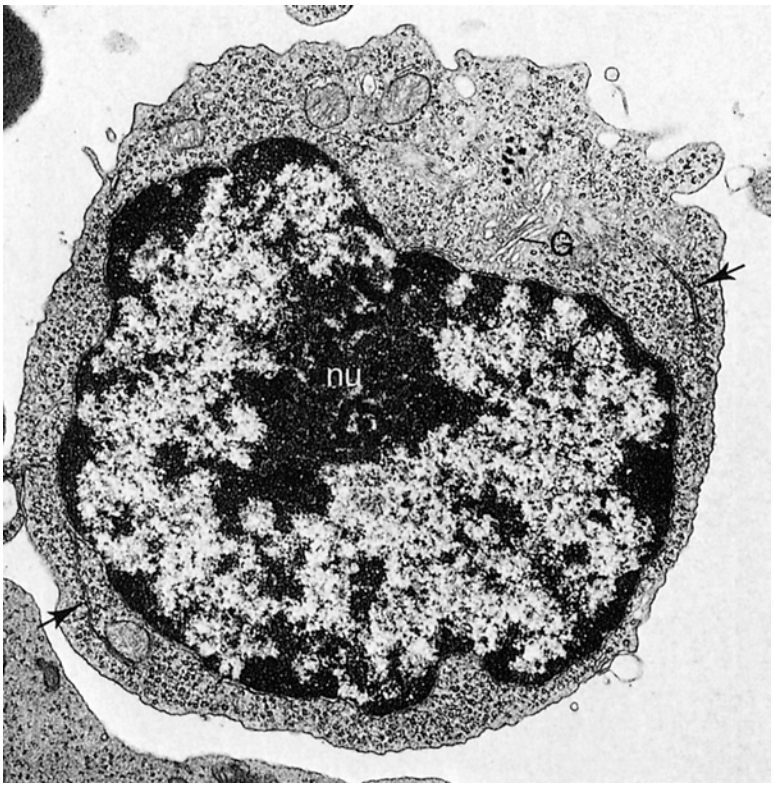


Figure 10–9 Electron micrograph of a lymphocyte (×14173). *Arrows* point to the rough endoplasmic reticulum. G, Golgi apparatus; nu, nucleus. (From Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

Lymphocytes have no function in the bloodstream, but in the connective tissue, these cells are responsible for the proper functioning of the immune system. To be immunologically competent, they migrate to specific body compartments to mature and to express specific surface markers and receptors. Lymphocytes destined to be B cells enter as yet unidentified regions of the **bone marrow**, whereas lymphocytes destined to be T cells migrate to the cortex of the **thymus**. Once they have become immunologically competent, lymphocytes leave their respective sites of maturation, enter the lymphoid system, and undergo mitosis, forming a group of identical cells known as a ***clone***. All members of a particular clone can recognize and respond to the same antigen.

After stimulation by a specific antigen, both B and T cells proliferate and differentiate into two subpopulations:

- Memory cells (whether B memory cells or T memory cells) do not participate in the immune response but remain as part of the clone with an "immunological memory," ready to undergo cell division and mount a response against a subsequent exposure to a particular antigen or foreign substance.
- Effector cells are classified as B cells and T cells (and their subtypes) and are detailed in the following section.

Effector Cells

Effector cells are immunocompetent lymphocytes that can perform their immune functions, that is, eliminating antigens and foreign or virally altered cells.

B cells are responsible for the **humorally mediated immune system**; that is, they differentiate into **plasma cells**, which produce **antibodies** against **antigens**. T cells are responsible for the **cellularly mediated immune system**. Some T cells differentiate into **cytotoxic T cells (T killer cells)** and **natural killer T cells (NKT cells)**, which make physical contact with and kill **foreign** or **virally altered cells**. In addition, certain T cells are responsible for the initiation and development **(T helper cells)** or for the suppression (**regulatory T cells [T reg cells]**, formerly known as *T suppressor cells*) of most humorally and cellularly mediated immune responses. They accomplish this by releasing signaling molecules known as ***cytokines (lymphokines)*** that elicit specific responses from other cells of the immune system (see Chapter 12).

Null Cells

Null cells are composed of two distinct populations:

- Circulating stem cells, which give rise to all of the formed elements of blood
- Natural killer (NK) cells, which can kill some foreign and virally altered cells on their own, without the influence of the thymus or T cells

CLINICAL CORRELATIONS

Lymphomas are cancers of lymphocytes (although some authors also consider noncancerous tumors of lymphocytes under the same heading). Even though there are various subcategories of lymphoma, historically they were subdivided into two major types, Hodgkin disease (Hodgkin lymphoma) and non-Hodgkin disease (non-Hodgkin lymphoma). The former accounted for 25% of lymphomas and the latter about 75% of all lymphomas. The general symptoms include swollen lymph nodes without accompanying pain, tiredness, itchiness, night sweat, elevated temperature, generalized malaise, and unexplained loss of weight.

1. Hodgkin disease occurs in two age groups, individuals in their late teens and early 30's as well as in people in their mid-50s to early 60s. In both groups, it usually originates in and spreads from lymph node to lymph node. As the disease progresses, it spreads into the liver, spleen, bone marrow, and the intestines. Histology presents with characteristically large, modified B cells (30 to 60 µm in diameter), known as Reed-Sternberg cells, with two distinctive nuclei that are mirror images of one another.

2. Non-Hodgkin disease occurs mostly in middle-aged and older individuals. The cancer is mostly of B cell origin, although in approximately 10% of the cases, T cells give rise to the malignancy. Unlike in Hodgkin disease, the cancer originates in lymph nodes in 75% of the cases and in other regions of the body such as the brain, intestines, stomach, and even the thyroid gland in 25% of the cases. Classically, non-Hodgkin disease may be of low-grade malignancy that takes years to develop or high-grade malignancy that develops over a period of weeks or months.

Platelets

Platelets (thromboplastids) are small, disk-shaped, nonnucleated cell fragments derived from megakaryocytes in the bone marrow.

Platelets are about 2 to 4 µm in diameter in blood smears (see Figs. 10–2 and 10–3). In light micrographs, they display a peripheral clear region, the **hyalomere**, and a central darker region, the **granulomere**. The platelet plasmalemma has numerous receptor molecules as well as a relatively thick (15 to 20 nm) **glycocalyx**, composed of glycoproteins, glycosaminoglycans, coagulation factors, and the extracellular moiety of the transmembrane glycoprotein Ib. There are between 250,000 and 400,000 platelets per mm^3 of blood, each with a life span of less than 14 days.

Platelet Tubules and Granules

Platelets possess three types of granules (alpha, delta, lambda) as well as two tubular systems (dense and surface opening).

Electron micrographs of platelets display 10 to 15 microtubules arranged parallel to each other and forming a ring within the hyalomere. The microtubules assist platelets in maintaining their discoid morphology. Associated with this bundle of microtubules are actin and myosin monomers, which can rapidly assemble to form a contractile apparatus. In addition, two tubular systems are present in the hyalomere, the **surface-opening (connecting)** and the **dense tubular systems** (Figs. 10–10 and 10–11). The surface-opening system is coiled, forming a labyrinthine complex within the platelet. Because this system communicates with the outside, the luminal aspect of this tubular system is a continuation of the outer surface of the platelet, thus increasing the platelet surface area by a factor of seven or eight.

The ultrastructure of the granulomere displays the presence of a small number of mitochondria, glycogen deposits, peroxisomes, and three types of granules: **alpha-granules (α-granules)**, **delta granules (δ-granules)**, and **lambda-granules** (**λ-granules**, lysosomes). The tubules and granules, as well as their contents and functions, are listed in Table 10–4. The granulomere also houses a system of enzymes that permits platelets to catabolize glycogen, consume oxygen, and generate ATP.

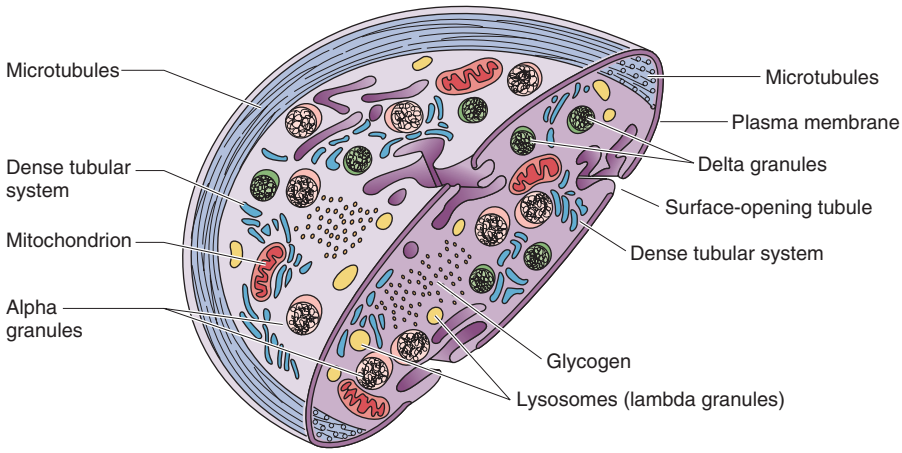


Figure 10–10 Diagram of platelet ultrastructure. Note that the periphery of the platelet is occupied by actin filaments that encircle the platelet and maintain the discoid morphology of this structure.

TABLE 10–4 Platelet Tubules and Granules

Structure (Size)	Location	Contents	Function
Surface-opening tubule system	Hyalomere		Expedites rapid uptake and release of molecules from activated platelets
Dense tubular system	Hyalomere		Probably sequesters calcium ions to prevent platelet "stickiness"
α-Granules (300–500 nm)	Granulomere	Fibrinogen, platelet-derived growth factor, platelet thromboplastin, thrombospondin, coagulation factors	Contained factors facilitate vessel repair, platelet aggregation, and coagulation of blood
δ-Granules (dense bodies) (250–300 nm)	Granulomere	Calcium, ADP, ATP, serotonin, histamine, pyrophosphatase	Contained factors facilitate platelet aggregation and adhesion, as well as vasoconstriction
λ-Granules (lysosomes) (200–250 nm)	Granulomere	Hydrolytic enzymes	Contained enzymes aid clot resorption

ADP, Adenosine diphosphate; ATP, adenosine triphosphate.

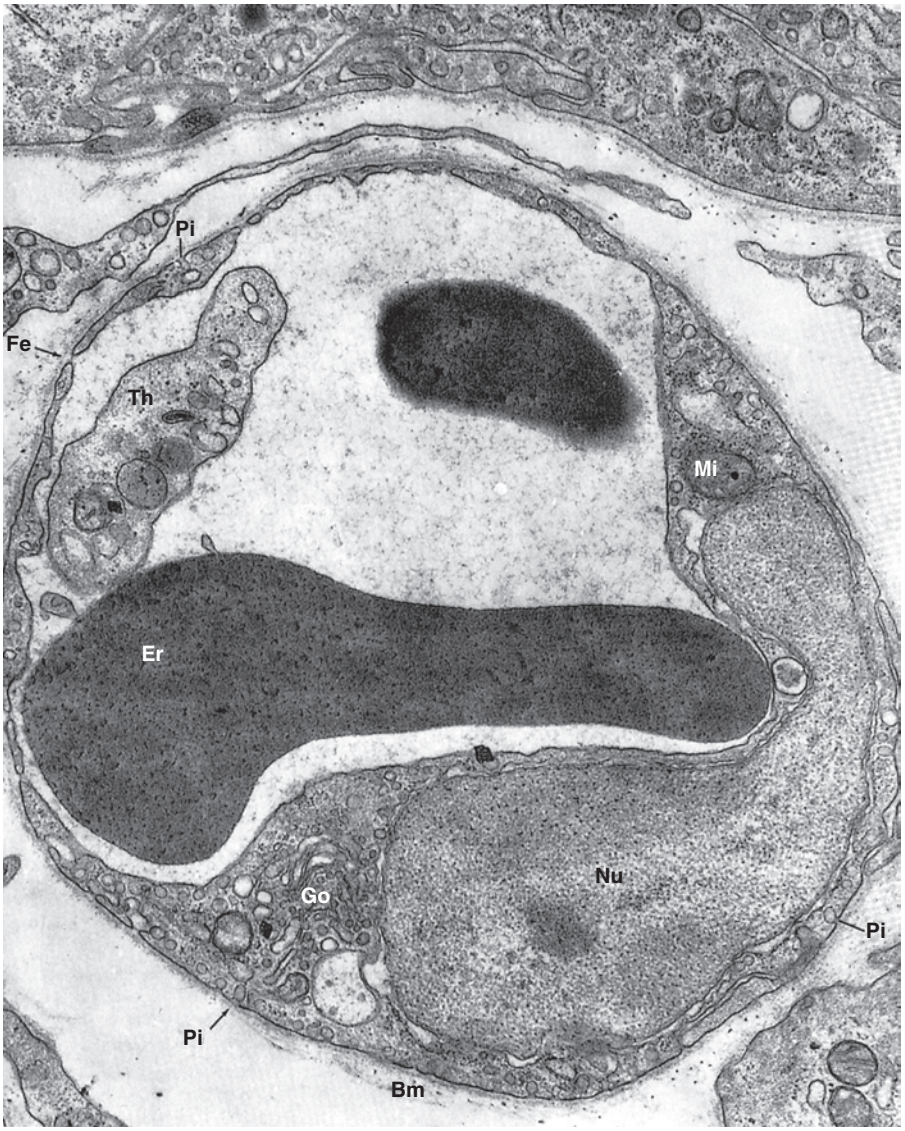


Figure 10–11 Electron micrograph of a platelet and two erythrocytes in the gastric mucosa capillary (×22100). Bm, Basal lamina; Er, erythrocyte; Fe, fenestra; Go, Golgi apparatus; Nu, nucleus of the capillary; Pi, pinocytotic vesicles; Th, platelet. (From Rhodin JAG. *An Atlas of Ultrastructure*. Philadelphia: WB Saunders; 1963.)

Platelet Function

Platelets function in limiting hemorrhage to the endothelial lining of the blood vessel in case of injury.

If the endothelial lining of a blood vessel is disrupted and platelets come in contact with the subendothelial collagen, they become **activated**, release the contents of their granules, adhere to the damaged region of the vessel wall **(platelet adhesion)**, and adhere to each other **(platelet aggregation)**, as well as participating in **clot retraction** and **clot removal**. Interactions of tissue factors, plasma-borne factors, and platelet-derived factors form a blood clot (Figs. 10–12 and 10–13). Although the mechanism of platelet aggregation, adhesion, and blood clotting is beyond the scope of histology, some of its salient features are as follows:

1. Normally, the intact endothelium produces prostacyclins and NO, which inhibit platelet aggregation. It also blocks coagulation by the presence of thrombomodulin and heparin-like molecule on its luminal plasmalemma. These two membrane-associated molecules inactivate specific coagulation factors.
2. The process of coagulation is a bimodal system, and platelets are the connecting link between them.

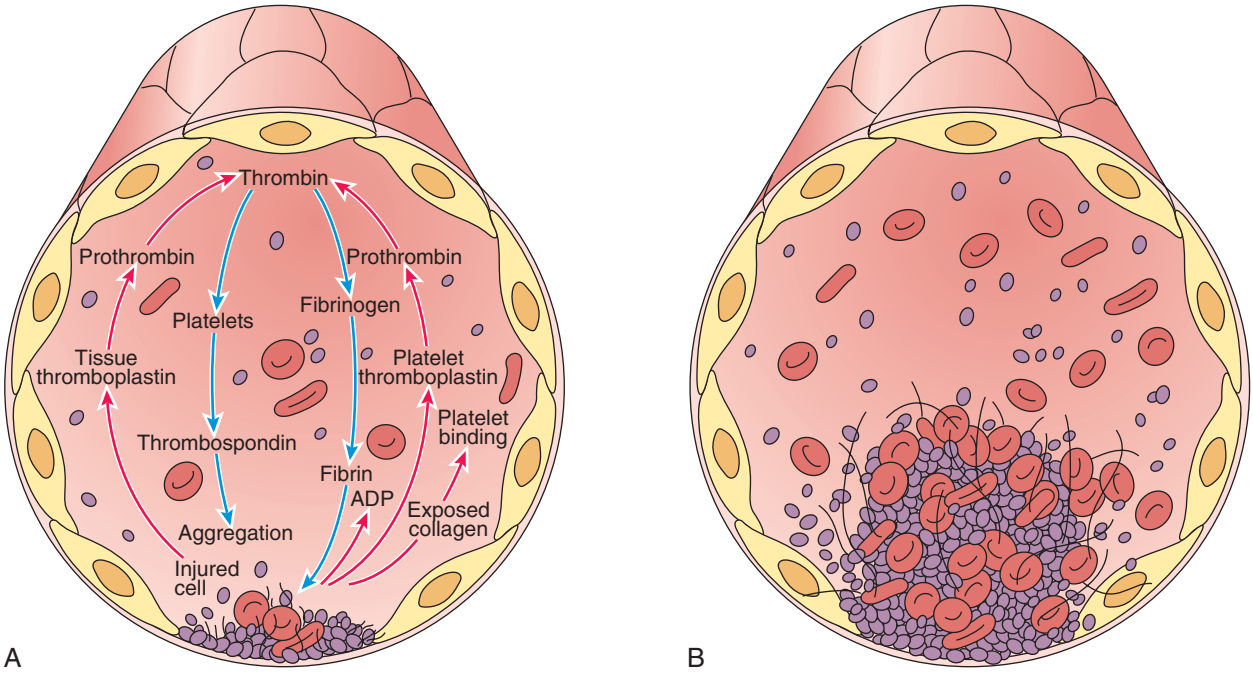


Figure 10–12 Schematic diagram of clot formation. **A,** Injury to the endothelial lining releases various clotting factors and ceases the release of inhibitors of clotting. **B,** The increase in the size of the clot plugs the defect in the vessel wall and stops the loss of blood. ADP, Adenosine diphosphate. (From Fawcett DW. *Bloom and Fawcett's A Textbook of Histology*. 12th ed. New York: Chapman and Hall; 1994.)

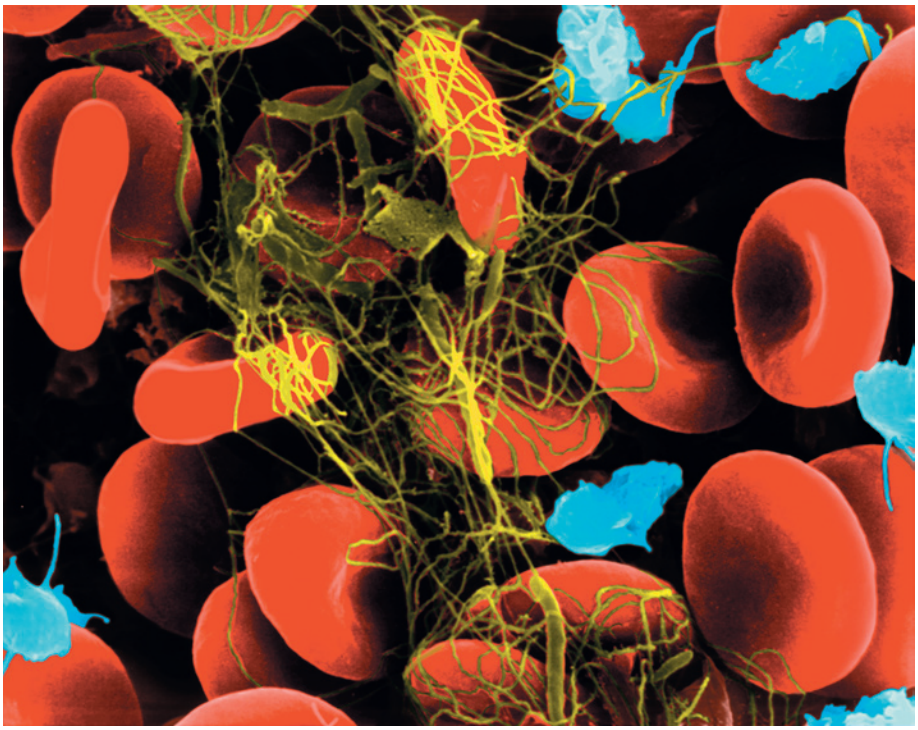


Figure 10–13 This close-up view of a clot forming in human blood shows beautifully how the different blood components are crammed into the plasma. (The scanning electron micrographs have been colored to emphasize the different structures.) Red blood cells *(red)* are entangled with the fibrin *(yellow)* that makes up the scaffolding of the clot. The platelets *(blue)*, which initiate clotting, are fragments of larger cells (megakaryocytes). (Courtesy Dennis Kunkel, PhD.)

Injured endothelial cells cease the production and expression of the inhibitors of coagulation and platelet aggregation, and they release von Willebrand factor (vWF) and tissue factor (also known as *thromboplastin*). They also release endothelin, a powerful vasoconstrictor that reduces the loss of blood.

3. The glycoprotein Ib molecules of platelet membranes avidly adhere to vWF, which then adheres to the exposed collagen fibers of the vessel wall. This contact between vWF and the glycoprotein Ib induces the platelets to release the contents of their granules and adhere to one another. These three events are collectively called *platelet activation*.
4. The release of some of their granular contents, especially calcium ions, adenosine diphosphate, and thrombospondin, makes glycocalyx of the platelets "sticky," causing circulating platelets to adhere to the collagen-bound platelets and to degranulate.
5. Arachidonic acid, formed in the activated platelet plasmalemma, is converted to thromboxane A_2, a potent vasoconstrictor and an avid platelet activator.
6. The aggregated platelets act as a plug, blocking hemorrhage, and the platelets maintain their contact with each other by exposing additional integrin molecules, glycoproteins IIb/IIIa, whose extracellular moieties make firm bonds with those of adjacent platelets, eventually forming the rather weak primary hemostatic plug. Less than a half an hour after its formation, enough fibrinogen, a normal constituent of blood, contacts the primary hemostatic plug that its adhering platelets are induced to contract forming a dense, stable secondary hemostatic plug that is fixed to the site of injury by the fibrin filaments resulting from the coagulation process. To prevent the formation of an overly large secondary hemostatic plug, the endothelial cells release prostacyclins and NO. As this is occurring, the adhering platelets express platelet factor 3 on their plasmalemma, providing the necessary phospholipid surface for the proper assembly of the coagulation factors (especially of thrombin).
7. As part of the complex cascade of reactions involving the various coagulation factors, tissue factor and platelet thromboplastin both act on circulating prothrombin, converting it into thrombin. Thrombin is an enzyme that facilitates platelet aggregation. In the presence of calcium (Ca^{2+}), it also converts fibrinogen to fibrin. The mechanism of blood coagulation is described in the next section.
8. The fibrin monomers thus produced polymerize and form a fibrin reticulum that attach to the secondary hemostatic plug, thus establishing a reticulum of clot, entangling additional platelets, erythrocytes, and leukocytes into a stable, gelatinous blood clot (thrombus). The erythrocytes facilitate platelet activation, whereas neutrophils and endothelial cells limit both platelet activation and thrombus size.
9. Approximately 1 hour after clot formation, actin and myosin monomers form thin and thick filaments, which interact by using ATP as their energy source. As a result, the clot contracts to about half its previous size, pulling the cut edges of the damaged vessel closer together and minimizing blood loss.
10. When the vessel is repaired, the endothelial cells release tissue plasminogen activators and urokinase-type plasminogen activator, which convert circulating plasminogen to plasmin, the enzyme that initiates lysis of the thrombus, a process that is assisted by the hydrolytic enzymes of λ-granules.

CLINICAL CORRELATIONS

1. In a patient with a thromboembolism, the most common type of embolism, clots break free and circulate in the bloodstream until they reach a vessel whose lumen is too small to accommodate them. If a clot is large enough to occlude the bifurcation of the pulmonary artery (saddle embolus), it can result in sudden, unexpected death. If a clot obstructs branches of the coronary artery, a myocardial infarct may occur.

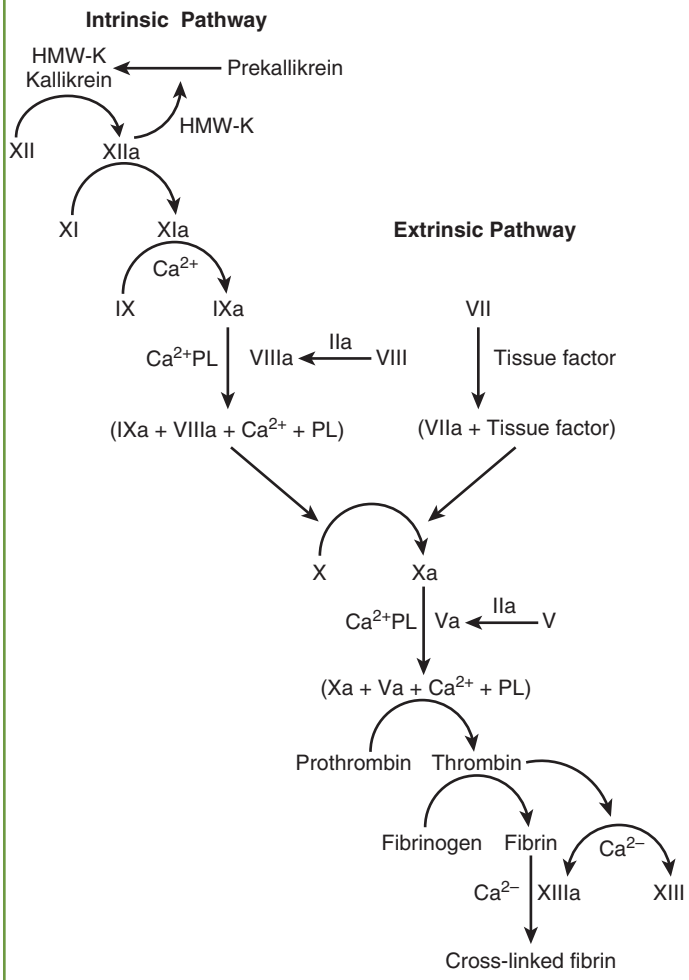
2. Several types of coagulation disorders that result in excessive bleeding are known. The disorder may be acquired (as in vitamin K deficiency) or hereditary (as in hemophilia) or may be caused by low levels of blood platelets (thrombocytopenia). Vitamin K is required by the liver as a cofactor in the synthesis of the clotting factors VII, IX, and X and prothrombin. Absence of or reduced levels of these factors result in partial or complete dysfunction of the clotting process.

3. In patients with thrombocytopenia, the blood level of platelets is decreased. The condition becomes serious when the platelet level is below 50,000/mm^3. Although bleeding is common in these patients, the bleeding is generalized and occurs from small vessels, resulting in purplish splotches on the skin. This condition is believed to be an autoimmune disease, in which antibodies are formed to one's own platelets, and these antibodies destroy the platelets.

Blood Coagulation Cascade

The coagulation of blood (clotting of blood) involves the interactions of a number of factors that circulate in the blood but it also requires the presence of phospholipid complexes on the plasma membranes of activated platelets and calcium ions (factor IV). Coagulation occurs by way of two pathways (Table 10–5), the tissue factor

TABLE 10–5 Diagram of the Blood Coagulation Pathways



Coagulation

Number	Common Name
I	Fibrinogen
Ia	Fibrin
II	Prothrombin
IIa	Thrombin
III	Tissue factor
IV	Ca^{2+} ions
V	Proaccelerin (labile factor)
Va	Accelerin
VII	Proconvertin (stable factor)
VIIa	Convertin
VIII	Antihemophilic factor A
IX	Christmas factor
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent
XIa	Plasma thromboplastin
XII	Hageman factor
XIII	Fibrin stabilizing factor (HMWK)

Contact Activation Pathway (Intrinsic Pathway)

1. High-molecular-weight kininogen (HMW-K) in concert with factor XII activates prekallikrein to form kallikrein.

2. Kallikrein and HMW-K activate factor XII to form factor XIIa.

3. Factor XIIa converts factor XI to factor XIa.

4. Factor XIa, in the presence of Ca^{2+} ions, converts factor IX to factor IXa.

5. Factor IXa, in the presence of Ca^{2+} ions and platelet phospholipids (PL) activate factor X to become factor Xa.

Tissue Factor Pathway (Extrinsic Pathway)

6. When the extrinsic pathway is activated, endothelial cells, and some connective tissue cells release tissue factor, which activates factor VII to factor VIIa.

7. Factor VIIa in concert with tissue factor and in the presence of Ca^{2+} ions activates factor X to factor Xa.

Common Pathway

8. The common pathway starts with the activation of factor X to factor Xa.

9. Factor Xa, in concert with factor Va in the presence of Ca^{2+} ions and platelet membrane phospholipids form a prothrombinase complex that converts prothrombin (factor II) to thrombin (factor IIa).

10. Thrombin converts fibrinogen (factor I) to fibrin monomers (factor Ia).

11. Factor XIIIa, in the presence of Ca^{2+} ions, facilitates the cross linking of fibrin monomers to form a fibrin plug.

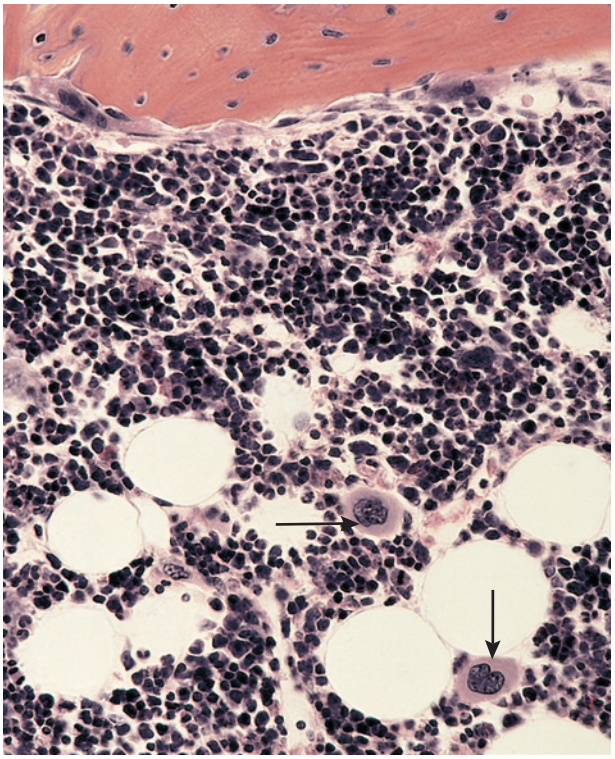


Figure 10–14 Photomicrograph of human bone marrow displaying two megakaryocytes *(arrows)*. Observe that marrow has a much greater population of nucleated cells than does peripheral blood. Also note the presence of epithelial reticular cells that resemble adipocytes. At the top of the photomicrograph, the decalcified bone with osteocytes located in lacunae is evident (×270).

pathway (extrinsic pathway) and contact activation pathway (intrinsic pathway). The final few steps of the two pathways are identical to each other and therefore are referred to as the *common pathway* (Fig. 10–14). It is important to note that the tissue factor pathway is the *principal coagulation pathway*, and the contact activation pathway is of secondary importance.

- The tissue factor (extrinsic) pathway is a rapid-onset pathway that occurs within seconds subsequent to an injury of a blood vessel if, *in addition* to the endothelium, the wall of the vessel is damaged. Connective tissue cells exposed to blood and endothelial cells release tissue factor (factor III, also known as *tissue thromboplastin*).
- **The contact activation (intrinsic) pathway** is a **slow-onset** pathway that occurs minutes after the endothelium *(but not the vessel wall)* is injured. Because the endothelium is damaged, collagens of the vessel wall are contacted by **factor XII (Hageman factor)**, which initiates blood coagulation.
- The **common pathway** is where the two other pathways converge and **fibrin monomers** are formed. These monomers join to produce the fibrin reticulum that attach to the secondary hemostatic plug and form the reticulum of the clot (see previous text).

CLINICAL CORRELATIONS

1. The most common type of hemophilia is caused by factor VIII deficiency (classic hemophilia), a recessive hereditary trait transmitted by mothers to their male children. Because the trait is carried on the X chromosomes, girls would not be affected unless both parents had deficient X chromosomes. Affected persons are likely to bleed after trauma, usually involving damage to larger vessels.

2. Gray platelet syndrome (also known as alpha granule deficiency) is caused by a lack or deficiency in the number of α-granules in the platelets. This autosomal recessive mutation results in the thrombocytopenia with enlarged platelets with reduced granulomeres. Individuals with this syndrome present with increased bleeding disorders as well as with myelofibrosis (increased collagen deposits in the bone marrow that reduces available space for hemopoiesis) due to the release of α-granule enzymes into the bone marrow during platelet formation by megakaryocytes.

BONE MARROW

Bone marrow, a gelatinous, vascular connective tissue located in the marrow cavity, is richly endowed with cells that are responsible for hemopoiesis.

The medullary cavity of long bones and the interstices between trabeculae of spongy bones house the soft, gelatinous, highly vascular and cellular tissue known as ***marrow***. Bone marrow is isolated from bone by the endosteum (composed of osteoprogenitor cells, osteoblasts, and occasional osteoclasts). Bone marrow constitutes almost 5% of the total body weight. It is responsible for the formation of blood cells **(hemopoiesis)** and their delivery into the circulatory system, and it performs this function from the fifth month of prenatal life until the person dies. Bone marrow also provides a microenvironment for much of the maturation process of B lymphocytes and for the initial maturation of T lymphocytes.

The marrow of the newborn is called ***red marrow*** because of the great number of erythrocytes being produced there. By age 20 years, however, the diaphyses of long bones house only **yellow marrow** because of the accumulation of large quantities of fat and the absence

of hemopoiesis in the shafts of these bones. However, flat bones, short bones, irregular bones, and the epiphyses of long bones of the adult still house red marrow.

The vascular supply of bone marrow is derived from the nutrient arteries that pierce the diaphysis via the nutrient foramina, tunnels leading from the outside surface of bone into the medullary cavity. These arteries enter the marrow cavity and give rise to a number of small, peripherally located vessels that provide numerous branches both centrally, to the marrow, and peripherally, to the cortical bone. Vessels entering the cortical bone are distributed through the haversian and Volkmann canals to serve the compact bone.

The centrally directed branches of the bone marrow deliver their blood to the extensive network of large venous spaces known as ***sinusoids*** (45 to 80 μm in diameter). The sinusoids drain into a **central longitudinal vein**, which is drained by veins leaving the bone via the nutrient canal.

It is interesting that the veins are *smaller* than the arteries, thus establishing high hydrostatic pressure within the sinusoids, and this increased pressure maintain their patency. The veins, arteries, and sinusoids form the **vascular compartment**, and the intervening spaces are filled with pleomorphic clusters of hemopoietic cells that merge with each other, forming the **hemopoietic compartment** (see Fig. 10–14).

The sinusoids are lined by endothelial cells and are surrounded by slender threads of **reticular fibers** and a large number of **adventitial reticular cells**. Processes of adventitial reticular cells touch the sparse basement membrane of the endothelial cells, covering a large portion of the sinusoidal surface. Additional processes of these cells are directed away from the sinusoids and are in contact with similar processes of other adventitial reticular cells, forming a three-dimensional network surrounding discrete clusters of hemopoietic cells known as ***hemopoietic cords (hemopoietic islands)***.

Hemopoietic cords are composed of blood cells in various stages of maturation as well as **macrophages**, which not only destroy the extruded nuclei of erythrocyte precursors, malformed cells, and excess cytoplasm but also regulate the differentiation of hemopoietic cell differentiation and maturation, as well as transmit iron to developing erythroblasts to be utilized in the synthesis of the heme portion of hemoglobin. Frequently, processes of macrophages penetrate the spaces between endothelial cells to enter the sinusoidal lumina.

As adventitial reticular cells accumulate fat in their cytoplasm, they begin to resemble adipose cells. The volume occupied by these very large cells reduces the hemopoietic compartment in size, transforming the red marrow to yellow marrow.

CLINICAL CORRELATIONS

In certain leukemias or in severe bleeding, adventitial reticular cells may lose their lipids and decrease in size, transforming yellow marrow to red marrow, thus making more space available for hemopoiesis.

Prenatal Hemopoiesis

Prenatally, hemopoiesis is subdivided into four phases: mesoblastic, hepatic, splenic, and myeloid.

Blood cell formation begins 2 weeks after conception **(mesoblastic phase)** in the mesoderm of the yolk sac, where mesenchymal cells aggregate into clusters known as ***blood islands***. The peripheral cells of these islands form the vessel wall, and the remaining cells become **erythroblasts**, which differentiate into nucleated **erythrocytes** containing HbF (fetal hemoglobin). It is only around the time of birth that the erythrocytes house HbA_1, and HbA_2 (adult hemoglobin) as well as a very small percent of HbF.

The mesoblastic phase begins to be replaced by the **hepatic phase** by the sixth week of gestation. The circulating erythrocytes still have nuclei, and non-erythroid progenitors appear by the eighth week of gestation. The **splenic phase** begins during the second trimester, and both hepatic and splenic phases continue until the end of gestation.

Hemopoiesis in the bone marrow **(myeloid phase)** begins by the end of the second trimester. As the skeletal system continues to develop, the bone marrow assumes an increasing role in blood cell formation. Although, postnatally, the liver and the spleen are not active in hemopoiesis, they can revert to forming new blood cells if the need arises.

Postnatal Hemopoiesis

Postnatal hemopoiesis occurs almost exclusively in bone marrow.

Because all blood cells have a finite life span, they must be replaced continuously. This replacement is accomplished by hemopoiesis, starting from a common population of stem cells within the bone marrow (Fig. 10–15). On a daily basis, more than 10^{11} blood cells are produced in the marrow to replace cells that leave the bloodstream, die, or are destroyed. During hemopoiesis, stem cells undergo multiple cell divisions and differentiate through several intermediate stages, eventually giving rise to the mature blood cells discussed earlier. Table 10–6 outlines the numerous intermediate cells in the formation of each type of mature blood cell. The entire process is regulated by various growth factors and cytokines that act at different steps to control the type of cells formed and their rate of formation.

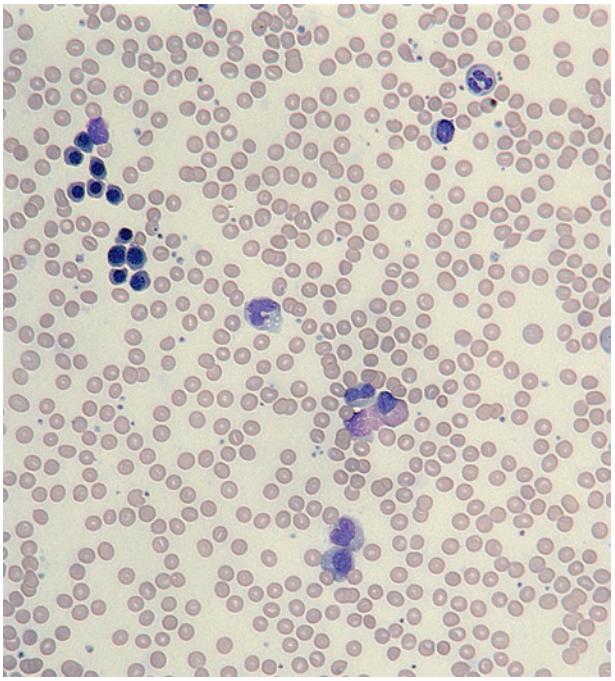


Figure 10–15 Photomicrograph of a human bone marrow smear (×270).

Stem Cells, Progenitor Cells, and Precursor Cells

The least differentiated of the cells responsible for the formation of the formed elements of blood are stem cells; stem cells give rise to progenitor cells whose progeny are the precursor cells.

All blood cells arise from **pluripotential hemopoietic stem cells (PHSCs)** (also known as ***hemopoietic stem cells* [*HSC*]**), which account for about 0.01% of the nucleated cell population of bone marrow. They are usually amitotic but may undergo bursts of cell division, giving rise to more PHSCs as well as to two types of **multipotential hemopoietic stem cells (MHSCs)**, also known as ***multipotent progenitors***. The two populations of MHSCs are **colony-forming unit–lymphocyte (CFU-Ly)**, also known as ***common lymphoid progenitors***, and **CFU-GEMM (colony-forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte)**, also known as ***common myeloid progenitors***. These two populations of MHSCs are responsible for the formation of a series of **progenitor cells** (also known as ***committed precursors***) each of which gives rise to one specific type of the various types of blood cells and platelets.

- CFU-GEMM cells (common myeloid progenitors) are predecessors of the myeloid cell lines (erythrocytes, granulocytes, monocytes, dendritic cell, mast cells, and platelets).
- CFU-Ly (common lymphoid progenitors) are predecessors of the lymphoid cell lines (T cells, B cells, NK cells, and perhaps dendritic cells).

Both PHSCs and MHSCs resemble lymphocytes and constitute a small fraction of the null-cell population of circulating blood.

Stem cells are commonly in the G_0 stage of the cell cycle but can be driven into the G_1 stage by various growth factors and cytokines. Early stem cells may be recognized because they express the specific marker molecules CD34, CD59, CD133, Thy1, and c-*kit* on their plasma membranes. **Homeobox genes** may be active in the differentiation of the early stages of hemopoietic cells, specifically *Hox1* in the myeloid (*but not* erythroid) cell lines and certain members of *Hox2* group in the erythroid (but not myeloid) cell lines.

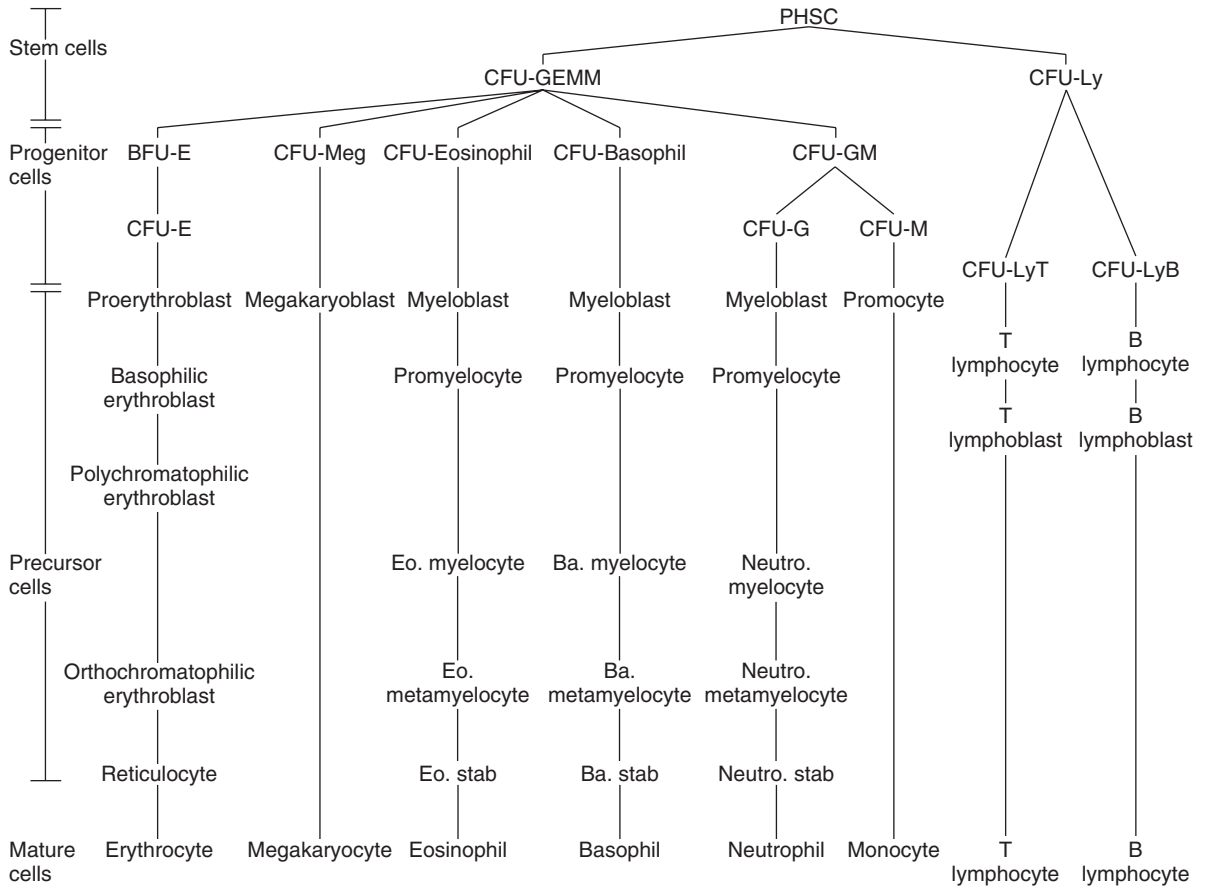
- CD34 assists in attaching the cell to the extracellular matrix as well as to stromal cells of bone marrow.
- CD59 inhibits complement from forming the membrane attack complex (discussed in Chapter 12).
- CD133 appears to assist in the organization of the three dimensional morphology of the cell membrane and in that fashion it may assist the function of CD59.
- Thy1 also appears to assist CD34 in its function of attaching the cell to the extracellular matrix as well as to stromal cells of bone marrow.
- The c-kit is the membrane-bound receptor for stem cell factor (also known as *steel factor* or *c-kit ligand*, discussed below).

Progenitor cells also resemble small lymphocytes but are **unipotential** (i.e., committed to forming a single cell line, such as eosinophils). Their mitotic activity and differentiation are controlled by specific hemopoietic factors. These cells have only limited capacity for self-renewal.

Precursor cells arise from progenitor cells, are incapable of self-renewal, and *have specific morphological characteristics that permit them to be recognized as the first cell of a particular cell line*. Precursor cells undergo cell division and differentiation, eventually giving rise to a clone of mature cells. As cell maturation and differentiation proceed, succeeding cells become smaller, their nucleoli disappear, their chromatin network becomes denser, and the morphological characteristics of their cytoplasm approximate those of the mature cells (Fig. 10–16).

Researchers studying hemopoiesis have isolated from circulating blood individual lymphocyte-like cells that, under proper conditions, occasionally give rise to groups *(colonies)* of cells composed of granulocytes, erythrocytes, monocytes, lymphocytes, and platelets. Thus, it has been shown that all blood cells are derived from a single **PHSC**. More frequently, however, individual stem cells isolated from circulating blood give rise to only erythrocytes or eosinophils or another type of blood cell. Because these experiments used the

TABLE 10–6 Cells of Hemopoiesis*



*Note that the lineage of NK cells, mast cells, and dendritic cells are not included in this table.
Ba., Basophil; BFU, burst-forming unit (E, erythrocyte); CFU, colony-forming unit (E, erythrocyte; G, granulocyte; GM, granulocyte-monocyte; Ly, lymphocyte; Meg, megakaryoblast); Eo., eosinophil; Neutro., neutrophil; PHSC, pluripotential hemopoietic stem cell.
Modified from Gartner LP, Hiatt JL, Strum J. *Histology*. Baltimore: Williams & Wilkins; 1988.

spleen as the site of hemopoiesis, the individual lymphocyte-like cells were originally called ***colony-forming units–spleen*** *(CFU-S)*, but have been renamed to describe their function to **PHSCs** (also known as ***HSCs***). Careful observations have shown that, as stated previously, there are two types of multipotential cells (CFU-GEMM and CFU-Ly), which give rise to the myeloid series of cells and lymphocytes, respectively. Newer research has demonstrated that each **precursor cell** has a unipotential **progenitor cell** as its predecessor (see Table 10–6). Precursor cells undergo a series of cell divisions and differentiations to yield the mature cell.

Hemopoietic Growth Factors (Colony-Stimulating Factors)

Hemopoiesis is regulated by a number of cytokines and growth factors, such as interleukins, colony-stimulating factors, macrophage inhibiting protein-α, and steel factor.

Hemopoiesis is regulated by numerous growth factors produced by various cell types. Each factor acts on specific stem cells, progenitor cells, and precursor cells, generally inducing rapid mitosis, differentiation, or both (Table 10–7). Some of these growth factors also promote the functioning of mature blood cells. Most hemopoietic growth factors are glycoproteins.

Three routes are used to deliver growth factors to their target cells: (1) transport via the bloodstream (as endocrine hormones), (2) secretion by stromal cells of the bone marrow near the hemopoietic cells (as paracrine hormones), and (3) direct cell–cell contact (as surface signaling molecules).

Certain growth factors—principally, stem cell factor (also known as *steel factor* or *c-kit ligand*), GM-CSF, PU.1 transcription factor, and four ILs (IL-1, IL-3, IL-6, and IL-7)—stimulate proliferation of pluripotential and multipotential stem cells, thus maintaining their populations. Additional cytokines, granulocyte

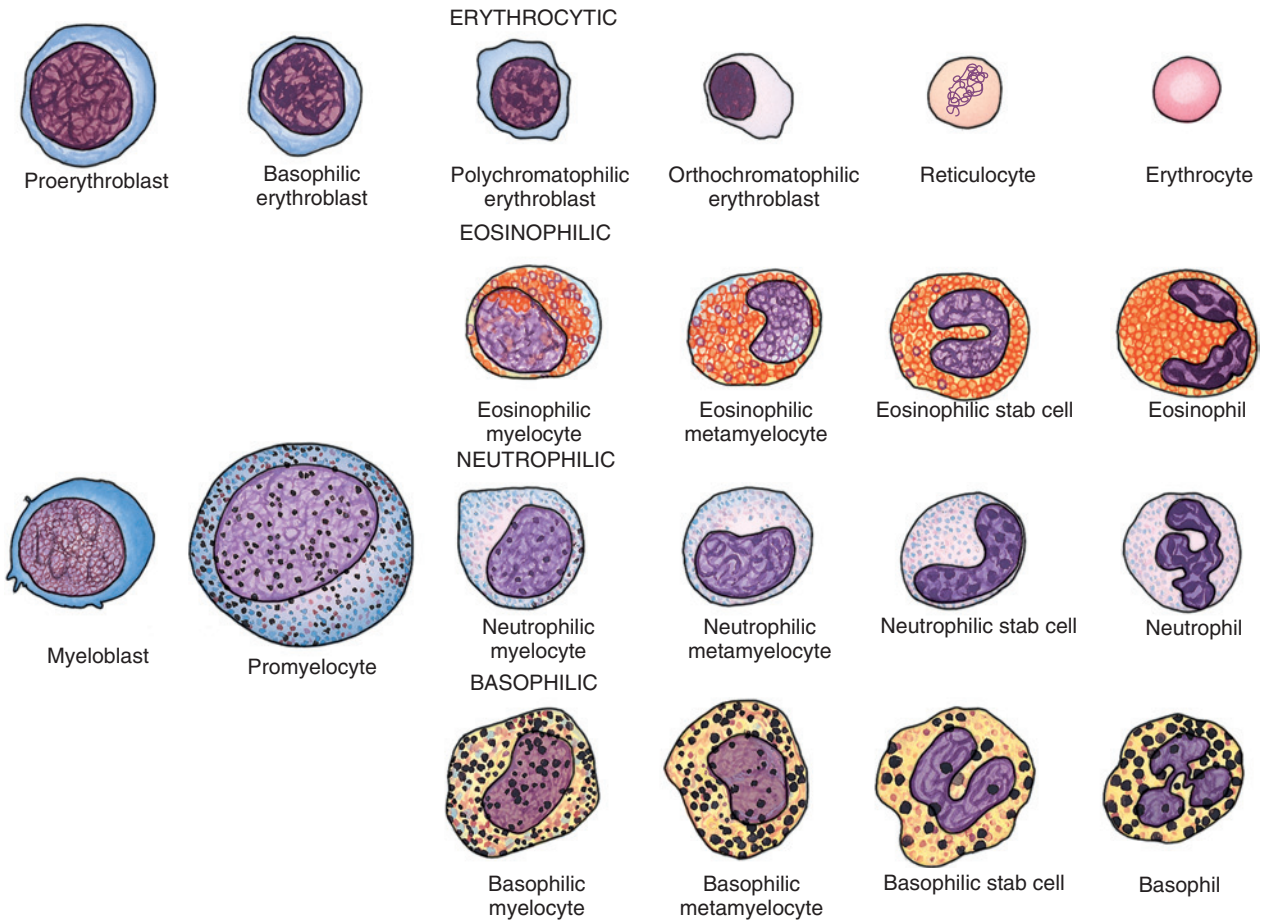


Figure 10–16 Schematic diagram of precursor cells in formation of erythrocytes and granulocytes. The myeloblast and promyelocyte intermediaries in the formation of eosinophils, neutrophils, and basophils are indistinguishable for the three cell types.

CLINICAL CORRELATIONS

1. Patients who require bone marrow transplants after therapeutic procedures (e.g., irradiation or chemotherapy) must be matched for the MHC (major histocompatibility complex) of the donor. Unless an identical twin is available for the transplantation, grafting failure is common. This can be circumvented by freezing the patient's own bone marrow in liquid nitrogen and reintroducing it (as in an autologous transplant) to the patient after treatment with irradiation or chemotherapy. Because the number of stem cells per unit volume of bone marrow is relatively small, large volumes of marrow have to be harvested from the patient. Newer procedures that permit the isolation of pluripotential hemopoietic stem cells by the use of monoclonal antibodies against the CD34 molecule, which is expressed only by these cells, permit the use of small volumes of bone marrow enriched in pluripotential hemopoietic stem cells. These procedures are being investigated clinically, involving patients with various types of malignancies.

2. Perhaps in the relatively near future, people with hereditary blood cell disorders (e.g., sickle cell anemia) may be treated by the use of genetically engineered stem cells. Pluripotential hemopoietic stem cells isolated from the patient may be transfected with the normal gene (e.g., for hemoglobin) and reintroduced as an autologous transplant. These genetically engineered cells bearing the normal gene would proliferate, and their progeny would produce normal blood cells. Although the patient would still produce some defective cells, it is hoped that enough normal cells would be produced to minimize the hereditary defect.

TABLE 10–7 Hemopoietic Growth Factors

Factors	Principal Action	Site of Origin
Stem cell factor (steel factor, c-kit ligand)	Stimulate proliferation of pluripotential and multipotential stem cells and the formation of mast cells	Stromal cells of bone marrow
GM-CSF	Promotes CFU-GM mitosis and differentiation; facilitates granulocyte activity	T cells; endothelial cells
G-CSF	Promotes CFU-G mitosis and differentiation; facilitates neutrophil activity	Macrophages; endothelial cells
M-CSF	Promotes CFU-M mitosis and differentiation	Macrophages; endothelial cells
IL-1	In conjunction with IL-3 and IL-6, it promotes proliferation of PHSC, CFU-GEMM, and CFU-Ly; suppresses erythroid precursors	Monocytes; macrophages, endothelial cells
IL-2	Stimulates activated T- and B-cell mitosis; induces differentiation of NK cells	Activated T cells
IL-3	In conjunction with IL-1 and IL-6, it promotes proliferation of PHSC, CFU-GEMM, and CFU-Ly as well as all unipotential precursors (except for LyB and LyT); also promotes formation of BFU-E	Activated T and B cells
IL-4	Stimulates T- and B-cell activation and development of mast cells and basophils; also promotes formation of BFU-E	Activated T cells
IL-5	Promotes CFU-Eo mitosis and activates eosinophils	T cells
IL-6	In conjunction with IL-1 and IL-3, it promotes proliferation of PHSC, CFU-GEMM, and CFU-Ly; also facilitates CTL and B-cell differentiation	Monocytes and fibroblasts
IL-7	Promotes differentiation of CFU-LyB; CFU-LyT, and enhances differentiation of NK cells	Stromal cells
IL-8	Induces neutrophil migration and degranulation	Leukocytes, endothelial cells, and smooth muscle cells
IL-9	Induces mast cell activation and proliferation; modulates IgE production; promotes T helper cell proliferation	T helper cells
IL-10	Inhibits cytokine production by macrophages, T cells, and NK cells; facilitates CTL differentiation and proliferation of B cells and mast cells	Macrophages and T cells
IL-12	Stimulates NK cells; enhances TCL and NK cell function	Macrophages
IL-15	NK cell maturation	Macrophages
γ-Interferons	Activate B cells and monocytes; enhance CTL differentiation; augment the expression of class II HLA	T cells and NK cells
Erythropoietin	CFU-E differentiation; BFU-E mitosis	Endothelial cells of the peritubular capillary network of kidney; hepatocytes
Thrombopoietin	Proliferation and differentiation of CFU-Meg and megakaryoblasts	Hepatocytes and liver sinusoidal lining cells; kidney proximal tubule cells, and stromal cells of bone marrow
GATA3 transcription factor	Differentiation of B and T lymphocytes	Expressed in the relevant cells
Ikaros family of transmission factors	Differentiation of B and T lymphocytes	Expressed in the relevant cells
Pax5 transcription factor	B lymphocyte maturation	Expressed in the relevant cells
PU.1 transcription factor	Development of granulocytes, macrophage and B lymphocytes	Expressed in the relevant cells

BFU-E, Burst forming unit-erythrocyte; CFU-E, colony forming unit-erythrocyte; CTL, cytotoxic T cell; CFU, colony-forming unit (Eo, eosinophil; G, granulocyte; GEMM, granulocyte, erythrocyte, monocyte, megakaryocyte; GM, granulocyte-monocyte; Ly, lymphocyte; CSF, colony-stimulating factor (G, granulocyte; GM, granulocyte-monocyte; M, monocyte); IL, interleukin; NK, natural killer; PHSC, pluripotential hemopoietic stem cell.

colony-stimulating factor (G-CSF), monocyte colony-stimulating factor (M-CSF), IL-2, IL-5, IL-6, IL-11, IL-12, macrophage inhibitory protein-α (MIP-α), thrombopoietin, and erythropoietin, are believed to be responsible for the mobilization and differentiation of these cells into unipotential progenitor cells.

CSFs are also responsible for the stimulation of cell division and for the differentiation of unipotential cells of the granulocytic and monocytic series. **Erythropoietin** activates cells of the erythrocytic series, whereas **thrombopoietin** stimulates platelet production.

Steel factor (stem cell factor), which, as indicated above, acts on pluripotential, multipotential, and unipotential stem cells, is produced by stromal cells of the bone marrow and is inserted into their own cell membranes. Stem cells must come in contact with these stromal cells before they can become mitotically active. It is believed that hemopoiesis cannot occur without the presence of cells that express stem cell factors, which is why postnatal blood cell formation is restricted to the bone marrow (and liver and spleen, if necessary).

Hemopoietic cells are programmed to die by undergoing **apoptosis** unless they come into contact with growth factors. Such dying cells display clumping of the chromatin in their shrunken nuclei and a dense, granular-appearing cytoplasm. On their cell surface, they express specific macromolecules that are recognized by receptors of the macrophage plasma membrane. These phagocytic cells engulf and destroy the apoptotic cells.

It has been suggested that there are factors responsible for the release of mature (and almost mature) blood cells from the marrow. These proposed factors have not yet been characterized completely, but they most probably include ILs, CSF, and steel factor.

CLINICAL CORRELATIONS

Pathologically increased secretion of erythropoietin can cause secondary polycythemia, an increase in the total number of red blood cells in the blood, increasing its viscosity, reducing its flow rate, and thus impeding circulation. The increased secretion is usually caused by tumors of erythropoietin-secreting cells. Patients may have an erythrocyte count of 10 million red blood cells/mm^3.

Erythropoiesis

Erythropoiesis, the formation of red blood cells, is under the control of several cytokines, namely steel factor, interleukin-3, interleukin-4, and erythropoietin.

The process of **erythropoiesis**, RBC formation, generates 2.5×10^{11} erythrocytes every day. In order to produce such a tremendous number of cells, two types of unipotential progenitor cells arise from the CFU-GEMM: the **burst-forming units–erythrocyte (BFU-E)** and **colony-forming units–erythrocyte (CFU-E)**.

If the circulating RBC level is low, the kidney produces a high concentration of **erythropoietin**, which, in the presence of IL-3, IL-4, and stem cell factor, induces CFU-GEMM to differentiate into BFU-E. These cells undergo a "burst" of mitotic activity, forming a large number of CFU-E. Interestingly, this transformation requires the loss of IL-3 receptors.

CFU-E requires a low concentration of erythropoietin not only to survive but also to form the first recognizable erythrocyte precursor, the **proerythroblast** (Fig. 10–17; see Fig. 10–16). The proerythroblasts and their progeny (Figs. 10–18, 10–19, and 10–20) form spherical clusters around macrophages **(nurse cells)**, which phagocytose extruded nuclei and excess or

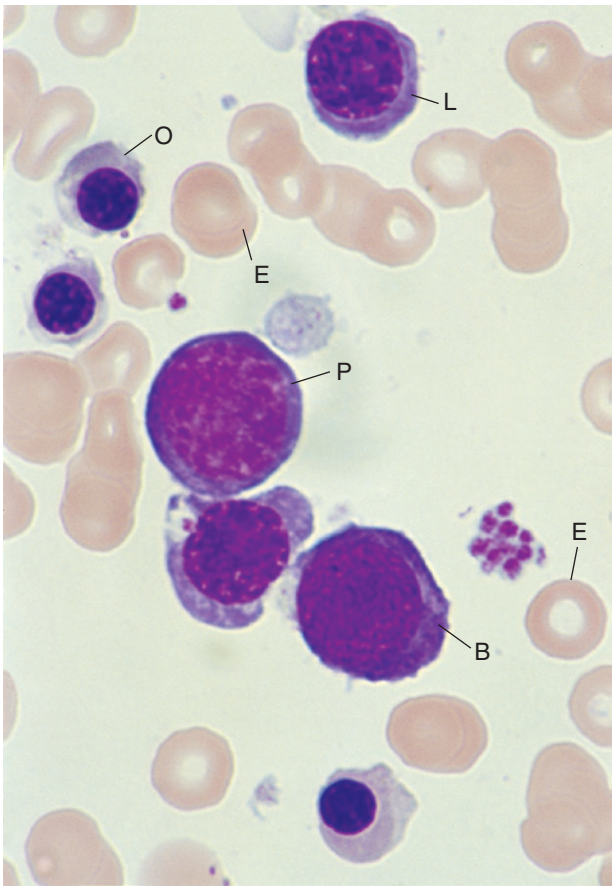


Figure 10–17 Photomicrograph of bone marrow displaying all of the stages of red blood cell formation, except for reticulocytes. B, Basophilic erythroblast; E, erythrocyte; L, polychromatophilic erythroblast; O, orthochromatophilic erythroblast; P, proerythroblast (×1325).

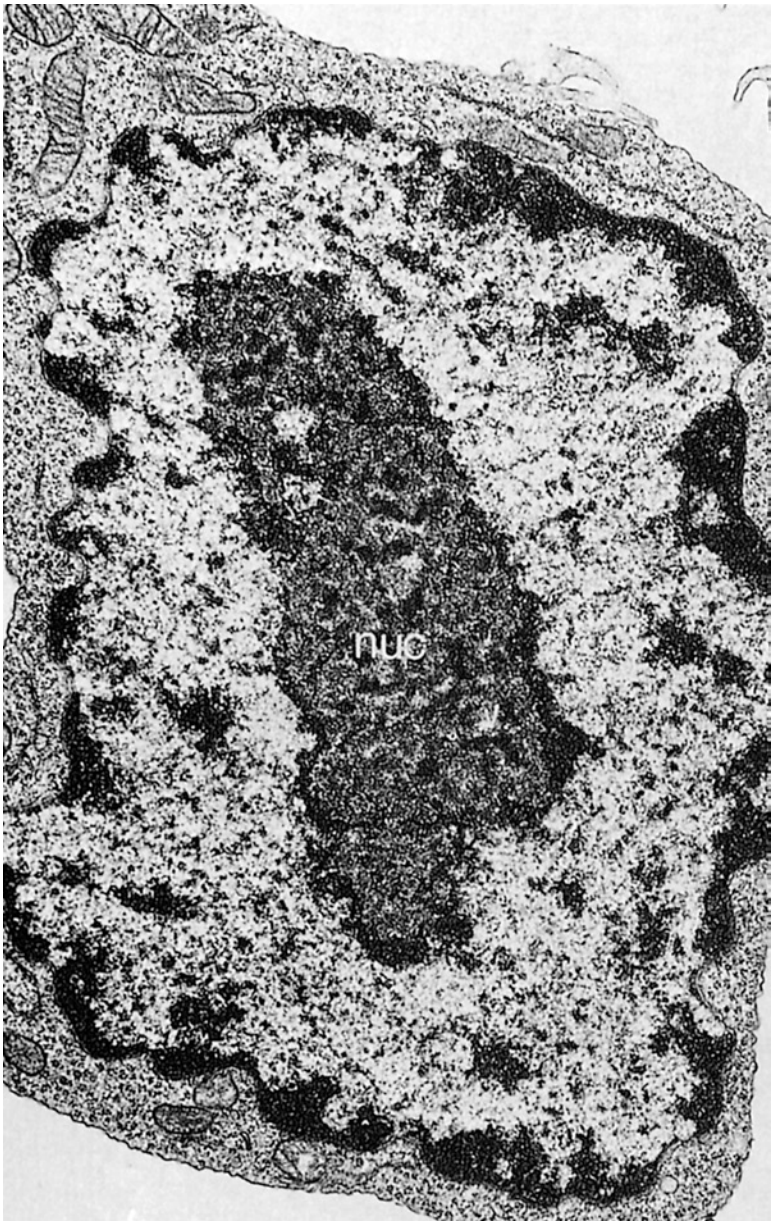


Figure 10–18 Electron micrograph of a proerythroblast, displaying its nucleus as well as the perinuclear cytoplasm. Note that the nucleoplasm is relatively smooth in appearance and that the cytoplasm is rich in mitochondria and free ribosomes, indicating that the cell is active in protein synthesis (×14000). nuc, Nucleolus. (From Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

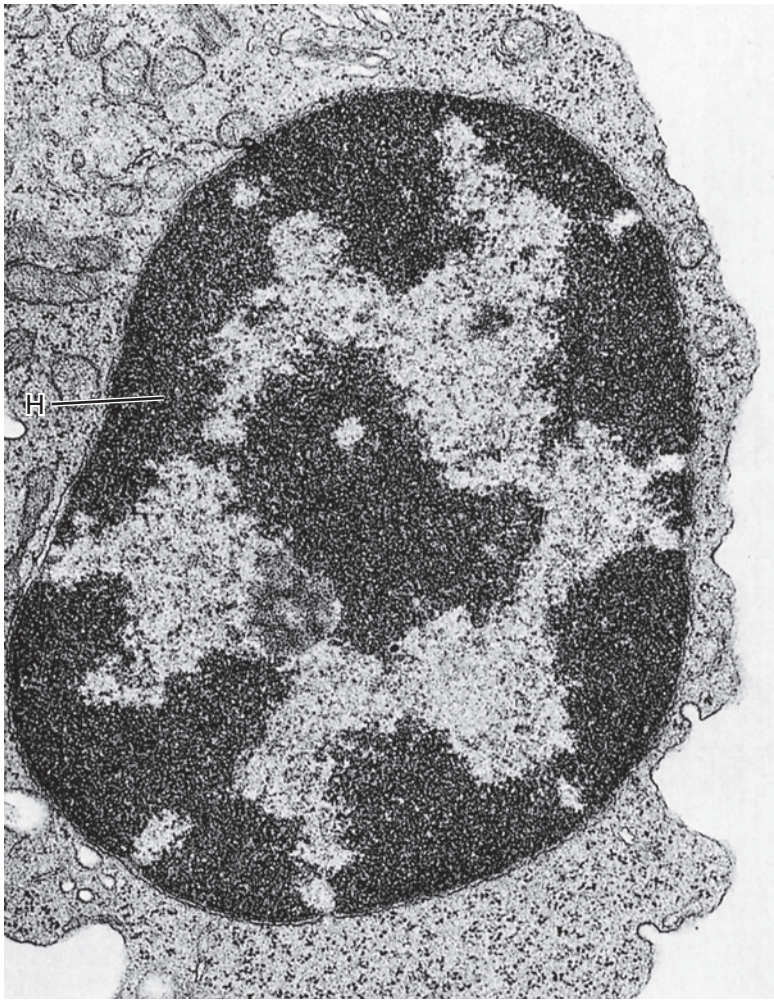


Figure 10–19 Electron micrograph of an orthochromatophilic erythroblast. Observe that the nucleus possesses a lot of heterochromatin (×21300). (From Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

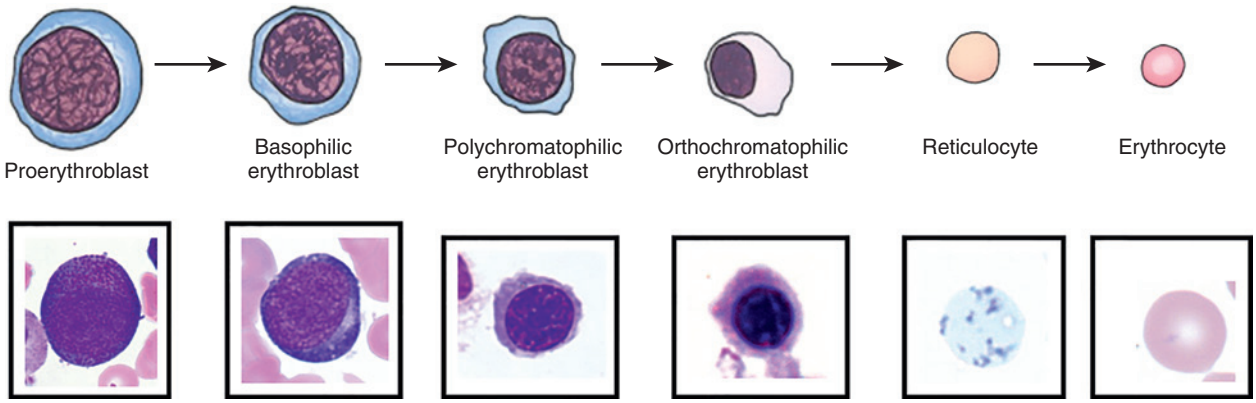


Figure 10–20 Diagram and photomicrographs of the erythrocytic series.

TABLE 10–8 Cells of the Erythropoietic Series

Cell	Size (μm)	Nucleus* and Mitosis	Nucleoli	Cytoplasm*	Electron Micrographs
Proerythroblast	14–19	Round, burgundy-red; chromatin network: fine; mitosis	3–5	Gray-blue, peripheral clumping	Scant RER; many polysomes, few mitochondria; ferritin
Basophilic erythroblast	12–17	Same as above but chromatin network is coarser; mitosis	1–2?	Similar to above but slight pinkish background	Similar to above but some hemoglobin is present
Polychromatophilic erythroblast	12–15	Round and densely staining; very coarse chromatin network; mitosis	None	Yellowish-pink in bluish background	Similar to above but more hemoglobin is present
Orthochromatophilic erythroblast	8–12	Small, round, dense; eccentric or is being extruded; no mitosis	None	Pink in a slight bluish background	Few mitochondria and polysomes; much hemoglobin
Reticulocyte	7–8	None	None	Similar to mature RBC but when stained with cresyl blue; display bluish reticulum in pink cytoplasm	Clusters of ribosomes; cell is filled with hemoglobin
Erythrocyte	7.5	None	None	Pink cytoplasm	Only hemoglobin

*Colors as appear using Romanovsky-type stains (or their modifications).
RBC, Red blood cell; RER, rough endoplasmic reticulum.

deformed erythrocytes. Nurse cells may also provide growth factors to assist erythropoiesis. The properties of the cells in the erythropoietic series are presented in Table 10–8.

CLINICAL CORRELATIONS

Iron deficiency anemia, the most common form of anemia resulting from nutritional deficiency, affects about 10% of the United States population. Although the cause may be low dietary intake of iron, that is usually not the case in the United States; instead, it is caused by either malabsorption or chronic blood loss. The erythrocytes of an iron-deficient person are smaller than usual; the patient presents a whitish pallor, and the nails appear spoon shaped with accentuated longitudinal ridges. The patient complains of generalized weakness, constant tiredness, and lack of energy.

Granulocytopoiesis

Granulocytopoiesis, the formation of the granulocytes neutrophils, eosinophils, and basophils, and mast cells is under the influence of several cytokines, including stem cell factor, G-CSF and GM-CSF, PU.1 transcription factor, as well as IL-3, IL-5, IL-6, IL-8, and TNF-α.

Although the granulocytic series is usually discussed under a single heading, as it is here, the three types of granulocytes are actually derived from their own unipotential (or bipotential, as with neutrophils) stem cells (see Table 10–6). Each of these stem cells is a descendant of the pluripotential stem cell CFU-GEMM. Thus, CFU-Eo, of the eosinophil lineage, and CFU-Ba, of the basophil lineage, each undergo cell division, giving rise to the precursor cell, or **myeloblast**. Neutrophils originate from the bipotential stem cell, **CFU-GM**, whose mitosis produces two unipotential stem cells, **CFU-G** (of the neutrophil line) and **CFU-M**, responsible for the monocyte lineage. Similar to CFU-Ba and CFU-Eo, CFU-G divides to give rise to myeloblasts.

The proliferation and differentiation of these stem cells are under the influence of G-CSF, IL-3, and GM-CSF. Therefore, these three factors facilitate the development of neutrophils, basophils, and eosinophils. In turn, IL-3, IL-6, IL-8, PU.1 transcription factor, and TNF-α are cofactors necessary for the synthesis and release of G-CSF and GM-CSF. In addition, if IL-5 is present, it drives the differentiation toward the formation of eosinophils. In the absence of IL-5, basophils form. **Myeloblasts** (Fig. 10–21; see also Fig. 10–16) are precursors of all three types of granulocytes, and they cannot be differentiated from one another. It is not known whether a single myeloblast can produce all three types of granulocytes or there is a specific myeloblast for each type of granulocyte. Myeloblasts undergo mitosis, giving rise to promyelocytes, which in turn divide to form myelocytes. It is at the myelocyte step

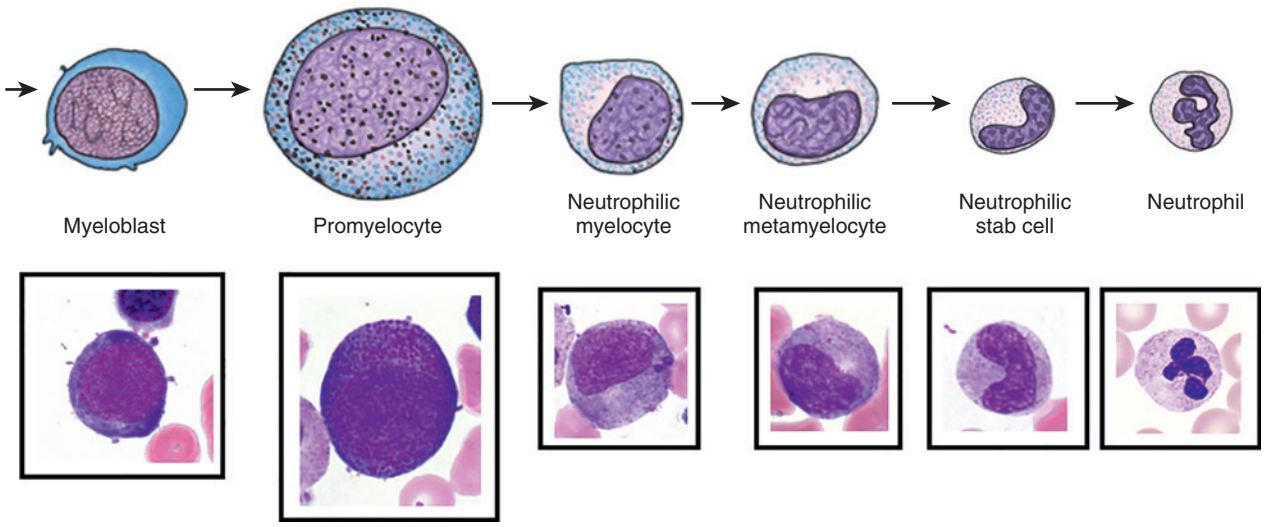


Figure 10–21 Diagram and photomicrographs of the neutrophilic series.

that specific granules are present and the three granulocyte lines may be recognized. Each day, the average adult produces approximately 800,000 neutrophils, 170,000 eosinophils, and 60,000 basophils.

Table 10–9 details the neutrophil lineage. The eosinophil and basophil lineages appear to be identical to the neutrophil lineage except for the differences in their specific granules (Figs. 10–22 to 10–24; see Fig. 10–21). **Mast cells** also arise from myeloblasts but require **stem cell factor** for their differentiation.

Newly formed neutrophils leave the hemopoietic cords by *piercing* the endothelial cells lining the sinusoids rather than by *migrating* between them. Once neutrophils enter the circulatory system, they **marginate**; that is, they adhere to the endothelial cells of the blood vessels and remain there until they are needed. The process of margination requires the sequential expression of various transmembrane adhesion molecules and integrins by the neutrophils as well as specific surface receptor molecules by the endothelial cells, the description of which is beyond the scope of this textbook. Because of the process of margination, there are always many more neutrophils in the circulatory system than in the circulating blood.

CLINICAL CORRELATIONS

Acute myeloblastic leukemia results from uncontrolled mitosis of a transformed stem cell whose progeny do not differentiate into the mature cell. The cells involved may be the CFU-GM, CFU-Eo, or CFU-Ba, whose differentiation stops at the myeloblast stage. The disease affects young adults between 15 and 40 years of age and is treated by intensive chemotherapy and, more recently, by bone marrow transplantation.

Monocytopoiesis

Monocytes share their bipotential cells with neutrophils. CFU-GM undergoes mitosis and gives rise to CFU-G and **CFU-M (monoblasts)**. The progeny of CFU-M are **promonocytes**, large cells (16 to 18 µm in diameter) that have a kidney-shaped, acentrically located nucleus. The cytoplasm of promonocytes is bluish and houses numerous azurophilic granules.

Electron micrographs of promonocytes disclose a well-developed Golgi apparatus, abundant RER, and numerous mitochondria. The azurophilic granules are lysosomes, about 0.5 µm in diameter. Every day, the average adult forms more than 10^{10} monocytes, most of which enter the circulation. Within a day or two, the newly formed monocytes enter the connective tissue spaces of the body and differentiate into **macrophages**, **monocyte-derived dendritic cells**, **osteoclasts**, and **microglia**.

Platelet Formation

The formation of platelets is under the control of thrombopoietin, which induces the development and proliferation of giant cells known as megakaryoblasts.

The unipotential platelet progenitor, **CFU-Meg**, gives rise to a very large cell, the **megakaryoblast** (25 to 40 µm in diameter), whose single nucleus has several lobes. These cells undergo **endomitosis**, whereby the cell does not divide; instead, it becomes larger and the nucleus becomes polyploid, as much as 64 N. The bluish cytoplasm accumulates azurophilic granules. These cells are stimulated to differentiate and proliferate by thrombopoietin.

Megakaryoblasts differentiate into **megakaryocytes** (see Fig. 10–14), which are large cells (40 to 100 µm in

TABLE 10–9 Cells of the Neutrophilic Series

Cell	Size (μm)	Nucleus* and Mitosis	Nucleoli	Cytoplasm*	Granules	Electron Micrographs
Myeloblast	12–14	Round, reddish-blue; chromatin network: fine; mitosis	2–3	Blue clumps in a pale blue background; cytoplasmic blebs at cell periphery	None	RER, small Golgi, many mitochondria and polysomes
Promyelocyte	16–24	Round to oval, reddish blue; chromatin network: coarse; mitosis	1–2	Bluish cytoplasm; no cytoplasmic blebs at cell periphery	Azurophilic granules	RER, large Golgi, many mitochondria, numerous lysosomes (0.5 μm in diameter)
Neutrophilic myelocyte	10–12	Flattened, acentric; chromatin network: coarse; mitosis	0–1	Pale blue cytoplasm	Azurophilic and specific granules	RER, large Golgi, numerous mitochondria, lysosomes (0.5 μm) and specific granules (0.1 μm)
Neutrophilic metamyelocyte	10–12	Kidney shaped, dense; chromatin network: coarse; no mitosis	None	Pale blue cytoplasm	Azurophilic and specific granules	Organelle population is reduced, but granules are as above
Neutrophilic band (stab; juvenile)	9–12	Horseshoe shaped; chromatin network: very coarse; no mitosis	None	Pale blue cytoplasm	Azurophilic and specific granules	Same as above
Neutrophil	9–12	Multilobed; chromatin network: very coarse; no mitosis	None	Pale bluish-pink	Azurophilic and specific granules	Same as above

*Colors as appear using Romanovsky-type stains (or their modifications).
RER, Rough endoplasmic reticulum.

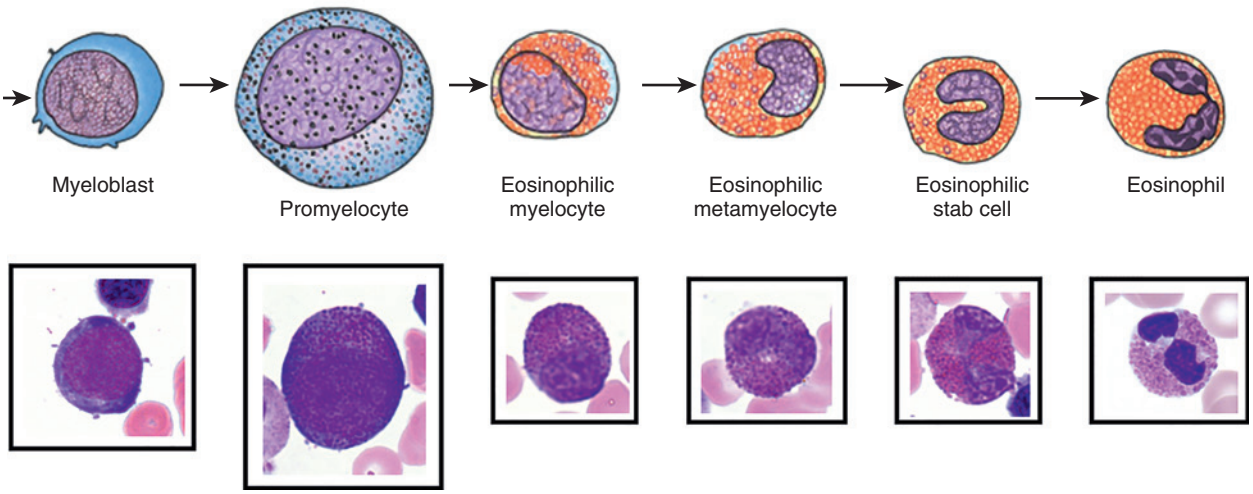


Figure 10–22 Diagram and photomicrographs of the eosinophilic series.

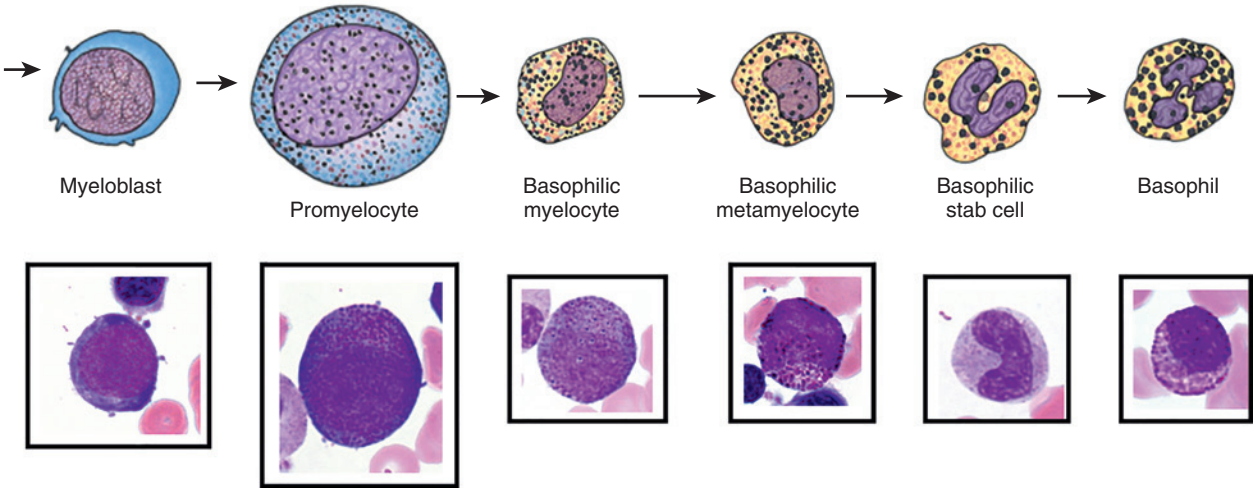


Figure 10–23 Diagram and photomicrographs of the basophilic series.

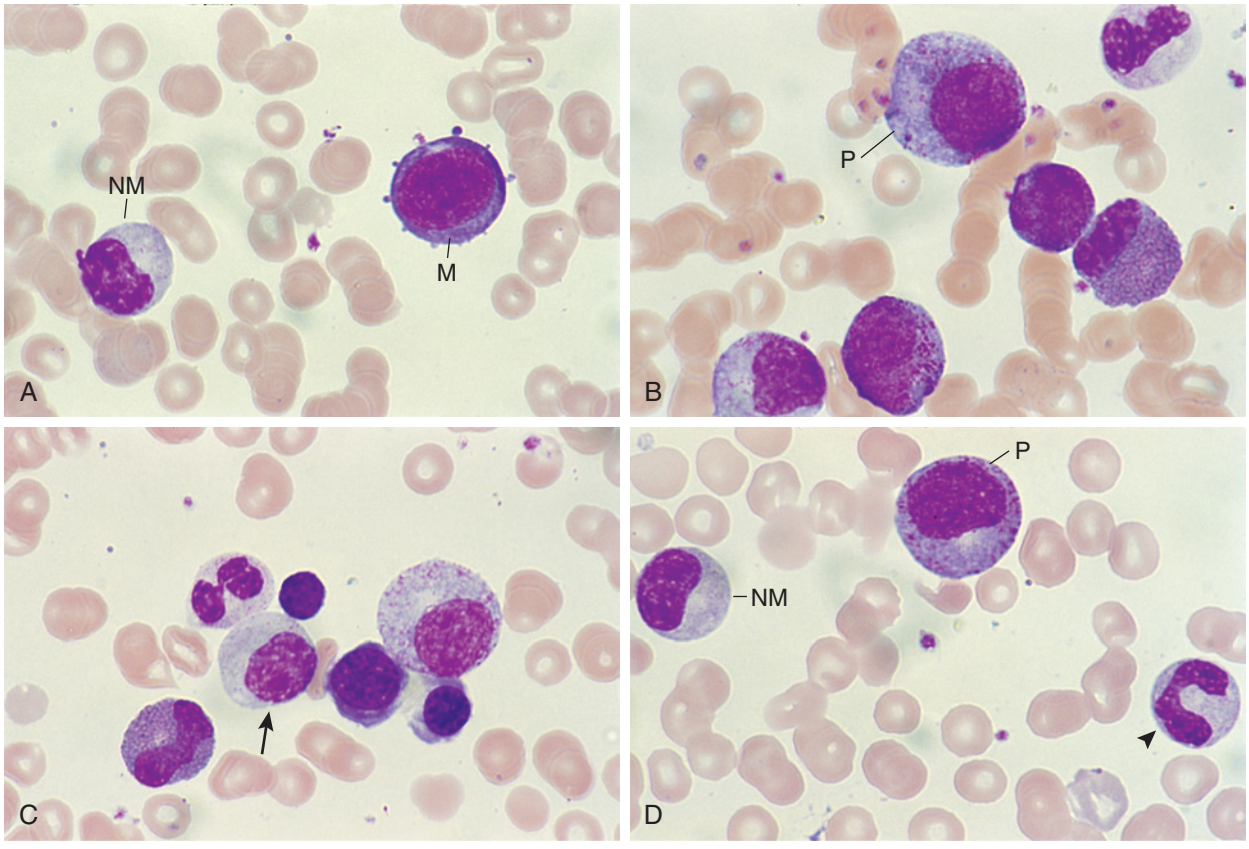


Figure 10–24 Photomicrographs of granulocytopoiesis displaying the various intermediary cell types. **A,** Myeloblast (M), neutrophilic metamyelocyte (NM). **B,** Promyelocyte (P). **C,** Neutrophilic myelocyte *(arrow)*. **D,** Neutrophilic metamyelocyte (D); neutrophilic stab cell *(arrowhead)*. P, Promyelocyte. (×1234).

Figure 10–25 Electron micrograph of a megakaryocyte displaying segmentation in the formation of platelets. Although this cell possesses a single nucleus, it is lobulated, which gives the appearance of the cell possessing several nuclei (×3166). (From Hopkins CR. *Structure and Function of Cells*. Philadelphia: WB Saunders; 1978.)

diameter), each with a single lobulated nucleus. Electron micrographs of megakaryocytes display a well-developed Golgi apparatus, numerous mitochondria, abundant RER, and many lysosomes (Fig. 10–25).

Megakaryocytes are located next to sinusoids, into which they protrude their cytoplasmic processes. These cytoplasmic processes fragment along complex, narrow invaginations of the plasmalemma, known as ***demarcation channels***, into clusters of **proplatelets**. Shortly after the proplatelets are released, they disperse into individual platelets. Each megakaryocyte can form several thousand platelets. The remaining cytoplasm and nucleus of the megakaryocyte degenerate and are phagocytosed by macrophages.

Lymphopoiesis

Pluripotential hemopoietic stem cells give rise to the myeloid series of cells via CFU-GEMM as well as to the lymphoid series of cells via CFU-Ly.

The multipotential stem cell **CFU-Ly** divides in the bone marrow to form the two unipotential progenitor cells, CFU-LyB, CFU-LyT, as well as CFU-NK cells, none of which are immunocompetent. The differentiation of CFU-Ly to CFU-LyB and CFU-LyT requires the expression of **zinc finger proteins**, namely the **Ikaros family of transcription factors** as well as the expression of a moderate level of **PU.1 transcription factor**.

In birds, **CFU-LyB** migrates to a diverticulum attached to the gut, known as the ***bursa of Fabricius*** (thus the name, *B cell*). Here, CFU-LyB divides several times, giving rise to **immunocompetent** B lymphocytes expressing specific surface markers, including antibodies. A similar event occurs in mammals, but in the absence of a bursa, this development of immunocompetence occurs in a bursa-equivalent location in the bone marrow. The process of B cell maturation is partially controlled by **IL-7** and **Pax5 transcription factor**.

CFU-LyT cells undergo mitosis, forming immunoincompetent T cells, which travel to the cortex of the thymus, where they proliferate, mature, and begin to express cell surface markers. As these surface markers appear on the T-cell plasmalemma (e.g., T-cell receptors and clusters of differentiation markers), the cells become immunocompetent T lymphocytes. Most of these newly formed T cells are destroyed in the thymus and are phagocytosed by resident macrophages. The process of T cell maturation is partially controlled by **IL-7** and **GATA3 transcription factor**.

Both B lymphocytes and T lymphocytes proceed to lymphoid organs (e.g., the spleen and lymph nodes), where they form clones of immunocompetent T and B cells in well-defined regions of the organs. It has also been demonstrated that the differentiation of both B cells and T cells, as well as their activation relies on the presence of specific micro-ribonucleic acids.

NK cells also migrate to a yet unknown region of the bone marrow where they will become immunocompetent. The process of NK cell maturation is partially controlled by IL-12 and **IL-15**.

The lymphocytic series is discussed in more detail in Chapter 12.

PATHOLOGICAL CONSIDERATIONS

See Figures 10–26 through 10–28.

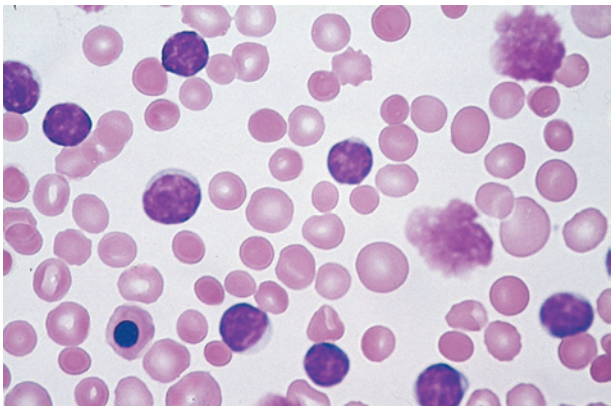


Figure 10–26 Photomicrograph of a peripheral bloodstream of an individual with chronic lymphocytic leukemia. Note the presence of numerous lymphocytes as well as the characteristic "smudge cells," which are dead tumor cells in the *upper right* corner and in the *middle right* of the field. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:594.)

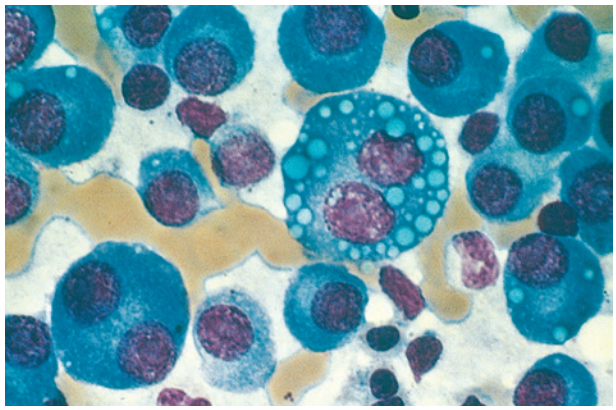


Figure 10–27 Photomicrograph of a bone marrow aspirate of an individual with multiple myeloma. Note the presence of numerous mast cells, some with more than one nucleus housing very large nucleoli. The cytoplasmic granules are filled with immunoglobulin. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:600.)

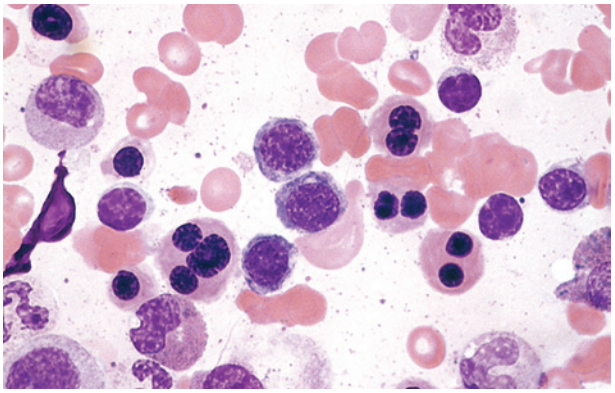


Figure 10–28 Photomicrograph of a bone marrow aspirate of an individual with a form of myelodysplasia. Observe that some of the erythrocytic precursors possess two or more nuclei, some of which are multilobed. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:615.)

REVIEW QUESTIONS

1. A patient whose colloid osmotic pressure is 15 mm Hg or less but has a normal blood volume is lacking
   a. gamma globulins.
   b. alpha globulins.
   c. beta globulins.
   d. albumins.
   e. fibrinogen.
2. A patient whose plasma gamma globulin levels are highly elevated
   a. has a high colloid osmotic pressure.
   b. is in the process of a humorally mediated immune reaction.
   c. is in the process of a cellularly mediated immune reaction.
   d. is immunosuppressed.
   e. has a viral infection.
3. Human erythrocytes do not have mitochondria but have the ability to produce ATP because these cells possess
   a. carbonic anhydrase.
   b. chloride shift.
   c. band 3 proteins.
   d. spectrin.
   e. glycolytic pathway.
4. A patient with sickle cell anemia has a point mutation in which the amino acid valine is incorporated into the chain instead of
   a. glutamate.
   b. tyrosine.
   c. glycine.
   d. proline.
   e. lysine.
5. Patients diagnosed with secondary polycythemia have
   a. low levels of erythropoietin.
   b. higher than normal levels of neutrophils.
   c. a decreased blood viscosity.
   d. higher than normal erythrocyte levels.
   e. lower than normal platelet levels.
6. A patient presents with hereditary spherocytosis. This condition is caused by a defective
   a. hemoglobin A_1.
   b. substitution of valine for glutamate.
   c. impaired formation of β chains of the hemoglobin molecule.
   d. abnormal spectrin molecule.
   e. abnormal band 3 molecule.
7. Erythroblastosis fetalis is a condition that may be fatal to a newborn because of
   a. Rh blood group.
   b. A blood group.
   c. B blood group.
   d. M blood group.
   e. N blood group.
8. In a healthy patient, the most populous leukocytes are
   a. monocytes.
   b. lymphocytes.
   c. basophils.
   d. eosinophils.
   e. neutrophils.
9. A pediatrician sees a male child with hereditary deficiency of NADPH oxidase. This child is
   a. anemic.
   b. unable to bind IgE to his basophils and mast cells.
   c. unable to fight parasitic worm invaders.
   d. subject to persistent infections.
   e. unable to mount a humorally mediated immune response.
10. A patient with vitamin K deficiency has
    a. normal levels of prothrombin.
    b. normal levels of factor VII.
    c. normal levels of factor IX.
    d. normal levels of factor X.
    e. normal levels of fibrinogen.

ANSWERS

1. D. The patient is lacking albumins, large proteins whose presence in the bloodstream prevent the fluid component of plasma from leaving the capillaries. The other proteins listed have only very minor effects on blood fluid volume.
2. B. Gamma globulins (immunoglobulins) are manufactured by plasma cells, and a high plasma immunoglobulin level indicates that the patient is undergoing a humorally mediated immune reaction. Albumin, not gamma globulin, is responsible for the colloid osmotic pressure. Cellularly mediated immune response is dependent on the presence of cytotoxic T lymphocytes, and they do not make immunoglobulins. The patient is responding to an antigenic challenge and is not immunosuppressed. Because viruses act intracellularly, a viral infection does not elicit the formation and release of immunoglobulins.
3. E. The enzymes of the glycolytic pathway are present in the cytosol of erythrocytes, and these enzymes can generate ATP without the presence of oxygen. Carbonic anhydrase facilitated the formation of carbonic acid from carbon dioxide and water. The chloride shift is accomplished by the integral protein band 3, a coupled anion transporter that facilitates the exchange of intracellular bicarbonate for the extracellular chloride ion; most of the carbon dioxide is carried to the lungs for release in the form of bicarbonate. Spectrin is a tetrameric protein that acts as a major component of the scaffolding beneath the erythrocyte cell membrane and assists in maintaining the biconcave disk shape of the red blood cell.
4. A. Patients with sickle cell anemia possess hemoglobin S (HbS) instead of HbA because valine is substituted for glutamate. The hemoglobin of these patients causes a change in the shape of the erythrocyte under conditions of reduced oxygen tension (as during strenuous exercise), and the red blood cells are incapable of transporting the normal amount of oxygen to the tissues of the body.
5. D. Patients with secondary polycythemia usually have tumors of erythropoietin-producing cells and therefore they have higher than normal erythropoietin levels, which result in red blood cell counts that can reach as high as 10 million RBC/mm^3. Their neutrophil levels are normal, but the viscosity of their blood is increased, reducing flow rate and impeding circulation. Their platelet levels are unaffected.
6. D. Hereditary spherocytosis is a disease that is caused by the synthesis of abnormal spectrin molecules that exhibit defective binding to band 4.1 proteins of the erythrocyte cell membrane. Substitution of valine for glutamate results in sickle cell amenia. Impaired formation of β chains of the hemoglobin molecule results in thalassemia. Band 3 molecules are coupled anion transporters that facilitate the exchange of intracellular bicarbonate for the extracellular chloride ion.
7. A. When an Rh^- pregnant woman delivers her first Rh^+ baby, enough of the baby's blood is likely to enter her circulation to induce the formation of anti-Rh antibodies. During a subsequent pregnancy with an Rh^+ fetus, these antibodies attack the erythrocytes of the fetus, causing erythroblastosis fetalis, a condition that may be fatal to the newborn. Prenatal and postnatal transfusions of the fetus are necessary to prevent brain damage and death of the newborn unless the mother has been treated with anti-Rh agglutinins—Rh_O(D) immune globulin (RhoGAM)—before or shortly after the birth of the first Rh^+ baby. The other blood groups listed do not cause erythroblastosis fetalis.
8. E. Neutrophils constitute approximately 60% to 70% of the white blood cell count in a healthy patient. Monocytes are 3% to 8%, lymphocytes are the second most numerous making up 20% to 25% of the white blood cell population, and eosinophils are only 2% to 4% of the total population.
9. D. This patient is subject to persistent bacterial infections because his neutrophils cannot form a respiratory burst response to bacterial challenges. His neutrophils cannot generate superoxide, hydrogen peroxide, or hypochlorous acid during the phagocytosis of bacteria. He is not anemic and is able to bind IgE antibodies to his basophils and to his mast cells. Moreover, this child is able to fight parasitic invaders and can mount a normal humorally mediated immune response.
10. E. A patient who has vitamin K deficiency has normal fibrinogen levels. Vitamin K is required by the liver as a cofactor in the synthesis of clotting factors VII, IX, and X and prothrombin. The absence or reduced levels of these factors result in partial or complete dysfunction of the clotting process.

Circulatory System

11

The circulatory system is composed of two separate but related components: the cardiovascular system and the lymphatic vascular system. The function of the **cardiovascular system** is to carry blood in both directions between the heart and the tissues. The function of the **lymphatic vascular system** is to collect **lymph**, the excess extracellular fluid (tissue fluid), and to deliver it back to the cardiovascular system. Thus, the lymphatic system provides one-way transport, whereas the cardiovascular system provides two-way circulation.

CARDIOVASCULAR SYSTEM

The cardiovascular system is composed of two circuits: the pulmonary circuit to the lungs and the systemic circuit to the tissues of the body.

The **cardiovascular system** is composed of the **heart**, a muscular organ that pumps the blood into two separated circuits: the **pulmonary circuit**, which carries blood to and from the lungs, and the **systemic circuit**, which distributes blood to and from all of the organs and tissues of the rest of the body. The components of these circuits are

- **Arteries**. A series of vessels that transport blood away from the heart by branching into vessels of smaller and smaller diameters, eventually to capillaries to supply all regions of the body with blood.
- **Capillaries**. Thin-walled vessels with the smallest diameter, forming capillary beds, where gases, nutrients, metabolic wastes, hormones, and signaling substances are interchanged or passed between the blood and the tissues of the body to sustain normal metabolic activities.
- **Veins**. Vessels that drain capillary beds and form larger and larger vessels returning blood to the heart.

General Structure of Blood Vessels

Arteries generally have thicker walls and are smaller in diameter than their venous counterparts.

Most blood vessels have several features that are structurally similar, although dissimilarities exist and are the bases for classifying the vessels into different identifiable groups. For example, the walls of high-pressure vessels (e.g., subclavian arteries) are thicker than vessels conducting blood at low pressure (e.g., subclavian veins). However, arterial diameters continue to decrease at each branching, whereas vein diameters increase at each convergence, thus altering the respective layers of the walls of the vessels. Therefore, the descriptions used as distinguishing characteristics for a particular type of artery or vein are not always absolute. Indeed, the walls of the capillaries and venules are completely modified and less complex compared with those in larger vessels. Generally, arteries have thicker walls and are smaller in diameter than are the corresponding veins. Moreover, in histological sections, arteries are round and usually have no blood in their lumina, whereas veins are flattened and frequently contain blood in their lumina.

Vessel Tunics

Walls of blood vessels are composed of three layers: the tunica intima, the tunica media, and the tunica adventitia.

Three separate concentric layers of tissue, or **tunics**, make up the wall of the typical blood vessel (Fig. 11–1). The innermost layer, the **tunica intima**, is composed of a single layer of flattened, squamous endothelial cells, which form a tube lining the lumen of the vessel, and the underlying subendothelial connective tissue. The intermediate layer, the **tunica media**, is composed mostly of smooth muscle cells oriented concentrically around the lumen. The outermost layer, the **tunica adventitia**, is composed mainly of fibroelastic connective tissue whose fibers are arranged longitudinally.

The tunica intima houses in its outermost layer the **internal elastic lamina**, a thin band of elastic fibers that is well developed in medium-sized arteries. The outermost layer of the tunica media houses another band of elastic fibers, the **external elastic lamina**, although it is not distinguishable in all arteries.

Tunica Intima

The tunica intima is composed of a simple squamous epithelium, known as the endothelium*, and the subendothelial connective tissue, including the internal elastic lamina.*

The endothelial cells (simple squamous epithelium) lining the lumen of the blood vessel rest on a basal lamina. These flattened cells are elongated into a sheet such that their long axis is more or less parallel to the long axis of the vessel, which nearly permits each endothelial cell to surround the lumen of a small-caliber vessel. In larger-bore vessels, many individual endothelial cells are required to line the circumference of

SUMMARY

The **circulatory system** has two components: the **cardiovascular system** and the **lymphatic vascular system**. The cardiovascular system is composed of the **heart** and two separate circuits: the **pulmonary circuit** that carries deoxygenated blood from the heart to the lungs and oxygenated blood from the lungs to the heart, and the **systemic circuit** that distributes oxygenated blood from the heart to the rest of the body and returns deoxygenated blood from the tissues of the body to the heart. Blood leaves the heart via vessels that belong to the **arterial system**, whose members decrease in size the farther away they are from the heart, and return to the heart via vessels that belong to the **venous system** whose members increase in size as they approach the heart. The smallest branches of the arterial and venous system are connected to each other by way of **capillaries** that form extensive **capillary beds**. The members of the arterial system are named by more or less arbitrary criteria as **elastic (conducting) arteries**, **muscular (distributing) arteries**, and **arterioles**, where arterioles regulate **blood pressure**. The members of the venous system are the **large veins**, **medium-sized veins**, **small veins**, and **venules**. It is at the level of venules that materials are exchanged between connective tissue spaces and lumina of these vessels, and it is here that leukocytes migrate in and out of blood vessels. The **lymphatic vascular system** is composed of lymph vessels that remove excess extracellular fluid from the interstitial tissue spaces and return it to the cardiovascular system.

KEY WORDS

Aorta; arterial system; arterioles; blood pressure; capillaries; capillary beds; cardiovascular system; circulatory system; conducting arteries; distributing arteries; elastic arteries; heart; large veins; lymphatic vascular system; medium-sized veins; muscular arteries; pulmonary circuit; small veins; systemic circuit; venous system; venules

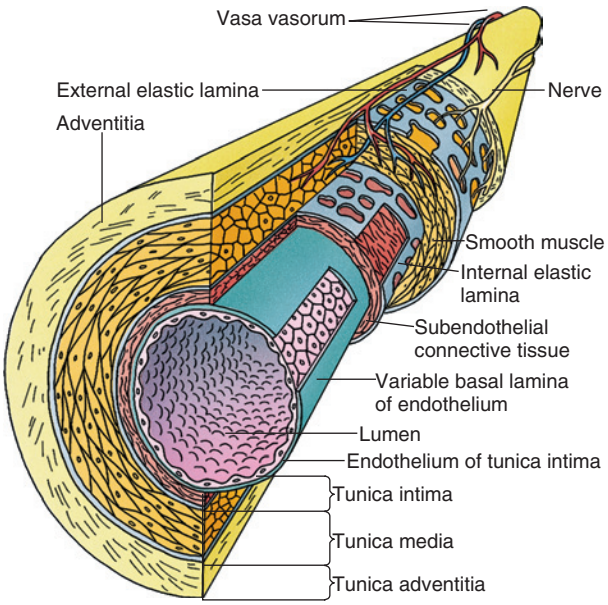


Figure 11–1 Diagram of a typical artery.

the lumen. Endothelial cells provide an exceptionally smooth surface; they

- Secrete types II, IV, and V collagens, laminin, endothelin, nitric oxide, von Willebrand factor (vWF), tissue factor, and P-selectin
- Possess membrane-bound enzymes, such as **angiotensin-converting enzyme (ACE)**, which cleave **angiotensin I** to generate **angiotensin II** (See the later section on the regulation of arterial blood pressure.)
- Possess enzymes that inactivate bradykinin, serotonin, prostaglandins, thrombin, and norepinephrine
- Bind lipoprotein lipase, the enzyme that degrades lipoprotein triglycerides into glycerol and fatty acids

A **subendothelial layer** lies immediately beneath the endothelial cells. It is composed of loose connective tissue. Beneath the subendothelial connective tissue layer is an **internal elastic lamina** that is especially well developed in muscular arteries. Separating the tunica intima from the tunica media, the internal elastic lamina is composed of **elastin**, which is a fenestrated sheet that permits the diffusion of substances into the deeper regions of the arterial wall to nourish the cells there.

Tunica Media

The tunica media, usually the thickest layer of the vessel wall, is composed of helically disposed layers of smooth muscle and the external elastic lamina, when present.

The tunica media is the thickest layer of the vessel. The **concentric cell layers** forming the tunica media is composed mostly of helically arranged smooth muscle cells. Interspersed within the layers of smooth muscle cells are some elastic fibers, type III collagen, and proteoglycans. The fibrous elements form lamellae within the ground substance secreted by smooth muscle cells. Larger muscular arteries have an **external elastic lamina**, which is more delicate than the internal elastic lamina and separates the tunica media from the overlying tunica adventitia. Capillaries and postcapillary venules do not have a tunica media; in these small vessels, **pericytes** replace the tunica media (see later section on capillaries).

Tunica Adventitia

The tunica adventitia, the outermost layer of the vessel wall, blends into the surrounding connective tissue.

Covering the vessels on their outside surface is the **tunica adventitia**, composed of a dense, irregular, collagenous connective tissue consisting mostly of fibroblasts, types I and III collagen fibers, and longitudinally oriented elastic fibers. This layer becomes continuous with the connective tissue elements surrounding the vessel.

Vasa Vasorum

Vasa vasorum (vessels of the vessel) furnish the muscular walls of blood vessels with a blood supply.

The thickness and muscularity of larger vessels prevent the cells composing the tunics from being nourished by diffusion from the lumen of the vessel. The deeper cells of the tunica media and tunica adventitia are nourished by the **vasa vasorum (vessels of the vessel)**, small arteries that enter the vessel walls and branch profusely to serve the cells located primarily in the tunica media and tunica adventitia. Compared with arteries, veins have more cells that cannot be supplied with oxygen and nutrients by diffusion because venous blood contains less oxygen and nutrients than arterial blood. For this reason, the vasa vasorum are more prevalent in the walls of veins than arteries.

Nerve Supply to Vessels

Sympathetic nerves supply vasomotor innervation to the smooth muscles of the tunica media.

A network of **vasomotor nerves** of the sympathetic component of the autonomic nervous system supplies smooth muscle cells of blood vessels. These unmyelinated, postganglionic sympathetic nerves are responsible for **vasoconstriction** of the vessel walls. Because the nerves seldom enter the tunica media of the vessel, they do not synapse directly on the smooth muscle cells. Instead, they release the neurotransmitter **norepinephrine**, which diffuses into the media and acts on smooth muscle cells nearby. These impulses are

propagated throughout all of the smooth muscle cells via their gap junctions, thereby orchestrating contractions of the entire smooth muscle cell layer and thus reducing the diameter of the vessel lumen.

Arteries are more heavily endowed with vasomotor nerves than the veins are, but veins also receive vasomotor nerve endings in the tunica adventitia. The arteries supplying skeletal muscles also receive cholinergic (parasympathetic) nerves to bring about vasodilation.

Arteries

Arteries are blood vessels that carry blood away from the heart.

Arteries are efferent vessels that transport blood away from the heart to the capillary beds. The two major arteries that arise from the right and left ventricles of the heart are the **pulmonary trunk** and the **aorta**, respectively.

The **pulmonary trunk** branches, shortly after exiting the heart, into right and left pulmonary arteries that enter the lungs for distribution (Chapter 15 describes the branching and blood supply to the lungs.) The right and left coronary arteries, which supply the heart muscle, arise from the aorta as it exits the left ventricle.

The **aorta**, upon leaving the heart, courses in an obliquely posterior arch to descend in the thoracic cavity, where it sends branches to the body wall and the viscera; it then enters the abdominal cavity, where it sends branches to the body wall and viscera. The abdominal aorta terminates by bifurcating into the right and left common iliac arteries in the pelvis.

Three major arterial trunks—the right brachiocephalic artery, the left common carotid artery, and the left subclavian artery—arise from the arch of the aorta to supply the superior extremities and the head and neck. It is interesting to note that the right common carotid artery arises from the right brachiocephalic trunk, whereas the left common carotid artery arises directly from the aortic arch. Continued branching of all of these arteries into large numbers of smaller and smaller arteries continues until the vessel walls contain a single layer of endothelial cells. The resulting vessels, called ***capillaries***, are the smallest functional vascular elements of the cardiovascular system.

Classification of Arteries

Arteries are of three types: elastic arteries (conducting arteries), muscular arteries (distributing arteries), and arterioles.

Arteries are classified into three major types based on their relative size, morphological characteristics, or both (Table 11–1). From largest to smallest, they are as follows:

- Elastic (conducting) arteries
- Muscular (distributing) arteries
- Arterioles

Because the vessels decrease in diameter in a continuous fashion, there are gradual changes in morphological characteristics in going from one type to another. Therefore, some vessels having characteristics of two categories cannot be assigned to a specific category with certainty.

TABLE 11–1 Characteristics of Various Types of Arteries

Artery	Tunica Intima	Tunica Media	Tunica Adventitia
Elastic artery *(conducting)* (e.g., aorta)	Endothelium with Weibel-Palade bodies, basal lamina, subendothelial layer, incomplete internal elastic lamina	40–70 fenestrated elastic membranes, smooth muscle cells interspersed between elastic membranes, thin external elastic lamina, vasa vasorum in outer half	Thin layer of fibroelastic connective tissue, vasa vasorum, lymphatic vessels, nerve fibers
Muscular artery *(distributing)* (e.g., femoral artery)	Endothelium with Weibel-Palade bodies, basal lamina, subendothelial layer, thick internal elastic lamina	Up to 40 layers of smooth muscle cells, thick external elastic lamina	Thin layer of fibroelastic connective tissue; vasa vasorum not very prominent; lymphatic vessels, nerve fibers
Arteriole	Endothelium with Weibel-Palade bodies; basal lamina, subendothelial layer not very prominent; some elastic fibers instead of a defined internal elastic lamina	One or two layers of smooth muscle cells	Loose connective tissue, nerve fibers
Metarteriole	Endothelium, basal lamina	Smooth muscle cells form precapillary sphincter	Sparse, loose connective tissue

Elastic Arteries

Concentric layers of elastic membranes, known as fenestrated *membranes, occupy much of the tunica media.*

The aorta and the branches originating from the aortic arch (the common carotid artery and the subclavian artery), the common iliac arteries, and the pulmonary trunk are **elastic (conducting) arteries** (Fig. 11–2). The walls of these vessels may be yellow in the fresh state because of the abundance of elastin.

The **tunica intima** of the elastic arteries is composed of an endothelium that is supported by a narrow layer of underlying subendothelial connective tissue containing a few fibroblasts, occasional smooth muscle cells, and collagen fibers. Thin laminae of elastic fibers, the **internal elastic lamina**, are also present.

The endothelial cells of the elastic arteries are 10 to 15 μm wide and 25 to 50 μm long; their long axes are oriented parallel to the longitudinal axis of the vessel. These cells are connected to each other mostly by occluding junctions. Their plasma membranes contain small vesicles thought to be related to transport of water, macromolecules, and electrolytes. Occasional blunt processes may extend from the plasma membrane through the internal elastic lamina to form gap junctions with smooth muscle cells located in the tunica media. The endothelial cells contain **Weibel-Palade bodies**, membrane-bound inclusions, 0.1 μm in diameter and 3 μm long, that have a dense matrix housing tubular elements containing the glycoprotein **vWF** (von Willebrand factor) as well as **tissue factor** and **P-selectin**. The vWF facilitates the coagulation of platelets during clot formation and is manufactured by most endothelial cells but is stored in arteries only. Tissue factor enhances the process of coagulation, and P-selectin induces leukocytes to leave the bloodstream, to enter the connective tissue spaces, and to function in the immune process.

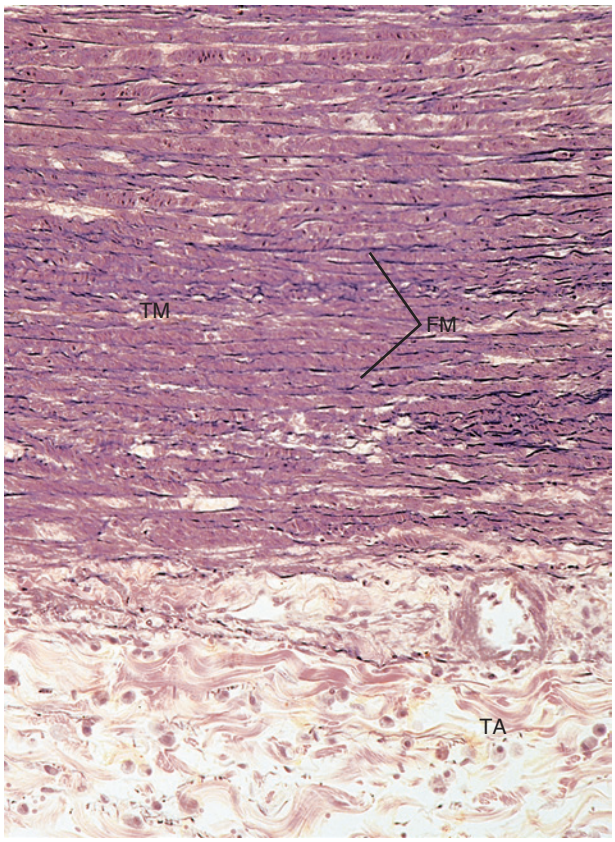


Figure 11–2 Light micrograph of an elastic artery (×132). Observe the fenestrated membranes (FM), the tunica media (TM), and the tunica adventitia (TA). The tunica intima is not shown.

CLINICAL CORRELATIONS

Patients with **von Willebrand disease**, an inherited disorder that results in impaired adhesion of platelets, have prolonged coagulation times and excessive bleeding at an injury site.

The **tunica media** of the elastic arteries consists of many fenestrated lamellae of elastin, known as the ***fenestrated membranes***, alternating with circularly oriented layers of smooth muscle cells. The number of lamellae of elastin increases with age; there are approximately 40 lamellae of fenestrated membranes in newborns and 70 in adults. These fenestrated membranes also increase in thickness because of the continued deposition of elastin, which constitutes much of the tunica media; smooth muscles cells are less abundant in elastic arteries than in most muscular arteries. The extracellular matrix, secreted by the smooth muscle cells, is composed mostly of chondroitin sulfate, collagen, and reticular and elastic fibers. An **external elastic lamina** is also present in the tunica media.

The **tunica adventitia** of elastic arteries is relatively thin and is composed of loose fibroelastic connective tissue housing some fibroblasts. Vasa vasorum also are abundant throughout the adventitia. Capillary beds arise from the vasa vasorum and extend to the tissues of the tunica media, where they supply the connective tissue and smooth muscle cells with oxygen and nutrients. Fenestrations in the elastic laminae permit some diffusion of oxygen and nutrients to the cells in the tunica media from the blood flowing through the lumen, although most of the nourishment is derived from branches of the vasa vasorum.

Muscular Arteries

Muscular arteries are characterized by a thick tunica media that is composed mostly of smooth muscle cells.

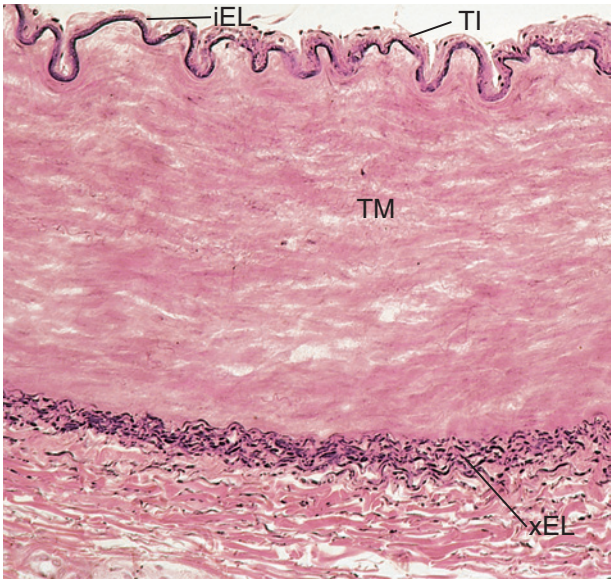


Figure 11–3 Light micrograph of a muscular artery (×132). Note the tunica adventitia, the internal (iEL) and external (xEL) elastic laminae within the thick tunica media (TM). TI, Tunica intima.

Muscular (distributing) arteries include most vessels arising from the aorta, except for the major trunks originating from the arch of the aorta and the terminal bifurcation of the abdominal aorta, which are identified as elastic arteries. Indeed, most of the named arteries, even those with a diameter of only slightly greater than 0.1 mm, are classified as muscular arteries (e.g., brachial, ulnar, renal). The identifying characteristic of muscular arteries is a relatively thick tunica media composed mostly of layers of smooth muscle cells (Fig. 11–3).

The **tunica intima** in the muscular arteries is thinner than that in the elastic arteries, but the subendothelial layer contains a few smooth muscle cells; also, in contrast with that of elastic arteries, the **internal elastic lamina** of the muscular arteries is prominent and displays an undulating surface to which the endothelium conforms. Occasionally, the internal elastic lamina is duplicated; this is called ***bifid internal elastic lamina***. As in elastic arteries, the endothelial cells have processes that pass through fenestrations within the internal elastic lamina and make gap junctions with smooth muscle cells of the tunica media that are near the interface with the tunica intima. It is believed that these gap junctions may couple metabolically the endothelium and the smooth muscle cells.

The **tunica media** of the muscular arteries is composed predominantly of smooth muscle cells, although these cells are considerably smaller than those located in the walls of the viscera. The orientation of most of the smooth muscle cells is circular, where the tunica media interfaces with the tunica intima; however, a few bundles of smooth muscle fibers are arranged longitudinally in the tunica adventitia. Small muscular arteries have 3 or 4 layers of smooth muscle cells, whereas larger muscular arteries may have as many as 40 layers of circularly arranged smooth muscle cells. The number of cell layers decreases as the diameter of the artery diminishes.

Each smooth muscle cell is enveloped by an **external lamina** (similar to a **basal lamina**), although muscle cell processes extend through intervals in the basal lamina to form gap junctions with other muscle cells, ensuring coordinated contractions within the tunica media. Interspersed within the layers of smooth muscle cells are elastic fibers, type III collagen fibers, and chondroitin sulfate, all secreted by the smooth muscle cells. Type III collagen fibers (30 nm in diameter) are located in bundles within the intercellular spaces.

An **external elastic lamina** is identifiable in histological sections of larger muscular arteries as several layers of thin elastic sheets; in electron micrographs, these sheets display fenestrations.

The **tunica adventitia** of the muscular arteries consists of elastic fibers, collagen fibers (60 to 100 nm in diameter), and ground substance composed mostly of dermatan sulfate and heparan sulfate. This extracellular matrix is produced by fibroblasts in the adventitia. The collagen and elastic fibers are oriented longitudinally and blend into the surrounding connective tissues. Located at the outer regions of the adventitia are vasa vasorum and unmyelinated nerve endings. Neurotransmitter released at the nerve endings diffuses through fenestrations in the external elastic lamina into the tunica media to depolarize some of the superficial smooth muscle cells. Depolarization is propagated to all of the muscle cells of the tunica media via gap junctions.

CLINICAL CORRELATIONS

Aneurysm, a sac-like dilation of the wall of an artery (or less often of a vein), results from weakness in the vessel wall and is usually age related. The aneurysm occurs in regions of the vessel wall where, frequently as a result of atherosclerosis, Marfan syndrome, syphilis, and Ehlers-Danlos syndrome, elastic fibers are displaced by collagen fibers. The abdominal aorta is the most common vessel with this type of aneurysm. When discovered, the ballooned area can be repaired, but if it is not discovered and it ruptures, there is rapid massive blood loss that may result in the death of the individual.

Arterioles

Arteries with a diameter of less than 0.1 mm are considered to be arterioles.

Arterioles are the terminal arterial vessels that regulate blood flow into capillary beds. In histological sections,

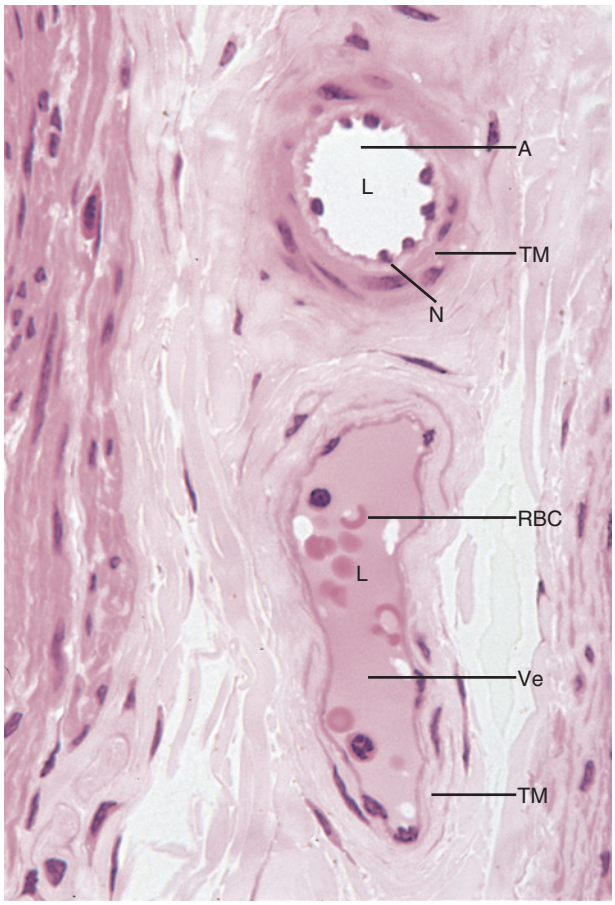


Figure 11–4 Light micrograph of an arteriole and a venule containing blood cells (×540). The arteriole (A) is well defined with a thick tunica media (TM). Nuclei of endothelial cells bulge into the lumen (L). The venule (Ve) is poorly defined with a large, poorly defined lumen containing red blood cells (RBCs). The tunica media of the venule is not as robust as that in the arteriole.

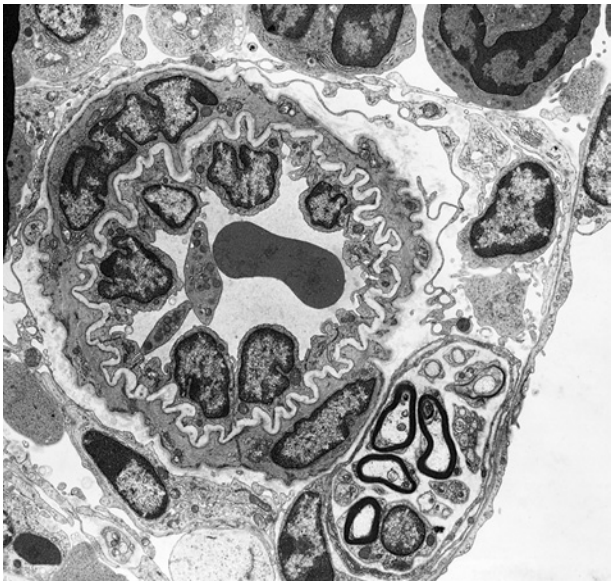


Figure 11–5 Electron micrograph of an arteriole (From Yamazaki K, Allen TD. Ultrastructural morphometric study of efferent nerve terminals on murine bone marrow stromal cells, and the recognition of a novel anatomical unit: the "neuro-reticular complex." *Am J Anat*. 1990;187:261-276. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

the width of the wall of an arteriole is approximately equal to the diameter of its lumen (Fig. 11–4). The endothelium of the **tunica intima** is supported by a thin subendothelial connective tissue layer consisting of type III collagen and a few elastic fibers embedded in ground substance. A thin, fenestrated **internal elastic lamina** is present in larger arterioles but absent in small and terminal arterioles (Fig. 11–5). In small arterioles, the **tunica media** is composed of a single smooth muscle cell layer that completely encircles the endothelial cells (Fig. 11–6). In larger arterioles, the tunica media consists of two to three layers of smooth muscle cells. Arterioles do not have an external elastic lamina. The **tunica adventitia** of arterioles is scant and is represented by fibroelastic connective tissue housing a few fibroblasts.

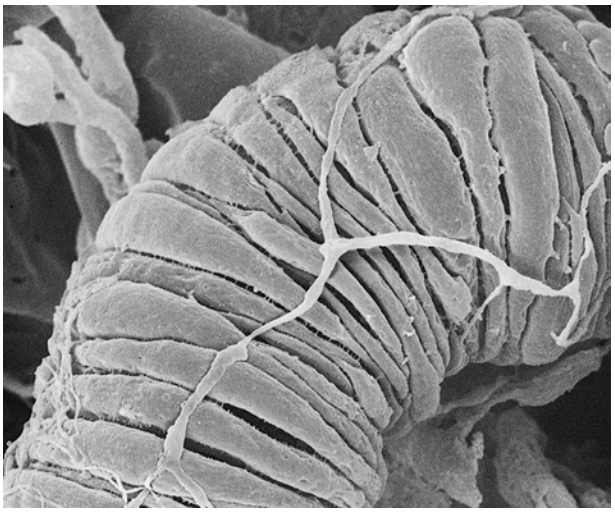


Figure 11–6 Scanning electron micrograph of an arteriole illustrating its compact layer of smooth muscle and its attendant nerve fibers (×4200). (From Fujiwara T, Uehara Y. The cytoarchitecture of the wall and innervation pattern of the microvessels in the rat mammary gland: a scanning electron microscopic observation. *Am J Anat*. 1984;170:39-54. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Arteries that supply blood to capillary beds are called ***metarterioles***. They are approximately 8 µm in diameter and differ structurally from arterioles in that their smooth muscle layer is not continuous; rather, the individual muscle cells **(precapillary sphincters)** are spaced apart, and each encircles the endothelium of a capillary arising from the metarteriole. It is believed that this arrangement permits these smooth muscle cells to function as a sphincter upon contraction, thus controlling blood flow into the capillary bed.

Specialized Sensory Structures in Arteries

Specialized sensory structures in the arteries include the carotid sinus, the carotid body, and aortic bodies.

Three types of specialized sensory structures are located in the major arteries of the body: **carotid sinuses**, **carotid bodies**, and **aortic bodies**. Nerve endings in these structures monitor blood pressure and blood composition, providing essential inputs to the brain for controlling heartbeat, respiration, and blood pressure.

Carotid Sinus

The carotid sinus is a baroreceptor located in the wall of the internal carotid artery just distal to the bifurcation of the common carotid artery.

The carotid sinus is a baroreceptor; that is, it perceives changes in blood pressure. This structure is a specialization within the wall of the internal carotid artery just above the bifurcation of the common carotid artery. At this site, the adventitia of this vessel is relatively thicker and heavily endowed with sensory nerve endings from the glossopharyngeal nerve (cranial nerve IX). The tunica media at this site is relatively thinner, thus permitting it to be distended during increases in blood pressure; this distention stimulates the nerve endings. The afferent impulses, received at the vasomotor center in the brain, trigger adjustments in vasoconstriction, resulting in maintenance of proper blood pressure. Additional small baroreceptors are located in the aorta and in some of the larger vessels.

Carotid Body

The carotid body functions as a chemoreceptor monitoring changes in oxygen and carbon dioxide levels as well as hydrogen ion concentration.

Located at the bifurcation of the common carotid artery is a small, oval structure known as the ***carotid body***. The carotid body possesses specialized chemoreceptor nerve endings responsible for monitoring changes in oxygen and carbon dioxide levels as well as blood H^+ concentration. The carotid body, 3 to 5 mm in diameter, is composed of multiple clusters of pale-staining cells embedded in connective tissue. Two types of parenchymal cells are clearly distinguishable in electron micrographs: **glomus (type I) cells** and **sheath (type II) cells**.

Glomus cells have a large nucleus and the usual array of organelles. They are distinguished by the presence of dense-cored vesicles, 60 to 200 nm in diameter, that resemble vesicles located in the chromaffin cells of the suprarenal medulla. Cell processes also contain longitudinally oriented microtubules, dense-cored vesicles, and a few small electron-lucent vesicles. These processes contact other glomus cells and capillary endothelial cells.

Sheath cells are more complex and have long processes that almost completely ensheath the processes of the glomus cells. The nuclei of these cells are irregular and contain more heterochromatin compared with the nuclei of glomus cells; moreover, sheath cells contain no dense-cored vesicles. As nerve terminals enter clusters of glomus cells, they lose their Schwann cells and become covered by the sheath cells in much the same way as glial cells would ensheath fibers in the central nervous system (CNS).

Carotid bodies contain catecholamines (as do the cells of the suprarenal medulla and paraganglia), but whether they produce hormones is unclear. The glossopharyngeal and vagus nerves supply the carotid body with numerous afferent fibers. In some of the synapses, the glomus cells appear to function as presynaptic cell bodies, but the specific relationships are as yet not understood.

Aortic Bodies

Aortic bodies are located at the arch of the aorta between the right subclavian and the right common carotid arteries and between the left common carotid artery and the left subclavian artery. Their microscopic anatomy resembles that of the carotid bodies and, therefore, it is assumed that they also are chemoreceptors.

Regulation of Arterial Blood Pressure

Arterial blood pressure is regulated by the vasomotor center in the brain.

The heart, which serves as the cardiovascular pump, rests between each stroke, thus developing a pressurized burst of blood that first enters into the elastic arteries, then moves into the muscular arteries and arterioles, and finally into capillaries, which serve the tissues. The **vasomotor center** in the brain responds to the continual monitoring of blood pressure by controlling **vasomotor tone**, the constant state of contraction of the vessel walls, which is modulated via vasoconstriction and vasodilation. **Vasoconstriction** is accomplished via **vasomotor nerves** of the sympathetic nervous system, whereas **vasodilation** is a function of the parasympathetic system. During vasodilation, acetylcholine from the nerve terminals in the vessel walls initiates release of **nitric oxide** from the endothelium to diffuse into the smooth muscle

cells, which activates the cyclic guanosine monophosphate system of smooth muscle cells, resulting in relaxation of the muscle cells, thus dilating the vessel lumen.

Smooth muscle cells of the arteries have receptors for substances besides the neurotransmitter norepinephrine. When the blood pressure is low, the kidneys secrete the enzyme **renin**, which cleaves **angiotensinogen** circulating in the blood, forming **angiotensin I**. This mild vasoconstrictor is converted to **angiotensin II** by **ACE (angiotensin converting enzyme)**, which is located on the luminal plasmalemmae of capillary endothelia (especially capillaries of the lungs). Angiotensin II is a potent vasoconstrictor that initiates smooth muscle contraction, thereby reducing vessel lumen diameter, resulting in increased blood pressure. Dehydration as well as severe hemorrhage induces the posterior pituitary to release **antidiuretic hormone**, also known as ***vasopressin***, another powerful vasoconstrictor.

The structure of elastic arteries permits distention of their walls during **systole** (heart contraction), followed by recoil of their walls during **diastole** (heart relaxation), which assists in delivering a more constant blood pressure and flow of blood. Muscular arteries branching from the elastic arteries distribute blood to the body and are subject to constant changes in diameter resulting from vasoconstriction and vasodilation. To assist in accommodating for these events, the tunica adventitia blends loosely into the surrounding connective tissue, thus preventing restraint on the vessel during contractions and expansions for changes in blood pressure.

Artery location also dictates the thickness of the various tunics. For example, the thickness of the tunica media in the arteries of the leg is greater than that found in the arteries of the upper extremity. This is in response to the continued pressure on the blood column in the vessel resulting from gravitational forces. Moreover, the coronary arteries, serving the heart, are high-pressure arteries and, as such, have a thick tunica media. Conversely, arteries in the pulmonary circulation are under low pressure; thus, the tunica media in these vessels are thinner.

CLINICAL CORRELATIONS

Normal and Pathological Vascular Changes

The largest arteries continue to grow until about age 25 years, although there is progressive thickening of their walls and an increase in the number of elastic laminae. In the muscular arteries, from middle age on, deposits of collagen and proteoglycans increase in the walls, thus reducing their flexibility. The coronary vessels are the first to display the effects of aging, with the intima displaying the greatest age-related changes. These natural changes are not unlike the regressive changes observed in arteriosclerosis.

Arteriosclerosis

Small arteries and arterioles, especially those of the kidneys, are prone to the most common type of arteriosclerosis (hardening of the arteries), displaying a hyaline or concentric thickening that is often associated with hypertension and diabetes.

Atherosclerosis

The largest of the arteries including the coronary arteries, the carotid arteries, and the major arteries of the brain among others, are susceptible to **atherosclerosis**, a disease that is the forerunner to heart attack and stroke. Atherosclerosis is distinguished by infiltrations of soft, noncellular lipid material into the tunica intima; these infiltrations can reduce the luminal diameter appreciably even by age 25 years. It is not clear whether these conditions are physiological or a manifestation of a disease process. The fibrous plaques that form in the intima of older persons, however, are pathological.

The smooth muscle cell layer in the tunica media of a healthy person undergoes renewal, but when the endothelium is injured, platelets that accumulate at the lesion site release **platelet-derived growth factor** (PDGF), stimulating proliferation of smooth muscle cells. As a consequence, these cells begin to get packed with cholesterol-rich lipids, which stimulate the muscle cells to manufacture additional collagen and proteoglycans, resulting in a cycle whereby the tunica intima becomes thickened. This further damages the endothelium leading to necrosis, which attracts more platelets, and, finally, clotting, forming a thrombus that may occlude the vessel at that site or, if released into the general circulation, it may occlude a more vulnerable vessel (e.g., a coronary vessel or a cerebral vessel).

The pathogenesis is still unclear, although current research theories point to the role of cholesterol, lipoproteins, and certain mitogens. A correlation between blood cholesterol levels and heart disease has been demonstrated, and it has been noted that **C-reactive protein** (**CRP**), synthesized by the liver, can be used as a marker for inflammation, which appears to be a far more accurate indicator of the risk for cardiovascular disease. Statins, which have been used extensively for reducing cholesterol levels in the blood, thereby reducing the risk of heart disease, have also been shown to reduce the levels of CRP. This fact is important because there are data supporting that the response to inflammation is as critical to heart disease as are high levels of cholesterol. Thus, there appears to be a commonality between inflammation and cardiovascular disease.

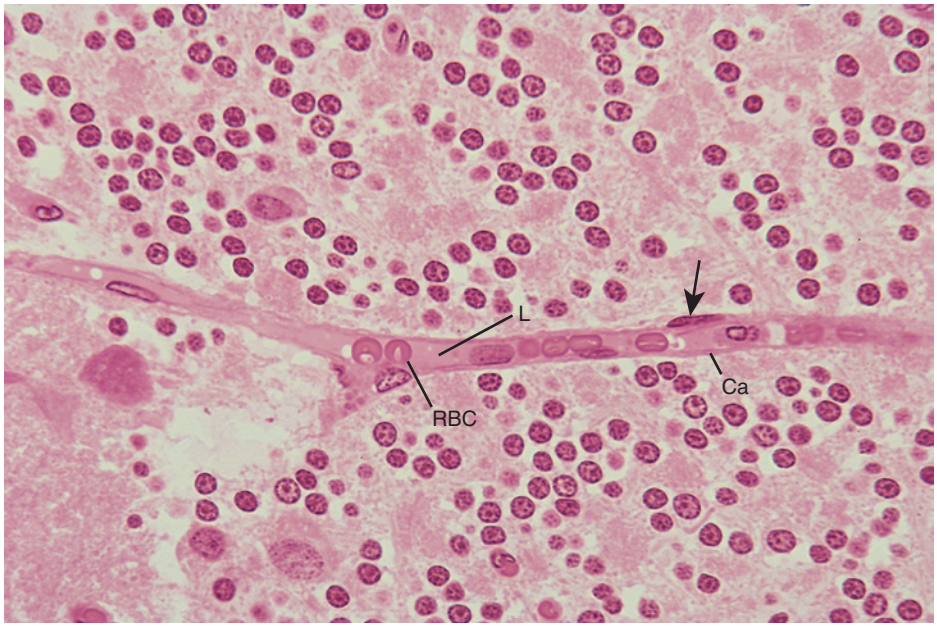


Figure 11–7 Photomicrograph of a capillary in the monkey cerebellum (×270). A capillary (Ca) is present in the field of view and red blood cells (RBCs) are evident in its lumen (L). Note the nucleus (*arrow*) of an endothelial cell bulging into the lumen.

Capillaries

Capillaries arise from the terminal ends of arterioles and also from metarterioles (Fig. 11–7). By branching and anastomosing, capillaries form capillary beds (capillary networks) between the arterioles and the venules.

General Structure of Capillaries

Capillaries, composed of a single layer of endothelial cells, are the smallest blood vessels.

Capillaries are the smallest of the vascular channels, on the average approximately 50 µm in length with a diameter of 8 to 10 µm. These slender, small vessels are formed by a single layer of squamous endothelial cells rolled into a tube with the long axis of these cells lying in the same direction as the blood flow. These endothelial cells are flattened, with the attenuated ends tapering to a thickness to 0.2 µm or less, although an elliptical nucleus bulges out into the lumen of the capillary. The cytoplasm contains a Golgi complex, a few mitochondria, some rough endoplasmic reticulum (RER), and free ribosomes (Figs. 11–8 and 11–9). Intermediate filaments (9 to 11 nm), located about the perinuclear zone, are composed of **desmin** and/or **vimentin**. These filaments provide structural support to the endothelial cells, but the significance of their variation is unclear.

The large number of pinocytotic vesicles associated with the endothelial cell plasmalemma is an identifying

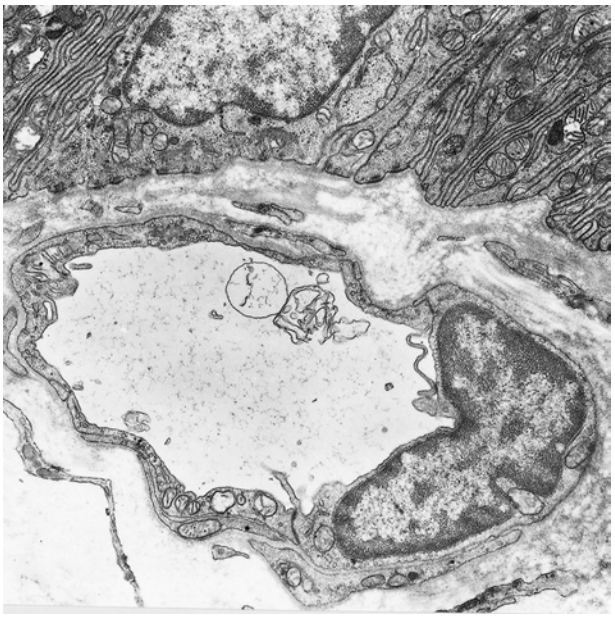


Figure 11–8 Electron micrograph of a continuous capillary in the rat submandibular gland (×13000). The pericyte shares the endothelial cell's basal lamina. (From Sato A, Miyoshi S. Morphometric study of the microvasculature of the main excretory duct subepithelia of the rat parotid, submandibular, and sublingual salivary glands. *Anat Rec*. 1990;226:288-294. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

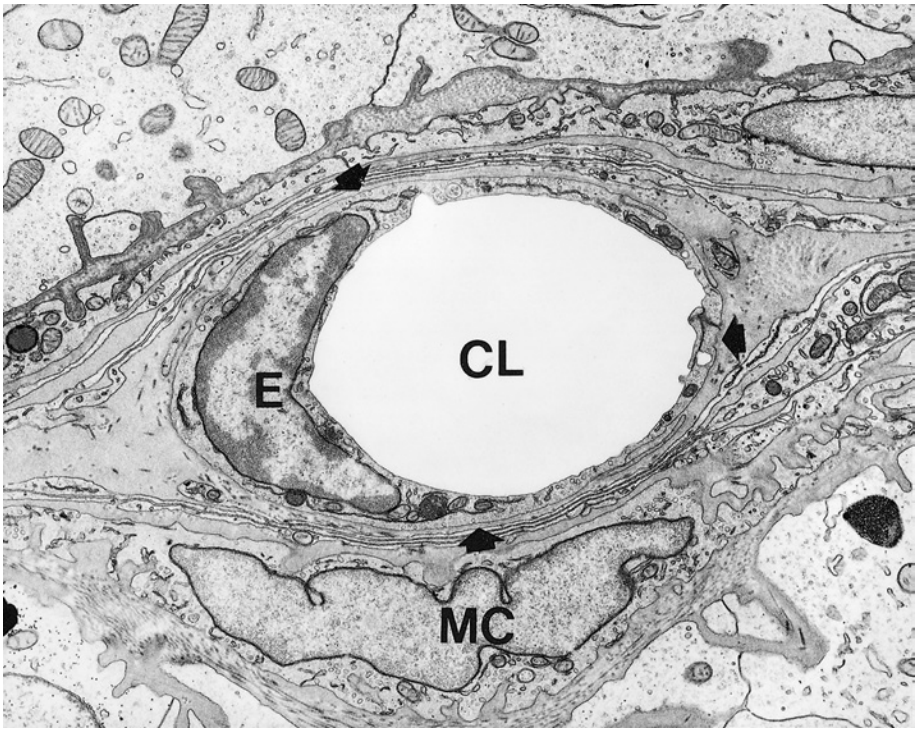


Figure 11–9 Electron micrograph of a testicular capillary. CL, Capillary lumen; E, nucleus of endothelial cell; MC, myoid cell. *Arrows* represent the basal lamina. (From Meyerhofer A, Hikim APS, Bartke A, Russell LD. Changes in the testicular microvasculature during photoperiod-related seasonal transition from reproductive quiescence to reproductive activity in the adult golden hamster. *Anat Rec*. 1989;224:495-507. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

characteristic of capillaries. These vesicles may be in singular array, two single vesicles may be fused together, or several vesicles may be fused, forming a transient channel. Where the endothelial cells are the thinnest, a single array of fused vesicles may span from the adluminal plasmalemma across the cytoplasm to the abluminal plasmalemma of the endothelial cell.

The endothelial cells of capillaries are rolled into a tube, giving the lumen a diameter that ranges from 8 to 10 μm but remains constant throughout the entire length of a capillary. This diameter is sufficient to permit individual cells of the blood to pass without being hindered. Not all of the capillary beds are open at any one time; however, increased demand initiates the opening of more beds, thus increasing blood flow to meet physiological needs. The external surfaces of the endothelial cells are surrounded by a basal lamina secreted by the endothelial cells (see Fig. 11–9). When viewed in the cross section, it is evident that the circumference of small capillaries is formed by a single endothelial cell, whereas portions of two or three endothelial cells contribute to forming the circumference of larger capillaries. At these cellular junctions, the endothelial cells tend to overlap, forming a **marginal fold** that projects into the lumen. Endothelial cells are joined together by **fasciae occludentes**, or **tight junctions**, although occasional gap junctions and desmosomes are also evident.

Pericytes, located along the outside of the capillaries and small venules, appear to be surrounding them, and share the basal laminae of the endothelial cells (Figs. 11–10 and 11–11). These cells have long primary processes, which are located along the long axis of the capillary and from which secondary processes arise to wrap around the capillary, forming a few **gap junctions** with the endothelial cells. Pericytes possess a small Golgi complex, mitochondria, RER, microtubules, and filaments extending into the processes. These cells also contain tropomyosin, isomyosin, and protein kinase, which are all related to the contractile process that regulates blood flow through the capillaries. Furthermore, as discussed in Chapter 6, after injury, pericytes

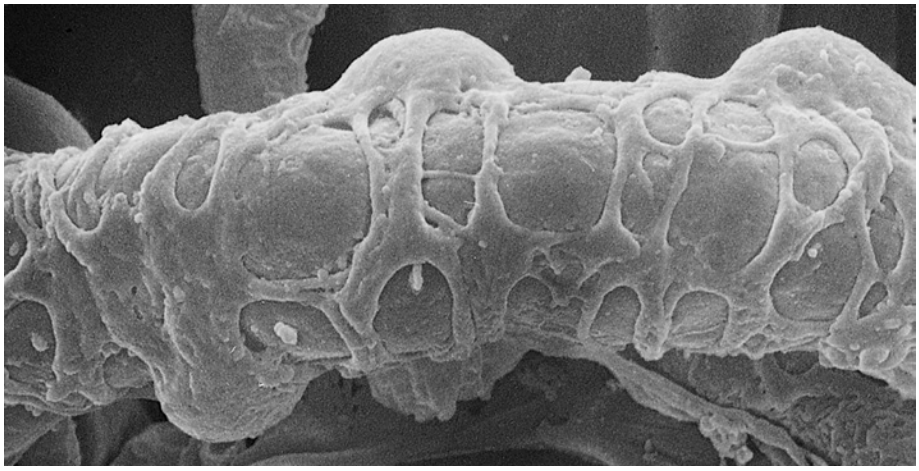


Figure 11–10 Scanning electron micrograph of a capillary displaying pericytes on its surface (×5000). (From Fujiwara T, Uehara Y. The cytoarchitecture of the wall and innervation pattern of the microvessels in the rat mammary gland: a scanning electron microscopic observation. *Am J Anat*. 1984;170:39-54. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

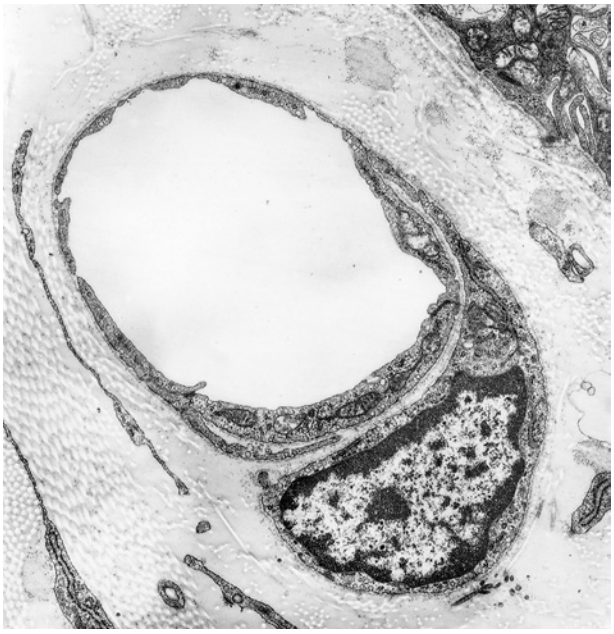


Figure 11–11 Electron micrograph of a fenestrated capillary and its pericyte in a cross section. Note that the capillary endothelial cells and the pericyte share the same basal lamina. (From Sato A, Miyoshi S. Morphometric study of the microvasculature of the main excretory duct subepithelia of the rat parotid, submandibular, and sublingual salivary glands. *Anat Rec*. 1990;226:288-294. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

may undergo differentiation to become smooth muscle cells and endothelial cells in the walls of arterioles and venules.

Classification of Capillaries

Capillaries are of three types: continuous, fenestrated, and sinusoidal (Fig. 11–12); they differ in their location and structure.

Continuous Capillaries (Somatic Capillaries)

Continuous capillaries (somatic capillaries) have no pores or fenestrae in their walls.

Continuous capillaries (also known as ***somatic capillaries***) are present in muscle, nervous, and connective tissues as well as in the lungs and exocrine glands. The intercellular junctions between their endothelial cells are **fasciae occludentes**, which prevent passage of many molecules. Substances such as amino acids, glucose, nucleosides, and purines move across the capillary wall via carrier-mediated transport as evidenced by the numerous pinocytotic vesicles associated with these types of capillaries. The cells exhibit a polarity with the transport systems, such that Na^+-K^+ ATPase is located in the adluminal cell membrane only. In the CNS, the blood–brain barrier regulation resides within the endothelial cells but is influenced by signaling molecules formed by the astrocytes associated with the capillaries. It is interesting to note that continuous capillaries of the CNS have a much reduced number of pinocytotic vesicles.

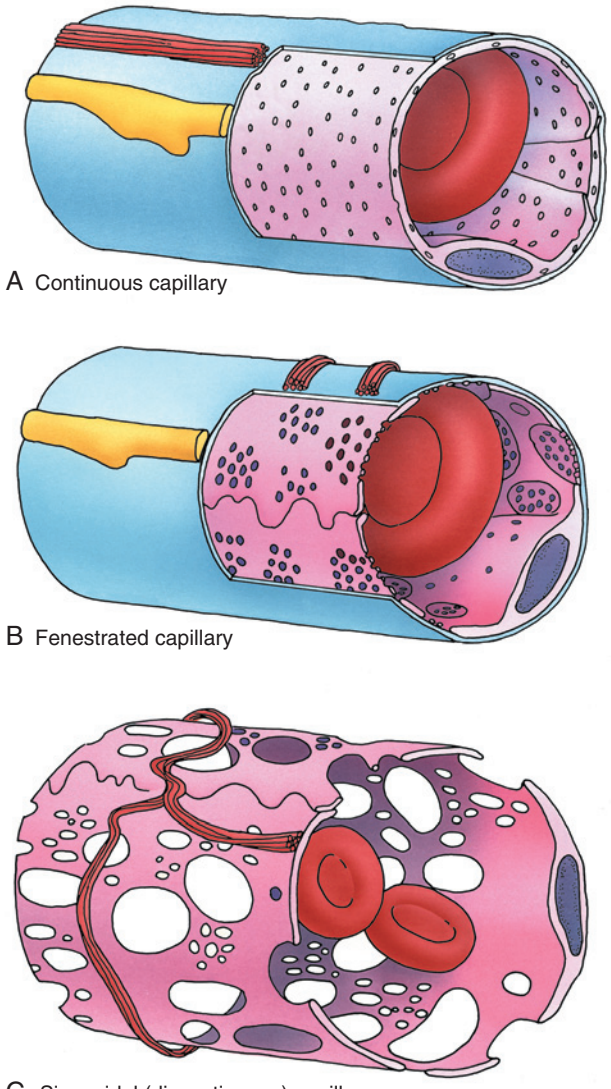


Figure 11–12 Diagram of the three types of capillaries. **A,** Continuous. **B,** Fenestrated. **C,** Sinusoidal.

Fenestrated Capillaries (Visceral Capillaries)

Fenestrated capillaries (visceral capillaries) possess pores (fenestrae) in their walls that are covered by pore diaphragms.

Fenestrated capillaries (also known as *visceral capillaries*) have **pores (fenestrae)** in their walls that are 60 to 80 nm in diameter and covered by a pore diaphragm. These capillaries are found in the pancreas, intestines, kidneys, and endocrine glands.

The pores in fenestrated capillaries are bridged by an ultrathin diaphragm, although the **renal glomerulus** is composed of fenestrated capillaries whose fenestrae lack diaphragms. When viewed after processing with platinum-carbon shadowing, the diaphragm displays eight fibrils radiating out from a central area and forming wedge-like channels, each with an opening of about 5.5 nm. These pore–diaphragm complexes are regularly spaced about 50 nm apart but are located in clusters; thus, most of the endothelial wall of the fenestrated capillary is without fenestrae (see Fig. 11–12*B*).

Sinusoidal Capillaries

Sinusoidal capillaries may possess discontinuous endothelial cells and basal lamina and contain many large fenestrae without diaphragms, enhancing exchange between blood and tissue.

The vascular channels in certain organs of the body, including the bone marrow, liver, spleen, lymphoid organs, and some endocrine glands, are called ***sinusoids***, irregular blood pools or channels that conform to the shape of the structure in which they are located. The peculiar conformation of a sinusoid is determined by its being shaped between the parenchymal components of the organ during organogenesis.

Because of their location, sinusoidal capillaries have an enlarged diameter of 30 to 40 μm (Fig. 11–12*C*). They also contain many large fenestrae that lack diaphragms; the endothelial wall may be discontinuous, as is the basal lamina, permitting enhanced exchange between the blood and the tissues. Sinusoids are lined by endothelium. In certain organs, the endothelium is thin and continuous (as in some lymphoid organs); in others, it may have continuous areas intermingled with fenestrated areas (as in endocrine glands). Macrophages may be located either in or along the outside of the sinusoidal wall.

Regulation of Blood Flow into a Capillary Bed

Arteriovenous Anastomoses (Arteriovenous Shunts)

Arteriovenous anastomoses (arteriovenous shunts) are direct vascular connections between arterioles and venules that bypass the capillary bed.

Terminals of most arterioles **(terminal arterioles)** end in capillary beds, which deliver their blood to venules for the return back to the venous side of the cardiovascular system. In many parts of the body, however, the arteriole is connected to a venous channel by a vessel that forms an **arteriovenous anastomosis (AVA, arteriovenous shunt)**. The structures of the arterial and venous ends of the AVA are similar to those of an artery and vein, respectively, whereas the intermediate segment has a thickened tunica media, and its subendothelial layer is composed of plump polygonal cells that are modified, longitudinally arranged smooth muscle cells.

When the AVAs are closed, the blood passes through the capillary bed; when shunts are open, a large amount

of blood bypasses the capillary bed and flows through the AVA. These shunts are useful in thermoregulation and are abundant in skin. The intermediate segments of the AVAs are richly innervated with adrenergic and cholinergic nerves. Whereas most peripheral nerves are controlled somewhat by local environmental stimuli, the nerves of the AVAs are controlled by the thermoregulatory system in the brain.

Glomera

Nail beds and the tips of the fingers and toes are vascularized by **glomera** (singular, **glomus**). The glomus is a small organ that receives an arteriole devoid of an elastic lamina and acquires a richly innervated smooth muscle cell layer, which surrounds the vessel lumen, thus directly controlling blood flow to the region before emptying into a venous plexus. The glomera complex is not fully understood.

Central Channel

Metarterioles form the proximal portion of a central channel, and thoroughfare channels form the distal portion of a central channel.

Blood flow from the arterial system is controlled either by **terminal arterioles** directly, discussed previously, or by **metarterioles** (with precapillary sphincters). **Metarterioles** and their continuations, the **central channel**, pass through capillary beds conveying blood from arterioles to venules. The distal portion of the central channel drains into a **thoroughfare channel** that ends in a **small venule (postcapillary venule)**. Metarterioles possess precapillary sphincters, whereas central channels do not. If the precapillary sphincters are open, blood from the metarterioles pass into the capillary bed, and the capillaries convey their blood to the central channel from which the blood enters the thoroughfare channel and then into a small venule. If the precapillary sphincters are not open, blood bypasses the capillary bed and goes directly from the metarteriole into the central channel then into the thoroughfare channel and from there into a small venule (postcapillary venule) of the venous system (Fig. 11–13).

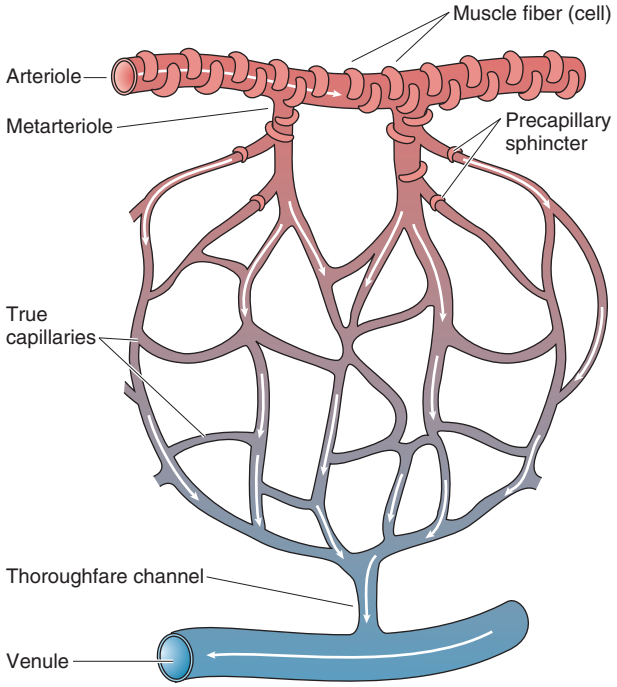


Figure 11–13 Diagram of the control of blood flow through a capillary bed. The central channel, composed of the metarteriole on the arterial side and the thoroughfare channel on the venous side, can bypass the capillary bed by closure of the precapillary sphincters.

Histophysiology of Capillaries

Capillaries are regions where blood flow is very slow, permitting exchange of material between the circulating blood and the extravascular connective tissue.

The endothelial cells of capillaries may contain two distinct pore systems: **small pores** (~9 to 11 nm in diameter) and **large pores** (~50 to 70 nm in diameter). The smaller pores are believed to be discontinuities between endothelial cell junctions. The large pores are represented by fenestrae and transport vesicles. Oxygen, carbon dioxide, and glucose may diffuse or be transported across the plasmalemma, then diffuse through the cytoplasm and, finally, through the abluminal plasmalemma into the extravascular space. Water and hydrophilic molecules (~1.5 nm) simply diffuse through these intercellular junctions.

Water-soluble molecules greater than 11 nm in diameter are transported from the *adluminal* plasmalemma to the *abluminal* plasmalemma by the numerous pinocytotic vesicles adjacent to the cell membrane. This process is called ***transcytosis*** (Fig. 11–14) because the material traverses the entire cell instead of remaining within the cell. In continuous capillaries, substances are taken up by open vesicles located on the *adluminal* plasmalemma. The vesicles are then transported across the cytoplasm to the *abluminal* plasmalemma, where the vesicles fuse with it to deliver their contents into the extravascular space. This is an efficient process because the number of vesicles in these endothelial cells may exceed 1000/μm^2. It appears that these vesicles are members of a stable population of vesicles arising from the Golgi complex via a fusion–fission mechanism of renewal.

Leukocytes leave the bloodstream to enter the extravascular space by passing through the junctions via a mechanism called ***diapedesis***. **Histamine** and

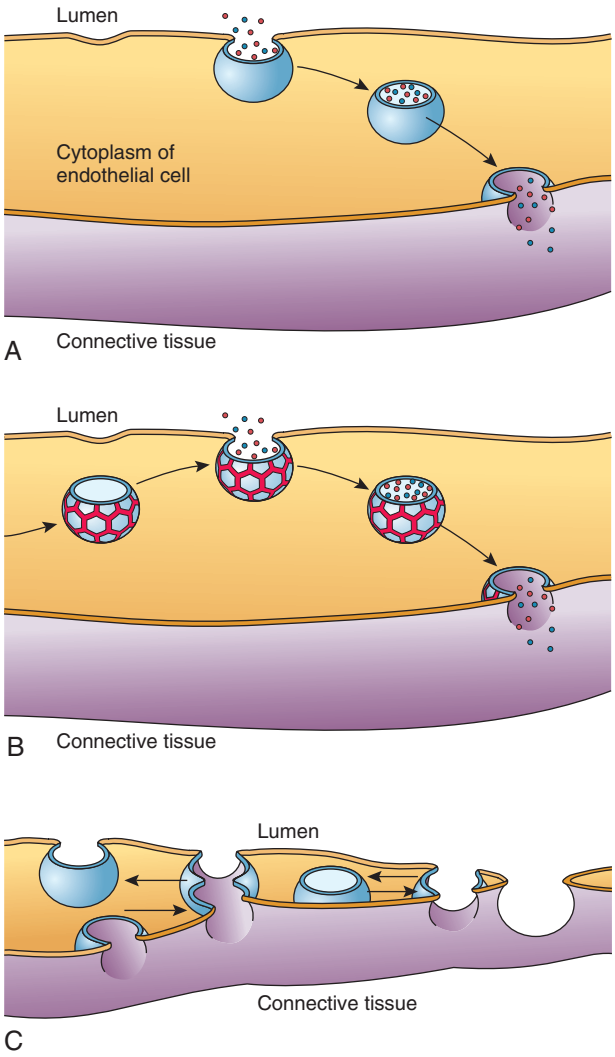


Figure 11–14 Diagram of the various methods of transport across capillary endothelia. **A,** Pinocytotic vesicles, which form on the luminal surface, traverse the endothelial cell, and their contents are released on the opposite surface into the connective tissue spaces. **B,** *Trans* Golgi network–derived vesicles possessing clathrin coats and receptor molecules fuse with the luminal surface of the endothelial cells and pick up specific ligands from the capillary lumen. They then detach and traverse the endothelial cell, fuse with the membrane of the opposite surface, and release their contents into the connective tissue spaces. **C,** In regions where the endothelial cells are highly attenuated, the pinocytotic (or *trans* Golgi network–derived) vesicles may fuse with each other to form transient fenestrations through the entire thickness of the endothelial cell, permitting material to travel between the lumen and the connective tissue spaces. (Modified from Simionescu N, Simionescu M. In Ussing H, Bindslev N, Sten-Knudsen O, eds. *Water Transport Across Epithelia*. Copenhagen: Munksgaard International Publishers Ltd; 1981.)

bradykinin, whose levels are increased during the inflammatory process, increase capillary permeability, thus causing excessive fluid passage into the extravascular spaces. This excess extravascular fluid causes the tissues to swell and is known as ***edema***.

The endothelial cells of capillaries also secrete a number of substances, including **type II collagen**, **type IV collagen**, **type V collagen**, **fibronectin**, and **laminin**, all of which are released into and become part of the extracellular matrix. Additionally, endothelial cells produce several other important substances related to **clotting**, **vascular smooth muscle tone**, **lymphocyte circulation**, and **neutrophil movement**.

A vasoconstrictor substance, **endothelin I**, secreted by the capillary endothelial cells, attaches to vascular smooth muscle cells. It acts as a hypertensive agent, keeping the smooth muscle cells contracted for long periods and thus elevating blood pressure. Although endothelin I is much more effective than angiotensin II, it is unclear how widespread its effects really are.

Adhesion molecules (L-selectin and β_2-integrins), expressed on the plasma membranes of migrating leukocytes, bind to receptors (such as **P-selectin**) on the plasma membranes of capillary endothelial cells at sites of inflammation. The bound leukocytes then enter the connective tissue spaces, where they perform their functions in the inflammatory process. **Prostacyclin**, a powerful vasodilator and inhibitor of platelet aggregation, is also released by endothelial cells of capillaries.

In addition to these functions, capillaries also serve a maintenance role in converting such substances as serotonin, norepinephrine, bradykinin, prostaglandins, and thrombin into inactive compounds.

Enzymes on the luminal surface of endothelial cells of the capillaries in adipose tissue degrade lipoproteins into monoglycerides and fatty acids for storage within adipocytes.

Veins

Veins are vessels that return blood to the heart.

At the discharging ends of capillaries are small venules, the beginning of the venous return, which conducts blood away from the organs and tissues and returns it to the heart. These venules empty their contents into larger veins, and the process continues as the vessels become larger and larger back to the heart. Veins not only outnumber arteries but also usually have larger luminal diameters; therefore, at any one time, almost 70% of the total blood volume is in these vessels. In histological sections, veins parallel arteries. However, their walls are usually collapsed because they are thinner and less elastic than arterial walls because the venous return is a low-pressure system. Of the three layers, the tunica adventitia is usually the thickest in veins,

TABLE 11–2 Characteristics of Veins

Type	Tunica Intima	Tunica Media	Tunica Adventitia
Large veins	Endothelium; basal lamina, valves in some; subendothelial connective tissue	Connective tissue, smooth muscle cells	Smooth muscle cells oriented in longitudinal bundles; cardiac muscle cells near their entry into the heart; collagen layers with fibroblasts
Medium and small veins	Endothelium, basal lamina; valves in some; subendothelial connective tissue	Reticular and elastic fibers, some smooth muscle cells	Collagen layers with fibroblasts
Venules	Endothelium, basal lamina (pericytes, postcapillary venules)	Sparse connective tissue and a few smooth muscle cells	Some collagen and a few fibroblasts

and veins have a richer supply of vasa vasorum than do arteries.

Classification of Veins

Veins are classified into three groups on the basis of their diameter and wall thickness: small, medium, and large.

The structure of veins is not necessarily uniform, even for veins of the same size or for the same vein along its entire length. Veins are described as having the same three layers (i.e., tunicae intima, media, and adventitia) as arteries (Table 11–2). Although the muscular and elastic layers are not as well developed, the connective tissue components in veins are more pronounced than in arteries. In certain areas of the body, where the structures housing the veins protect them from pressure (e.g., retina, meninges, placenta, penis), the veins have little or no smooth muscle in their walls; moreover, the boundaries between the tunica intima and the tunica media of most veins are not clearly distinguishable.

Venules and Small Veins

Venules are similar to but larger than capillaries; larger venules possess smooth muscle cells instead of pericytes.

As the blood pools from the capillary bed, it is discharged into **small venules (postcapillary venules)**, which are 15 to 20 μm in diameter. Their walls are similar to those of capillaries, with a thin endothelium surrounded by reticular fibers and pericytes (see Fig. 11–4). The pericytes of postcapillary venules form an intricate, loose network surrounding the endothelium. Pericytes are replaced by smooth muscle cells in larger venules (>1 mm in diameter), first as scattered smooth muscle cells; then, as venule diameter increases, the smooth muscle cells become more closely spaced, forming a continuous layer in the largest venules and small veins. Materials are exchanged between the connective tissue spaces and vessel lumina, not only in the capillaries, but also in the postcapillary venules, whose walls are even more permeable. Indeed, this is the preferred location for emigration of the leukocytes from the bloodstream into the tissue spaces (Fig. 11–15). These vessels respond to pharmacological agents, such as histamine and serotonin.

The endothelial cells of venules located in certain lymphoid organs are cuboidal rather than squamous and are called ***high-endothelial venules***. These function in lymphocyte recognition and segregation by type-specific receptors on their luminal surface, ensuring that specific lymphocytes migrate into the proper regions of the lymphoid parenchyma.

Medium Veins

Medium veins are less than 1 cm in diameter.

Medium veins are those draining most of the body, including most of the regions of the extremities. Their tunica intima includes the endothelium and its basal lamina and reticular fibers. Sometimes an elastic network surrounds the endothelium, but these elastic fibers do not form laminae characteristic of an internal elastic lamina. The smooth muscle cells of the tunica media are in a loosely organized layer interwoven with collagen fibers and fibroblasts. The tunica adventitia, the thickest of the tunics, is composed of longitudinally arranged collagen bundles and elastic fibers along with a few scattered smooth muscle cells. Small and medium veins have a diameter that ranges between 1 and 9 mm.

Large Veins

Large veins return venous blood directly to the heart from the extremities, head, liver, and body wall.

Large veins include the vena cava and the pulmonary, portal, renal, internal jugular, iliac, and azygos veins. The tunica intima of the large veins is similar to that of

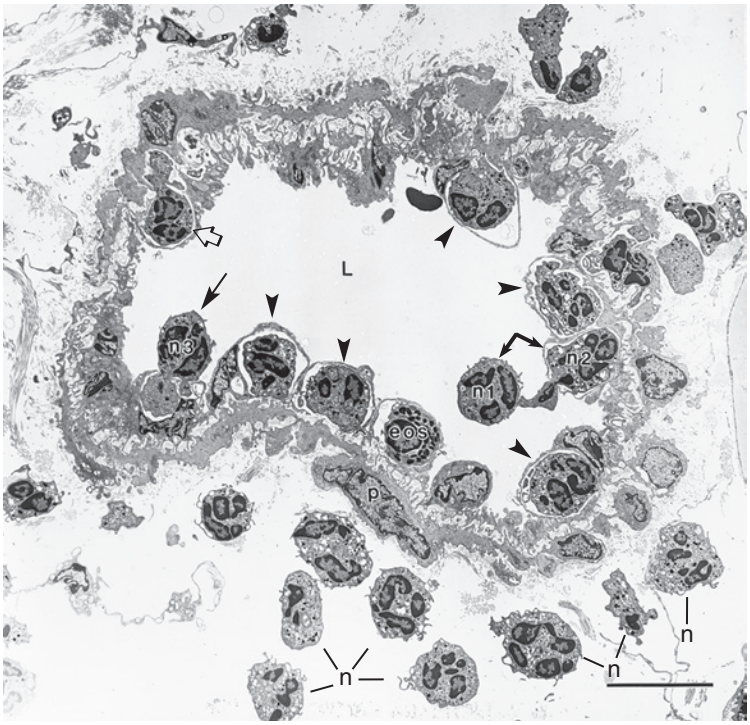


Figure 11–15 Large venule in guinea pig skin harvested 60 minutes after intradermal injection of 10^{-5} M *N*-formyl-methionyl-leucyl-phenylalanine (*F*-MLP). Many neutrophils and a single eosinophil (eos) are captured at various stages of attachment to and extravasation across vascular endothelium and underlying pericytes (p). Two neutrophils *(single joined arrow)*, one in another lumen and another partway across the endothelium, are tethered together. Another neutrophil *(long arrow)* has projected a cytoplasmic process into an underlying endothelial cell (EC). Other neutrophils *(arrowheads)* and the eosinophil have crossed the EC barrier but remain superficial to pericytes, forming dome-like structures that bulge into the vascular lumen. Still another neutrophil *(open arrow)* that has already crossed the endothelium has extended a process into the basal lamina and indents an underlying pericyte. Other neutrophils (some indicated by *n*) have crossed both the EC and pericyte barriers and have entered the surrounding connective tissues. L, Lumen; *bar*, 10 µm. (Modified from Feng D, Nagy JA, Pyne K, et al. Neutrophils emigrate from venules by a transendothelial cell pathway in response to FMLP. *J Exp Med*. 1998;187:903-915.)

the medium veins, except that large veins have a thick subendothelial connective tissue layer containing fibroblasts and a network of elastic fibers. Although only a few major vessels (e.g., the pulmonary veins) have a well-developed smooth muscle layer, most large veins are without a tunica media; in its place is a well-developed tunica adventitia. An exception are the superficial veins of the legs, which have a well-defined muscular wall, perhaps to resist the distention caused by gravity.

The tunica adventitia of large veins contains many elastic fibers, abundant collagen fibers, and vasa vasorum, whereas the inferior vena cava has longitudinally arranged smooth muscle cells in its adventitia. As the pulmonary veins and the vena cava approach the heart, their adventitia contains some cardiac muscle cells.

Valves of Veins

A venous valve is composed of two leaflets, each composed of a thin fold of the intima jutting out from the wall into the lumen.

Many medium veins have **valves** that function to prevent the backflow of blood. These valves are especially abundant in the veins of the legs, where they act against the force of gravity. A venous valve is composed of two leaflets, each having a thin fold of the intima jutting out from the wall into the lumen. The thin leaflets are structurally reinforced by collagen and elastic fibers that are continuous with those of the vessel's wall. As blood flows to the heart, the valve cusps are deflected in the direction of the blood flow toward the heart. Backward flow of blood forces the cusps to approximate each other, thus blocking backflow.

CLINICAL CORRELATIONS

Varicose veins are abnormally enlarged, tortuous veins usually affecting the superficial veins in the legs of older persons. This condition results from loss of muscle tone, degeneration of vessel walls, and valvular incompetence. Varicose veins may also occur in the lower end of the esophagus (**esophageal varices**) or at the terminus of the anal canal (**hemorrhoids**).

Heart

The heart is a four-chambered pump of the cardiovascular system.

The muscular wall **(myocardium)** of the heart is composed of cardiac muscle (see Chapter 8). The heart consists of four chambers: two **atria**, which receive blood, and two **ventricles**, which discharge blood from the heart (Fig. 11–16). The **superior** and **inferior venae cavae** return systemic venous blood to the **right atrium** of the heart. From here, the blood passes through the **right atrioventricular valve (tricuspid valve)** into the **right ventricle**. As the ventricles contract, blood from the right ventricle is pumped out the **pulmonary trunk**, a large vessel that bifurcates into the right and left pulmonary arteries to deliver deoxygenated blood to the lungs for gaseous exchange. Oxygenated blood from the lungs returns to the heart via the **pulmonary veins**, which empty into the **left atrium**. From here, the blood passes through the **left atrioventricular valve (bicuspid or mitral valve)** to enter the **left ventricle**. Again, ventricular contraction expels the blood from the left ventricle into the aorta, from which many branches emanate to deliver blood to the tissues of the body. On a daily basis, the heart pumps approximately 2000 gallons of blood, which translates into 50 million gallons of blood in an average life span.

The atrioventricular valves prevent regurgitation of the ventricular blood back into the atria, whereas the **semilunar valves**, located in the pulmonary trunk and the aorta near their origins, prevent backflow from these vessels into the heart.

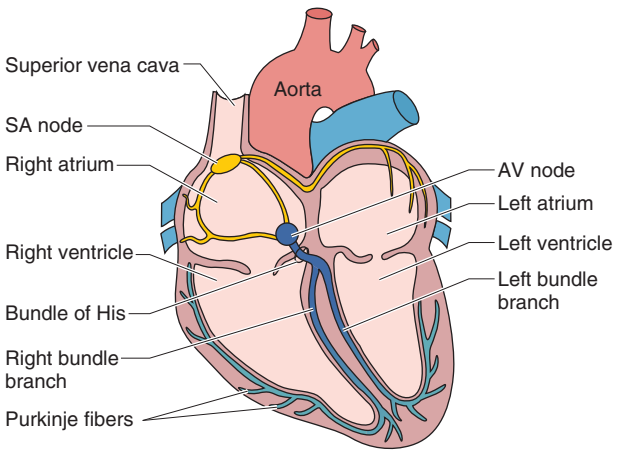


Figure 11–16 Locations of the sinoatrial (SA) and atrioventricular (AV) nodes, Purkinje fibers, and bundle of His of the heart.

Layers of the Heart Wall

The three layers that constitute the heart wall are the **endocardium**, **myocardium**, and **epicardium**, homologous to the tunica intima, tunica media, and the tunica adventitia, respectively, of the blood vessels.

Endocardium

The endocardium, a simple squamous epithelium and underlying subendothelial connective tissue, lines the lumen of the heart.

The **endocardium** is continuous with the tunica intima of the blood vessels entering and leaving the heart. It is composed of an **endothelium**, consisting of a simple squamous epithelium and an underlying layer of fibroelastic connective tissue with scattered fibroblasts. Lying deeper is a layer of dense connective tissue, heavily endowed with elastic fibers interspersed with smooth muscle cells. Deep to the endocardium is a **subendocardial layer** of loose connective tissue that contains small blood vessels, nerves, and Purkinje fibers from the conduction system of the heart. The subendocardial layer forms the boundary of the endocardium as it attaches to the endomysium of the cardiac muscle.

CLINICAL CORRELATIONS

1. Children who have had **rheumatic fever** may later develop **rheumatic heart valve disease** as a result of scarring of the valves stemming from the rheumatic fever episode. This condition develops because the valves cannot properly close (incompetence) or open (stenosis) because of reduced elasticity as a result of rheumatic fever. The **bicuspid (mitral) valve**, followed by the **aortic valves**, is the valve most commonly affected.
2. Recent investigations have demonstrated that in vivo administration of two microribonucleic acids (microRNAs), hsa-miR-590 and hsa-miR-199a, was able to cause regeneration of cardiac muscle cells subsequent to myocardial infarction in mice to the extent that these animals recovered almost completely and had a stable cardiac function.

Myocardium

The thick middle layer of the heart (the myocardium) is composed of cardiac muscle cells.

The **myocardium**, the middle and thickest of the three layers of the heart, contains cardiac muscle cells, arranged in complex spirals around the orifices of the chambers. Certain cardiac muscle cells attach the myocardium to the fibrous cardiac skeleton, others are specialized for endocrine secretions, and still others are specialized for impulse generation or impulse conduction.

The heart rate (~70 to 80 beats per minute) is controlled by the **sinoatrial node (pacemaker)** located at the junction of the superior vena cava and the right atrium (see Fig. 11–16). These specialized nodal cardiac muscle cells can spontaneously depolarize 70 to 80 times per minute, creating an impulse that spreads over the atrial chamber walls by internodal pathways to the **atrioventricular node**, located in the septal wall just above the tricuspid valve. Modified cardiac muscle cells of the atrioventricular node, regulated by impulses arriving from the sinoatrial node, transmit signals to the myocardium of the atria via the **atrioventricular bundle (bundle of His)**. Fibers from the atrioventricular bundle pass down the interventricular septum to conduct the impulse to the cardiac muscle, thus producing a rhythmic contraction. The atrioventricular bundle travels in the subendocardial connective tissue as large, modified cardiac muscle cells, forming **Purkinje fibers** (Fig. 11–17), which transmit impulses to the cardiac muscle cells located at the apex of the heart (Purkinje fibers are not be confused with the *Purkinje cells* in the cerebellar cortex). It should be noted that although the autonomic nervous system does not initiate the heartbeat, it does modulate the rate and stroke volume of the heartbeat. Stimulation of sympathetic nerves accelerates the heart rate, whereas stimulation of the parasympathetic nerves serving the heart slows the heart rate.

Specialized cardiac muscle cells, located primarily in the atrial wall and in the interventricular septum, produce **atrial natriuretic peptide (also known as *atriopeptin*, *cardiodilatin*, and *cardionatrin*)**, which is released into the surrounding capillaries (Fig. 11–18). Specialized cardiac muscle cells of the ventricle produce **B-type natriuretic peptide**, which is also released into the surrounding capillaries. These peptides aid in fluid maintenance and electrolyte balance and decrease blood pressure by decreasing blood volume.

CLINICAL CORRELATIONS

B-type natriuretic peptide (BNP) release by the specialized cardiac muscle cells of the ventricles has been correlated with **congestive heart failure**. Indeed, the more serious the condition, the more BNP is released.

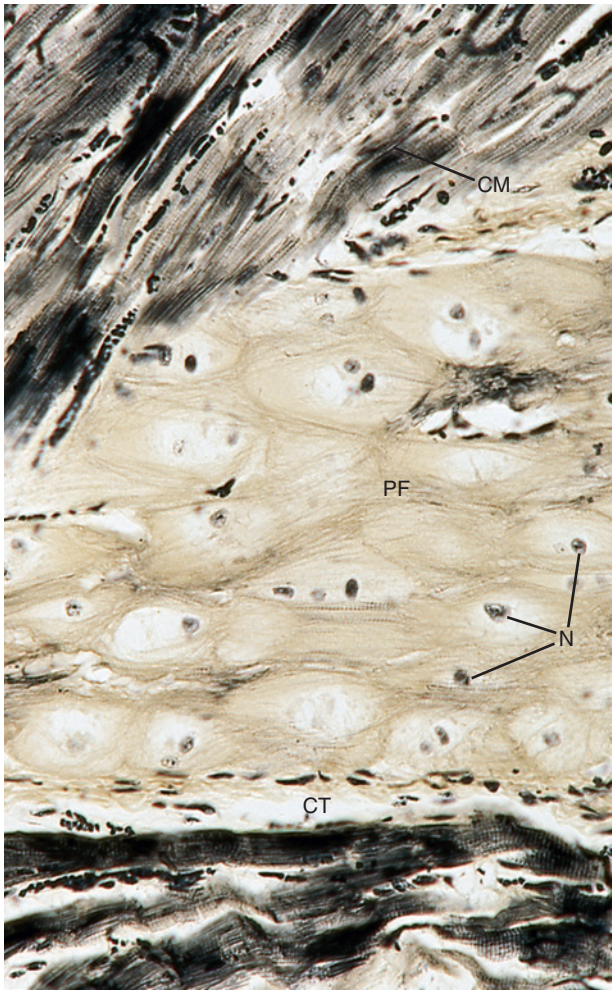


Figure 11–17 Light micrograph of Purkinje fibers. Cardiac muscle (CM) appears very dark, whereas Purkinje fibers (PF) with their solitary nuclei (N) appear light with this stain. Slender connective tissue elements (CT) surround the Purkinje fibers (×270).

Epicardium

The epicardium represents the homologue of the tunica adventitia in blood vessels.

Epicardium, the outermost layer of the heart wall, is also called the ***visceral layer of the pericardium*** (composed of a simple squamous epithelium known as a ***mesothelium*** and the underlying **subepicardium**). The subepicardial layer of loose connective tissue, the subepicardium, contains the coronary vessels, nerves, and ganglia. It also is the region where fat is stored on the surface of the heart. At the roots of the vessels entering and leaving the heart, the visceral pericardium becomes continuous with the serous layer of the parietal pericardium. These two layers of the pericardium

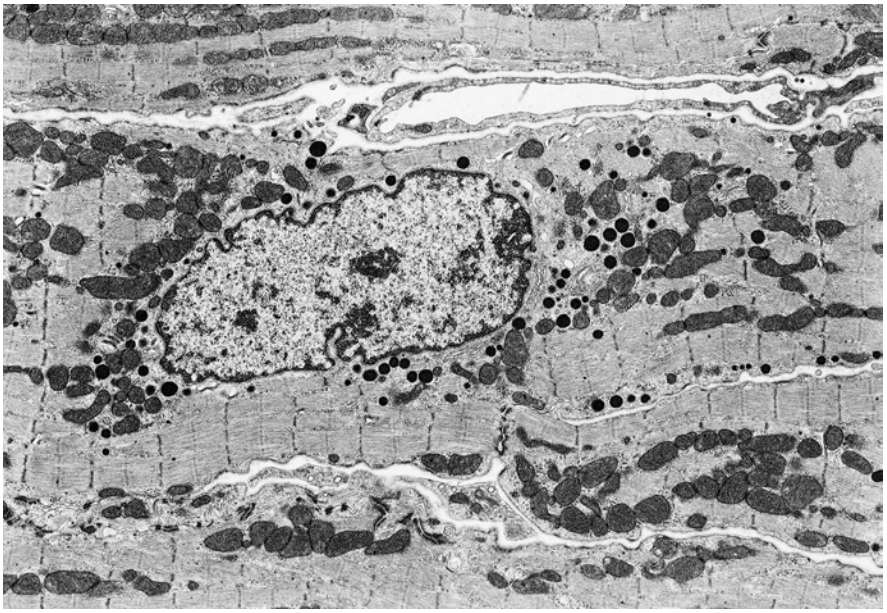


Figure 11–18 Electron micrograph of a cardiac muscle cell containing clusters of vesicles with atrial natriuretic peptide (ANP). (From Mifune H, Suzuki S, Honda J, et al. Atrial natriuretic peptide (ANP): a study of ANP and its mRNA in cardiocytes, and of plasma ANP levels in non-obese diabetic mice. *Cell Tissue Res*. 1992;267:267-272.)

enclose the pericardial cavity, a space containing a small amount of serous fluid for lubricating the serous layer of the pericardium and the visceral pericardium.

CLINICAL CORRELATIONS

Infection in the pericardial cavity, called ***pericarditis***, severely restricts the heart from beating properly because the space is obliterated by adhesions between the epicardium and the serous layer of the pericardium.

Cardiac Skeleton

The cardiac skeleton, composed of dense connective tissue, includes three main components:

- **Annuli fibrosi**, formed around the base of the aorta, pulmonary artery, and the atrioventricular orifices
- **Trigonum fibrosum**, formed primarily in the vicinity of the cuspal area of the aortic valve
- **Septum membranaceum**, constituting the upper portion of the interventricular septum

In addition to providing a structural framework for the heart and attachment sites for the cardiac muscle, the cardiac skeleton provides a discontinuity between the myocardia of the atria and ventricles, thus ensuring a rhythmic and cyclic beating of the heart, controlled by the conduction mechanism of the atrioventricular bundles.

LYMPHATIC VASCULAR SYSTEM

The lymphatic vascular system consists of vessels that collect the excess extracellular fluid (interstitial fluid) and return it to the cardiovascular system.

The **lymphatic vascular system** is composed of a series of vessels that remove excess extracellular fluid from the interstitial tissue spaces and return it to the cardiovascular system. Lymphatic vessels are present throughout the body, except in the CNS and a few other areas, including the orbit, internal ear, epidermis, cartilage, and bone. Unlike the cardiovascular system, which contains a pump (the heart) and circulates blood in a *closed* system, the lymphatic vascular system is an *open* system in that there is no pump and no circulation of fluid.

The lymphatic vascular system begins in the tissues of the body as blind-ended **lymphatic capillaries** (Fig. 11–19), which simply act as drain fields for excess extracellular fluid. The lymphatic capillaries empty their contents, known as ***lymph***, into **lymphatic vessels**, which empty into successively larger vessels until one of the two **lymphatic ducts** is reached. From either of these ducts, the lymph is emptied into the venous portion of the cardiovascular system at the junctions of the internal jugular and the subclavian veins.

Lymph nodes are interposed along the paths of lymphatic vessels, and lymph must pass through them to be filtered. **Afferent lymphatic vessels** deliver

CLINICAL CORRELATIONS

1. **Ischemic (coronary) heart disease**, especially prevalent in older persons, is related to **atherosclerosis of the coronary vessels** serving the myocardium. As atherosclerotic plaques reduce the lumina of the coronary vessels, the patient may experience referred pain and pressure, known as ***angina pectoris***, from lack of oxygen. Continued narrowing results in ischemia of the heart wall, which may be fatal if untreated. Angioplasty is the current mode of the initial invasive treatment for partially occluded arteries.
2. Prinzmetal angina (coronary vessel spasm) is a rare condition affecting about 4 people per 100,000, in which the coronary arteries undergo spasms and restrict blood flow to the heart. These spasms may occur randomly (though usually when the patient is resting) and cause angina-like chest pains. The condition is not related to coronary arterial disease or to atherosclerosis and is more common in females than in males. Smoking and hyperventilation are two of the few known aggravating conditions that bring about the spasms. Orally administered calcium channel blockers and nitrates appear to prevent the spasms from occurring. Without treatment, the possibility of cardiac arrest is increased.
3. Atrial fibrillation is a condition of arrhythmic heartbeat involving the atria only. The erratic atrial heartbeat disrupts normal blood flow and may cause the formation of small blood clots. If these small clots enter the circulation, they can plug smaller vessels, including those in the brain. It has been demonstrated that as many as 15% of stroke victims present with atrial fibrillation that may have been responsible for the stroke in these patients.

lymph into the lymph nodes, where lymph is distributed into labyrinthine channels (sinuses) lined by an endothelium and abundant macrophages. Here, the lymph is filtered and cleared of particulate matter. Lymphocytes are added to the lymph as it leaves by **efferent lymphatic vessels**, eventually reaching a lymphatic duct. Lymph nodes are discussed in Chapter 12.

Lymphatic Capillaries and Vessels

Lymphatic capillaries are composed of a single layer of attenuated endothelial cells with an incomplete basal lamina.

The blind-ended, thin-walled **lymphatic capillaries** are composed of a single layer of attenuated endothelial cells with an incomplete basal lamina (Fig. 11–20). The endothelial cells overlap each other in places but

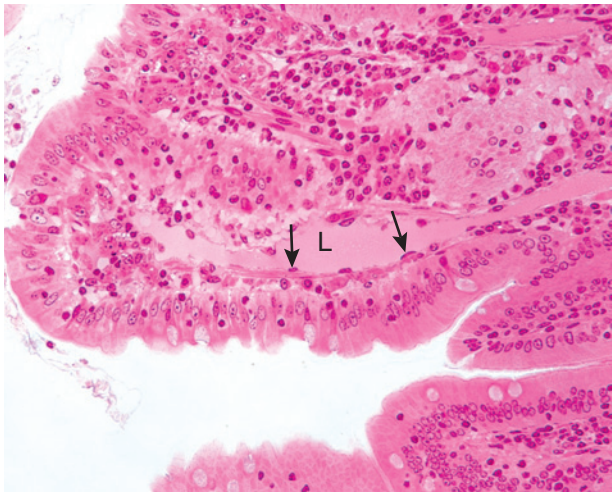


Figure 11–19 The lymph vessel in the villus core of the small intestine is known as a *lacteal* (L). Observe the endothelial lining of the lacteal *(arrows)*. The present photomicrograph is taken of the monkey duodenum (×270).

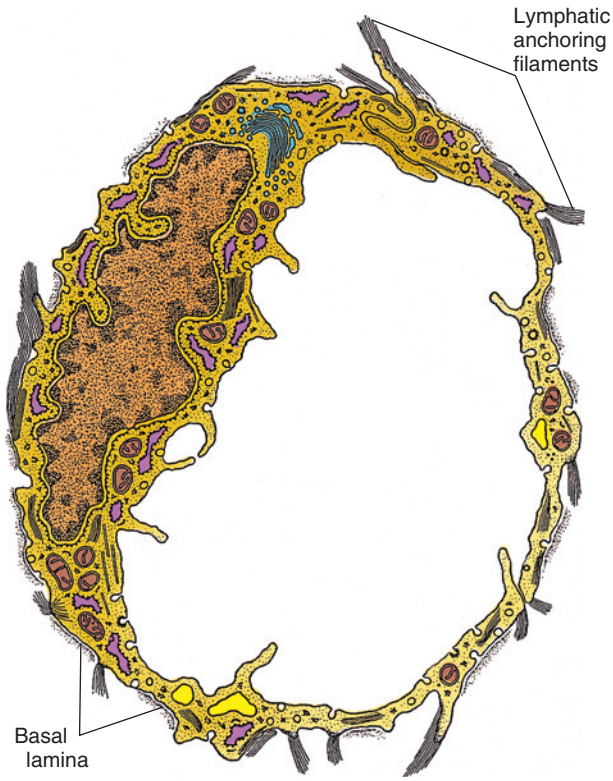


Figure 11–20 Diagram of the ultrastructure of a lymphatic capillary. (From Lentz TL. *Cell Fine Structure: an Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

have intercellular clefts that permit easy access to the lumen of the vessel. These cells do not have fenestrae and do not make tight junctions with each other. Bundles of **lymphatic anchoring filaments** (5 to 10 nm in diameter) terminate on the abluminal plasma membrane. It is thought that these filaments may play a role in maintaining the luminal patency of these flimsy vessels.

Small and medium lymphatic vessels are characterized by closely spaced valves. Large lymphatic vessels resemble small veins structurally, except that their lumina are larger and their walls thinner. Large lymphatic vessels have a thin layer of elastic fibers beneath their endothelium and a thin layer of smooth muscle cells. This smooth muscle layer is then overlaid with elastic and collagen fibers that blend with the surrounding connective tissue, much like a tunica adventitia. Although some histologists describe tunics similar to those in blood vessels, most do not concur because there are no clear boundaries between the layers and because the walls are so varied.

Lymphatic Ducts

Lymphatic ducts are similar to large veins; they empty their contents into the great veins of the neck.

The **lymphatic ducts**, which are similar in structure to large veins, are the final two collecting vessels of the lymphatic vascular system. The short **right lymphatic duct** empties its contents into the venous system at the junction of the right internal jugular and right subclavian veins. The larger, the **thoracic duct**, begins in the abdomen as the **cisterna chyli** and ascends through the thorax and neck to empty its contents at the junction of the left internal jugular and left subclavian veins. The right lymphatic duct collects lymph from the upper right quadrant of the body, whereas the thoracic duct collects lymph from the remainder of the body.

The tunica intima of lymphatic ducts is composed of an endothelium and several layers of elastic and collagen fibers. At the interface with the tunica media, a layer of condensed elastic fibers resembles an internal elastic lamina. Both longitudinal and circular layers of smooth muscle are present in the media. The tunica adventitia contains longitudinally oriented smooth muscle cells and collagen fibers that blend into the surrounding connective tissue. Piercing the walls of the thoracic duct are small vessels homologous to the vasa vasorum of the arteries.

CLINICAL CORRELATIONS

Malignant tumor cells (especially carcinomas) are spread throughout the body by lymphatic vessels. When the malignant cells reach a lymph node, they are slowed and multiply there, eventually leaving to metastasize to a secondary site. Therefore, in surgical removal of a cancerous growth, examination of the lymph nodes and removal of both enlarged lymph nodes in the pathway and associated lymphatic vessels are essential in preventing secondary growth of the tumor.

PATHOLOGICAL CONSIDERATIONS

See Figures 11–21 through 11–24.

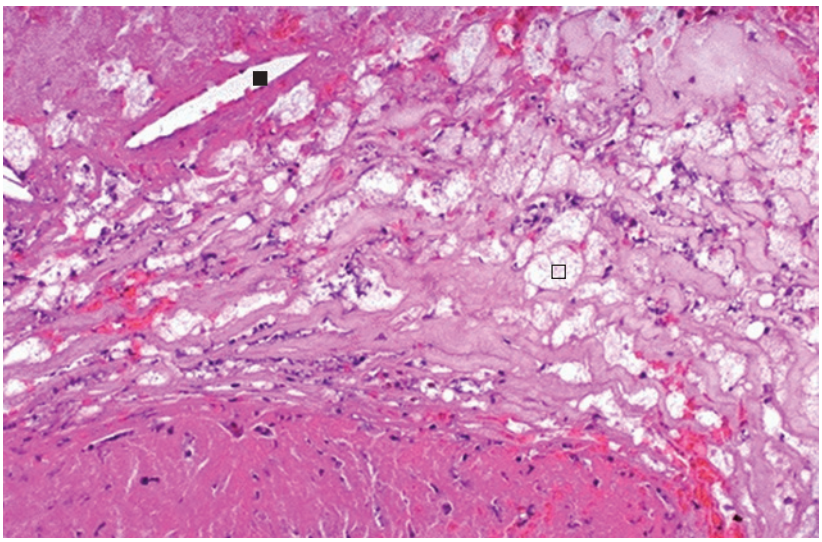


Figure 11–21 Photomicrograph of a coronary artery with atherosclerosis. Note that there are residual smooth muscle cells in the tunica media and the atheroma overlying it. Observe the presence of fat deposits *(open square)* and cholesterol clefts *(filled square)*. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:5.)

Figure 11–22 Photomicrograph of the aorta displaying a rupture as well as the aortic dissection along the tunica media caused by the pressure created by the escaped blood *(asterisk)*. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:11.)

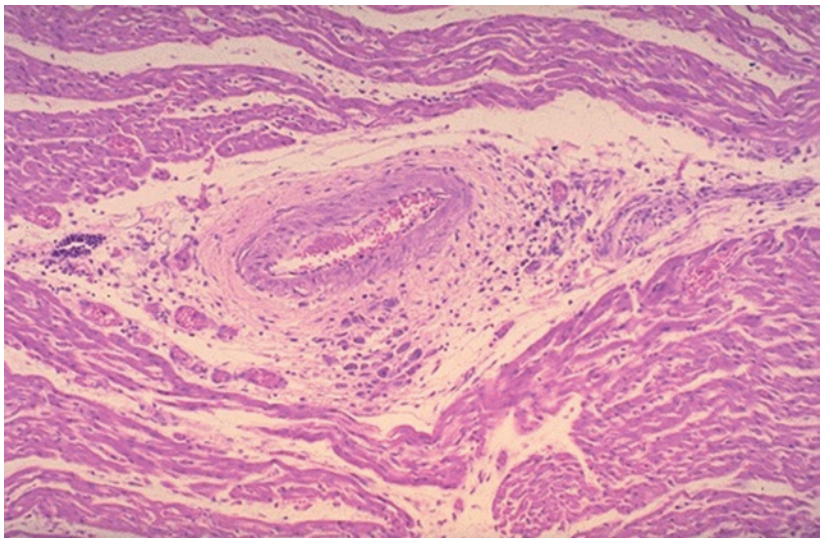


Figure 11–23 Photomicrograph of rheumatic heart disease. Note the presence of the characteristic Aschoff nodule at the lower middle of the field, composed of mostly mononuclear inflammatory cells. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:46.)

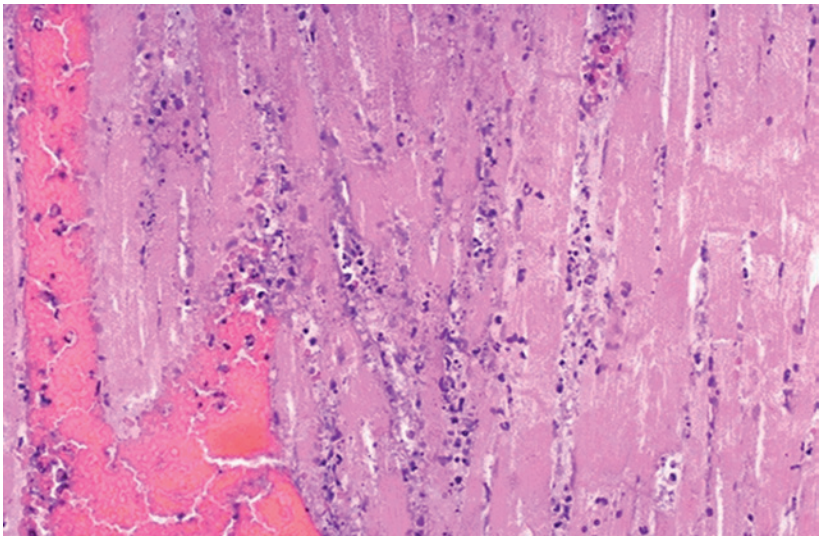


Figure 11–24 Photomicrograph of a myocardial infarction about 3 to 4 days after the damage. Note the necrotic cardiac muscle cells as well as the acute infiltration of inflammatory cells. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:41.)

REVIEW QUESTIONS

1. Fenestrated membranes are located in
   a. the tunica adventitia.
   b. the tunica intima.
   c. muscular arteries.
   d. conducting arteries.
   e. arterioles.
2. Weibel-Palade bodies of blood vessels
   a. are located in the smooth muscles of the tunica media.
   b. are located in the fibroblasts of the tunica media.
   c. contain von Willebrand factor.
   d. contain endothelial relaxing factor (NO).
   e. contain prothrombin.
3. The tetralogy of Fallot is a congenital malformation that does not include
   a. ventricular septal defect.
   b. right atrial hypertrophy.
   c. pulmonary stenosis.
   d. overriding aorta.
   e. right ventricular hypertrophy.
4. Varicose veins are
   a. caused by increased muscle tone.
   b. due to valvular hypermobility.
   c. due to increased muscularity of the vessel wall.
   d. present in the anal region as hemorrhoids.
   e. constricted veins present usually in the upper extremities.
5. Capillaries are slender vessels whose lumina are just wide enough to permit red blood cells to pass through them. Capillaries with fenestrae
   a. are continuous capillaries.
   b. are sinusoidal capillaries.
   c. have no diaphragm over their fenestrae in the glomerulus.
   d. are located in the central nervous system.
   e. are located in skeletal muscle.
6. High endothelial-venules are located in the
   a. medulla of lymph nodes.
   b. cortex of lymph nodes.
   c. paracortex of lymph nodes.
   d. red pulp of the spleen.
   e. white pulp of the spleen.
7. A child is seen by her pediatrician and is diagnosed with rheumatic fever. The doctor informs the parents that the possible consequence of this disease is
   a. myocardial infarct.
   b. occlusion of the coronary sinus.
   c. patent foramen ovale.
   d. scarring of the mitral valve.
   e. ventricular septal defect.
8. Purkinje fibers
   a. are present in the cerebellum.
   b. initiate heart beat.
   c. are continuous with the atrioventricular node.
   d. are continuous with the sinoatrial node.
   e. are continuous with the atrioventricular bundle.
9. Young women who have had several spontaneous abortions during their teenage years may have anti-cardiolipin antibodies. These young women may
   a. have thromboses in their renal vein.
   b. have myocardial infarcts as teenagers.
   c. have cardiomyopathy.
   d. have valvular insufficiency.
   e. have rhabdomyosarcoma.
10. Elephantiasis is a disease that affects which of the following?
    a. Capillaries
    b. Arteries
    c. Lymph vessels
    d. Venules
    e. Veins

ANSWERS

1. D. Fenestrated membranes are elastic sheets that are located in the tunica media of elastic (conducting) arteries such as the aorta. In muscular arteries (distributing arteries), the tunica adventitia houses a fibroelastic connective tissue as well as a not-very-distinguished vasa vasorum. The tunica intima of muscular arteries houses the well-developed internal elastic lamina. The smooth muscle layers of arterioles are well developed with respect to the size of these vessels.
2. C. Weibel-Palade bodies are membrane-bound inclusions in the endothelial cells of blood vessels. These long and narrow granules contain von Willebrand factor, a glycoprotein that facilitates the coagulation of blood by encouraging platelets to adhere to each other.
3. B. Right atrial hypertrophy is not a condition associated with the tetralogy of Fallot. This condition permits the mixing of oxygenated with nonoxygenated blood, resulting in a somewhat cyanotic condition and lack of energy for the afflicted individual.
4. D. Hemorrhoids are varicose veins of the anal region. There are internal and external hemorrhoids according to their location with respect to the external anal sphincter. They are usually caused by decreased muscle tone and valvular incompetence as well as by degeneration of the vessel wall. Varicose veins are dilated.
5. C. Fenestrated capillaries always have a thin diaphragm over their fenestrae, except in the glomerulus of the kidney. There are three types of capillaries: fenestrated, continuous, and sinusoidal. Both skeletal muscle and the central nervous system possess continuous capillaries.
6. C. High endothelial venules are lined by a simple cuboidal rather than a simple squamous epithelium. It is a region where lymphocytes preferentially leave the bloodstream to enter the paracortex of lymph nodes and the marginal zone of the spleen.
7. D. Rheumatic fever in children may develop into rheumatic heart valve disease as a result of the scarring of the valves of the heart. The most common sites of the scarring are the mitral and aortic valves. Myocardial infarct and occlusion of the coronary sinus do not occur because of rheumatic fever. Patent foramen ovale and ventricular septal defects are developmental anomalies and do not occur as a consequence of rheumatic fever.
8. E. Purkinje fibers are the terminal continuation of the atrioventricular bundle. Purkinje cells, not Purkinje fibers, are present in the cerebellum. The heart beat is initiated by the sinoatrial node, and it sends the information to the atrial muscles as well as to the atrioventricular node. The atrioventricular bundle (bundle of His) arises from the atrioventricular node; it bifurcates to form the Purkinje fibers, which transmit impulses to the cardiac muscle cells located at the apex of the heart.
9. A. Young women who have undergone several spontaneous abortions may form antibodies against their cardiolipin molecules; as a consequence, they form thromboses even in their renal or hepatic veins. As a consequence of the antibodies, they do not have myocardial infarcts. Individuals who have a hereditary incapability to form the enzyme tafazzin cannot synthesize cardiolipins; therefore, their mitochondria cannot synthesize an adequate amount of ATP. Many of these patients have cardiomyopathy. Their cardiac valves are normal. Rhabdomyosarcoma is a cancer of skeletal muscle.
10. C. Elephantiasis is a blockage of the lymph vessels by the parasite *Brugia timori* and other filarial parasites (although there are cases of elephantiasis when filarial parasites are not involved). Capillaries, arteries, venules, and veins are not affected by filarial parasites.

This page intentionally left blank

Lymphoid (Immune) System

12

The lymphoid system is responsible for the immunological defense of the body. Some of its component organs—**bone marrow**, **lymph nodes**, **thymus**, and **spleen**—are surrounded by connective tissue capsules, whereas its other components, members of the **diffuse lymphoid system**, are not encapsulated. The cells of the lymphoid system protect the body against foreign macromolecules, viruses, bacteria, and other invasive microorganisms, and they kill virally transformed cells.

There are two categories of lymphoid organs: primary and secondary. **Primary lymphoid organs** (bone marrow and thymus) participate in the development of immunocompetent lymphocytes. **Secondary lymphoid organs** (lymph nodes, spleen, tonsils, and diffuse lymphoid tissue) function in trapping antigens and providing sites for the interactions of antigen-presenting cells (APCs) and immunocompetent lymphocytes to mount an immune response and thereby remove the antigenic assault.

OVERVIEW OF THE IMMUNE SYSTEM

The immune system has two components: the innate immune system and the adaptive immune system.

The immune system provides the second and the third lines of defense against invading pathogens. The first line of defense is the epithelial barrier, namely skin and mucosa, which form a complete lining and covering of the body surfaces. Once this physical barrier is breached by a cut, tear, or abrasion or even if foreign substances are able to penetrate but have not yet penetrated the intact barrier, the second and the third lines of defense may become activated; these are the innate and the adaptive immune systems.

Because the immune system is very complex, by necessity, the following discussion presents a greatly condensed version of this immense topic. In order to make the reading of this material easier for the student, certain information is repeated in various regions of the presentation in order to spare the reader from having to refer back to the point where it was originally mentioned.

The **innate immune system** (natural immune system) is nonspecific and is composed of (1) a system of blood-borne macromolecules (C1 to C9) known as ***complement***; (2) **Toll-like receptors (TLRs)**, a family of integral proteins localized on either the plasmalemma or endosomal membranes of cells; (3) groups of cells known as ***macrophages*** and ***neutrophils***, which phagocytose invaders; and (4) another group of cells, **natural killer (NK) cells**, which kill tumor cells, virally infected cells, bacteria, and parasites.

The **adaptive immune system (acquired immune system)** is responsible for eliminating threats from specific invaders. Whereas a macrophage can phagocytose most bacteria, the adaptive immune system not only reacts against one specific antigenic component of a pathogen but also its ability to react against that particular component improves with subsequent confrontations with it.

Although the two systems differ in their mode of responses, they are intimately related to one another, and each affects the other's activities.

The Innate Immune System

The innate immune system responds rapidly, has no immunological memory, and depends on complement and Toll-like receptors for initiating inflammatory and/or immune responses.

Although the **innate immune system** is much older than the adaptive immune system, it responds rapidly, usually within a few hours, to an antigenic invasion; it responds in a nonspecific manner; and it has no immunologic memory. The critical components of the innate immune system are complement, antimicrobial peptides, cytokines, macrophages, neutrophils, NK cells, and TLRs. (Table 12–1 shows acronyms and abbreviations used in this chapter.)

Complement is a series of blood-borne proteins that attack microbes that found their way into the blood stream. As complement proteins precipitate on the surface of these invading pathogens, they form a **membrane attack complex (MAC)** that damages the microbe's cell membrane. Phagocytic cells, such as neutrophils and macrophages, of the host have receptors for a specific moiety of complement (namely, **C3b**) and the presence of C3b on the microbial surface facilitates phagocytosis of microbes by these host defense cells.

SUMMARY

The **lymphoid system**, composed of the following **lymphoid organs—bone marrow**, **lymph nodes**, **tonsils**, **thymus**, **spleen**, and the **diffuse lymphoid system**—is responsible for the **inflammatory** and **immune responses** of the body, responses that protect the individual from invading **pathogens** and **virally altered cells**, as well as from **foreign macromolecules**. The lymphoid organs that are responsible for the development of immunocompetent lymphocytes are known as the ***primary lymphoid organs*** (bone marrow and thymus), and lymphoid organs that function in trapping antigens and providing sites for the interactions of antigen-presenting cells with immunocompetent lymphocytes to mount an immune response are known as ***secondary lymphoid organs*** (lymph nodes, spleen, tonsils, and diffuse lymphoid tissue). The immune response may be a **humoral immune response** based on the formation of **antibodies** or a **cell-mediated immune response** based on the **cytotoxic T lymphocytes** that seek out and kill **foreign cells** or **virally altered cells**. All **lymphoid organs**, except for the **diffuse lymphoid tissue**, are surrounded by a connective tissue **capsule**.

KEY WORDS

Bone marrow; cell-mediated immune response; diffuse lymphoid tissue; humoral immune response; immune response; inflammatory response; lymph nodes; lymphoid organs; lymphoid system; pathogens; primary lymphoid organ; secondary lymphoid organ; spleen; thymus; tonsils; virally transformed cells

TABLE 12–1 Acronyms and Abbreviations

Acronym/ Abbreviation	Meaning of Acronym/Abbreviation
ADDC	Antibody-dependent cellular cytotoxicity
AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cell
BALT	Bronchus-associated lymphoid tissue
B lymphocyte	Bursa-derived lymphocyte (Bone marrow-derived lymphocyte)
C3b	Complement 3b
CD	Cluster of differentiation molecule (followed by an Arabic numeral)
Clip	Class II associated invariant protein
CSF	Colony stimulating factor
CTL	Cytotoxic T lymphocyte (T killer cell)
Fab	Antigen binding fragment of an antibody
Fas protein	CD95 (induces apoptosis)
Fc	Crystallized fragment (constant fragment of an antibody)
GALT	Gut-associated lymphoid tissue
G-CSF	Granulocyte—colony stimulating factor
GM-CSF	Granulocyte-monocyte stimulating factor
HEV	High endothelial venules
HIV	Human immunodeficiency virus
IFN-γ	Interferon gamma
Ig	Immunoglobulin (followed by a capital letter: A, D, E, G, or M)
IL	Interleukin (followed by an Arabic numeral)
M cell	Microfold cell
MAC	Membrane attack complex
MALT	Mucosa-associated lymphoid tissue
MHC I and MHC II	major histocompatibility class I molecules and class II molecules
MIIC vesicle	MHC class II–enriched compartment
NK cell	Natural killer cells
PALS	Periarterial lymphatic sheath
sIgs	Surface immunoglobulins
TAP	Transporter protein (1 and 2)
TCM	Central memory T cell
TCR	T-cell receptor
TEM	Effector T memory cell
T_H cell	T helper cell (followed by an Arabic numeral)
TLRs	Toll-like receptors
T lymphocyte	Thymus–derived lymphocyte
TNF-α	Tumor necrosis factor alpha
T reg cell	Regulatory T cell

Antimicrobial peptides, such as **defensins**, are synthesized and released by epithelial cells. They not only defend the body against Gram negative bacteria but also are chemoattractants for immature dendritic cells and T lymphocytes.

Cytokines are signaling molecules that are released by various cells of the innate and adaptive immune systems to effect responses from their target cells. Cytokines that are released by lymphocytes are known as **lymphokines**, usually named ***interleukins* (*IL* and a number)**, whereas cytokines that possess chemoattractant capabilities are usually referred to as ***chemokines***. Those cytokines that stimulate differentiation and mitotic activity of hemopoietic cells are known as ***colony-stimulating factors (CSFs)***, whereas cytokines displaying antiviral properties are referred to as ***interferons***.

Macrophages possess (1) receptors for the constant portions of antibodies (Fc receptors), (2) complement receptors, and (3) receptors that recognize carbohydrates that are not usually present on the surface of vertebrate cells. Macrophages are also APCs, that is, they present antigens to both T and B lymphocytes. They also release CSFs (G-CSF and GM-CSF) and numerous other signaling molecules that induce the formation of neutrophils and their release into the circulating blood.

Neutrophils leave the vascular system in the region of inflammation and enter the bacteria-laden connective tissue compartment, where they phagocytose and destroy bacteria. Bacterial killing is effected either in an oxygen-dependent manner, by the formation of hydrogen peroxide, hydroxyl radicals, and singlet oxygen within the phagolysosomes/endosomes, or via enzymatic digestion, using cationic proteins as well as myeloperoxidase and lysozymes.

NK cells are similar to cytotoxic T cells, which are members of the adaptive immune system, as discussed later, but NK cells do not have to enter the thymus to become mature killer cells. These are cells that use nonspecific markers to recognize their target cells, and they do so by the use of two different methods.

- NK cells possess Fc receptors, recognizing the constant portion of the IgG antibody and that acts as a signal to kill the target cell. This is known as the ***antibody-dependent cellular cytotoxicity (ADDC).***
- The NK cell surface also displays transmembrane proteins known as ***killer activating receptors*** that bind to certain markers on the surface of nucleated cells. In order to control this killing process, NK cells also possess killer inhibitory receptors that recognize major histocompatibility (MHC) type I molecules that are located on the plasma membranes of all cells. The presence of MHC I molecule prevents the killing of healthy cells by NK cells. The absence of MHC I molecules from the cell membrane or the presence of defective or altered MHC I molecules on the cell

membrane indicates to NK cells that these are either foreign cells or virally altered self cells (target cells) that have to be destroyed.

- The cytotoxic abilities of NK cells are enhanced, that is, NK cells become activated to become effector NK cells, by various cytokines, such as interleukins 12, 15, and 18 (IL-12, -15, and -18) as well as by type I interferons. IL-12 and IL-15 also induce NK cells to enter the cell cycle, thus increasing the number of effector NK cells.
- Effector NK cells release perforin molecules that attach to the plasmalemmae of target cells where they assemble to form pores. NK cells also release granzymes that pass through these pores to enter the target cell cytoplasm driving them into apoptosis.
- Effector NK cells also release interferon gamma (IFN-γ), which recruits and activates macrophages to the area of the response. The activated macrophages destroy invading microorganisms and provide time for the adaptive immune system to control the infection.

CLINICAL CORRELATIONS

Major histocompatibility (MHC) I molecules are required to be present on cell membranes of almost every nucleated cell for cytotoxic T lymphocytes (CTLs) to recognize them as targets for destruction. However, tumor cells and cells that are infected by viruses suppress the production of MHC I molecules in order to prevent their recognition as targets for CTLs. However, this evasive maneuver permits them to become targets of NK cells because their killer inhibitory receptors do not become activated. In addition to MHC I molecules, MHC II molecules are located on the surface of all antigen-presenting cells. (See later discussion of MHC molecules.)

TLRs (Toll-like receptors) are highly conserved integral proteins present in the plasma and endosomal membranes of cells of the innate immune system; humans have been shown to possess at least 11 different TLRs (Table 12–2), each with different roles. It appears that TLRs function in pairs, so that two TLR partners form a single active receptor. These may be the same TLRs (e.g., TLR4-TLR4; homodimers) or different TLRs (TLR1-TLR2; heterodimers). Some of the TLRs are present on cell membranes so that they have both intracellular and extracellular moieties, whereas other TLRs are located only intracellularly on the membranes of endosomes and rough endoplasmic reticulum and possess no extracellular moieties.

TLR pairs distinguish specific recurring molecular signatures of various pathogens. These molecular signatures are referred to as ***pathogen-associated molecular patterns (PAMPs)***. TLRs located on the cell membrane distinguish PAMPs belonging to bacteria, fungi, and protozoa, whereas intracellular TLRs recognize PAMPs of viral nucleic acids and of other pathogens that are capable of entering the cytoplasm. All TLRs (with the exception of TLR3) associate with and activate the NF-κB pathway that acts through several cytosolic proteins, including MyD88, that induces an intracellular cascade of TLR specific responses. This sequence of events results not only in the release of **cytokines** that induce systemic inflammation such as **interleukin 1**, **interleukin 12**, and **tumor necrosis factor-α** that are appropriate to the pathogen being detected, but also in the activation of B and T cells in order to mount a specific adaptive immune response.

All TLRs (with the exception of TLR3) associate with and activate the **NF-κB pathway (nuclear factor kappa-light chain enhancer of activated B cells)** that acts through several cytosolic proteins, including MyD88, that induces an intracellular cascade of TLR-specific responses. Members of the NF-κB protein family form heterodimers or homodimers with each other and are inactive in the presence of **IκB**, an inhibitory protein. If certain TLRs encounter and bind their ligands, the altered conformation phosphorylates a kinase. This phosphorylated kinase phosphorylates IκB, which loses its inhibitory capability, becomes ubiquitinated, and enters a proteasome to be destroyed. The now active NF-κB enters the nucleus, where it and a coactivator factor induce transcription of a target gene. The mRNA transcribed from the activated gene codes for signaling molecules whose actions result in an inflammatory reaction as well as an commencement of an **innate immune response**, including the conscription of NK cells and the initiation of an **adaptive immune response** (see the next section). Therefore, TLRs have the ability to modulate the immune response, suggesting that the innate immune system is not a static, one fits all, type of response but is dynamic in nature and is capable of regulating both the inflammatory and immune responses equally.

CLINICAL CORRELATIONS

1. Hypoactivity of TLRs can result in greater susceptibility to pathogens, whereas their hyperactivity may be responsible for some autoimmune diseases such as systemic lupus erythematosus, cardiovascular diseases, and rheumatoid arthritis.
2. Interestingly, it has been noted that high levels of TLR4 are expressed in mice that are sleep deprived and when injected with malignant cells, the tumors that developed in sleep-deprived animals were larger and more aggressive and grew at a more rapid rate than in mice that were permitted to sleep normally. Additionally, instead of eliminating the tumor cells, the macrophages that the sleep-deprived animals recruited to the site of the tumor elicited the development of a vascular supply that encouraged tumor growth.

TABLE 12–2 Toll-Like Receptors, Their Locations, and Their Putative Functions

Domains	Toll-Like Receptor Pair	Located on/in Cell	Functions
Intracellular and extracellular (on cell membrane)	TLR1–TLR2	Monocytes, macrophages, dendritic cells, B cells, mast cells	Binds to bacterial lipoprotein; also binds to certain proteins of parasites
	TLR2–TLR2	Monocytes, macrophages, dendritic cells, B cells, mast cells	Binds to peptidoglycan of bacteria
	TLR2–TLR6	Monocytes, macrophages, dendritic cells, B cells, mast cells	Binds to lipoteichoic acid of gram-positive bacterial wall; also binds to zymosan, a fungally derived polysaccharide
	TLR4–TLR4	Monocytes, macrophages, mast cells, lining cells of the digestive system	Binds to LPS (lipoprotein saccharide) of gram-negative bacteria
	TLR5–??*	Monocytes, macrophages, dendritic cells, mast cells, lining cells of the digestive system	Binds to flagellin of bacterial flagella
	TLR11–??	Macrophages, epithelial cells of kidney and bladder	Host recognition of *Toxoplasmosis gondii*
Intracellular only	TLR3–??*	Dendritic cells and B cells	Binds to double stranded viral RNA (dsRNA)
	TLR7–??*	Monocytes, macrophages, dendritic cells, B cells	Binds to single stranded viral RNA (ssRNA)
	TLR8–??*	Monocytes, macrophages, dendritic cells, mast cells	Binds to single stranded viral RNA (ssRNA)
	TLR9–??*	Monocytes, macrophages, dendritic cells, B cells	Binds to bacterial and viral DNA
	TLR10–??*	Monocytes, macrophages, B cells	Unknown

*Currently, TLR partner is unknown.

The Adaptive Immune System

The adaptive immune system responds slower than the innate immune system, has immunological memory, and depends on B and T lymphocytes to mount an immune response.

The **adaptive immune response** exhibits four distinctive properties: **specificity**, **diversity**, **memory**, and **self/nonself recognition**—that is, the ability to distinguish between structures that belong to the organism, **self**, and those that are foreign, **nonself**. Two additional characteristics of the adaptive immune system are

- Clonal expansion, the ability to increase the number of cells that can react to a renewed antigenic challenge
- Contraction and homeostasis, the ability of the immune system to respond simultaneously to multiple antigenic challenges.

T lymphocytes, **B lymphocytes**, and specialized macrophages known as ***APCs (antigen-presenting cells)*** participate in the (adaptive) immune response. These cells communicate with members of the innate immune system as well as with each other not only by signaling molecules **(cytokines)**, which are released in response to encounters with foreign substances called ***antigens*** but also by the presence markers on the cell membrane, such as cluster of differentiation molecules (CD molecules), T-cell receptors (TCRs), and surface immunoglobulins.

Recognition of a substance as foreign by the immune system stimulates a complex sequence of reactions that result either in the production of **immunoglobulins**, (also known as ***antibodies***), which bind to the antigen, or in the induction of a **group of cells** that specialize in killing foreign cell, invading pathogens, or altered self-cell (e.g., tumor cell). The immune response that depends on the formation of antibodies is called the ***humoral immune response***, whereas the cytotoxic response is known as the ***cell-mediated immune response***.

The cells that constitute the functional components of the innate and adaptive immune system (T cells, B cells, macrophages, and their subcategory, APCs) are all formed in the bone marrow. B cells become immunocompetent in the bone marrow, whereas T cells migrate to the thymus to become immunocompetent; therefore, bone marrow and the thymus are called the ***primary (central) lymphoid organs***. After lymphocytes become immunocompetent in the bone marrow or in the thymus, they migrate to the **secondary (peripheral) lymphoid organs**, namely diffuse lymphoid tissue (mucosa associated lymphoid tissue), lymph nodes,

spleen, and tonsils where they come into contact with antigens.

Immunogens and Antigens

Immunogens are molecules that always elicit an immune response; antigens are molecules that bind to antibodies but do not necessarily elicit an immune response.

A foreign structure that can elicit an immune response in a particular host is known as an ***immunogen***; an **antigen** is a molecule that can react with an antibody irrespective of its ability to elicit an immune response. Although not all antigens are immunogens, in this textbook, the two terms are considered synonymous, and only the term *antigen* is used.

The region of the antigen that reacts with an **antibody**, or **TCR (T-cell receptor)**, is known as its ***epitope***, or antigenic determinant. Each epitope is a small portion of the antigen molecule and consists of only 8 to 12 or 15 to 22 hydrophilic amino acid or sugar residues that are accessible to the immune apparatus. Large foreign invaders such as bacteria have several epitopes, each capable of binding to a different antibody. Although the term is not frequently used, it should be mentioned that the portion of an antibody that has an affinity to epitopes is referred to as a ***paratope***.

CLINICAL CORRELATIONS

The complexity of a foreign substance is also important in determining its antigenicity. Hence, large polymeric molecules that have relatively simple chemical compositions, such as certain human-made plastics, have minimal immunogenicity and are therefore used in the manufacture of artificial implants (as in hip replacement).

Clonal Selection and Expansion

During embryonic development, an extremely large number of small clusters (clones) of lymphocytes are formed; each clone can recognize one specific foreign antigen (epitope).

The immune system can recognize and combat an astonishing number of different antigens. The explanation for this capability is that during embryonic development an enormous number ($\sim 10^{15}$) of lymphocyte **clones** are formed by rearrangement of the 400 or so genes encoding immunoglobulins or TCRs. All of the cells of a particular clone have identical surface markers and can react with a specific antigen even though they have not yet been exposed to that antigen. The cell-surface proteins that enable lymphocytes to interact with antigens are **membrane-bound antibodies** (**B-cell receptors** or **surface immunoglobulins [sIgs]**) in the case of B cells and **TCRs** in the case of T cells. Although the molecular structures of antibodies and TCRs differ, they are functionally equivalent in their ability to recognize and interact with specific epitopes.

The first time the organism encounters an antigen, the adaptive immune response is slow to begin and not very robust; this response is called the ***primary immune response***. Subsequent exposures to the same antigen elicit the **secondary immune response**, which begins rapidly and is much more intense than the primary response. The increased potency of the secondary reaction is due to the process of **immunological memory**, which is inherent to the adaptive immune system. Both B and T cells are said to be **virgin cells (naive cells)** before exposure to antigens. After a virgin cell comes in contact with an antigen, it proliferates to form activated cells and memory cells.

Activated cells, also known as ***effector cells***, are responsible for carrying out an immune response. Effector cells derived from B cells are called ***plasma cells*** and produce and release antibodies. Effector cells derived from T cells either secrete cytokines or destroy foreign cells or altered self-cells.

Memory cells, similar to virgin lymphocytes, express either B-cell receptors (sIgs) or TCRs, which can interact with specific antigens. Memory cells are not directly involved in the immune response during which they are generated. However, these cells live for months or years and have a much greater affinity for antigens than do virgin lymphocytes. Moreover, formation of memory cells after first exposure to an antigen increases the size of the original clone, a process called ***clonal expansion***. Because of the presence of an expanded population of memory cells with an increased affinity for the antigen, subsequent exposure to the same antigen induces a secondary response **(anamnestic response)** that is much faster, more potent, and longer in duration than the primary response.

Immunological Tolerance

Macromolecules of the self are not viewed as antigens and therefore do not elicit an immune response.

The immune system can recognize macromolecules that belong to the self and does not attempt to mount an immune response against them. This lack of action is due to **immunological tolerance**. The mechanism of immunological tolerance depends on killing or disabling those cells that would react against the self. During embryonic development, if a lymphocyte encounters the substance to which it is designed to react, the cell is either killed **(clonal deletion)** so that this particular clone does not form, or the lymphocyte is disabled **(clonal anergy)** and cannot mount an immune response, even though it is present.

CLINICAL CORRELATIONS

Autoimmune diseases involve a malfunction of the immune system that results in the loss of immunological tolerance. One example is Graves disease, in which the receptors for thyroid-stimulating hormone (TSH) on the follicular cells of the thyroid gland are perceived to be antigens. Antibodies formed against TSH receptors bind to these receptors and stimulate the cells to release an excess amount of thyroid hormone. Patients with Graves disease have an enlarged thyroid gland and exophthalmos (protruding eyeballs).

Immunoglobulins

Immunoglobulins are antibodies (also known as gamma globulins*) that are manufactured by plasma cells; a typical immunoglobulin has one pair of heavy and one pair of light chains attached to each other by disulfide bonds.*

Immunoglobulins (antibodies, gamma globulins) are glycoproteins that inactivate antigens (including viruses) and elicit an extracellular response against invading microorganisms. The response may involve phagocytosis in the connective tissue spaces by macrophages (or neutrophils) or the activation of the blood-borne **complement system**.

CLINICAL CORRELATIONS

The complement system is composed of 20 plasma proteins that assemble in a specific sequence and fashion on the surface of invading microorganisms to form a membrane attack complex (MAC) that lyses the foreign cell. The key component of the complement system is protein C3. Deficiency of protein C3 predisposes a person to recurring bacterial infections.

Immunoglobulins are manufactured in large number by plasma cells, which release them into the lymph or blood vascular system. There are five classes of antibodies (IgA, IgD, IgE, IgG, and IgM). Members of the various classes of antibodies share certain characteristics and, because immunoglobulin G (IgG) is the typical antibody, it will be described as a template for the all classes of immunoglobulins. Each IgG is a Y-shaped molecule, composed of two long, identical 55- to 70-kilodalton (kD) polypeptides, known as ***heavy chains***, and two shorter, identical 25-kD polypeptides, the **light chains**. The four chains are bound to each other by several disulfide bonds and noncovalent bonds in such a way that the stem of the Y is composed only of heavy chains, and the diverging arms consist of both light and heavy chains (Fig. 12–1).

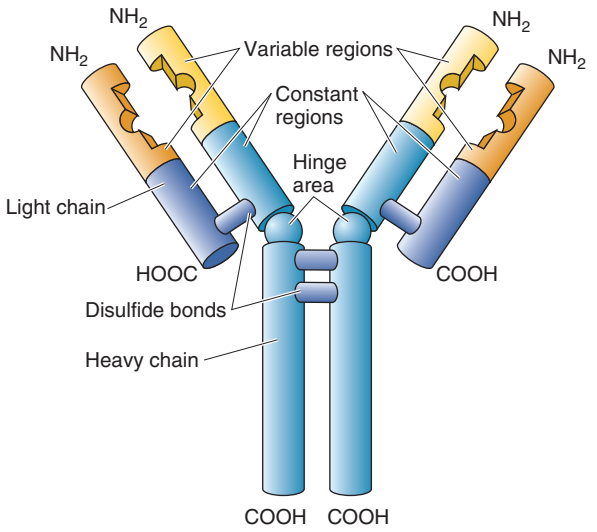


Figure 12–1 Schematic diagram of an antibody indicating its regions.

The region in the vicinity of the disulfide bonds between the two heavy chains, the **hinge region**, is flexible and permits the arms to move away from or toward each other. The distal regions on the tips of the arms (the four amino-terminal segments) are responsible for binding to the epitope; hence, each antibody molecule can bind two *identical* epitopes.

The enzyme papain cleaves the antibody molecule at its hinge regions (see Fig. 12–1), forming three fragments: one **Fc fragment** composed of the stem of the Y and containing equal parts of the two heavy chains, and two **Fab fragments**, each composed of the remaining part of one heavy chain and one entire light chain. Fc fragments are easily crystallized (hence, the *c* designation), whereas the Fab fragment is the antigen-binding region of the antibody (hence, the *ab* designation).

The amino acid sequence of the Fc fragment is mostly constant in its class; thus, the stem of an antibody has the ability to bind to Fc receptors of many different cells. The amino acid sequence of the Fab region is variable, and it is the alterations of that sequence that determine the **specificity** of the antibody molecule for its particular antigen.

Each antibody is specific against a particular epitope; thus, the Fab regions of all antibodies against that particular epitope are identical. It is believed that there are 10^6 to 10^9 different types of antibodies in a person, each specific against one particular antigen. Each type of antibody is manufactured by members of the same **clone**. Thus, there are 10^6 to 10^9 clones whose members discern and react to a particular epitope (or a small number of similar epitopes).

As noted earlier, small amounts of immunoglobulins are made by B cells and inserted into their plasmalemma; these are known as *sIgs (surface immunoglobulins)* or **B-cell receptors**; they function as antigen-receptor molecules. They are slightly different from antibodies in that they possess a membrane-binding component composed of two pairs of membrane-spanning chains, Igβ and Igα, which bind the heavy chains of the antibody molecule to the cell membrane.

Classes of Immunoglobulins

There are five classes (isotypes) of immunoglobulins in humans, namely IgG, IgM, IgA, IgD, and IgE.

Humans have five **isotypes** (classes) of immunoglobulins:

- IgG, the monomeric form of immunoglobulin described previously
- IgM, which resembles five IgG molecules bound to each other (pentameric form of immunoglobulin)
- IgA, which resembles two IgG molecules bound to each other (dimeric form of immunoglobulin)
- IgD, present in very low concentration in the blood, but is found on the B-cell surface as a monomeric form of immunoglobulin known as *surface IgD (sIG)*
- IgE, a monomeric form of immunoglobulin present on the surface of basophils and mast cells.

The classes of immunoglobulins are also determined by the amino acid sequences of their heavy chains. The various heavy chains are designated by the Greek letters α, δ, γ, ε, and μ. The characteristics of the five isotypes of immunoglobulins are detailed in Table 12–3.

Cells of the Adaptive and Innate Immune Systems

The cells of the adaptive *and* innate immune system are B lymphocytes, T lymphocytes, NK cells, macrophages, and APCs.

B Lymphocytes

B lymphocytes originate and become immunocompetent in the bone marrow; they are responsible for the humorally mediated immune system.

B lymphocytes, also known as ***B cells***, are small lymphocytes (see Chapter 10) that both originate and become **immunocompetent** in the bone marrow. However, in birds, in which B cells were first identified, they become immunocompetent is a diverticulum of the cloaca, known as the ***bursa of Fabricius*** (hence, the designation "B" cells). During the process of becoming immunocompetent, each cell goes from an immature pre–B cell stage to a **transitional B cell**, which manufactures a series of identifying immunoglobulin chains. Transitional B cells migrate to the spleen to be killed or to be permitted to develop into a **mature B cell**. Each mature B cell manufactures 50,000 to 100,000 **IgM** and **IgD** immunoglobulins (sIgs) and inserts these in its plasma membrane so that the epitope-binding sites of the antibodies face the extracellular space. The Fc region of the antibody is embedded in the phospholipid bilayer by the assistance of two pairs of transmembrane proteins, Igβ and Igα, whose carboxyl termini are in contact with certain intracellular protein complexes. Every member of a particular clone of B cells has antibodies that bind to the same epitope.

When the surface immunoglobulin reacts with its epitope, the Igβ and Igα transduce (relay) the information to the intracellular protein complex with which they are in contact, initiating a chain of events that results in **activation** of the B cell. There are a number of different types of B cells: B-1 B cells, B-2 B cells, B memory cells, spleen follicular B cells, and spleen marginal zone B cells.

- B-1 B cells are derived from hemopoietic stem cells that are develop in the fetal liver. They arise early in development of the individual and populate, at least in rodents, the mucosa, and the peritoneum. They manufacture IgM that they place on their plasmalemmae, but they have a limited ability to produce antibody diversity and respond mostly to carbohydrates of the most common microorganisms without the need to interact with T cells. They constitute approximately 50% of the mucosal IgA-secreting B cells but do not form memory B-1 B cells. Interestingly, in rodents but not in humans, they sport cluster of differentiation molecule 5 (CD 5) on their cell membranes.
- B-2 B cells (which in this textbook are referred to simply as *B cells*) are the most numerous of the B cell population. They possess CD40 molecules on their cell membrane with which they contact and signal T_H2 cells to release signaling molecules that inhibit T_H1 cells from entering the cell cycle and also to prompt B cells to form B memory cells and plasma cells. Additional cytokines are also release by T_H2 cells that allow the B cell to manufacture a different class of immunoglobulin. This capability is known as ***class switching (isotype switching)*** and is determined by the particular cytokines that are present in the B cell's microenvironment. These cytokines are released by T-helper cells as a function of the type of pathogens present:
- During parasitic worm invasion, T cells release interleukin-4 (IL-4) and IL-5, and B cells differentiate into plasma cells and, after class switching, form IgE to elicit mast cell degranulation on the surface of the parasites.
- During bacterial and viral invasions, T cells release IFN-γ and interleukin-6 (IL-6), and B cells switch to forming IgG, which opsonizes bacteria, fixes complement, and stimulates NK cells to kill virally altered

TABLE 12–3 Properties of Human Immunoglobulins

Class	Cytokines*	No. of Units†	Ig in Blood (%)	Crosses Placenta	Binds to Cells	Biological Characteristics
IgA	TGF-β	1 or 2	10–15	No	Temporarily to epithelial cells during secretion	Also known as *secretory antibody* because it is secreted into tears, saliva, the lumen of the gut, and the nasal cavity as dimers; individual units of the dimer are held together by J protein manufactured by plasma cells and protected from enzymatic degradation by a secretory component manufactured by the epithelial cell; combats antigens and microorganisms in the lumen of gut, nasal cavity, vagina, and conjunctival sac; secreted into milk, thus protecting neonates with passive immunity; monomeric form in bloodstream; assists eosinophils in recognizing and killing parasites
IgD		1	<1	No	B-cell plasma membrane	Surface immunoglobulin; assists B cells in recognizing antigens for which they are specific; functions in the activation of B cells subsequent to antigenic challenge to differentiate into plasma cells
IgE	IL-4, IL-5	1	<1	No	Mast cells and basophils	Reaginic antibody; when several membrane-bound antibodies are cross-linked by antigens, IgE facilitates degranulation of basophils and mast cells, with subsequent release of pharmacological agents, such as heparin, histamine, eosinophil and neutrophil chemotactic factors, and leukotrienes; elicits immediate hypersensitivity reactions; assists eosinophils in recognizing and killing parasites
IgG	IFN-γ, IL-4, IL-6	1	80	Yes	Macrophages and neutrophils	Crosses placenta and thus protects fetuses with passive immunity; secreted in milk and thus protects neonates with passive immunity; fixes complement cascade; functions as opsonins, that is, by coating microorganisms, facilitates their phagocytosis by macrophages and neutrophils, cells that possess Fc receptors for the Fc region of these antibodies; also participates in antibody-dependent cell-mediated cytotoxicity by activating NK cells; produced in large quantities during secondary immune responses
IgM		1 or 5	5–10	No	B cells (in monometric form)	Pentameric form is maintained by J-protein links, which bind Fc regions of each unit; activates cascade of the complement system; is the first isotype to be formed in the primary immune response

*Cytokines responsible for switching to this isotype.
†A unit is a single immunoglobulin composed of two heavy and two light chains; thus, IgA exists as both a monomer and as a dimer.
Fc, Crystallizable fragment; IFN, interferon; Ig, immunoglobulin; IL, interleukin; NK, natural killer; TGF, tumor growth factor.

cells (antibody-dependent cell-mediated cytotoxicity [ADCC]).

- During viral or bacterial invasion of mucosal surfaces, T cells release tumor growth factor-β (TGF-β), and B cells switch to IgA formation, which is secreted onto the mucosal surface.

B cells are considered to be members of the APC population because they are able to complex epitopes with class II MHC molecules and present them to T_H1 cells. It is believed that they present epitopes only during an anamnestic not the primary immune response. When they act as APCs, not only do they synthesize and

secrete interleukin-12, a cytokine that prompts T_H1 cells to proliferate and induce NK cells to become active, but they also differentiate into plasma cells and increase their population of B memory cells.

- B memory cells are long-lived cells that not only increase the size of the clone that is specific against a particular antigen but also react faster and more vigorously than the cells comprising the original clone. Therefore, at a second exposure to the same antigen these cells are ready to react quickly and more forcefully in a process known as a ***secondary response (anamnestic response).***
- Follicular B cells are the most populous of the B cells of the primary and secondary follicles of the spleen. These are almost mature cells, and they express IgM, IgD, and CD21 molecules on their cell membranes. These cells are T cell dependent, and they migrate in and out of the various lymphoid organs, where they are always located in the B cell follicles. Because they migrate, they are also known as ***recirculating B cells.***
- Marginal zone B cells have a limited range of antibody diversity, are T cell independent, and are located very close to marginal sinuses of the spleen. They can migrate to lymph nodes in humans and have the ability to react to self antigens as well as to bacterial polysaccharide antigens. They possess IgM, CD1, CD9, and CD 21 molecules on their cell membranes, and during an antigenic challenge, they differentiate into plasma cells that release IgM.

Plasma cells are B cells that have undergone differentiation into antibody-forming cells and possess no surface antibody. All antibodies manufactured by plasma cells derived from a single clone of B cells manufacture the identical antibodies that are specific against one particular antigen (or to antigens that are very similar to that specific antigen). Because plasma cells release the antibodies that they manufactured into connective tissue from where the antibodies enter blood vessels or lymph vessels, B cells are responsible for the **humorally mediated immune response**.

As naive B cells first become activated, they make IgM, which, when bound to the surface of an invading pathogen, is able to activate the complement system (complement fixation). IgM molecules can also bind to viruses, preventing them from contacting the cell surface, thus protecting the cells from viral invasion.

Certain antigens (e.g., polysaccharides of microbial capsules) can elicit a humoral immune response without a T-cell intermediary. These are known as ***thymic-independent antigens***. They cannot induce formation of B-memory cells and can elicit only IgM-antibody formation. However, most antigens require participation of a T-cell intermediary before they can induce a humoral immune response (see later section on humoral immune response).

T Lymphocytes

T lymphocytes originate in the bone marrow and migrate to the thymus to become immunocompetent; they are responsible for the cellularly mediated immune response.

T lymphocytes (T cells) are also formed in the bone marrow, but they migrate to the thymic cortex, where they become immunocompetent by expressing specific molecules on their cell membranes that permit them to perform their functions. The process whereby T cells become immunocompetent is discussed in this chapter (see the section on the thymus).

Although histologically T cells appear to be identical to B cells, there are important differences between them:

- T cells have TCRs rather than sIgs on their cell surfaces.
- Although TCRs belong to the immunoglobulin superfamily, they are never secreted.
- T cells recognize only epitopes presented to them by APCs.
- T cells respond to protein antigens only.
- For them to respond even to protein antigens, the epitopes have to be presented to them bound to MHC molecules present on the surface of APCs.
- Because of the MHC constraint, T cells are said to be MHC restricted (see the later section on MHC restriction and T cells).
- T cells perform their functions at short distances only.

T cells express **clusters of differentiation proteins (CD molecules or CD markers)** on their plasmalemma. These accessory proteins bind to specific ligands on target cells. Although almost 300 CD molecules are known, Table 12–4 lists only those that are immediately pertinent to the subsequent discussion of cellular interactions in the immune process. The membrane-bound portion of the **TCR** associates with the membrane proteins, **CD3**, and either **CD4 or CD8**, forming the **TCR complex**. Several other membrane proteins play roles in signal transduction and in strengthening the interaction between the TCR and an epitope, thus facilitating antigen-stimulated T-cell activation.

Similar to sIgs on B cells, **TCRs** on the plasmalemma of T cells function as antigen receptors. The **constant regions** of the TCR are membrane bound, whereas the variable **amino-terminal regions** containing the antigen-binding sites extend from the cell surface. There are two types of TCRs, depending on their protein chain compositions: **gamma and delta (γ and δ)**, known as **γ/δ T cells**, and **alpha and beta (α and β)**, known as **α/β T cells**. There is another category of T cells, known as ***NKTs***.

- γ/δ T cells form a small population of cells. They react much faster to antigenic challenges, although mostly in the mucosa of the gastrointestinal tract and

TABLE 12–4 Selected Surface Markers Involved in the Immune Process

Protein	Cell Surface	Ligand and Target Cell	Function
CD3	All T cells	None	Transduces epitope–MHC complex binding into intracellular signal, activating T cell
CD4	T-helper cells	MHC II on APCs	Coreceptor for TCR binding to epitope–MHC II complex, activation of T-helper cell
CD8	Cytotoxic T cells and T reg cells	MHC I on most nucleated cells	Coreceptor for TCR binding to epitope–MHC I complex; activation of cytotoxic T cell
CD28	T-helper cells	B7 on APCs	Assists in the activation of T-helper cells
CD40	B cells	CD40 receptor molecule expressed on activated T-helper cells	Binding of CD40 to CD40 receptor permits T-helper cell to activate B cell to proliferate into B memory cells and plasma cells

APC, Antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptors.

typically to microbial pathogenic invasion. Unlike their α/β counterparts, they do not form memory T cells and are not MHC restricted. It is believed that γ/δ T cells recognize microbial nonprotein antigens, and these antigens do not require APCs to present them. Although these cells become "educated" in the cortex of the thymus to become immunocompetent, they spend considerably less time there than do their α/β counterparts.

- Natural killer T cells spend very little time in the thymus and possess some α/β TCRs on their surfaces that are designed to recognize lipid antigens bound to CD1 molecules (similar to class I MHC molecules) presented to them by APCs. Therefore, NKT cells are said to be CD1 restricted but not MHC restricted. NKT cells secrete IL-4, IL-10, and IFN-γ. It is believed that these cells kill bacteria whose cell walls are rich in lipids.
- The majority of T cells are α/β T cells; they have the ability to form memory T cells. Although they react much slower than their γ/δ counterparts, they are the most common T cells to respond to antigenic challenges. Maturation of these cells is described in the following paragraphs.

Maturation of α/β T Cells

Because **α/β T cells** spend a considerable time in the thymus, only their maturation is presented in this textbook. While in the thymus, the α/β T cells are exposed to various signaling molecules and growth factors produced by reticular epithelial cells of the thymus that control their development into immunocompetent T cells.

1. Progenitor T lymphocytes formed in the bone marrow are immunoincompetent (i.e., they cannot participate in an immune response). These cells travel to the medulla of the thymus, where they leave the postcapillary venule at the corticomedullary junction and enter the thymic cortex, where they are known as *thymocytes*. The thymocytes migrate to the outer region of the cortex. These thymocytes possess Notch-1 receptors on their surfaces but because they have neither CD4 nor CD8 molecules, they are referred to as *double-negative T cells*. The thymus possesses various types of epithelial reticular cells (see the section on the thymus), some of which release signaling molecules that are recognized by the Notch-1 receptors. It should be stressed that double-negative cells do not express CD3 or TCR molecules on their cell membranes.
2. The signaling molecules activate Notch-1 on the surface of the double-negative cells, inducing these cells to manufacture both CD4 and CD8 molecules and place them on their plasma membranes. Because both CD4 and CD8 molecules are present on these cells, they are now referred to as *double-positive T cells*. Additionally, these double-positive cells also begin to express TCRs and CD3 molecules on their surfaces. As double-positive cells proliferate, a large number of cells are formed, and they all express different variable regions in their α/β TCR molecules.
3. Epithelial reticular cells of the thymus present various self epitope–MHC complexes to the double-positive T cells to *conserve only those double positive T cells that bind very weakly* to self peptides presented by self MHC molecules and eliminate all others. Therefore, this is a positive selection of thymocytes because they have to demonstrate only a weak recognition of self epitope–MHC molecule complexes. An amazing 90% of double-negative T cells are killed in the thymic cortex. The reason why the killing of these cells is essential is that *only those T cells may be allowed to survive that recognize* ***only*** *foreign epitopes presented by self MHC molecules.*
4. Because there are two types of MHC molecules, MHC I and MHC II, the epithelial reticular cells

present either self epitope–MHC I or self epitope–MHC II complexes to the double-positive T cells. The double-positive T cells that are exposed to MHC I molecules cease to express CD4 molecules on their surfaces but continue to express CD8 molecules and are referred to as *single-positive T cells* (also known as *CD 8 cells*). Similarly, the double-positive T cells that are exposed to MHC II molecules cease to express CD8 molecules on their surfaces but continue to express CD4 molecules and are referred to as *single-positive T cells* (also known as *CD 4 cells*).

5. The single-positive T cells (I T cells) that are not forced into apoptosis are immunocompetent, and they leave the thymic cortex and migrate into the medulla of the thymus. These I T cells also possess CD45RA molecules on their cell membranes.
6. Once in the medulla, epithelial reticular cells residing there present self epitope–MHC II complexes to these I T cells. The I T cells that show a strong response to these complexes are also forced into apoptosis to prevent the mounting of an immune response by these cells to the self (i.e., to prevent an autoimmune response). Therefore, this is a negative selection of thymocytes because they do not recognize the epitope–MHC complex as self. But not all of these I T cells that show a strong response to the self epitope–MHC complex are forced into apoptosis. In an unknown fashion, some of these I T cells escape the "death sentence" and differentiate into regulatory T cells (T reg cells) that suppress an immune response (see the section on effector T cells).
7. Epithelial reticular cells of the medulla possess the capability to force the I T cells into apoptosis that would mount an immune response against tissue-specific antigens, such as insulin. The epithelial reticular cells are able to do this because they release autoimmune regulator (AIRE), a transcription factor that permits these tissue specific antigens to be expressed in the thymus and thus be presented to the T cells.
8. The I T cells that remain alive use the vascular system to leave the thymic medulla and enter the various lymphoid organs located throughout the body. After they leave the thymic medulla, they are referred to as *naïve T cells*.

A TCR can recognize an epitope only if the epitope is a polypeptide (composed of amino acids) and if the epitope is bound to an **MHC complex molecule**, such as those in the plasmalemma of an APC. There are two classes of these glycoproteins: MHC class I and MHC class II molecules. Most nucleated cells express MHC I molecules on their surfaces, whereas APCs can express both MHC I and MHC II on their plasmalemmas. The MHC molecules are unique in each individual (except for identical twins), and, to be activated, T cells must recognize not only the foreign epitope but also the MHC molecule as self. If a T cell recognizes the epitope but not the MHC molecule, it does not become stimulated; hence, the T cell's capacity to act against an epitope is **MHC restricted**.

There are three types of T cells, some with two or more subtypes:

- Naïve T cells
- Memory T cells
- Effector T cells

Naïve T Cells

Naïve T cells possess CD45RA molecules on their cell surfaces, and they leave the thymus programmed as immunologically competent cells, but they are not yet ready to function in that capacity until they become activated T cells. To become activated, naive T cells have to contact their specific antigen after they leave the thymic medulla. When a T lymphocyte becomes activated, it will undergo cell division and will form both memory T cells and effector T cells.

Memory T Cells

Memory T cells are of two types, central memory T cells and effector memory T cells; they are responsible for the immunological memory of the adaptive immune system.

Memory T cells express CD45R0 molecules on their cell membranes; they form the immunological memory of the adaptive immune system because they form a clone whose members are identical and have the capability of combating a particular antigen. These memory cells can become activated and express effector capabilities. There are two types of memory T cells, those that express CR7+ molecules on their surfaces and are known as *TCM* (central memory T cells) and *CR7– cells*, namely, effector T memory cells (TEM). **TCMs** populate and remain in the T cell–rich zones of lymph nodes (in the paracortex), are incapable of immediate effector function, interact with and stimulate APCs, and cause them to release IL-12. This signaling molecule binds to IL-12 receptors of TCMs and stimulates them to differentiate into **TEMs**. TEM express receptors that permit these cells to migrate to regions of inflammation, where they have immediate effector function by proliferating and differentiating into effector T cells.

Effector T Cells

Effector T cells are of three types, T helper cells, cytotoxic T lymphocytes, and regulatory T cells; these are the cells that are able to respond to an immunologic challenge.

Effector T cells are derived from effector T memory cells. They are immunologically competent cells that are capable of responding to and mounting an immune response. There are three types of effector T cells: T helper cells, T killer cells (cytotoxic T lymphocytes [CTLs]), and T reg cells; T helper and T reg cells have their own cell subtypes.

T Helper Cells

The three subtypes of T helper cells display CD4 molecules on their cell membranes and are responsible for the recognition of foreign antigens as well as for mounting an immunological response against them.

T helper cells possess **CD4 molecules** (in addition to the CD3 and TCR) as their cell membrane markers, are capable of interacting with other cells of the innate and adaptive immune systems, synthesize and release various cytokines, and can activate cells of the cell-mediated immune system to mount a response against invading pathogens and eliminate them. T helper cells also play a major role in stimulating the humorally mediated immune system by interacting with B cells and stimulating them to become antibody-producing plasma cells. There are a number of subtypes of T helper cells, T_H0, T_H1, T_H2, T17, and $T_H\alpha\beta$. An additional subtype, T_H3 cell, has been reclassified as the inducible T reg cell (see later).

- T_H0 cells are precursor cells that have the capability of manufacturing and releasing a large number of cytokines. These cells can differentiate into either T_H1 or T_H2 cells, depending on the signaling molecules that they receive from APCs, and then their repertoire of cytokine release becomes limited.
- T_H1 cells secrete IFN-γ, TFN-α, and IL-2, where IFN-γ is the cytokine release that is characteristic of these cells.
  - IL-2 stimulates CD4 and CD8 T cell proliferation as well as cytotoxicity by CD8 T cells (CTLs).
  - IFN-γ stimulates macrophages so that they can destroy pathogens, such as mycobacteria, protozoa, and fungi that they have phagocytosed; this cytokine also activates NK cells of the innate immune system to become cytotoxic.
    - Macrophages release IL-12 that, which induces the proliferation of T_H1 cells and inhibits the proliferation of T_H2 cells.
    - Macrophages that phagocytosed bacteria express CD40 molecules on their surfaces, and T_H1 cells express CD40 ligand whose interaction not only increases the macrophage's phagocytic capability but also induces the macrophage to release TNF, IL-1, and IL-12.
  - T_H1 cells are crucial for the control of intracellular pathogens and are also responsible for the induction of the cell-mediated immune response, as in acute allograft rejection and in the cases of multiple sclerosis.
- T_H2 cells secrete IL-4, -5, -6, -9, -10, and -13, and many of these interleukins facilitate the production of antibodies by plasma cells. T_H2 cells elicit a response against a parasitic (IgE) or mucosal (IgA) infection.
  - IL-4 stimulates B cells to proliferate and to switch to IgE synthesis; thus, it plays an important role in allergic reactions.
  - IL-5 induces the production of eosinophils.
  - IL-6 induces the proliferation of B cells.
  - IL-9 prompts T_H2 cells to proliferate and enhances mast cell activity.
  - IL-10, acting in concert with IL-4, suppresses the differentiation of T_H0 cells to T_H1 cells.
  - IL-13 enhances the functions of IL-4.
- T_H17 cells secrete IL-17, a cytokine that not only attracts neutrophils to the site of antigenic attack but also boosts their ability to phagocytose and destroy the bacterial pathogens. Additionally, T_H17 cells secrete IL-21 and IL-22.
  - IL-21 stimulates the activities of B cells, T cells, and NK cells.
  - IL-22 facilitates the inflammatory response and enhances the integrity of the epithelial barrier.
- $T_H\alpha\beta$ cells secrete IL-10 and are active in providing immunity against viruses.
  - IL-10 activates NK cells to force virally infected cells into apoptosis.
  - $T_H\alpha\beta$ cells, when overly exuberant in combating autoantigens, are responsible for the initiation of a type 2 hypersensitivity.

Cytotoxic T Lymphocytes

Cytotoxic T lymphocytes (CTLs, T killer cells) display CD 8 molecules on their cell membranes and are responsible for killing foreign cells, tumor cells, and virally altered cells.

CTLs (T killer cells) possess **CD8 molecules** (in addition to CD3 and TCRs) on their cell membranes. They recognize epitopes that are displayed on the cell membranes of foreign cells, tumor cells, as well as cells that have been altered by viruses and display viral epitopes on their plasmalemmae and kill these cells. The epitopes on the target cell surface must be presented to the CTL by **class I MHC molecules**. The killing of these cells is performed in one of two ways via the perforin/granzyme pathway or the Fas/FasL pathway:

- CTLs place perforins into the cell membranes of the virally altered cell.
- Perforins stimulate the formation of pores in the plasmalemma.
- CTLs transfer granzymes into the cytoplasms of the virally altered cell.

- Granzymes stimulate caspases to induce apoptosis, thus killing the virally altered cell.
- CTLs express Fas L, also known as *death ligand* (*CD95L*), on their cell membranes.
- Fas, also known as *CD95 (death receptor),* on the surface of the target cell is activated.
- When Fas is activated, it stimulates an apoptotic cascade, resulting in the death of the target cell.
- After killing it, the CTL can detach from the target cell and find additional target cells, killing them in the same fashion just described.

Regulatory T cells

Regulatory T cells (T reg cells) possess CD 4 molecules on their cell membranes and function in suppressing the immune response.

T reg cells display CD4 molecules on their cell membranes and function in suppressing the immune response. Historically, the role of suppressing the immune response was ascribed to a theoretical T suppressor cell; however, many immunologists did not accept the existence of these cells. Recent investigations, however, showed that there are specific cells that suppress the immune function, and these cells were named **T reg cells**. There are two types of T reg cells, natural (constitutive) T reg cells and inducible (adaptive) T reg cells, and both express **CD4 molecules** on their plasma membranes.

- Natural T reg cells (nT reg cells) develop in the thymus under the indirect influence of epithelial reticular cells (most probably those of Hassall corpuscles) that release thymic stromal lymphopoietin, a cytokine that causes dendritic cells in the vicinity to prompt naïve T cells to express CD25 and FoxP3 (forkhead family transcription factor) molecules on their plasma membrane, thus converting naïve T cells to nT reg cells. These natural T reg cells leave the thymus, and when their TCRs bind to an APC or effector T cell, they suppress the immune response by secreting IL-10 and TGF-β. Usually, the nT reg cells perform their functions once the pathogen is eliminated or if the response is against the self (autoimmune response).
- Inducible T reg cells (also known as *T_H3 cells*) originate outside of the thymus and are also derived from naïve T cells. They also have CD4, CD25, and FoxP3 on their cell membranes and secrete cytokines, such as IL-10 and TGF-β, that inhibit the formation of T_H1 cells, thereby suppressing the immune response.

It is possible that the two types of T reg cells have overlapping function and that they act in concert to suppress the autoimmune response to self molecules.

Natural Killer T Cells

NKT cells (natural Killer T cells) are effector T cells that resemble NK cells but must enter the thymic cortex to become immunocompetent effector cells. They release the following cytokines: IFN-γ, IL-4, and IL-10. Similar to NK cells, they can be activated almost immediately. These are very unusual cells because they are able to recognize *lipid antigens* that are presented to them on the surfaces of immature dendritic cells. In order for NKT cells to recognize antigenic lipids, the lipids must be co-presented to them with **CD1 molecules**, and they recognize the antigen-CD1 complex via their TCRs (as indicated previously, CD1 molecules resemble class I MHC molecules). There are four isoforms of CD1 molecules, and they are located either on the cell surface or are monitoring lysosomal and late endosomal compartments.

Major Histocompatibility Molecules

Major histocompatibility molecules present epitopes of pathogens to T cells. There are two classes of MHC molecules, MHC I and MHC II.

The prime importance of **MHC molecules** is to permit APCs and cells under viral attack (or cells already virally transformed) to present the epitopes of the invading pathogen to the **T cells**. These epitopes are short polypeptides that fit into a groove on the surface of the MHC molecule. It should be noted that in humans, MHC molecules are frequently referred to as ***human leukocyte antigen*** *(HLA)* molecules; therefore, there are class I HLA and class II HLA molecules that correspond to the MHC complex.

Although there are three classes of MHC molecules, only class I and class II are presented in this textbook because class III MHC molecules do not function in presenting epitopes:

- MHC I molecules (class I MHC molecules; class I HLA molecules) function in presenting short polypeptide fragments (8 to 12 amino acids in length) derived from endogenous proteins (i.e., proteins manufactured by the cell).
- MHC II molecules (class II MHC molecules; class II HLA molecules) function in presenting longer polypeptide fragments (13 to 25 amino acids in length) derived from exogenous proteins (i.e., proteins that were phagocytosed by these cells from the extracellular space).

As indicated previously, almost every nucleated cell synthesizes and displays MHC I molecules, but only APCs synthesize and display MHC II molecules in addition to their MHC I molecules.

In humans, the MHC molecules are unique in each individual (except for identical twins), and, to be activated, T cells must recognize not only the foreign epitope but also recognize that the MHC molecule belongs to that particular individual, that is, the MHC molecule is "self." If a T cell recognizes the epitope as foreign but not the MHC molecule as "self," it does not

become stimulated; hence, the T cell's capacity to act against an epitope is **MHC restricted**.

Loading Epitopes on MHC I Molecules

Epitopes derived from endogenous proteins are transported by specialized transporter proteins into the rough endoplasmic reticulum cisternae.

Proteins manufactured by a cell, whether they belong to the cell or to a virus or a parasite that has overtaken the protein synthetic machinery of the cell, are known as ***endogenous proteins***. The quality of the proteins that the cell manufactures is controlled by **proteasomes**, which are modified to splice defective or foreign proteins into the proper-sized polypeptide fragments (8 to 12 amino acids in length) that fit in the groove of an MHC I molecule. These fragments, known as ***epitopes***, are transported by specialized **transporter proteins** (**TAP1** and **TAP2**) into the cisternae of the rough endoplasmic reticulum, where they are complexed to MHC I molecules that were manufactured on the rough endoplasmic reticulum surface. The **MHC I–epitope complex** is transported to the Golgi apparatus and is packaged, within the *trans*-Golgi network, into clathrin-coated vesicles for transport to and insertion into the cell membrane. In this fashion, **CTLs** can "look" at the cell surface and "see" whether the cell is producing self or nonself proteins.

Loading Epitopes on MHC II Molecules

Epitopes derived from proteins endocytosed by macrophages and APCs are loaded onto MHC II molecules within specialized intracellular compartments known as the major histocompatibility complex II compartment (MIIC).

Macrophages and other APCs endocytose proteins from their extracellular milieu by the formation of pinocytotic vesicles or phagosomes. The contents of these vesicles, known as ***exogenous proteins***, are delivered into early endosomes, where they are enzymatically cleaved into polypeptide fragments. The polypeptide fragments are transported to late endosomes, where they are further cleaved to be the proper size (13 to 25 amino acids long) so that they can fit into the groove of the MHC II molecule.

MHC II molecules are synthesized on the rough endoplasmic reticulum. As they are assembled in the rough endoplasmic reticulum cisternae, a protein known as ***CLIP*** (***cl****ass II–associated* ***i****nvariant* ***p****rotein*) is loaded into the groove of the MHC II molecule, preventing the accidental loading of the molecule with an endogenous epitope. The **MHC II–CLIP complex** is transported into the Golgi apparatus and is sorted into clathrin-coated vesicles within the *trans* Golgi network for delivery to MHC class II–enriched compartments **(MIIC vesicle)**, dedicated vesicles that specialize in loading epitopes onto the MHC II molecule.

The MIIC vesicle receives not only MHC II–CLIP complex but also the epitopes from the processed antigens from the late endosomes. Within the MIIC vesicle, the CLIP is enzymatically dissociated from the MHC II molecule and is replaced by an epitope. The **MHC II–epitope complex** is then transported to and inserted into the cell membrane.

In this fashion, **T-helper cells** can "look" at the cell surface and "see" whether the cell is encountering nonself proteins.

MHC restriction and T Cells

As indicated previously, for T cells to perform their immunologic function, they must be presented epitopes that are complexed with MHC molecules.

- MHC I molecules are recognized by cytotoxic T cells (CD8+ cells).
- MHC II molecules are recognized by T_H1 and T_H2 cells (CD4+ cells).
- Both MHC I and MHC II molecules are recognized by T memory cells (CD45R0) cells).

Antigen-Presenting Cells

APCs express both MHC I and MHC II on their plasmalemmae, and they phagocytose, catabolize, process, and present antigens.

The **APCs** phagocytose, catabolize, and process antigens, attach their epitopes to MHC II molecules, and present this complex to T cells. Most APCs are derived from monocytes and therefore belong to the mononuclear phagocyte system. APCs include **macrophages**, **dendritic cells** (e.g., Langerhans cells of the epidermis and oral mucosa), and two types of **non–monocyte-derived APCs** (B cells and those epithelial reticular cells of the thymic medulla that are responsible for elimination of naïve T cells that would recognize the self as well as those that are responsible for nT reg cell development).

Similar to T-helper cells, APCs manufacture and release **cytokines**. These signaling molecules are needed to activate target cells to perform their specific functions, not only in the immune response but also in other processes. Table 12–5 lists some of these cytokines but includes only properties that relate specifically to the immune response.

Interaction Among the Lymphoid Cells

Cells of the lymphoid system interact with each other to effect an immune response. The process of interaction is regulated by recognition of surface molecules; if the molecules are not recognized, the cell is eliminated to prevent an incorrect response. If the surface molecules are recognized, the lymphocytes proliferate and

TABLE 12–5 Origin and Selected Functions of Some Cytokines

Cytokine	Cell Origin	Target Cell	Function
IL-1a and IL-1b	Macrophages and epithelial cells	T cells and macrophages	Activate T cells and macrophages
IL-2	T_H1 cells	Activated T cells and activated B cells	Promotes proliferation of activated T cells and B cells
IL-4	T_H2 cells	B cells	Promotes proliferation of B cells and their maturation to plasma cells; also facilitates switch from production of IgM to IgG and IgE
IL-5	T_H2 cells	B cells	Promotes B-cell proliferation and maturation; also facilitates switch from production of IgM to IgE
IL-6	Antigen presenting cells and T_H2 cells	T cells and activated B cells	Activates T cells; promotes B-cell maturation to IgG-producing plasma cells
IL-10	T_H2 cells	T_H1 cells	Inhibits development of T_H1 cells and inhibits them from secreting cytokines
IL-12	B cells and macrophages	NK cells and T cells	Activates NK cells and induces the formation of T_H1-like cells
TNF-α	Macrophage	Macrophages	Self-activates macrophages to release IL-12
	T_H1 cells	Hyperactive macrophages	Stimulates hyperactive macrophages to produce oxygen radicals, thereby facilitating bacterial killing
IFN-α	Cells under viral attack	NK cells and macrophages	Activates macrophages and NK cell
IFN-β	Cells under viral attack	NK cells and macrophages	Activates macrophages and NK cells
IFN-γ	T_H1 cells	Macrophages and T cells	Promotes cell killing by cytotoxic T cells and phagocytosis by macrophages

Ig, Immunoglobulin; IL, interleukin; INF, interferon; NK, natural killer; T_H, T-helper; TNF, tumor necrosis factor.

differentiate. The initiation of these two responses is called ***activation***. At least two signals are required for activation:

- Recognition of the antigen (or epitope)
- Recognition of a second, costimulatory signal, which may be mediated by a cytokine or by a membrane-bound signaling molecule

T-Helper Cell–Mediated (T_H2 cells) Humoral Immune Response

Except for thymus-independent antigens, B cells can respond to an antigen only if instructed to do so by the T_H2 subtype of T-helper cells (Fig. 12–2). When the B cell binds antigens on its sIgs, it internalizes the antigen–antibody complex, removes the epitope, attaches it to MHC II molecules, places the epitope–MHC II complex on its surface, and presents it to a T_H2 cell.

Signal 1. The T_H2 cell not only must recognize the epitope with its TCR but also must recognize the MHC II molecule with its CD4 molecule.

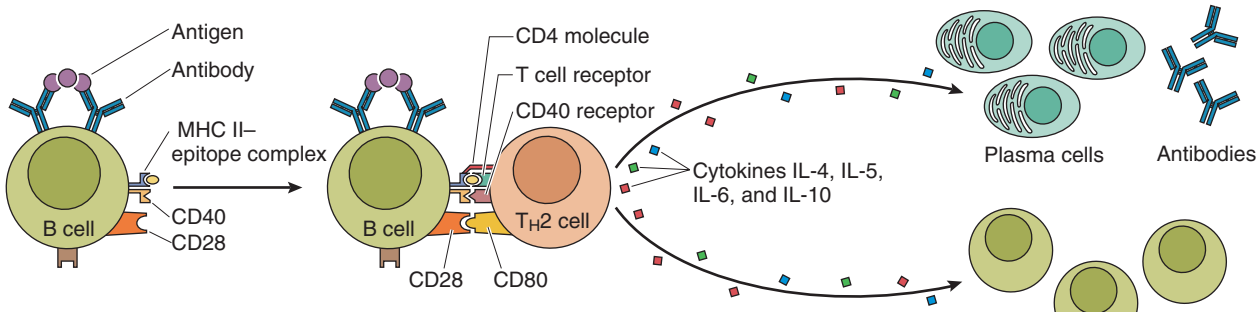
Signal 2. The T_H2 cell's CD40 receptor must bind to the B cell's CD40 molecule, and the T_H2 cell's CD28 has to contact the B cell's CD80 molecule.

If both signaling events are properly executed, the B cell becomes activated and rapidly proliferates. During proliferation, the T_H2 cell releases IL-4, IL-5, IL-6, and IL-10. The first three of these cytokines facilitate the differentiation of the newly formed B cells into **B memory cells** and antibody-secreting **plasma cells**, whereas IL-10 inhibits the proliferation of T_H1 cells. The interaction of CD40 with the CD40 ligand facilitates isotype switching from IgM to IgG, and IL-4 facilitates the isotype switching to IgE.

T-Helper Cell–Mediated (T_H1) Killing of Virally Transformed Cells

In most cases, CTLs need to receive a signal from a T_H1 cell to be capable of killing virally transformed cells. Before that signal can be given, however, the T_H1 cell must be activated by an APC that offers the proper epitope (Fig. 12–3).

Signal 1. The TCR and the CD4 molecule of a T_H1 cell must recognize the epitope–MHC II complex on the surface of an APC. If these events occur, the APC expresses a molecule called ***B7*** on its surface.



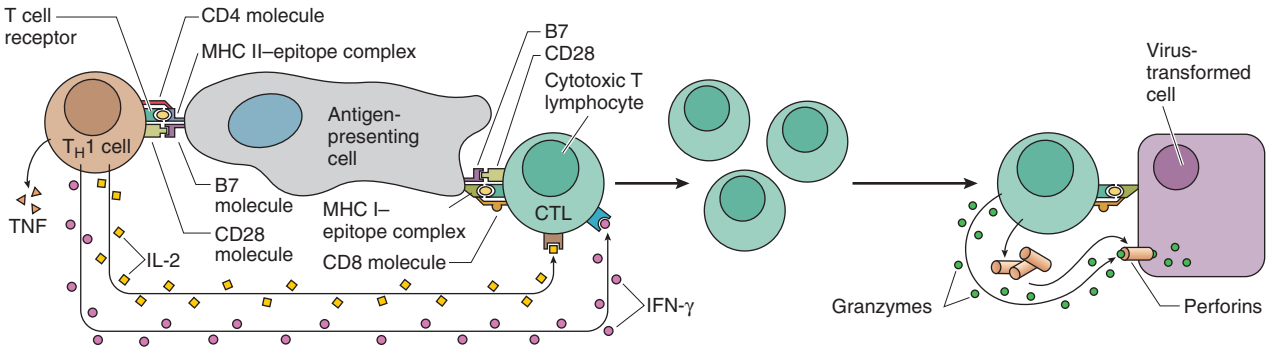
B cell becomes activated by the cross-linking of surface antibodies by the antigen. B cell places MHC II–epitope complex on its surface.

T_H2 cell recognizes the MHC II–epitope complex presented by the B cell using its TCR and CD4 molecules. Additionally, the T_H2 CD40 receptor binds to the CD40 molecule on the B cell plasmalemma and CD28 binds to CD80.

Binding of CD40 to CD40 receptor causes proliferation of B cells. The T_H2 cell releases cytokines IL-4, IL-5, IL-6, and IL-10. Binding of CD28 of B cell to CD80 of T_H2 cell activates more T_H2 cells.

IL-4, IL-5, and IL-6 facilitate the activation and differentiation of B cells into B memory cells and antibody-forming plasma cells. IL-10 inhibits the proliferation of T_H1 cells.

Figure 12–2 Schematic diagram of the interaction between B cells and T-helper cell (T_H2 cell) in a thymus-dependent, antigen-induced, B-memory and plasma cell formation. CD, Cluster of differentiation; IL, interleukin; MHC, major histocompatibility complex; TCR, T-cell receptor.



T_H1 cell TCR binds to MHC II–epitope complex of antigen-presenting cell. The CD4 molecule of the T_H1 cell recognizes MHC II. These two events cause the APC to express B7 molecules on its surface, which bind to CD28 of the T_H1 cell, causing it to release IL-2, IFN-γ, and TNF.

The same APC also has **MHC I–epitope** complex expressed on its surface that is bound by a CTL's CD8 molecule and T-cell receptor. Additionally, the CTL has CD28 molecules bound to the APC's B7 molecule. The CTL also possesses IL-2 receptors, which bind the IL-2 released by the T_H1 cell, causing the CTL to undergo proliferation, and IFN-γ causes its activation.

The newly formed CTLs attach to the MHC I–epitope complex via their TCR and CD8 molecules and secrete **perforins** and **granzymes**, killing the virus-transformed cells. Killing occurs when granzymes enter the cell through the pores established by perforins and act on the intracellular components to drive the cell into apoptosis.

Figure 12–3 Schematic diagram of the T-helper cell (T_H1 cell) activation of cytotoxic T cells in killing virus-transformed cells. APC, Antigen-presenting cell; CD, cluster of differentiation; CTL, cytotoxic T lymphocyte; IFN-γ, interferon-gamma; MHC, major histocompatibility complex; TCR, T-cell receptor; TNF, tumor necrosis factor.

Signal 2. The CD28 molecule of the T_H1 cell binds to the B7 molecule of the APC.

The T_H1 cell is now activated and releases IL-2, IFN-γ, and TNF. **IFN-γ** causes activation and proliferation of the CTL, ***if*** that CTL is bound to the same APC ***and if*** the following conditions are met:

Signal 1. The TCR and the CD8 molecule of the CTL must recognize the epitope–MHC I complex of the APC; also, the CD28 molecule of the CTL must bind with the B7 molecule of the APC.
Signal 2. IL-2 released by the T_H1 cell binds to the IL-2 receptors of the CTL.

The CTL is now activated and rapidly proliferates. The newly formed CTLs seek out virally transformed cells by binding with their TCR and CD8 to the transformed cell's epitope–MHC I complex. Target cell killing may occur in one of the following ways:

1. Binding (in the presence of calcium) causes release of perforins, a group of glycoproteins that are closely related to the C9 fraction of the complement membrane attack complex. Perforins embed themselves into the cell membranes of the transformed cells and, by aggregating, form hydrophilic pores. These pores may become so large and so abundant that the target cell cannot maintain its cytoplasmic integrity, and the cells undergo necrosis. It is interesting to note that the CTL is protected from autodestruction by perforin in two ways:
   a. Because the CTL moves away from the target cell as the process of perforin release is taking place
   b. Because of cathepsin B, a proteolytic enzyme that is co-released by the vesicle that released the perforin. This enzyme remains by the CTL cell membrane and degrades perforin molecules that attempt to embed themselves into the CTL's membrane.
2. Binding (in the presence of calcium) causes the release of perforins and granzymes. Granzymes are released from the storage granules of the CTL; these enzymes enter the transformed cells via the perforin-formed pores and drive the cells into apoptosis, killing them within a few minutes.
3. Binding can also bring the CTL's Fas ligand (a transmembrane protein; a member of the tumor necrosis family, also known as *CD95L*) in contact with the target cell membrane's "death receptor," known as the *Fas receptor* (*CD95*). When a threshold number of these Fas ligands and Fas proteins bond, the clustering of the Fas proteins induces the intracellular protein cascade that leads to apoptosis.

Note that certain highly vigorous APCs can act as the first signal. In such an instance, the CTL does not require a T-helper cell intermediary but can release IL-2 and can activate itself.

T_H1 Cells Assist Macrophages in Killing Bacteria

Bacteria that are phagocytosed by macrophages can readily proliferate within the phagosome (becoming infected) because macrophages cannot destroy these microorganisms unless they are activated by T_H1 cells (Fig. 12–4).

Signal 1. The TCR and CD4 molecules of the T_H1 cell must recognize the epitope–MHC II complex of the macrophage that phagocytosed the bacteria.
Signal 2. The T_H1 cell expresses IL-2 receptors on its surface and releases IL-2, which binds to the receptors, thus activating itself.

The activated T_H1 cell rapidly proliferates, and the newly formed T_H1 cells contact macrophages that are infected with bacteria.

Signal 1. The TCR and CD4 molecules of the T_H1 cell must recognize the epitope–MHC II complex of the infected macrophage, and the T cell releases IFN-γ.
Signal 2. The IFN-γ activates the macrophage, which then expresses TNF-α receptors on its surface and releases the cytokine TNF-α.

When these two factors, IFN-γ and TNF-α, bind to their receptors on macrophages, they facilitate the production of oxygen radicals by the macrophage, resulting in the killing of bacteria.

CLINICAL CORRELATIONS

Human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS), binds to CD4 molecules of T-helper cells and injects its core into the cell. The virus incapacitates the cell, and as the virus spreads, it infects other T-helper cells, thereby reducing their number. As a result, infected persons eventually become incapable of mounting an immune response against bacterial or viral infections. Victims succumb to secondary infections owing to opportunistic microorganisms or to malignancies.

LYMPHOID ORGANS

The lymphoid organs are classified into two categories, primary and secondary, as described in the following:

1. Primary (central) lymphoid organs are responsible for the development and maturation of lymphocytes into mature, immunocompetent cells.

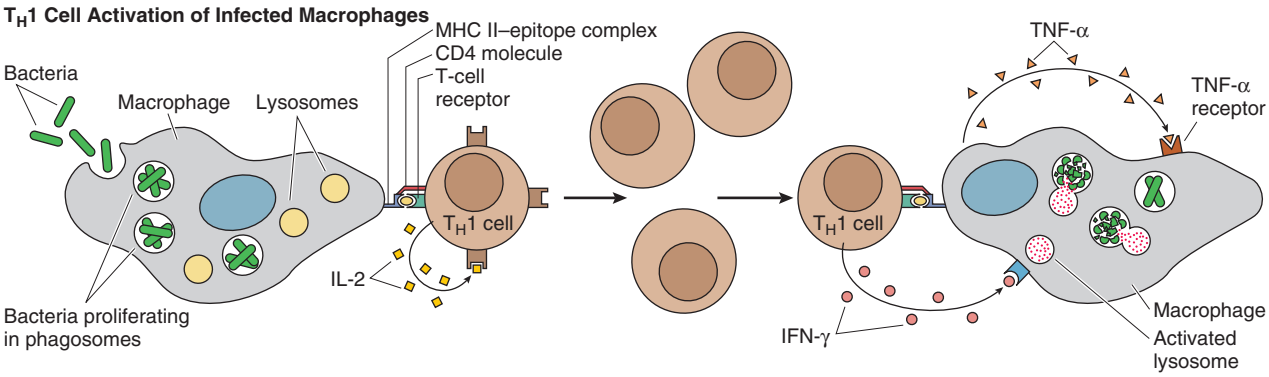


Figure 12–4 Schematic diagram of macrophage activation by T cells. CD, Cluster of differentiation; IL, interleukin; INF, interferon; TCR, T-cell receptor; TNF, tumor necrosis factor.

2. Secondary (peripheral) lymphoid organs are responsible for the proper environment in which immunocompetent cells can react with each other as well as with antigens and other cells to mount an immunological challenge against invading antigens or pathogens.

In humans, the fetal liver, prenatal and postnatal bone marrow, and thymus constitute the primary lymphoid organs. The lymph nodes, spleen, and mucosa-associated lymphoid tissues (MALT), as well as the postnatal bone marrow, constitute the secondary lymphoid organs.

Thymus

The thymus is a primary lymphoid organ that is the site of maturation of T lymphocytes.

The thymus, situated in the superior mediastinum and extending over the great vessels of the heart, is a small encapsulated organ composed of two **lobes**. Each lobe arises separately in the third (and possibly fourth) pharyngeal pouches of the embryo. The T lymphocytes that enter the thymus to become instructed to achieve immunological competence arise from mesoderm.

The thymus originates early in the embryo and continues to grow until puberty, when it may weigh as much as 35 to 40 g. After the first few years of life, the thymus begins to **involute** (atrophy) and becomes infiltrated by adipose cells. However, it may continue to function even in older adults.

The capsule of the thymus, composed of dense, irregular collagenous connective tissue, sends septa into the lobes, subdividing them into incomplete **lobules** (Fig. 12–5). Each lobule is composed of a cortex and a medulla, although the medullae of adjacent lobules are confluent with each other.

Cortex

Immunological competency of T cells, elimination of self-intolerant T lymphocytes, and MHC recognition occur in the thymic cortex.

The **cortex** of the thymus appears much darker histologically than does the medulla because of the presence of a large number of **T lymphocytes (thymocytes)** (Fig. 12–6; see Fig. 12–5). Immunologically incompetent T cells leave the bone marrow and migrate to the periphery of the thymic cortex, where they undergo extensive proliferation and instruction to become immunocompetent T cells (see earlier). In addition to the thymocytes, the cortex houses macrophages, dendritic cells, and **epithelial reticular cells (thymic epithelial cells)** (Table 12–6). It is believed that in humans epithelial reticular cells are derived from the endoderm of the third (and possibly fourth) pharyngeal pouch. Three types of epithelial reticular cells are present in the thymic cortex.

Type I cells separate the cortex from the connective tissue capsule and trabeculae and surround vascular elements in the cortex. These cells form occluding

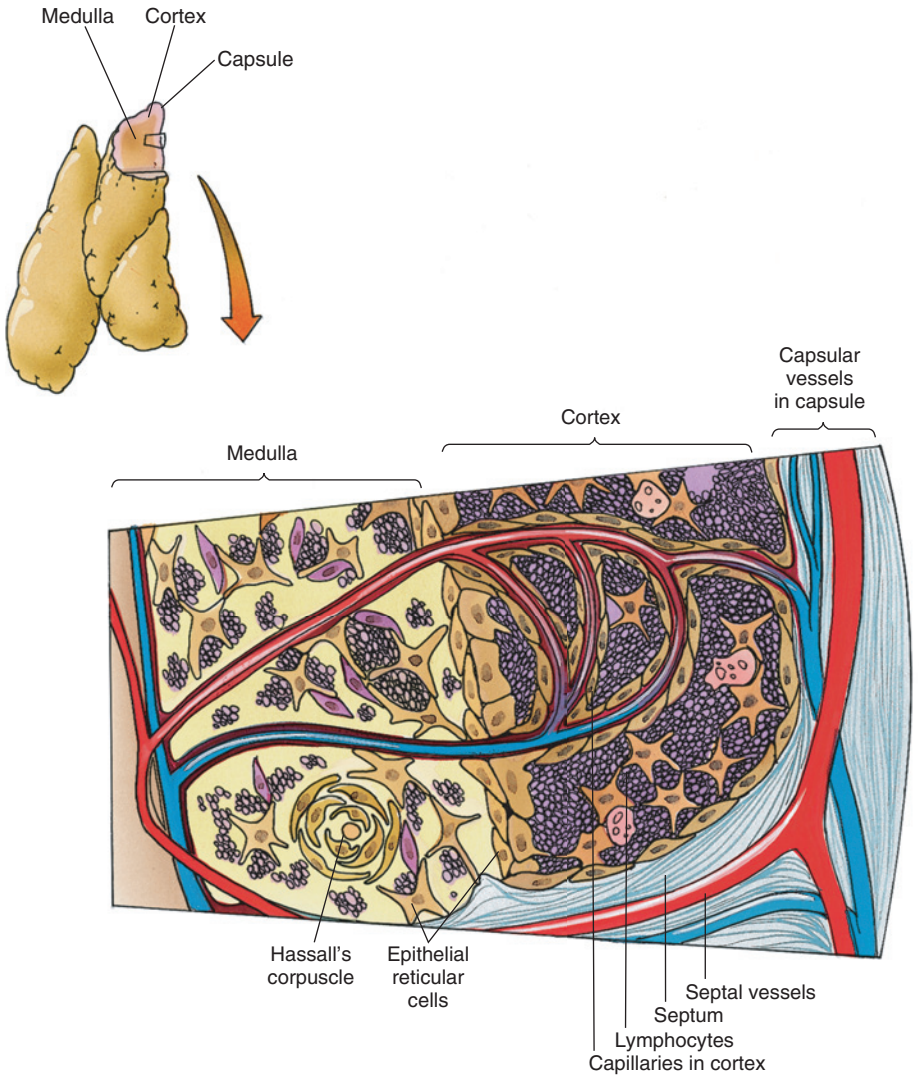


Figure 12–5 Diagram of the thymus demonstrating its blood supply and its histological arrangement.

TABLE 12-6 Epithelial Reticular Cells of the Thymus and Their Function

Cell Type	Location	Function
I	Cortex	Separate the cortex from the connective tissue capsule and trabeculae and surround vascular elements in the cortex. These cells form occluding junctions with each other, completely isolating the thymic cortex from the remainder of the body and participate in the formation of the blood-thymus barrier.
II	Mid-cortex	Their processes form a cytoreticulum that subdivides the thymic cortex into small, lymphocyte-filled compartments. They express MHC I and MHC II molecules complexed with self epitopes and test developing T cells for self recognition.
III	Cortical aspect of corticomedullary junction	Participate in the formation of occluding junctions with each other and with type IV epithelial reticular cells of the medulla, thereby isolating the cortex from the medulla. They express MHC I and MHC II molecules complexed with self epitopes and test developing T cells for self-recognition.
IV	Medullary aspect of corticomedullary junction	Participate in the formation of occluding junctions with each other and with type III epithelial reticular cells of the cortex, thereby isolating the cortex from the medulla.
V	Medulla	Form the cytoreticulum of the medulla and provide compartment for T cells.
VI	Medulla	Form whorl-shaped thymic corpuscles (Hassall corpuscles) where T cells are eliminated; they also synthesize and release thymic stromal lymphopoietin the signaling molecule that participates in the development of T reg cells.

MHC, Major histocompatibility complex.

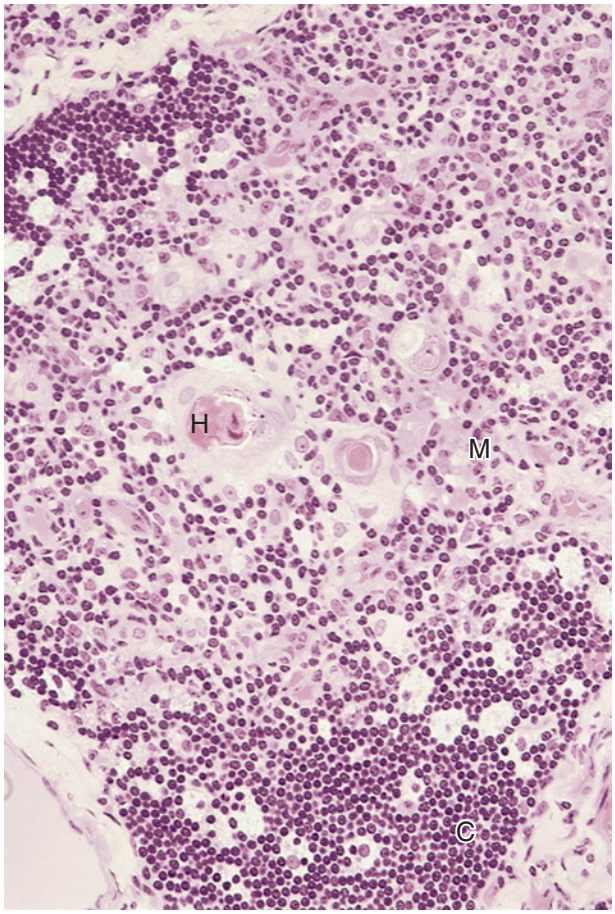


Figure 12–6 Photomicrograph of a lobule of the thymus. The peripheral cortex (C) stains darker than the central medulla (M) that is distinguished by the presence of Hassall corpuscles (H) (×124).

junctions with each other, completely isolating the thymic cortex from the remainder of the body. The nuclei of type I cells are polymorphous and have a well-defined nucleolus.

Type II cells are located in the midcortex. These cells have long, wide, sheath-like processes that form desmosomal junctions with each other. Their processes form a cytoreticulum that subdivides the thymic cortex into small, lymphocyte-filled compartments. The nuclei of type II cells are large, pale structures with little heterochromatin. The cytoplasm is also pale and is richly endowed with tonofilaments.

Type III cells are located in the deep cortex and at the corticomedullary junction. The cytoplasm and the nuclei of these cells are denser than those of type I and type II epithelial reticular cells. The rough endoplasmic reticulum of type III cells displays dilated cisternae, which is indicative of protein synthesis. Type III epithelial reticular cells also have wide, sheath-like processes that form lymphocyte-filled compartments. These cells participate in the formation of occluding junctions with each other and with epithelial reticular cells of the medulla; this isolates the cortex from the medulla.

These three types of epithelial reticular cells completely isolate the thymic cortex and thus prevent developing T cells from contacting foreign antigens. Type II and III cells as well as bone marrow–derived interdigitating cells (APCs) also present **self-antigens**, **MHC I** molecules, and **MHC II** molecules to the developing T cells. Developing T lymphocytes whose TCRs recognize self-proteins or whose CD4 or CD8 molecules cannot recognize the MHC I or MHC II molecules undergo apoptosis before they can leave the cortex. It is interesting that 98% of developing T cells die in the cortex and are phagocytosed by resident macrophages, which are referred to as ***tingible body macrophages***. The surviving cells enter the medulla of the thymus as I T lymphocytes *(single positive T cells)*, and, from there (or from the corticomedullary junction), they are distributed to secondary lymphoid organs via the vascular system.

Medulla

The medulla is characterized by the presence of Hassall corpuscles; all thymocytes of the medulla are immunocompetent T cells.

The thymic **medulla** stains much lighter than the cortex because its lymphocyte population is not nearly as profuse and because it houses macrophages, dendritic cells, a small population of B cells, and a large number of endothelially derived epithelial reticular cells (see Figs. 12–5 and 12–6). There are three types of epithelial reticular cells in the medulla (see Table 12-6).

Type IV cells are found in close association with type III cells of the cortex and assist in the formation of the corticomedullary junction. The nuclei of these cells have a coarse chromatin network, and their cytoplasm is dark-staining and richly endowed with tonofilaments.

Type V cells form the cytoreticulum of the medulla. The nuclei of these cells are polymorphous, with a well-defined perinuclear chromatin network and a conspicuous nucleolus.

Type VI cells compose the most characteristic feature of the thymic medulla. These large, pale-staining cells coalesce around each other, forming whorl-shaped **thymic corpuscles (Hassall corpuscles)**, whose numbers increase with a person's age (see Figs. 12–5 and 12–6). Type VI cells may become highly cornified and even calcified. The function of thymic corpuscles is not understood completely, although they might be the site of T lymphocyte cell death in the medulla, and it has been demonstrated that it is the type VI epithelial reticular cells of Hassall

corpuscles that manufacture thymic stromal lymphopoietin. It has also been suggested that, once medullary epithelial reticular cells cease to express the transcription factor AIRE, they become type VI cells and form Hassall corpuscles.

Vascular Supply

The cortical vascular supply forms a very powerful blood–thymus barrier to prevent developing T cells from contacting blood-borne macromolecules.

The thymus receives numerous small arteries, which enter the capsule and are distributed throughout the organ via the trabeculae between adjacent lobules. Branches of these vessels do not gain access to the cortex directly; instead, from the trabeculae, they enter the corticomedullary junction, where they form capillary beds that penetrate the cortex.

The capillaries of the cortex are of the **continuous** type, possess a thick basal lamina, and are invested by a sheath of type I epithelial reticular cells that form a **blood–thymus barrier**. Thus, the developing T cells of the cortex are protected from contacting blood-borne macromolecules. However, self-macromolecules are permitted to cross the blood–thymus barrier (probably controlled by the epithelial reticular cells), possibly to eliminate those T cells that are programmed against self-antigens. The cortical capillary network drains into small venules in the medulla.

Newly formed, immunologically incompetent T cells arriving from the bone marrow leave the vascular supply at the corticomedullary junction and migrate to the periphery of the cortex. As these cells mature, they move deeper into the cortex and enter the medulla as **single positive T cells (I T cells)** that are inactive immunocompetent cells. As they leave the thymic medulla via veins draining the thymus, they are referred to as *naïve T cells*.

Histophysiology of the Thymus

The primary function of the thymus is to instruct immunoincompetent T cells to achieve immunocompetence.

The developing T cells proliferate extensively in the cortex, begin to express their surface markers, and are tested for their ability to recognize **self-MHC molecules** and **self-epitopes**. T cells that are unable to recognize self-MHC I and self-MHC II molecules are destroyed by being driven into apoptosis. Additionally, T lymphocytes whose TCRs are programmed against self-macromolecules are also destroyed.

The epithelial reticular cells of the thymus produce the transcription factor known as ***AIRE*** and **thymic stromal lymphopoietin**. These factors facilitate dendritic cell activation as well as T-cell proliferation, expression of their surface markers, and differentiation into T-cell subtypes. Additionally, hormones from extrathymic sources, especially the gonads and the pituitary, thyroid, and suprarenal glands, influence T-cell maturation. The most potent effects are due to (1) **adrenocorticosteroids**, which decrease T-cell numbers in the thymic cortex; (2) **thyroxin**, which stimulates the cortical epithelial reticular cells to facilitate T cell and NK cell activity; and (3) **somatotropin**, which promotes T-cell development in the thymic cortex.

CLINICAL CORRELATIONS

Congenital failure of the thymus to develop is called ***DiGeorge syndrome.*** Patients with this disease cannot produce T cells. Hence, their cellularly mediated immune response is nonfunctional, and these patients die at an early age from infection. Because these patients also lack parathyroid glands, death also may be caused by tetany.

Lymph Nodes

Lymph nodes are small, encapsulated, oval structures that are interposed in the path of lymph vessels to serve as filters for the removal of bacteria and other foreign substances.

Lymph nodes are located in various regions of the body but are most prevalent in the neck, in the axilla, in the groin, along major vessels, and in the body cavities. Their parenchyma is composed of collections of T and B lymphocytes, APCs, macrophages, dendritic cells, follicular dendritic cells, and stromal cells. These lymphoid cells react to the presence of antigens by mounting an immunological response in which macrophages phagocytose bacteria and other microorganisms that enter the lymph node by way of the lymph.

Each lymph node is a relatively small, soft structure that is less than 3 cm in diameter and that has a fibrous connective tissue capsule, usually surrounded by adipose tissue (Fig. 12–7). It has a convex surface that is perforated by **afferent lymph vessels** that have **valves**, which ensure that lymph from those vessels enters the substance of the node. The concave surface of the node, the **hilum**, is the site of arteries and veins entering and exiting the node. Additionally, lymph leaves the node via the **efferent lymph vessels**, which are also located at the hilum. The efferent lymph vessels have valves that prevent regurgitation of lymph back into the node.

CLINICAL CORRELATIONS

In the presence of antigens or bacteria, lymphocytes of the lymph node rapidly proliferate, and the node may increase to several times its normal size, becoming hard and palpable and at times painful to the touch.

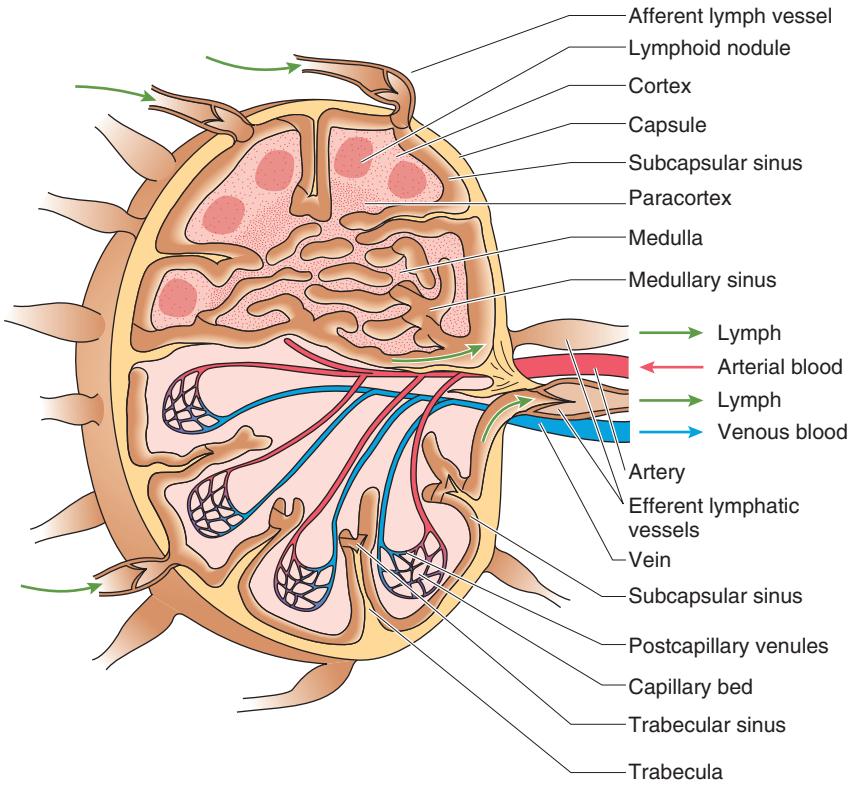


Figure 12–7 Schematic diagram of a typical lymph node.

Histologically, a lymph node is subdivided into three regions: cortex, paracortex, and medulla. All three regions have a rich supply of sinusoids, enlarged endothelially lined spaces through which lymph percolates.

Cortex

The cortex of the lymph node is subdivided into compartments that house B-cell–rich primary and secondary lymphoid nodules.

The dense, irregular, collagenous connective tissue **capsule** sends **trabeculae** into the substance of the lymph node, subdividing the outer region of the **cortex** into incomplete compartments. These partitioned regions extend along the entire periphery of the node all the way to the vicinity of the hilum (Fig. 12–8; see Fig. 12–7). The capsule is thickened at the hilum, and, as vessels enter the substance of the node, they are surrounded by a connective tissue sheath derived from the capsule. Suspended from the capsule and trabeculae is a three-dimensional network of **reticular connective tissue** that forms the architectural framework of the entire lymph node.

The afferent lymph vessels pierce the capsule on the convex surface of the node and empty their lymph into the **subcapsular sinus**, which is located just deep to the capsule. This sinus is continuous with the **cortical sinuses (paratrabecular sinuses)** that parallel the trabeculae and deliver the lymph into the **medullary sinuses**, eventually to enter the **efferent lymphatic vessels**. These sinuses have a network of **stellate reticular cells** whose processes contact those of other cells and the endothelium-like simple squamous epithelium. Collagen fibers, coated by processes of reticular cells form a loose web in the lumina of these sinuses, thereby reducing the velocity of flow of the lymph and thereby providing **macrophages**, attached to the stellate reticular cells, to avidly phagocytose foreign particulate matter. Additional channels are present that allow lymph to flow from the subcapsular sinus as well as from the paratrabecular sinuses to **perivenular channels** that surround **high endothelial venules (HEVs)** of the paracortex. These channels are known as ***fibroblastic reticular cell conduits*** because they are formed by **fibroblastic reticular cells** and fibrillin-coated collagen fibers surrounded by a basal lamina. These conduits are surrounded by dendritic cells as well as by a tightly packed population of **naive T cells** that have entered the paracortex via the HEVs. The naive T cells are mostly $\mathbf{T_H}$ **cells**, although **CTLs** are also present whose population becomes greatly enlarged during viral pathogenesis.

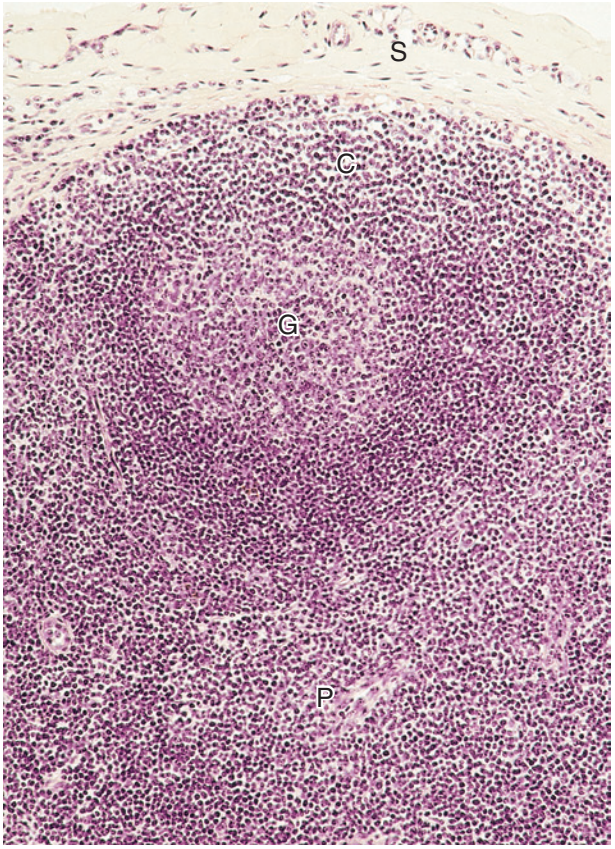


Figure 12–8 Photomicrograph of the lymph node cortex, displaying the subcapsular sinus (S), a secondary lymphoid nodule with its corona (C), germinal center (G), and the paracortex (P) (×132).

Lymphoid Nodules

There are two types of lymphoid nodules: primary and secondary; secondary lymphoid nodules have a germinal center.

The incomplete compartments within the cortex house **primary lymphoid nodules**, which are spherical aggregates of **B lymphocytes** (both virgin B cells and B memory cells) clustered around **follicular dendritic cells (FDCs)** whose processes contact those of their neighboring FDCs to form a three-dimensional web-like network. These B lymphocytes are in the process of entering or leaving the lymph node (see Figs. 12–7 and 12–8). In addition to the B cells, some T cells are also present. Frequently, the centers of the lymphoid nodules are stained paler and house **germinal centers**, and these lymph nodules are then known as ***secondary lymphoid nodules***. Secondary lymphoid nodules form only in response to an antigenic challenge; it is believed that they are the sites of **B memory cell** and **plasma cell** generation.

The region of the lymphoid nodule peripheral to the germinal center is composed of a dense accumulation of small lymphocytes that are migrating away from their site of origin within the germinal center. This peripheral region is called the ***corona (mantle)***.

Germinal centers display three zones: a dark zone, a basal light zone, and an apical light zone. The **dark zone** is the site of the intense proliferation of closely packed B cells (that do not possess sIgs). These cells, known as ***centroblasts***, migrate into the **basal light zone**, express sIgs, switch immunoglobulin class, and are known as ***centrocytes***. These cells are exposed to antigen-bearing **follicular dendritic cells** and undergo hypermutation to become more proficient at forming antibodies against the antigen. Cells that do not synthesize the proper sIgs are forced into apoptosis, and their remnants are destroyed by macrophages. The newly formed centrocytes that are permitted to survive enter the **apical light zone**, where they become either **B memory cells** or **plasma cells** and subsequently leave the secondary follicle.

Paracortex

The region of the lymph node between the cortex and the medulla is the paracortex. It houses mostly T cells and fibroblastic reticular cells and is the thymus-dependent zone of the lymph node.

The **paracortex** is a T cell–rich region that is located between the medulla and the follicle-rich region of the cortex. APCs (e.g., Langerhans cells from skin or dendritic cells from the mucosa) migrate to the **paracortex** region of the lymph node to present their epitope–MHC II complex to T helper cells. If T helper cells become activated, they proliferate, increasing the width of the paracortex to such an extent that it may intrude deep into the medulla. Newly formed T cells then migrate to the medullary sinuses, leave the lymph node, and proceed to the area of antigenic activity.

HEVs (postcapillary venules) are located in the paracortex. Lymphocytes leave the vascular supply by migrating between the cuboidal cells of this unusual endothelium and enter the substance of the lymph node. B cells migrate to the outer cortex, whereas most T cells remain in the paracortex.

The lymphocyte plasma membrane expresses surface molecules, known as ***selectins***, that aid the cell in recognizing the endothelial cells of HEVs and permit it to roll along the surface of these cells. When the lymphocyte contacts additional signaling molecules that are located on the endothelial cell plasmalemma, the selectins become activated, bind firmly to the endothelial cell, and stop the rolling action of the lymphocyte. Then, via **diapedesis**, the lymphocyte migrates between the cuboidal endothelial cells to leave the lumen of the postcapillary venule and enter the lymph node parenchyma.

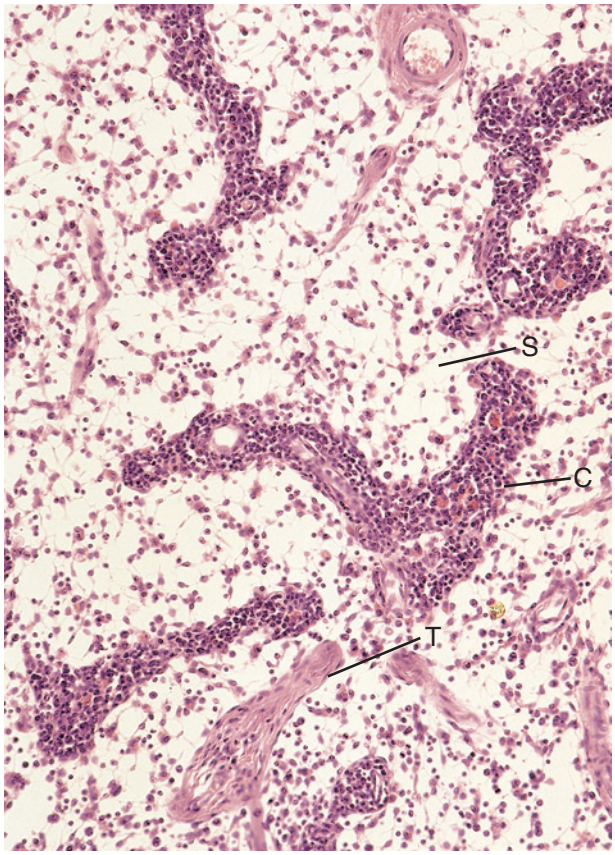


Figure 12–9 Photomicrograph of the lymph node medulla with its medullary sinusoids (S) and medullary cords (C), and trabecula (T) (×132).

Medulla

The medulla is composed of large, tortuous lymph sinuses surrounded by lymphoid cells that are organized in clusters known as medullary cords.

The cells of the medullary cords (lymphocytes, plasma cells, and macrophages) are enmeshed in a network of reticular fibers and reticular cells (Fig. 12–9; see Fig. 12–7). The lymphocytes migrate from the cortex to enter the medullary sinuses from which they enter the efferent lymphatic vessels to leave the lymph node. Histological sections of the medulla also display the presence of trabeculae, arising from the thickened capsule of the hilum, conveying blood vessels into and out of the lymph node.

Vascularization of the Lymph Node

The arterial supply enters the substance of lymph nodes at the hilum. The vessels course through the medulla within trabeculae and become smaller as they repeatedly branch. Eventually, they lose their connective tissue sheath, travel within the substance of medullary cords, and contribute to the formation of the medullary capillary beds. The small branches of the arteries continue in the medullary cords until they reach the cortex. Here, they form a cortical capillary bed, which is drained by **postcapillary venules**. Blood from postcapillary venules drains into larger veins, which exit the lymph node at the hilum.

Histophysiology of Lymph Nodes

Lymph nodes filter lymph, segregate T and B cells from each other, and act as sites for antigen recognition.

Lymph nodes function in attracting B cells and T cells to their proper residence in the cortex, providing a location for combating antigens and removing foreign particulate matter from the lymph that enters the node.

In order to be able to attract B and T cells to their proper location in the cortex, stromal cells release factors known as ***chemotactic chemokines lymphocytes*** that are specific for attracting **B cells** and **follicular dendritic cells** and those that are specific for attracting **T cells** and **dendritic cells**. Both types of chemokines are released by these stromal cells, and the chemokines attach to the luminal surface of endothelial cells of **postcapillary venules** (high endothelial cell venules; HEVs). As the T cells and dendritic cells and B cells and follicular dendritic cells reach these chemokine-coated endothelial cells, they attach to the endothelial cells, penetrate the intercellular spaces, leave the lumen of the blood vessel, enter the stroma of the lymph node cortex, and follow their designated chemokines to the paracortex (in the case of T cells and dendritic cells) and to the lymph nodules (in the case of B cells and follicular dendritic cells). In the paracortex, certain dendritic cells and certain stromal cells express **AIRE**, a transcription factor that functions in forcing the T cells into **apoptosis** that were able to escape from the thymus even though they are anti-self.

As lymph enters the lymph node, the flow rate is reduced, which gives the macrophages that reside in (or have their processes intrude into) the sinuses more time to phagocytose foreign particulate matter. In this fashion, 99% of the impurities found in lymph are removed.

Lymph nodes also function as sites of antigen recognition because APCs that contact antigens migrate to the nearest lymph node and present their epitope–MHC complex to lymphocytes. Additionally, antigens percolating through the lymph node are trapped by **follicular dendritic cells**, and B lymphocytes that are in the lymphoid nodule or migrate into the lymphoid nodule recognize the antigen.

If an antigen is recognized and a B cell becomes activated, that B cell migrates to a **primary lymphoid nodule** and proliferates, forming a germinal center, and

the primary lymphoid nodule becomes known as a ***secondary lymphoid nodule***. The newly formed cells differentiate into B memory cells and plasma cells, leave the cortex to enter the medulla, and form the medullary cords. About 10% of the newly formed plasma cells stay in the medulla and release antibodies into the medullary sinuses. The remainder of the plasma cells enter the sinuses and go to the bone marrow, where they continue to manufacture antibodies until they die. Some B memory cells stay in the primary lymphoid nodules of the cortex, but most leave the lymph node to take up residence in other secondary lymphatic organs of the body. Therefore, if there is a second exposure to the same antigen, a large number of memory cells are available so that the body can mount a prompt and potent secondary response.

Most of the T cell immune responses depend on epitope-bearing dendritic cells that migrate into the lymph nodes, progress to the paracortex and there present their epitope-MHC complex to T cells. The T cells that recognize the epitope–MHC complex become activated and initiate a cell-mediated immune response.

CLINICAL CORRELATIONS

Lymph nodes are located along the paths of lymph vessels and form a chain of lymph nodes so that lymph flows from one node to the next. For this reason, infection can spread, and malignant cells may metastasize through a chain of nodes to remote regions of the body.

Spleen

The spleen, the largest lymphoid organ in the body, is invested by a collagenous connective tissue capsule; it has a convex surface and a concave aspect, known as the hilum.

The **spleen**, the largest lymphoid organ in the body, weighs approximately 150 g in an adult individual and is located in the peritoneum in the upper left quadrant of the abdominal cavity. Its dense, irregular fibroelastic connective tissue capsule, occasionally housing **smooth muscle cells**, is surrounded by visceral peritoneum. The simple squamous epithelium of the peritoneum provides a smooth surface for the spleen. The spleen functions not only in the immunological capacity of antibody formation and T-cell and B-cell proliferation but also as a filter of the blood and in destroying old erythrocytes and old platelets. During fetal development, the spleen is a hemopoietic organ; if necessary, it can resume that function in an adult. Additionally, in some animals (but not in humans), the spleen also acts as a reservoir of red blood cells, which may be released into circulation as the need arises.

The spleen has a convex surface as well as a concave aspect, which is known as the ***hilum***. The capsule of the spleen is thickened at the hilum, and it is here that arteries and their accompanying nerve fibers enter and veins and lymph vessels leave the spleen.

The trabeculae, arising from the capsule, carry blood vessels into and out of the parenchyma of the spleen (Fig. 12–10). Histologically, the spleen has a three-dimensional network of **reticular fibers** and associated reticular cells. The reticular fiber network is attached to the capsule as well as to the trabeculae and forms the architectural framework of this organ (Fig. 12–11).

The interstices of the reticular tissue network are occupied by **venous sinuses**, trabeculae conveying blood vessels, and the splenic parenchyma. The cut surface of a fresh spleen shows gray areas surrounded by red areas; the former are called ***white pulp***, and the latter are known as ***red pulp***. White pulp is composed mostly of lymphocytes, and red pulp is composed mostly of venous sinuses. Unlike lymph nodes, the spleen does not have a cortex or a medulla or afferent lymph vessels serving it.

Central to the appreciation of the organization and function of the spleen is an understanding of its blood supply.

Vascular Supply of the Spleen

The spleen is supplied by the splenic artery and is drained by the splenic vein; both vessels enter and leave the spleen at the hilum.

The splenic artery branches repeatedly as it pierces the connective tissue capsule at the hilum of the spleen. Branches of these vessels, **trabecular arteries**, are conveyed into the substance of the spleen by trabeculae of decreasing sizes (see Fig. 12–10). When the trabecular arteries are reduced to about 0.2 mm in diameter, they leave the trabeculae. The tunica adventitia of these vessels become loosely organized, and they become infiltrated by a sheath of lymphocytes, the **periarterial lymphatic sheath (PALS)**. Because the vessel occupies the center of the PALS, it is called the ***central artery (central arteriole)***. Branches of the central artery, known as ***follicular arterioles***, serve the lymphoid nodules of the spleen.

At its termination, the central artery loses its lymphatic sheath and subdivides into several short, parallel branches, known as ***penicillar arteries***, which enter the red pulp. The penicillar arteries have three regions: (1) **pulp arteriole**, (2) **sheathed arteriole** (a thickened region of the vessel surrounded by a sheath of macrophages, the Schweigger-Seidel sheath), and (3) **terminal arterial capillaries**.

Although it is known that the terminal arterial capillaries deliver their blood into the splenic sinuses, the method of delivery is not completely understood,

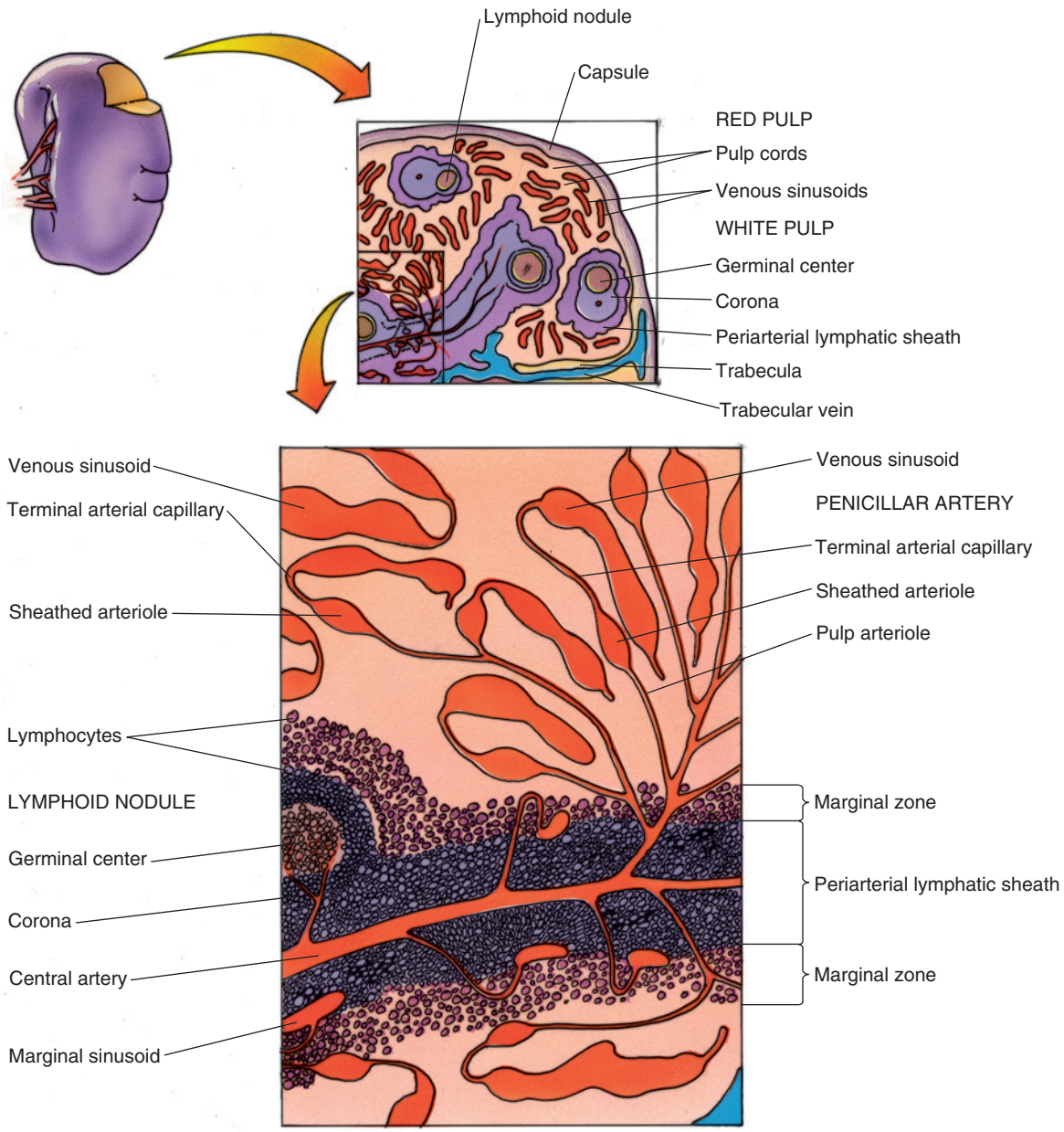


Figure 12–10 Schematic diagram of the spleen. Top, Low-magnification view of white pulp and red pulp. Bottom, Higher magnification view of the central arteriole and its branches.

which has prompted the formulation of three theories of circulation in the spleen: (1) closed circulation, (2) open circulation, and (3) a combination of the two theories.

Proponents of the **closed circulation theory** believe that the endothelial lining of the terminal arterial capillaries is continuous with the sinus endothelium (Fig. 12–12). Investigators who subscribe to the **open circulation theory** believe that the terminal arterial capillaries terminate prior to reaching the sinusoids, and blood from these vessels percolates through the red pulp into the sinuses. Still other investigators believe that some vessels connect to the sinusoids, whereas other vessels terminate as open-ended channels in the red pulp, suggesting that the spleen has both an **open and a closed system of circulation**.

Splenic sinuses are drained by small **veins of the pulp**, which are tributaries of larger and larger veins that merge to form the **splenic vein**, a tributary of the **portal vein**.

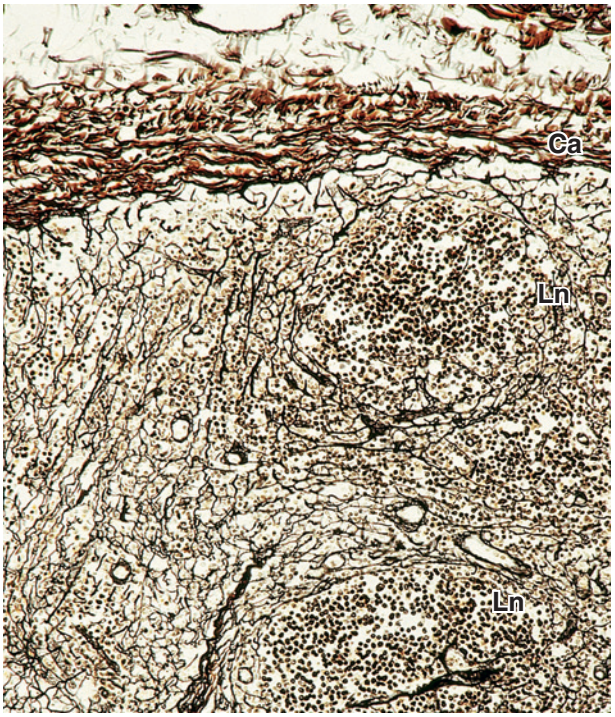


Figure 12–11 Photomicrograph of the reticular fiber architecture of the spleen. Note the capsule (Ca) and lymphoid nodule (Ln). Silver stain (×132).

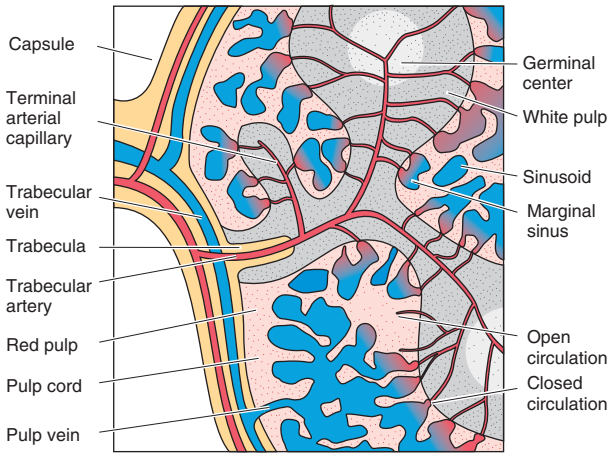


Figure 12–12 Diagram of open and closed circulation in the spleen.

White Pulp and Marginal Zone

The white pulp is composed of the periarterial lymphatic sheath, housing T cells, and lymphoid nodules, housing B cells; the marginal zone also houses B cells that are specialized to recognize thymic-independent antigens.

The structure of the **white pulp** is closely associated with the central artery. The PALS that surrounds the central artery is composed of T lymphocytes.

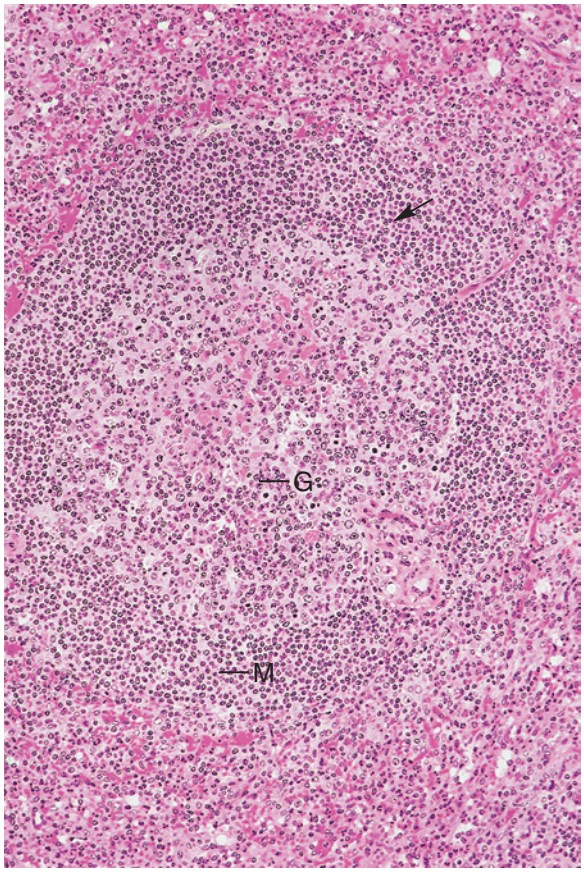


Figure 12–13 Photomicrograph of the white pulp and marginal zone of the spleen (×116). G, **G**erminal center; M, marginal zone. The central artery is represented by an *arrow*.

Frequently, enclosed within the PALS are **lymphoid nodules (lymphoid follicles)**, which are composed of B cells and displace the central artery to a peripheral position; as indicated previously, follicular arterioles branch from the central artery to serve the lymphoid nodules. Lymphoid nodules may display **germinal centers**, indicative of antigenic challenge (Fig. 12–13; see Fig. 12–10). The PALS and lymphoid nodules constitute the white pulp, and, as in the lymph node, the T and B cells are stationed in specific locations. Between the white pulp and the red pulp is an intermediate zone, known as the *marginal zone*, where T cells and B cells are present but occupy prescribed locations (see later). In order to ensure that B cells and T cells migrate to their appropriate locations in the spleen, splenic stromal cells release B and T cell-specific **chemotactic chemokines for lymphocytes (CCLs)**. Those CCLs that are specific to T cells attract them to the PALS and to T cell–rich regions of the marginal zone, whereas those CCLs that are specific to B cells attract them to the lymphoid nodules and to B cell–rich regions of the marginal zone.

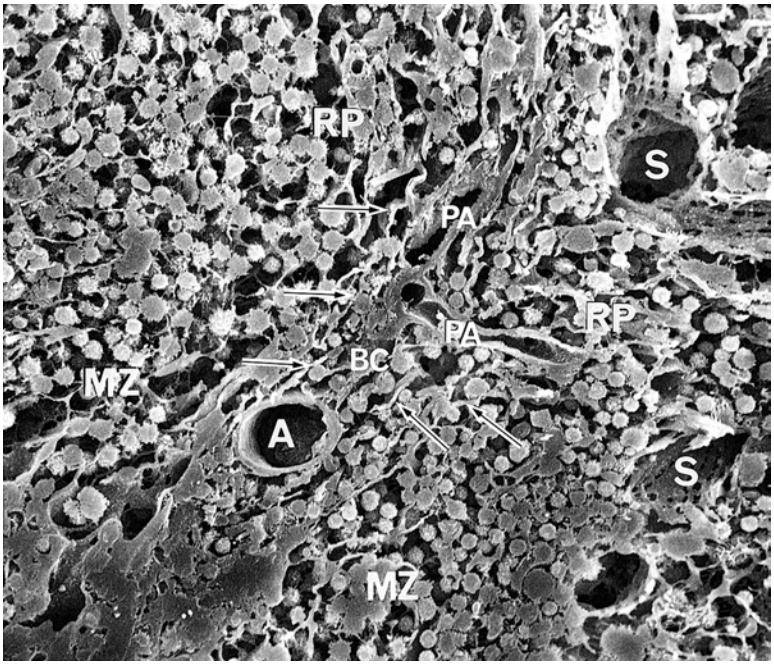


Figure 12–14 Scanning electron micrograph of the marginal zone and adjoining red pulp of the spleen (×680). *Arrows* point to periarterial flat reticular cells. A, Central artery; BC, marginal zone bridging channel; MZ, marginal zone; PA, penicillar artery; RP, red pulp; S, venous sinus. (From Sasou S, Sugai T. Periarterial lymphoid sheath in the rat spleen: **a** light, transmission, and scanning electron microscopic study. *Anat Rec*. 1992;232:15-24. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

The white pulp is surrounded by a **marginal zone**, 100 µm in width, that separates the white pulp from the red pulp (Fig. 12–14; see Figs. 12–10 and 12–13). This zone is composed of plasma cells, T and B lymphocytes, two types of macrophages (MZ macrophages and MZ metallophilic macrophages), and interdigitating dendritic cells. Additionally, numerous small vascular channels, **marginal sinuses**, are present in the marginal zone, especially surrounding lymphoid nodules. Slender blood vessels, radiating from the central arteriole, pass into the red pulp, recur, and deliver their blood into the marginal sinuses.

Because the spaces between the endothelial cells of these sinuses may be as wide as 2 to 3 µm, it is here that blood-borne cells, antigens, and particulate matter have their first free access to the parenchyma of the spleen. Thus, the following events occur at the marginal zone:

1. APCs sample the material traveling in blood, searching for antigens.
2. MZ macrophages attack microorganisms present in the blood and interact with B cells of the marginal zone activating them to present epitopes to marginal zone T cells.
3. MZ metallophilic macrophages recognize membrane-bound oligosaccharide ligands interact with B cells of the marginal zone, activating them to present epitopes to T cells located in the marginal zone as well as T cells located in the periarterial lymphatic sheath.
4. The circulating pool of T and B lymphocytes leaves the bloodstream to enter its preferred locations within the white pulp.
5. Lymphocytes may also come into contact with the interdigitating dendritic cells; if they recognize the epitope–MHC complex borne by these cells, the lymphocytes initiate an immune response within the white pulp.
6. B cells recognize and react to thymus-independent antigens (e.g., polysaccharides of bacterial cell walls) and are able to act on their own without the assistance of T helper cells.

Red Pulp

The red pulp of the spleen is composed of splenic sinuses and splenic cords (of Billroth).

The **red pulp** resembles a sponge in that the spaces within the sponge represent the sinuses and the sponge material among the spaces denotes the splenic cords (see Fig. 12–10).

The endothelial lining of **splenic sinuses** is unusual in that its cells are fusiform, resembling staves of a

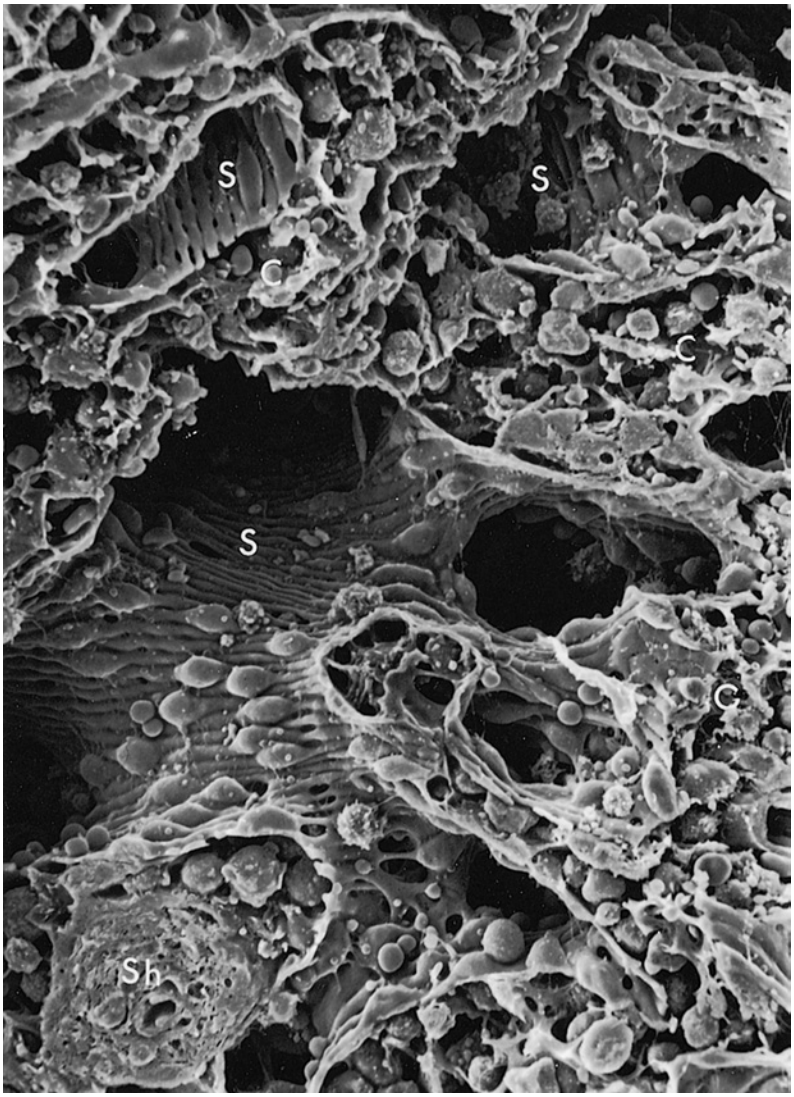


Figure 12–15 Scanning electron micrograph of sinusoidal lining cells bounded by splenic cords (×500). C, **S**plenic cords; S, venous sinuses; Sh, sheathed arteriole. (From Leeson TS, Leeson CR, Paparo AA. *Text-Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

barrel (Fig. 12–15). Moreover, spaces (2 to 3 μm wide) between adjoining cells are common. The sinuses are surrounded by reticular fibers (continuous with those of the splenic cords) that wrap around the sinuses as individual, thin strands of thread. The reticular fibers are arranged perpendicular to the longitudinal axis of the sinuses and are coated by **basal lamina**. Thus, splenic sinuses have a discontinuous basal lamina.

The **splenic cords** are composed of a loose network of reticular fibers, whose interstices are permeated by extravasated blood. The reticular fibers are enveloped by **stellate reticular cells**, which isolate the type III collagen fibers from blood, preventing a platelet reaction to the collagen (coagulation). **Macrophages** are particularly numerous in the vicinity of the sinusoids.

Histophysiology of the Spleen

The spleen filters the blood, forms lymphoid cells, eliminates or inactivates blood-borne antigens, destroys aged platelets and erythrocytes, and participates in hemopoiesis.

As blood enters the marginal sinuses of the marginal zone, it flows past a macrophage-rich zone. These cells phagocytose blood-borne antigens, bacteria, and other foreign particulate matter. Material that is not eliminated in the marginal zone is cleared in the red pulp at the periphery of the splenic sinuses.

Lymphoid cells are formed in the white pulp in response to an antigenic challenge. B memory cells and plasma cells are formed in lymphoid nodules, whereas

T cells of various subcategories are formed in the periarterial lymphatic sheaths. The newly formed B and T cells enter the marginal sinuses and migrate to the site of antigenic challenge or become part of the circulating pool of lymphocytes. Some plasma cells may stay in the marginal zone, manufacture antibodies, and release the immunoglobulins into the marginal sinuses. Most plasma cells, however, migrate to the bone marrow to manufacture and release their antibodies into the bone marrow sinuses.

Soluble blood-borne antigens are inactivated by the antibodies formed against them, whereas bacteria become **opsonized** and are eliminated by macrophages or neutrophils. Virus-transformed cells are killed by CTLs formed in the PALS of the white pulp.

Macrophages kill aged platelets and monitor erythrocytes as they migrate from the splenic cords between the endothelial cells into the sinuses (Fig. 12–16). Because older erythrocytes lose their flexibility (as do erythrocytes infected by the malarial parasite), they cannot penetrate the spaces between the endothelial cells and are phagocytosed by macrophages. The phagocytes also monitor the surface coats of red blood cells, which are destroyed in the following manners:

1. Old erythrocytes lose sialic acid residues from their surface macromolecules, exposing galactose moieties.
2. Galactose moieties that are exposed on erythrocyte membranes induce their phagocytosis.
3. Erythrocytes phagocytosed by macrophages are destroyed within phagosomes.
4. Hemoglobin is catabolized into its heme and globin portions.
5. The globin moiety is disassembled into its constituent amino acids, which become part of the circulating amino acid pool of the blood.
6. Iron molecules are conveyed to the bone marrow by transferrin and are used in the formation of new red blood cells.
7. Heme is converted to bilirubin and excreted by the liver in bile.
8. Macrophages also phagocytose damaged or defunct platelets and neutrophils.

During the second trimester of gestation, the spleen actively participates in **hemopoiesis**; after birth, however, blood cell formation occurs in the bone marrow only. If the necessity arises, the spleen can resume its hemopoietic function.

CLINICAL CORRELATIONS

Because the spleen is a friable (fragile) organ, major trauma to the upper left abdominal quadrant may cause rupture of the spleen. In severe cases, the spleen may be removed surgically, without compromising a person's life. Aged red blood cells are then phagocytosed by macrophages of the liver and bone marrow.

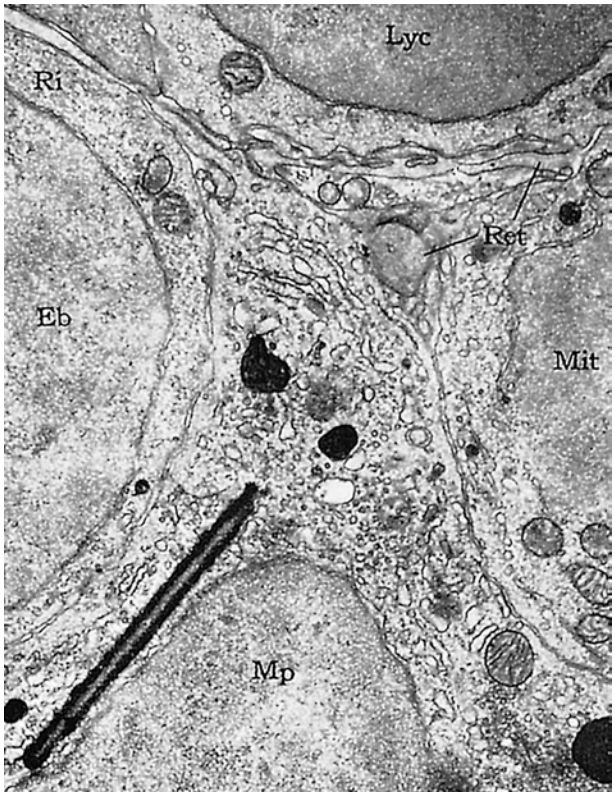


Figure 12–16 Electron micrograph of a macrophage containing phagocytosed materials including a crystalloid body. Mp, Macrophage; Mit, cell undergoing mitosis; Lyc, lymphocyte; Eb, erythroblast; Ret, reticular fibers in the interstitial spaces; Ri, ribosomes. (From Rhodin JAG. *An Atlas of Ultrastructure*. Philadelphia: WB Saunders; 1963.)

Mucosa-Associated Lymphoid Tissue

The mucosa-associated lymphoid tissue **(MALT)** is composed of a nonencapsulated, localized lymphocyte infiltration and lymphoid nodules in the mucosa of the gastrointestinal, respiratory, and urinary tracts. The best examples of these accumulations are those associated with the mucosa of the gut: **gut-associated lymphoid tissue (GALT)**, the **bronchus-associated lymphatic tissue (BALT)**, and the **tonsils**.

Gut-Associated Lymphoid Tissue

The most prominent accumulation of GALT is located in the ileum and is known as Peyer patches.

GALT is composed of lymphoid follicles along the length of the gastrointestinal tract. Most of the lymphoid follicles are isolated from each other; in the ileum, however, they form lymphoid aggregates, known as ***Peyer patches*** (Fig. 12–17). The lymphoid follicles of Peyer patches are composed of B cells surrounded by a looser region of T cells and numerous APCs.

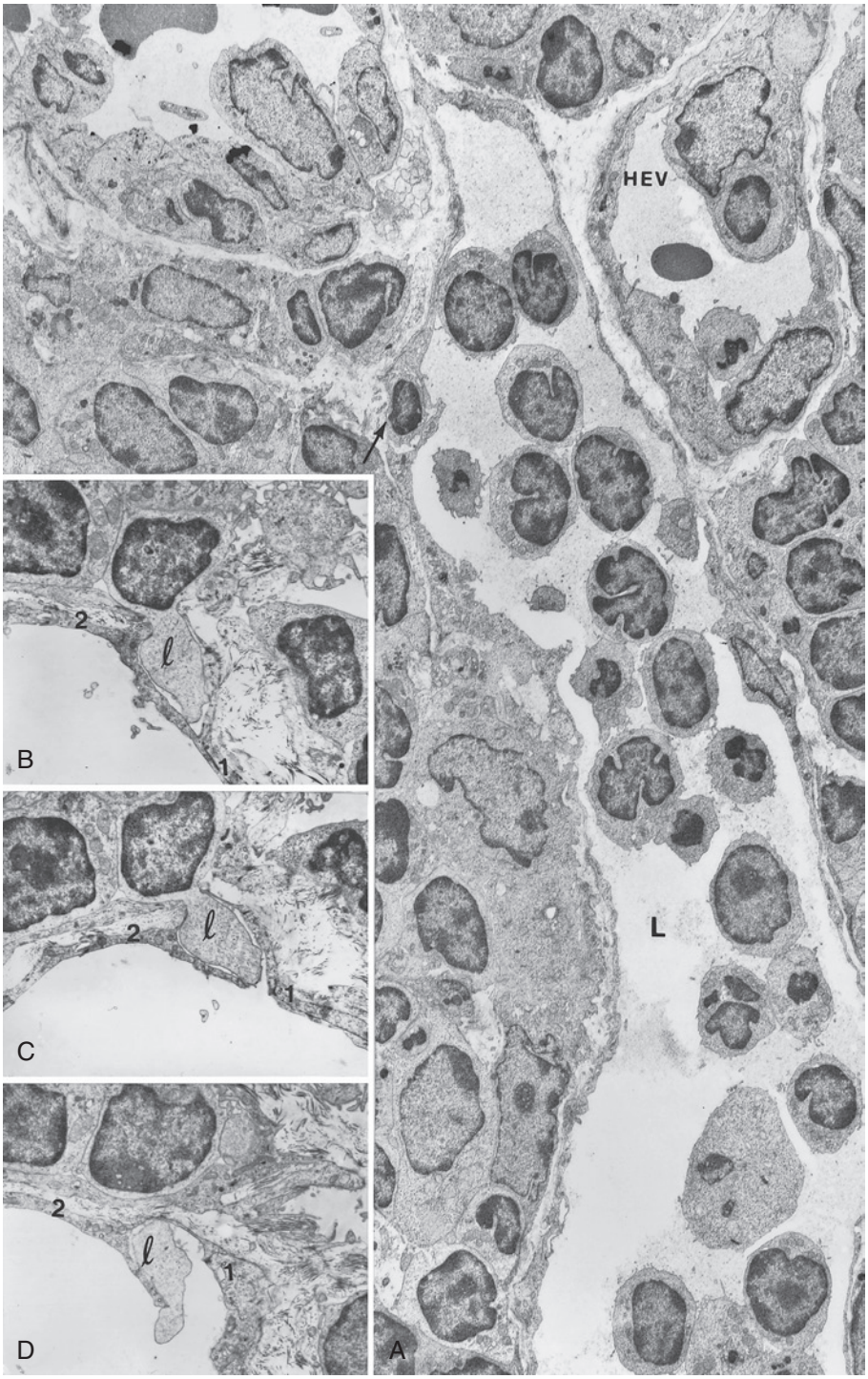


Figure 12–17 **A,** A transmission electron microscopy (TEM) micrograph of a rabbit's Peyer patches, showing absorbing peripheral lymphatic (ALPA) vessel (designated by the *L*) of the interfollicular area full of lymphocytes that has an intraendothelial channel that includes lymphocyte *(arrow)* in the endothelial wall (HEV, postcapillary high endothelium venula [×3000]). **B–D,** TEM micrographs of ultrathin serial sections that document various stages of lymphoctye migration through an intraendothelial channel composed of one and two endothelial cells (×9000). (From Azzali G, Arcari MA. Ultrastructural and three-dimensional aspects of the lymphatic vessels of the absorbing peripheral lymphatic apparatus in Peyer patches of the rabbit. *Anat Rec*. 2000;258:76. Reprinted with permission from Wiley Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Although the ileum is lined by a simple columnar epithelium, the regions immediately adjacent to the lymphoid follicles are lined by squamous-like cells, known as ***M cells (microfold cells)***. It is believed that M cells capture antigens and transfer them (without first processing them into epitopes) to macrophages located in Peyer patches (see Chapter 17 for more information concerning immunity in the gastrointestinal tract).

Peyer patches have no afferent lymphatic vessels, but they do have efferent lymph drainage. They receive small arterioles that form a capillary bed, drained by HEVs. Lymphocytes destined to enter Peyer patches have homing receptors that are specific for the HEVs of GALT.

Bronchus-Associated Lymphoid Tissue

BALT is similar to Peyer patches, except that it is located in the walls of bronchi, especially in regions where bronchi and bronchioles bifurcate. As in GALT, the epithelial cover over these lymphoid nodules changes from a pseudostratified ciliated columnar with goblet cells to **M cells**.

Afferent lymph vessels are absent, although lymph drainage has been demonstrated. The rich vascular supply of BALT indicates its possible systemic as well as localized role in the immune process. Most of the cells are B cells, although APCs and T cells are also present. Lymphocytes destined to enter BALT have homing receptors that are specific for the HEVs of this lymphoid tissue (see Chapter 15 for more information concerning immunity in the respiratory system).

Tonsils

The **tonsils** (palatine, pharyngeal, and lingual) collectively form Waldeyer ring. They are incompletely encapsulated aggregates of lymphoid nodules, many with germinal centers, that guard the entrance to the oral pharynx. Because of their locations, the tonsils are interposed into the path of airborne and ingested antigens. They react to these antigens by forming lymphocytes and mounting an immune response. B and T lymphocytes are distributed differently in each tonsil, and their specific locations are signaled by **stromal cell–released chemotactic chemokines for lymphocytes** specific for those cells. Collectively, tonsils respond to bacterial and viral infections by increasing in size and by forming IgA antibodies against the invading pathogens.

Palatine Tonsils

The bilateral **palatine tonsils** are located at the boundary of the oral cavity and the oral pharynx, between the palatoglossal and the palatopharyngeal folds. The deep aspect of each palatine tonsil is isolated from the surrounding connective tissue by a dense, fibrous **capsule**. The superficial aspect of the tonsils is covered by a stratified squamous nonkeratinized epithelium that dips into the 10 to 12 deep **crypts** that invaginate the tonsillar parenchyma. The crypts frequently contain food debris, desquamated epithelial cells, dead leukocytes, bacteria, and other antigenic substances.

The parenchyma of the tonsil is composed of numerous lymphoid nodules, many of which display germinal centers, indicative of B-cell formation.

Pharyngeal Tonsil

The single **pharyngeal tonsil** is in the roof of the nasal pharynx. It is similar to the palatine tonsil, but its incomplete capsule is thinner. Instead of crypts, the pharyngeal tonsil has shallow, longitudinal infoldings called ***pleats***. Ducts of seromucous glands open into the base of the pleats. Its superficial surface is covered by a pseudostratified ciliated columnar epithelium that is interspersed with patches of stratified squamous epithelium (Fig. 12–18).

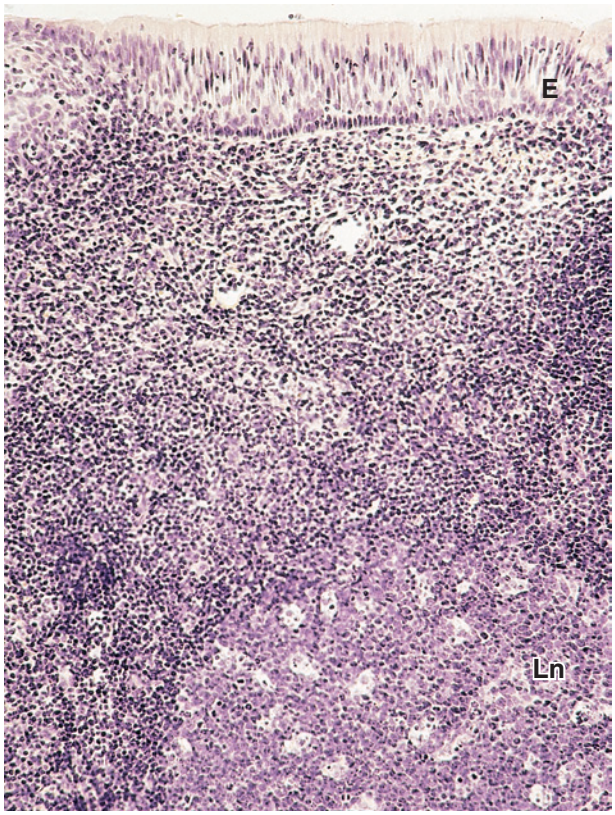


Figure 12–18 Photomicrograph of a lymphoid nodule of the pharyngeal tonsil displaying its pseudostratified ciliated columnar epithelium (E) and a germinal center of the secondary nodule (Ln) (×132).

The parenchyma of the pharyngeal tonsil is composed of lymphoid nodules, some of which have germinal centers. When this tonsil is inflamed, it is called the ***adenoid***.

Lingual Tonsil

The **lingual tonsil** is located on the dorsal surface of the posterior one third of the tongue and is covered on its superficial aspect by a stratified squamous nonkeratinized epithelium. The deep aspects of the lingual tonsil have a flimsy capsule that separates it from the underlying connective tissue. The tonsil has numerous crypts whose bases receive the ducts of mucous minor salivary glands.

The parenchyma of the lingual tonsil is composed of lymphoid nodules, which frequently have germinal centers.

PATHOLOGICAL CONSIDERATIONS

See Figures 12–19 through 12–21.

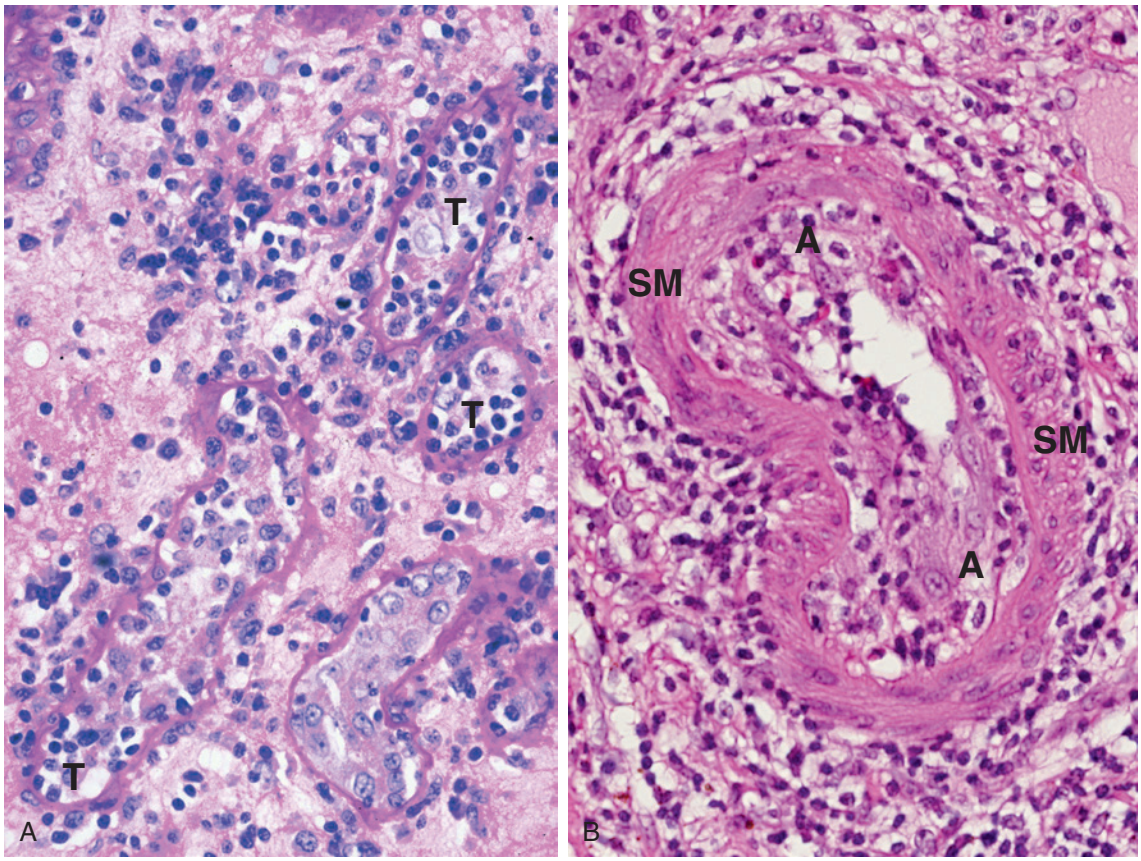


Figure 12-19 These two photomicrographs display T cell–mediated rejection of a kidney transplant. **A,** Banff type I T cell–mediated rejection is an acute rejection that is characterized by the presence of lymphocytes that infiltrate both the renal interstitium and the tubular epithelium. **B,** Banff type II T cell–mediated rejection is characterized by the infiltration of the endothelium of the blood vessels as well as by the enlargement and over-propagation of the endothelial cells. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:190.)

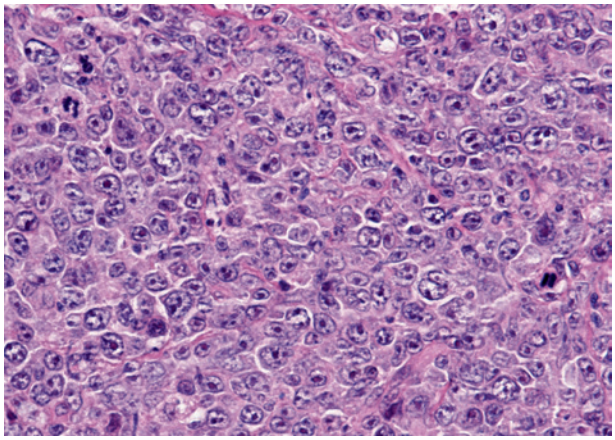


Figure 12-20 Photomicrograph of a diffuse large B-cell lymphoma. The B cells are large and pleomorphic and are widely distributed throughout the tissue. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:206.)

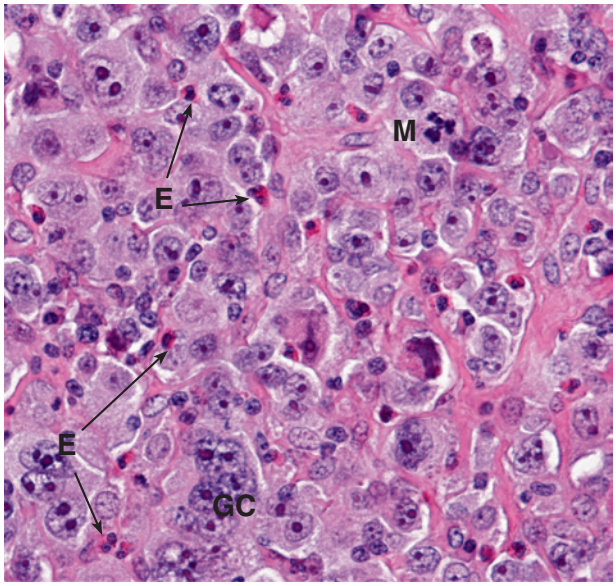
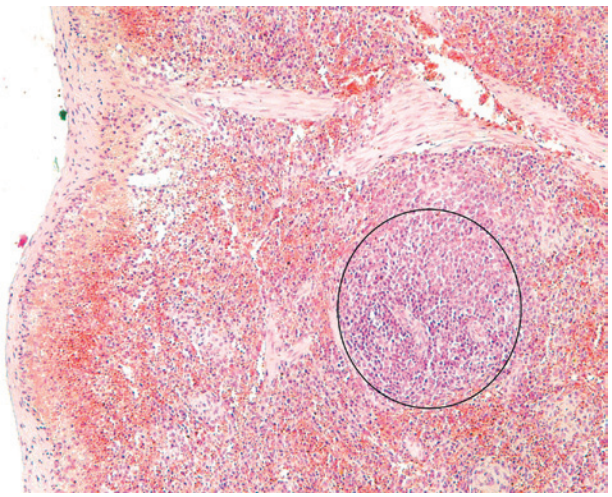


Figure 12-21 Photomicrograph of an anaplastic large cell lymphoma, characterized by large, atypical cells whose nuclei are highly irregular in morphology. The presence of aberrant mitotic figures (M) and numerous eosinophils (E) is common. Occasionally, giant cells (GC) are also evident. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:207.)

REVIEW QUESTIONS

1. Which of the following relates to the innate immune system?
   a. It is slower than the adaptive immune system.
   b. It has immunologic memory.
   c. It exhibits specificity.
   d. It depends on Toll-like receptors.
   e. It has diversity.
2. A child who is 6 years old sees his pediatrician for repeated infections as well as developing blood in his diarrhea. The physician orders a complete blood count, and the results show a very low platelet count and low lymphocyte levels. Because the little boy also has eczema, the pediatrician suspects Wiskott-Aldrich syndrome. Children with this disease have
   a. high T cell count and low B cell count.
   b. high B cell count and low T cell count.
   c. high T cell count and high B cell count.
   d. low T cell count and low B cell count.
   e. high erythrocyte count.
3. Which of the following is considered to be a primary (central) lymphoid organ?
   a. Thymus
   b. Lymph node
   c. Spleen
   d. Pharyngeal tonsil
   e. Palatine tonsil
4. Clonal expansion is dependent on which of the following cells?
   a. Effector cells
   b. Plasma cells
   c. Memory cells
   d. Virgin cells
   e. Naïve cells
5. Patients who have Graves disease have an autoimmune disorder in which the individual forms antibodies against his or her
   a. thyroid stimulating hormone.
   b. thyroxine.
   c. thyroid gland.
   d. parathyroid gland.
   e. TSH receptors.
6. The membrane attack system is the body's innate defense system against microbial attack. Which of the following components of the complement system plays a key role in this defense?
   a. C2
   b. C3
   c. C4
   d. Factor B
   e. Factor D
7. A patient is producing an antibody isotype that is composed of a dimer held together by J protein and is secreted into his saliva. Which of the following isotypes is being produced by this patient?
   a. IgA
   b. IgD
   c. IgE
   d. IgG
   e. IgM
8. Which of the following cells possess CD8 molecules?
   a. T_H0
   b. T_H1
   c. T_H2
   d. T reg
   e. CTL
9. Thymic corpuscles (Hassall corpuscles) are composed of
   a. type I reticular cells.
   b. type III reticular cells.
   c. type IV reticular cells.
   d. type V reticular cells.
   e. type VI reticular cells.
10. The region inside the circle is the
    a. cortex of a lymph node.
    b. medulla of a lymph node.
    c. cortex of the thymus.
    d. medulla of the thymus.
    e. white pulp of the spleen.



ANSWERS

1. D. The innate immune system relies on Toll-like receptors for initiating inflammatory and immune responses. It is the adaptive immune system that is slower acting, has immunologic memory, exhibits specificity, and has diversity.
2. D. Children with Wiskott-Aldrich syndrome have low T and B cell counts. They are at very high risk for infection, and they usually do not survive into their teens. Many of those who reach their teenage years succumb to leukemia, lymphoma, or other cancers. Because of their low T and B cell counts, these children are prone to respiratory infections and are also easy targets for viral, fungal, and bacterial infections. Only male children are susceptible to Wiskott-Aldrich syndrome.
3. A. The thymus and bone marrow are the two primary lymphoid organs because it is in those two regions that T cells and B cells become immunocompetent, respectively. After the lymphocytes become immunocompetent, they migrate to the secondary (peripheral) lymphoid organs, lymph nodes, diffuse lymphoid tissues, tonsils, and spleen.
4. C. Memory cells undergo cell division after first exposure to an antigen so that they are increased in number of memory cells in that particular clone. Because of the presence of an expanded population of memory cells with an increased affinity for the antigen, subsequent exposure to the same antigen induces a secondary (anamnestic) response that is much faster, more potent, and longer lasting than the primary response. Effector cells are responsive to the antigenic challenge, plasma cells manufacture antibodies, and virgin cells (also known as *naïve cells*) are B and T cells that have not as yet been exposed to antigens and none of these cells are responsible for clonal expansion.
5. E. Patients with Graves disease form antibodies against the TSH receptors of their own follicular cells. These individuals produce an excess amount of thyroid hormone and, therefore, they have an enlarged thyroid gland as well as exophthalmos (protruding eyeballs). They do not form antibodies against their thyroid-stimulating hormone or against thyroxine. Their thyroid and parathyroid glands are not perceived to be antigens.
6. B. C3 plays a key role in the complement system because deficiency of this protein leads to recurring bacterial infection. C3 has the ability to directly activate complement by its interaction with bacteria or even bacterial toxins. C2 and C4 deficiency can result in an autoimmune disease. Factors B and D are responsible for initiating the alternative pathway.
7. A. IgA, also known as the *secretory antibody*, is composed of a dimer that is held together by a J protein. IgA is secreted into the lumen of the gut, saliva, tears, milk, mucus of the nasal cavity, and vagina. IgD is a surface immunoglobulin that B cells secrete onto their own cell surface. IgE is reaginic antibody and is present on the surface of mast cells and basophils; when cross-linked by antigens, they facilitate the release of pharmacological agents from these cells. IgG crosses the placenta and is secreted into milk, thus it protects the fetus and the neonate by providing the fetus and the newborn with passive immunity. It is also responsible for antibody-dependent cell-mediated cytotoxicity by activating NK cells. IgM is a pentameric form of the antibody and is the first isotype to be formed during a primary response.
8. E. CTLs (cytotoxic T lymphocytes; T killer cells) possess CD8 molecules on their surface, and they can interact with MHC I molecules on virally altered cells. If their T-cell receptors recognize the epitopes displayed by the MHC I molecules on these virally altered cells, then the cytotoxic T lymphocyte will kill that cell. All T helper cells and T reg cells possess CD4 molecules on their cell surfaces, and they have the ability to recognize MHC II molecules located on antigen-presenting cells and B cells.
9. E. Type VI reticular cells compose thymic corpuscles. Type I and type III reticular cells are located in the thymic cortex. Type I cells are responsible for the formation of the blood–thymus barrier, whereas type II reticular cells subdivide the thymic cortex into lymphocyte–filled compartments. Type IV reticular cells are found in close association with type III reticular cells, and they form the corticomedullary junction. Type V reticular cells form the cytoreticulum of the medulla.
10. E. The region delineated by the circle is the white pulp of the spleen, as is evident by the surrounding red pulp and the dense irregular collagenous connective tissue capsule that sends trabeculae into the substance of this organ. Lymph nodes are usually surrounded by fat, and, in the cortex, a subcapsular sinus and cortical lymphoid nodules would be evident. The medulla of lymph nodes has no lymphoid nodules, and medullary cords and medullary sinuses would be present. The thymic cortex and thymic medulla have no lymphoid nodules.

Endocrine System

13

The **endocrine system** regulates metabolic activities in certain organs and tissues of the body, thereby helping to bring about homeostasis. The autonomic nervous system also regulates certain organs and tissues, but it does it via impulses that initiate the release of neurotransmitter substances that produce rapid responses in the tissues that are affected. Unlike the nervous system, the endocrine system produces a slow and diffused effect via chemical substances called ***hormones***, which are released into the bloodstream of capillary beds to influence target cells at remote sites. Although the nervous and endocrine systems function in different ways, the two systems interact to modulate and coordinate the metabolic activities of the body.

The **endocrine system** consists of **ductless glands**, distinct clusters of cells within certain organs of the body, and **endocrine cells** (known as the ***diffuse neuroendocrine system [DNES]***), isolated in the epithelial lining of the digestive tract and in the respiratory system. (The latter are discussed in Chapters 17 and 15, respectively.) The **endocrine glands**, the subject of this chapter, are abundantly and richly vascularized so that their secretory product may be released into slender connective tissue spaces between cells and the capillary beds from which they enter the bloodstream. The endocrine glands, discussed in this chapter, include the **pituitary gland**, **thyroid gland**, **parathyroid glands**, **suprarenal glands**, and **pineal body**. Other endocrine glands, such as the islets of Langerhans in the pancreas, are discussed in the pertinent chapters. Unlike the endocrine glands, which are ductless, the various exocrine glands (discussed in other chapters) empty their secretions in a duct system and exert only local effects.

HORMONES

Hormones are chemical messengers that are produced by endocrine glands and delivered by the bloodstream to target cells or organs on which they usually have regulatory effects.

The chemical nature of a hormone dictates its mechanism of action. Most hormones elicit several effects on their target cells (e.g., short and long-term effects). Although there are various ways of categorizing hormones, one of the simplest classification is to divide them into two types based on their water solubility (water soluble or lipid soluble) or into three types based on their chemical structure:

- Proteins and polypeptides—mostly water soluble (e.g., insulin, glucagon, follicle-stimulating hormone [FSH]).
- Amino-acid derivatives—mostly water soluble (e.g., thyroxine, epinephrine).
- Steroid and fatty acid derivatives—mostly lipid soluble (e.g., progesterone, estradiol, testosterone).

When a hormone enters the bloodstream and arrives in the vicinity of its target cells, it first binds to specific receptors on (or in) the target cell. Receptors for certain hormones (mostly protein and peptide hormones, i.e., water-soluble hormones) are located on the plasmalemma **(cell-surface receptors)** of the target cell, whereas other receptors are located in the cytoplasm and bind only to hormones that have diffused through the plasmalemma (namely, lipid-soluble hormones). The binding of a hormone to its receptor communicates a message to the target cell, initiating **signal transduction**, or translation of the signal into a biochemical reaction.

Hormones That Translocate to the Nucleus

Thyroid and steroid hormones bind to cytoplasmic receptors. The resulting hormone–receptor complex translocates to the nucleus, where it binds directly to deoxyribonucleic acid (DNA) close to a promoter site, thereby stimulating gene transcription. However, at least some steroid hormones bind to receptors that are located in the target cell plasma membrane, and thus the hormone's actions may be mediated directly without gene transcription or protein synthesis. Neither the hormone nor the receptor alone can initiate the target cell response.

Hormones That Bind to Cell-Surface Receptors

Hormones that bind to cell-surface receptors located in the plasmalemma can use one of several different mechanisms to elicit a response in their target cells. In each instance, the hormone–receptor complex is believed to induce a protein kinase to phosphorylate certain regulatory proteins, thereby generating a biological response

SUMMARY

The **endocrine system** is composed of the **pituitary**, **thyroid**, **parathyroid**, **suprarenal (adrenal)**, and **pineal glands**. None of these glands possess a system of ducts; therefore, the chemical products that they manufacture and store, known as ***hormones***, are released and delivered to the blood vascular system to be transported throughout the body. The hormones of the endocrine system regulate the metabolic activities of the body and interact with the nervous system to achieve homeostasis. Much of the activities of the endocrine system is controlled by the pituitary gland, which in turn is controlled by the **hypothalamus**. Because hormones travel via the bloodstream, they may reach most cells of the body but affect only those cells, known as ***target cells***, that possess the proper receptor that recognizes the hormone. These **hormone receptors** are located either on the plasma membrane of the target cell **(cell-surface receptors)** or within the cytoplasm **(intracellular receptors)**. The binding of a hormone to its receptor communicates a message to the target cell, initiating **signal transduction**, which is the translation of the chemical signal into a biochemical reaction by the target cell.

KEY WORDS

Adrenal gland; cell-surface receptor; endocrine system; hormone; hypothalamus; intracellular receptor; parathyroid gland; pineal gland; pituitary gland; signal transduction; suprarenal gland; target cells; thyroid gland

to the hormone. For example, some hormone–receptor complexes stimulate adenylate cyclase to synthesize cyclic adenosine monophosphate (cAMP), which stimulates protein kinase A in the cytosol. In such an instance, cAMP acts as a **second messenger**. Several additional second messengers have been identified, including (1) **cyclic guanosine 3′,5′ monophosphate**, (2) **metabolites of phosphatidylinositol**, (3) **calcium ions**, and (4) **sodium ions** (in neurons).

Some hormone receptor complexes are associated with **guanosine triphosphate–binding proteins (G proteins)**, which couple the receptor to the hormone-induced responses of the target cells. The receptors for epinephrine, thyroid-stimulating hormone (TSH), and serotonin, for example, use G proteins to activate a second messenger, which elicits a metabolic response. Other hormones, such as insulin and growth hormone, use **catalytic receptors** that activate protein kinases to phosphorylate target proteins.

Feedback Mechanism and Inactivation

Once a hormone activates its target cell, an inhibitory signal is generated and returned to the endocrine gland **(feedback mechanism)**, either directly or indirectly, to halt hormone secretion. The feedback mechanism also operates in another way: When the hormone level is inadequate to elicit a sufficient metabolic response in the target, a positive feedback signal is released, travels to the endocrine gland, and initiates an increase in hormone secretion. Through the feedback mechanism, therefore, regulation of the endocrine glands maintains homeostasis.

Many of the hormones that circulate in the bloodstream are in oversupply. They are usually bound to plasma proteins, which makes them biologically inactive, but they can be released from their bound state quickly, thus becoming active. Hormones become permanently inactivated in their target tissue; additionally, they may be degraded and destroyed in the liver and kidneys.

PITUITARY GLAND (HYPOPHYSIS)

The pituitary gland, composed of portions derived from oral ectoderm and from neural ectoderm, produces hormones that regulate growth, metabolism, and reproduction.

The **pituitary gland**, or **hypophysis**, is an endocrine gland that produces several hormones that are responsible for regulating growth, reproduction, and metabolism. It has two major subdivisions, that develop from different embryologic sources: (1) the **adenohypophysis (anterior pituitary)** develops from an evagination of the **oral ectoderm (Rathke pouch)** that lines the primitive oral cavity (stomadeum), and (2) the **neurohypophysis (posterior pituitary)** develops from neural ectoderm as a downgrowth of the diencephalon. Subsequently, both the adenohypophysis and the neurohypophysis are joined and encapsulated into a single gland. Because each subdivision has a distinctly different embryonic origin, the cellular constituents and the functions of each differ. Once the Rathke pouch begins to form, a continuous interaction between it and the developing diencephalon is required to form a downgrowth that will eventually become the neurohypophysis and to continue the development of the Rathke pouch to form the anterior pituitary. This evagination of the Rathke pouch occurs around the 4th to 5th week of embryogenesis and is controlled mostly by **bone morphogenetic protein 4**, **fibroblast growth factor 8**, and **Wnt5**. Once the pouch is established, formation of the anterior pituitary is stimulated by expression of **Rathke pouch homeobox gene**, **pituitary homeobox factor 2**, **Pax-6 protein (paired box protein-6)**, and homeobox protein **prophet of Pit-1**, as well as **sonic hedgehog protein**. The development of the neurohypophysis depends on various homeobox genes and transcription factors. One of the earliest genes that appears in the formation of the pars nervosa is **Rathke pouch homeobox gene** produced by cells of Rathke pouch. Additionally, the **WNT** (**wingless type**—homeobox gene first discovered in the fruit fly), **OTX2 (orthodenticle homeobox 2)**, and **SOX2** (also known as ***SRY-box 2***: ***sex determining region Y-box 2***) **signaling pathways** must be present for normal development of the neurohypophysis.

The pituitary gland lies below the hypothalamus of the brain, to which it is connected extending inferiorly from the diencephalon. It sits in the hypophyseal fossa, a bony depression in the sella turcica of the sphenoid bone that is lined by dura mater and covered over by a portion of the dura mater called the ***diaphragma sellae***. The gland measures approximately 1 cm × 1 to 1.5 cm; it is 0.5 cm thick and weighs about 0.6 g in men and slightly more in women who have never been pregnant and approximately 1.2 g in pregnant women as well as in women who have give birth two or more times.

The pituitary is connected to the **hypothalamus** of the brain by neural pathways; it also has a rich vascular supply from vessels that supply the brain, attesting to the intercoordination of the two systems in maintaining a physiological balance. The neural pathways consist of the **hypothalamohypophysial tract**, a group of axons whose soma reside in the **paraventricular** and **supraoptic nuclei** of the hypothalamus. The axons project to the pars nervosa of the pituitary gland and form dilatations that store and, when required, release the hormones produced in their soma.

- The **vascular supply** of the hypothalamus, via the **hypophyseal portal system**, drains into the capillary beds of the anterior pituitary (see below) and carries releasing hormones produced in the **dorsal**,

paraventricular, **medial paraventricular**, **arcuate**, **ventromedial**, and **periventricular nuclei** of the hypothalamus.

- It should also be noted that there is a **retrograde blood flow** from the pituitary to the hypothalamus that ensures a two-way communication between these interrelated systems.

In addition to controlling the pituitary, the hypothalamus also receives input from various areas of the central nervous system (i.e., information regarding plasma circulating levels of electrolytes and hormones) and controls the autonomic nervous system; therefore, the hypothalamus is the brain center for the maintenance of homeostasis.

Within each subdivision of the hypophysis are various regions having specialized cells that release different hormones. The subdivisions of the hypophysis, and the names of the regions are as follows (Figs. 13–1 and 13–2):

- Adenohypophysis (anterior pituitary)
  - Pars distalis (pars anterior)
  - Pars intermedia
  - Pars tuberalis
- Neurohypophysis (posterior pituitary)
  - Median eminence
  - Infundibulum
  - Pars nervosa

Interposed between the anterior and posterior lobes of the pituitary gland is the **pars intermedia**, remnants of the Rathke pouch (epithelial cells), which surround an amorphous colloid. The pars tuberalis forms a sleeve around the stem of the infundibulum.

Blood Supply and Control of Secretion

The hypophyseal portal system of veins delivers neurosecretory hormones from the primary capillary plexus of the the median eminence to the secondary capillary plexus of the pars distalis.

The arterial supply for the pituitary gland is provided from two pairs of vessels that arise from the internal carotid artery (see Fig. 13–2). The **superior hypophyseal arteries** supply the pars tuberalis and the infundibulum. They also form an extensive capillary network, the **primary capillary bed (plexus)**, in the median eminence. **Inferior hypophyseal arteries** primarily supply the posterior lobe, although they also send a few branches to the anterior lobe.

Hypophyseal portal veins drain the **primary capillary bed (plexus)** of the median eminence and deliver their blood into the **secondary capillary bed (plexus)**, located in the pars distalis (see Fig. 13–2). The capillaries of both plexuses are **fenestrated**.

Axons of neurons that originate in various portions of the hypothalamus terminate around the primary capillary plexus. The endings of these axons differ from other axons of the body because instead of delivering a signal to another cell, they liberate **hypothalamic secretory hormones** (either **releasing** or **inhibiting hormones [factors]**) directly into the connective tissue surrounding the primary capillary bed. These hormones are taken by the blood of the hypophyseal portal system and delivered to the secondary capillary bed of the pars distalis, where they leave the capillaries to regulate secretion of various anterior pituitary hormones. Because of this arrangement of vascular flow, the releasing hormones do not have to enter the systemic circulation, and therefore they are not diluted by the larger blood volume and can act in a timely manner in the regulation of the release of the anterior pituitary hormones. The following are the main releasing and inhibitory hormones (factors):

- Thyroid-stimulating hormone–releasing hormone (thyrotropin-releasing hormone [TRH]) stimulates the release of thyroid stimulating hormone (TSH).
- Corticotropin-releasing hormone (CRH) stimulates the release of adrenocorticotropin.
- Somatotropin-releasing hormone (SRH) stimulates the release of somatotropin (growth hormone).
- Luteinizing hormone releasing hormone (LHRH) stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH).
- Prolactin-releasing hormone (PRH) stimulates the release of prolactin.
- Prolactin inhibitory factor (PIF) inhibits prolactin secretion.

The physiological effects of pituitary hormones are summarized in Table 13–1.

Adenohypophysis

As indicated previously, the **anterior pituitary gland**, also referred to as the ***adenohypophysis***, develops from the Rathke pouch and consists of the following regions: pars distalis, pars intermedia, and pars tuberalis.

Pars Distalis

The parenchymal cells of the pars distalis consist of the chromophils and chromophobes.

The **pars distalis**, or anterior lobe of the pituitary gland, is covered by a fibrous capsule and is composed of cords of parenchymal cells that are surrounded by reticular fibers; these fibers also surround the large sinusoidal capillaries of the secondary capillary plexus. Scant connective tissue is located primarily around the hypophyseal arteries and the portal veins. The endothelial lining of the sinusoids is fenestrated, which facilitates the diffusion of releasing factors to the parenchymal cells and provides entry sites for their released secretions. The parenchymal cells of the pars distalis that have an affinity for dyes are called ***chromophils***;

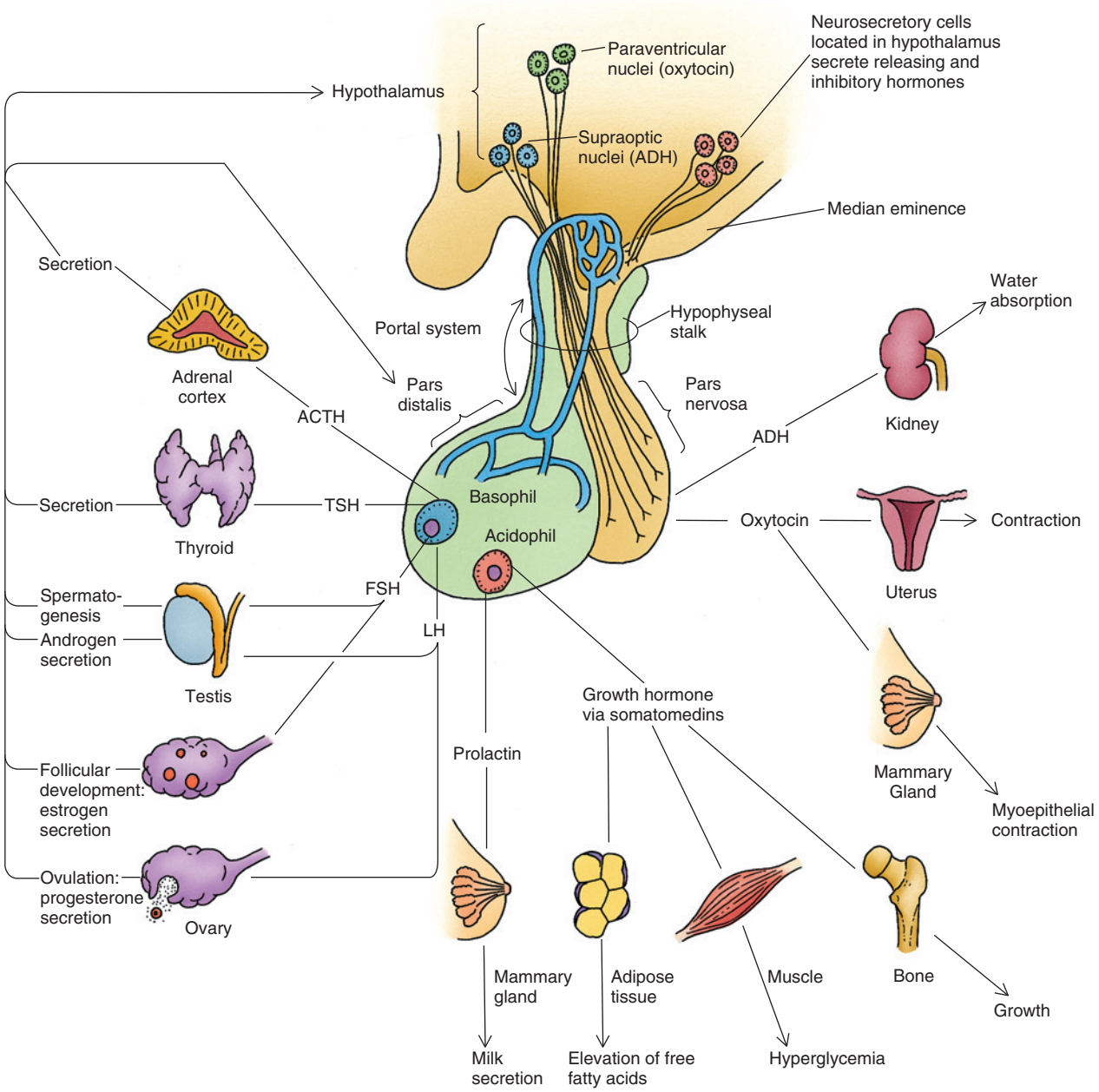


Figure 13–1 Schematic diagram of the pituitary gland and its target organs. ADH, Antidiuretic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

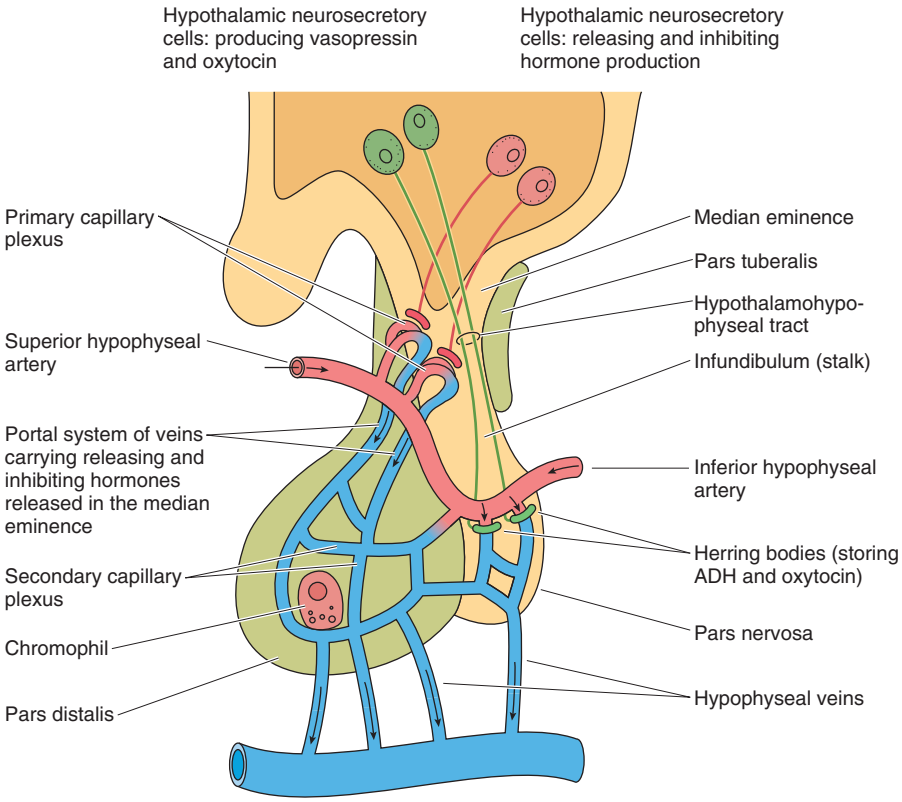


Figure 13–2 Schematic diagram of the pituitary gland and its circulatory system. ADH, Antidiuretic hormone.

TABLE 13–1 Physiological Effects of Pituitary Hormones

Hormone	Releasing/Inhibiting	Function
Pars Distalis		
Somatotropin (growth hormone)	*Releasing:* SRH (GHRH) *Inhibiting:* somatostatin	Generalized effect on most cells is to increase metabolic rates, stimulate liver cells to release insulin-like growth factors I and II, which increases proliferation of cartilage and assists in growth in long bones
Prolactin	*Releasing:* PRH *Inhibiting:* PIF	Promotes development of mammary glands during pregnancy; stimulates milk production after parturition (prolactin secretion is stimulated by suckling)
Adrenocorticotropic hormone (ACTH, corticotropin)	*Releasing:* CRH	Stimulates synthesis and release of hormones (cortisol and corticosterone) from suprarenal cortex
Follicle-stimulating hormone (FSH)	*Releasing:* GnRH and leptin *Inhibiting:* Inhibin (in males)	Stimulates secondary ovarian follicle growth and estrogen secretion; stimulates Sertoli cells in seminiferous tubules to produce androgen-binding protein
Luteinizing hormone (LH) in women	*Releasing:* GnRH	Assists FSH in promoting ovulation, formation of the corpus luteum, and secretion of progesterone and estrogen, forming a negative feedback to the hypothalamus to inhibit LHRH in women

Continued

TABLE 13–1 Physiological Effects of Pituitary Hormones—cont'd

Hormone	Releasing/Inhibiting	Function
Luteinizing hormone (LH) in men		Stimulates Leydig cells to secrete and release testosterone, which forms a negative feedback to the hypothalamus to inhibit LHRH in men
Thyroid-stimulating hormone (TSH) (thyrotropin)	*Releasing:* TRH *Inhibiting:* Negative feedback suppresses via CNS	Stimulates synthesis and release of thyroid hormone, which increases metabolic rate
Pars Nervosa		
Oxytocin		Stimulates smooth muscle contractions of the uterus during orgasm; causes contractions of pregnant uterus at parturition (stimulation of cervix sends signal to hypothalamus to secrete more oxytocin); suckling sends signals to hypothalamus, resulting in more oxytocin, causing contractions of myoepithelial cells of the mammary glands, assisting in milk ejection
Vasopressin (antidiuretic hormone [ADH])		Conserves body water by increasing resorption of water by kidneys; thought to be regulated by osmotic pressure; causes contraction of smooth muscles in arteries, thus raising the blood pressure; may restore normal blood pressure after severe hemorrhage

CNS, Central nervous system; CRH, corticotropin-releasing hormone; FSH, follicle-stimulating hormone; GHRH, growth hormone releasing hormone; GnRH, gonadotropin releasing hormone; PIF, prolactin inhibitory factor; PRH, prolactin-releasing hormone; SRH, somatotropin-releasing hormone; TRH, thyrotropin-releasing hormone.

whereas the parenchymal cells that have no affinity for dyes are called ***chromophobes***. Chromophils are further subdivided into **acidophils** (staining with acid dyes) and **basophils** (staining with basic dyes), which constitute the main secretory cells of the pars distalis (Fig. 13–3). However, it should be noted that these latter designations refer to the affinity of the secretory granules within the cells to the dyes, not to the parenchymal cell cytoplasm. The release or the continued storage of the hormones housed in the secretory granules of the chromophils is controlled by the **stimulatory** or **inhibitory hormones**, respectively. As indicated previously, these stimulatory and inhibitory hormones are manufactured by neurons located in the hypothalamus and delivered by the hypophyseal portal system into the secondary capillary bed of the pars distalis.

Chromophils

Secretory granules of chromophils have an affinity for histological dyes: those that stain orange-red with *acid* dyes and those that stain blue with *basic* dyes.

Acidophils

Acidophils, whose granules stain orange-red with eosin, are of two varieties: somatotrophs and mammotrophs.

The most abundant cells in the pars distalis are **acidophils**, whose granules are large enough to be seen by

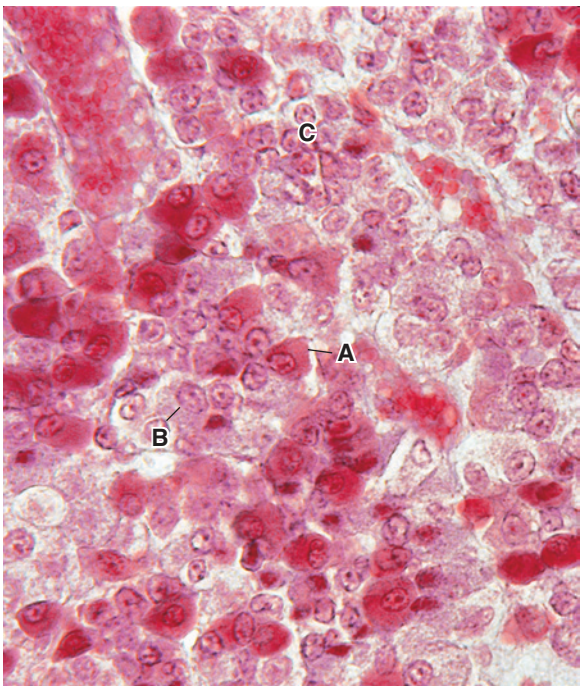


Figure 13–3 Light micrograph of the pituitary gland displaying chromophobes (C), acidophils (A), and basophils (B) (×470).

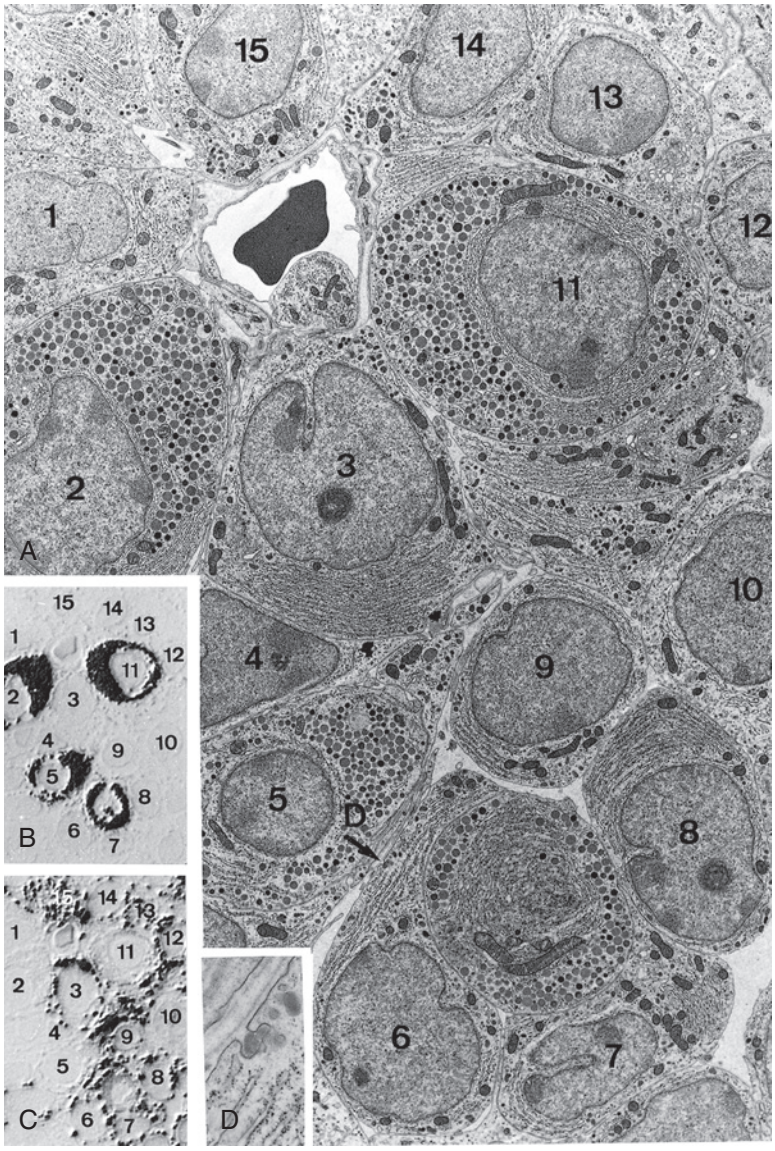


Figure 13–4 Light and electron micrograph of mouse adenohypophysis (×4000). Observe the mammotropes (cells 3, 6–9, 12–15) and somatotropes (cells 2, 5, 11) and note the secretory granules of these cells. (From Yamaji A, Sasaki F, Iwama Y, Yamauchi S. Mammotropes and somatotropes in the adenohypophysis of androgenized female mice: morphological and immunohistochemical studies by light microscopy correlated with routine electron microscopy. *Anat Rec*. 1992;233:103-110. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

the light microscope and stain orange to red with eosin (see Fig. 13–3).

Somatotrophs, one of the two varieties of the acidophils, have a centrally placed nucleus; a moderate Golgi complex; small rod-shaped mitochondria; abundant rough endoplasmic reticulum (RER); and numerous secretory granules, 300 to 400 nm in diameter (see Fig. 13–4). These cells secrete **somatotropin (growth hormone)**; thus, they are stimulated by **somatotropin releasing hormone (SRH)**, also known as ***growth hormone–releasing hormone***, and are inhibited by **somatostatin**. Somatotropin has a generalized effect of increasing cellular metabolic rates. This hormone also induces liver cells to produce **insulin-like growth factor I** and **insulin-like growth factor II**, which increase mitotic rates of epiphyseal plate chondrocytes,

promoting the elongation of long bones and hence stimulating growth. Approximately 50% of all chromophils are somatotrophs and reside mostly in the lateral aspect of the anterior pituitary.

Mammotrophs (lactotrophs), the other variety of acidophils, are arranged as individual cells rather than as clumps or clusters. These small, polygonal acidophils have the usual unremarkable organelle population; during lactation, however, the organelles enlarge, and the Golgi complex may become as large as the nucleus. These cells can be distinguished by their large secretory granules, formed by the fusion of smaller granules that are released by the *trans*-Golgi network (see Fig. 13–4). These fused granules, which may be 600 nm in diameter, contain the hormone **prolactin**, which promotes mammary gland development during pregnancy as well as lactation after birth.

During pregnancy, circulating estrogen and progesterone inhibit secretion of prolactin. At birth, estrogen and progesterone levels drop; thus, their inhibitory effect is lost. The number of mammotrophs also increases following birth. At the conclusion of nursing, the granules are degraded, and the excess mammotrophs regress. Release of prolactin from mammotrophs is stimulated by the **prolactin releasing hormone (PRH)** and, to a certain limited extent by **thyrotropin releasing hormone (TRH)**, especially when nursing is taking place, and is inhibited by **dopamine** (previously known as *prolactin-inhibiting hormone*). Approximately 10% of all chromophils are mammotrophs in females who have never been pregnant and in males; but in women who have had two or more pregnancies, these may constitute as much as 30% of the chromophil population.

Because the mammotroph population fluctuates, it is believed that there are stem cells present in the adenohypophysis that can differentiate into mammotrophs and probably into other acidophils and basophils. Some authors suggest that stem cells are members of the chromophobe cell population of the anterior pituitary whereas others suggest that they are members of the folliculostellate cell population.

Basophils

Basophils, the granules of which stain blue with basic dyes, are of three varieties: corticotrophs, thyrotrophs, and gonadotrophs.

Basophils stain blue with basic dyes (especially with periodic acid–Schiff reagent) and are usually located at the periphery of the pars distalis (see Fig. 13–3).

Corticotrophs, which are scattered throughout the pars distalis, are round to ovoid cells, each with an eccentric nucleus and relatively few organelles. Their secretory granules are 250 to 400 nm in diameter. Corticotrophs secrete **proopiomelanocortin (POMC)**, a large protein that is cleaved within the corticotrophs to produce **adrenocorticotropic hormone (ACTH)** and **lipotropic hormone (LPH)**. Further cleavage of ACTH forms **melanocyte-stimulating hormone (MSH)** whereas cleavage of LPH forms **β-endorphin**.

- ACTH acts on **melanocortin receptor type 2 receptor (MC2R)** in the adrenal gland cortex, prompting the release of glucocorticoids by cells of the zona fasciculata (see later section on zona fasciculata).
- MSH acts on melanocytes of the skin to produce melanin.
- LPH induces lipolysis and the synthesis of steroids; it also prompts melanocytes to manufacture melanin pigments.
- β endorphins are natural analgesics that bind to opioid receptors.

Corticotrophs comprise approximately 10% of the chromophil population and reside in the middle of the adenohypophysis.

Thyrotrophs are deeply embedded within cords of the parenchymal cells at a distance from sinusoids. These cells can be distinguished by their small secretory granules (150 nm in diameter), which contain **TSH**, also known as ***thyrotropin***. Secretion is stimulated by **TRH** and inhibited by the presence of thyroxine (T_4) and triiodothyronine (T_3) (thyroid hormones) in the blood. TSH functions in prompting the follicular cells of the thyroid gland to release thyroxine and triiodothyronine. Thyrotrophs are approximately 5% of the chromophil population and reside mostly in the anteromedial region of the adenohypophysis.

Gonadotrophs are round cells that have well-developed Golgi complexes and abundant RER and mitochondria. Their secretory granules vary in diameter from 300 to 400 nm. Gonadotrophs, situated near sinuses, secrete **FSH** and **LH**; sometimes LH is called ***interstitial cell–stimulating hormone (ICSH)***, because it stimulates steroid hormone production in interstitial cells of the testes. It remains unclear whether there are two subpopulations of gonadotrophs, one secreting FSH and the other LH, or whether both hormones are produced by one cell in different phases of the secretory cycle. Secretion is stimulated by **gonadotropin releasing hormone (GnRH)** as well as by the hormone **leptin** and is inhibited by various hormones that are produced by the ovaries and testes. Gonadotrophs comprise approximately 10% of the chromophil population and are distributed rather homogeneously throughout the adenohypophysis.

Chromophobes

Chromophobes have very little cytoplasm; therefore, they do not take up stain readily.

Groups of small, weakly staining cells in the pars distalis are called ***chromophobes*** (see Fig. 13–3). These cells

CLINICAL CORRELATIONS

Individuals with **Kallman syndrome** have very low levels of sex hormones, and as a consequence, female patients present with lack of secondary sexual development (i.e., breasts and pubic hair are lacking, and menstruation does not begin). Males present with micropenis and delay in the beginning of puberty. This syndrome is caused by an **X-linked recessive disorder** that also presents with additional developmental disorders, such as atrophy of the optic nerve, cleft palate, color blindness, cryptorchidism, and deafness (among others). The mutation occurs in the gene in which the protein product (known as **anosmin-1**) facilitates during embryogenesis the migration of GnRH cells from their original location in the olfactory placodes into the developing hypothalamus.

generally have less cytoplasm than chromophils do, and they may represent either nonspecific stem cells or partially degranulated chromophils, although some retain secretory granules. Because there is evidence for the cyclic nature of the secretory function of the chromophils, it is more probable that chromophobes are degranulated chromophils.

Folliculostellate Cells

Nonsecretory **folliculostellate cells** constitute a large population of cells in the pars distalis. Although their function is not clear, they have long processes that form gap junctions with those of other folliculostellate cells. It has been suggested that these cells may have multiple functions, including

- Phagocytic capabilities
- Providing physical support for chromophils and chromophobes
- Prompting chromophils to release their hormones
- Acting as stem cells to regenerate parenchymal cells of the anterior pituitary

Pars Intermedia

The pars intermedia lies between the pars distalis and the pars nervosa and contains cysts that are remnants of Rathke pouch.

The **pars intermedia** is characterized by many cuboidal cell-lined, colloid-containing cysts (Rathke cysts), which are remnants of the ectoderm of Rathke pouch. The pars intermedia, or more accurately in the adult human, the **zona intermedia**, sometimes houses cords of basophils along the networks of capillaries. These basophils synthesize the prohormone **proopiomelanocortin (POMC)**, a large protein that, as indicated previously, is cleaved within the corticotrophs to produce **ACTH** and **lipotropic hormone (LPH)**. Further cleavage of ACTH forms **MSH**, whereas cleavage of LPH forms **β-endorphin**.

Pars Tuberalis

The pars tuberalis surrounds the hypophyseal stalk and is composed of cuboidal to low-columnar basophilic cells.

The **pars tuberalis** surrounds the hypophyseal stalk but frequently is absent on its posterior aspect. Thin layers of pia arachnoid–like connective tissue separate the pars tuberalis from the infundibular stalk. The pars tuberalis is highly vascularized by arteries and the hypophyseal portal system, along which lie longitudinal cords of cuboidal to low-columnar epithelial cells. The cytoplasm of these basophilic cells contains small, dense granules; lipid droplets; interspersed colloid droplets; and glycogen. Although no specific hormones are known to be secreted by the pars tuberalis, some cells contain secretory granules that possibly contain **FSH** and **LH**.

Neurohypophysis

The **posterior pituitary gland (neurohypophysis)**, develops from a downgrowth of the hypothalamus. The neurohypophysis is divided into the median eminence, the infundibulum (continuation of the hypothalamus), and the pars nervosa (see Fig. 13–1) and is connected to the hypothalamus via the hypothalamohypophysial tract. The neurohypophysis and the median eminence are considered to be members of the **circumventricular organs (CVOs)**, similar to the pineal gland, subfornical organ, area postrema, and subcommissural organ, because their rich vascular supply with their fenestrated capillaries does not participate in the blood–brain barrier. The lack of the blood–brain barrier of CVOs allows macromolecules present in the bloodstream to contact the parenchymal cells of these organs; therefore, these organs can receive information from the blood stream that they can relay to other structures of the brain that otherwise could not be aware of this information. Additionally, the CVOs can secrete macromolecules into the vascular system to regulate various bodily functions.

Hypothalamohypophysial Tract

Axons of neurosecretory cells of supraoptic and paraventricular nuclei extend into the posterior pituitary as the hypothalamohypophysial tract.

Unmyelinated axons of neurosecretory cells enter the posterior pituitary to terminate in the vicinity of the capillaries, form the **hypothalamohypophysial tract**, and constitute the bulk of the posterior pituitary gland. The soma of these cells are located in the supraoptic and paraventricular nuclei and synthesize two hormones: **oxytocin** and **vasopressin (antidiuretic hormone**

[ADH]). Carrier proteins, **neurophysin I and neurophysin II**, also produced by the cells of these nuclei, bind to these hormones, respectively, as they travel down the axons to the posterior pituitary, where they are released into the bloodstream from the axon terminals. The neurosecretory cells of these two nuclei also release adenosine triphosphate (ATP) and acetylcholine, both of which secretory products use the hypothalamohypophysial tract to be transported into the pars nervosa.

Pars Nervosa

The pars nervosa of the posterior pituitary gland receives terminals of the neurosecretory hypothalamohypophysial tract.

Technically, the **pars nervosa** of the posterior pituitary gland is not an endocrine gland. The distal terminals of the axons of the **hypothalamohypophysial tract** (Fig. 13–5) end in the pars nervosa and store the neurosecretions that are produced by their cell bodies in the hypothalamus. These axons are supported by glia-like cells known as ***pituicytes***. Although only the nuclei of the pituicytes stain well enough to be evident by light microscopy, electron micrographs reveal that one population of axons contains membrane-bound granules of **vasopressin** and that another population contains **oxytocin**. As indicated previously, cell bodies of neurons that secrete vasopressin are located chiefly in the supraoptic nucleus of the hypothalamus, whereas cell bodies of neurons that secrete oxytocin are located mostly in the paraventricular nucleus of the hypothalamus. Each of these peptide hormones travels down the axons of its respective neurons in association with a precursor protein known as a *neurophysin I* or *neurophysin II*. By the time they reach the pars nervosa of the hypophysis, the hormones have matured and cleaved from their precursors Chrome-alum hematoxylin staining reveals blue-black distentions of the axons by light microscopy; these are called ***Herring bodies***, which represent accumulations of neurosecretory granules (see Fig. 13–5) not only at the termini but also along the length of the axons. In response to nerve stimulation, the contents of these granules are released into the perivascular space near the fenestrated capillaries of the capillary plexus.

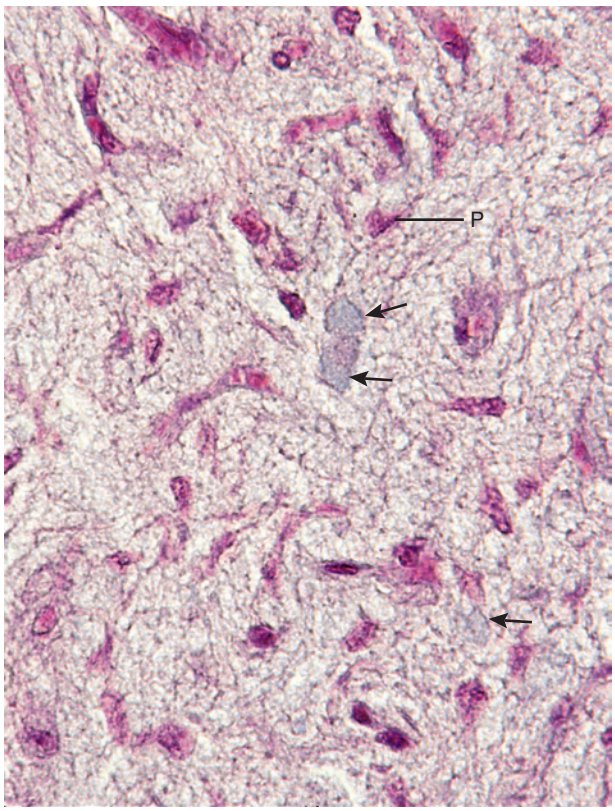


Figure 13–5 Light micrograph of the pars nervosa of the pituitary gland displaying pituicytes (P) and Herring bodies *(arrows)*. Herring bodies are the expanded terminals of the nerve fibers where the neurosecretory products vasopressin (antidiuretic hormone) and oxytocin are stored (×132).

The target for vasopressin (ADH) is the collecting ducts of the kidney, where it modulates plasma membrane permeability, which has the effect of lowering urine volume but increasing its concentration (see Chapter 19). The target for oxytocin is the myometrium of the uterus, where it is released in the late phases of pregnancy. During labor, oxytocin is believed to play a role in parturition by stimulating contraction of the smooth muscles of the uterus. Additionally, oxytocin functions in milk ejection from the mammary gland by stimulating contraction of the myoepithelial cells surrounding the glandular alveoli and the ducts of the mammary gland (see Chapter 20).

Pituicytes constitute about 25% of the volume of the pars nervosa. They are similar to neuroglial cells and help support the axons of the pars nervosa by ensheathing them as well as their dilatations. Pituicytes contain lipid droplets, lipochrome pigment, and intermediate filaments; they have numerous cytoplasmic processes that contact and form gap junctions with each other. Beyond supporting the neural elements in the pars nervosa, additional functions of pituicytes have not been elucidated. However, it is believed that they may contribute a trophic function to the normal operation of the neurosecretory axon terminals and neurohypophysis.

THYROID GLAND

The thyroid gland, located in the anterior portion of the neck, secretes the hormones thyroxine, triiodothyronine, and calcitonin.

The thyroid gland lies just inferior to the larynx, anterior to the junction of the thyroid and cricoid cartilages (Fig. 13–6). It is composed of a **right lobe** and a **left lobe**,

CLINICAL CORRELATIONS

Pituitary adenomas are common tumors of the anterior pituitary gland. Their growth and enlargement may suppress hormonal production in other secretory cells of the pars distalis. When left unattended, these adenomas may erode surrounding bone and other neural tissues.

Diabetes insipidus may be caused by lesions in the hypothalamus or pars nervosa that reduce the production of ADH by neurosecretory cells whose axon terminals are located in the neurohypophysis. This condition has nothing to do with the hormone insulin but results in renal dysfunction, which leads to inadequate water resorption by the kidneys, with a consequential polyuria (high urinary output) and dehydration. Unlike in diabetes mellitus where the urine tastes sweet (mellitus = honey) due to its high sugar content, the urine in diabetes insipidus is tasteless (insipidus = insipid).

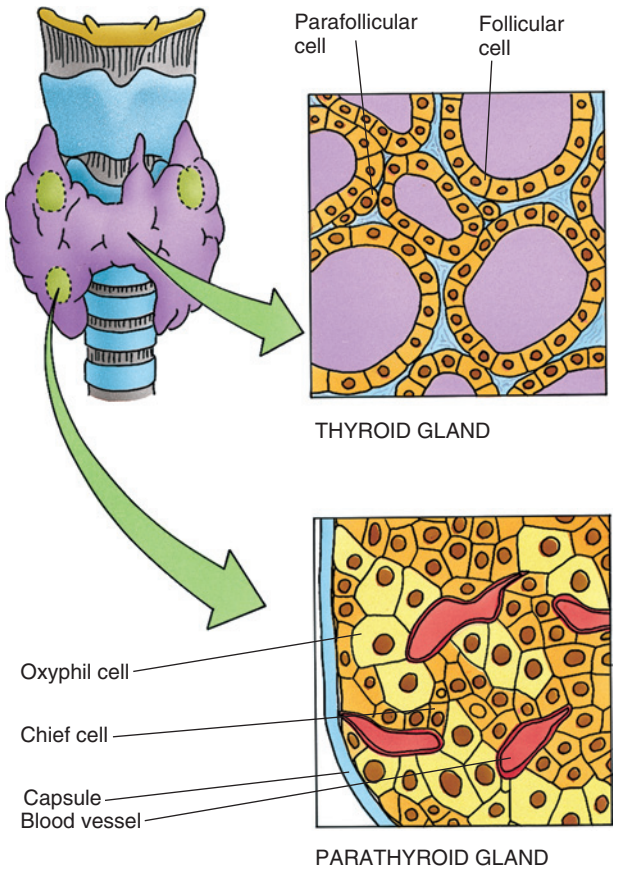


Figure 13–6 Schematic diagram of the thyroid and parathyroid glands.

which are connected across the midline by an **isthmus**. In some people, the gland has an additional **pyramidal lobe** that ascends from the left side of the isthmus. The pyramidal lobe is an embryological remnant of the path of descent of the thyroid primordium from its origin, an endodermal thickening of the foregut, through the forming tongue, by way of the thyroglossal duct.

The gland is surrounded by a slender, dense, irregular collagenous connective tissue capsule, a derivative of the deep cervical fascia. Septa derived from the capsule subdivide the gland into lobules. Embedded within the capsule, on the posterior aspect of the gland, are the parathyroid glands.

The thyroid gland produces thyroxin and triiodothyronine (**T4** and **T3**), the release of which are under the control of **TSH** (thyroid stimulating hormone) secreted by the anterior pituitary gland. Both T4 and T3 stimulate the rate of metabolism. Another hormone of the thyroid gland, **calcitonin**, aids in decreasing blood calcium levels and facilitates the storage of calcium in bones (Table 13–2).

Cellular Organization

The thyroid follicle is the structural and functional unit of the thyroid gland.

Unlike most of the endocrine glands, which store their secretory substances within the parenchymal cells, the thyroid gland stores its secretory substances in the lumina of **follicles** (Fig. 13–7). These cyst-like structures, ranging from 0.2 to 0.9 mm in diameter, are composed of a simple cuboidal epithelium, composed of **follicular cells** surrounding a central **colloid**-filled lumen and contacting the colloid as well as **parafollicular cells (clear cells; C cells)** that are located at the periphery of the follicle and do not contact the colloid. Parafollicular cells are derived from neural crest cells that migrate to the right and left 5th pharyngeal pouches and from there migrate into the developing thyroid gland. These parafollicular cells are considered to be members of the DNES cells. The hormones $\mathbf{T_4}$ and $\mathbf{T_3}$ are stored in the colloid bound to a large (660,000 D) secretory glycoprotein called ***thyroglobulin***. When the hormones are to be released, the hormone-bound thyroglobulin is endocytosed, by the follicular cells and the hormones are cleaved from it by lysosomal proteases.

Connective tissue septa derived from the capsule invade the parenchyma and provide a conduit for blood vessels, lymphatic vessels, and nerve fibers. These connective tissue septa surround 20 to 40 follicles, forming a lobule that is served by a single arteriole and each lobule acts independently from other lobules. Slender connective tissue elements, composed mostly of reticular fibers and housing a rich capillary plexus, arise from the connective tissue boundary of lobules to surround each follicle but are separated from the follicular and

TABLE 13–2 Hormones and Functions of the Thyroid, Parathyroid, Adrenal, and Pineal Glands

Hormone	Cell Source	Regulating Hormone	Function
Thyroid Gland			
Thyroxine (T_4) and triiodothyronine (T_3)	Follicular cells	Thyroid-stimulating hormone (TSH)	Facilitate nuclear transcription of genes responsible for protein synthesis; increase cellular metabolism and growth rates; facilitate mental processes; increase endocrine gland activity; stimulate carbohydrate and fat metabolism; decrease cholesterol, phospholipids, and triglycerides; increase fatty acids; decrease body weight; increase heart rate, respiration, muscle action
Calcitonin (thyrocalcitonin)	Parafollicular cells	Feedback mechanism with parathyroid hormone	Lowers plasma calcium concentration by suppressing bone resorption
Parathyroid Gland			
Parathyroid hormone (PTH)	Chief cells	Feedback mechanism with calcitonin	Increases calcium concentration in body fluids
Suprarenal (Adrenal) Glands			
Suprarenal Cortex			
Mineralocorticoids: aldosterone and deoxycorticosterone	Cells of the zona glomerulosa	Angiotensin II and adrenocorticotropic hormone (ACTH)	Control body fluid volume and electrolyte concentrations by acting on distal tubules of the kidney, causing excretion of potassium and resorption of sodium
Glucocorticoids: cortisol and corticosterone	Cells of the zona fasciculata (spongiocytes)	ACTH	Regulate metabolism of carbohydrates, fats, and proteins; decrease protein synthesis, increasing amino acids in blood; stimulate gluconeogenesis by activating liver to convert amino acids to glucose; release fatty acid and glycerol; act as anti-inflammatory agents; reduce capillary permeability; suppress immune response
Androgens: dehydroepiandrosterone and androstenedione	Cells of the zona reticularis	ACTH	Provides weak masculinizing characteristics
Suprarenal Medulla			
Catecholamines: epinephrine and norepinephrine	Chromaffin cells	Preganglionic, sympathetic, and splanchnic nerves	*Epinephrine* Operates the "fight or flight or freeze" mechanism preparing the body for severe fear or stress; increases cardiac heart rate and output, augmenting blood flow to the organs and release of glucose from the liver for energy *Norepinephrine* Causes an elevation in blood pressure by vasoconstriction
Pineal Gland			
Melatonin	Pinealocytes	Norepinephrine	May influence cyclic gonadal activity

parafollicular cells by a thin **basal lamina**. Occasionally, follicular cells of neighboring follicles may come into contact with each other and disrupt the continuity of the basal lamina.

Follicular Cells (Principal Cells)

Follicular (principal) cells are squamous to low-columnar in shape.

Follicular cells range from squamous to low columnar in shape and are tallest when stimulated. These cells have a round to ovoid nucleus with two nucleoli, basophilic cytoplasm. Frequently, their RER is distended and displays zones that are ribosome free. These cells also have numerous apically located lysosomes, rod-shaped mitochondria, a supranuclear Golgi complex, and numerous short microvilli that extend into the colloid (Fig. 13–8). Numerous small vesicles, dispersed

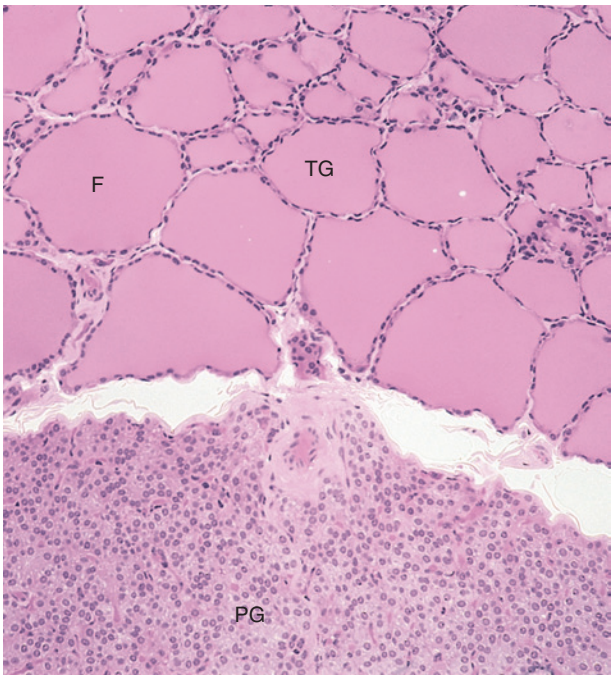


Figure 13–7 Light micrograph of the thyroid and parathyroid glands. Observe the colloid-filled follicles (F) of the thyroid gland (TG) in the upper portion of the figure. The lower portion of this figure is the parathyroid gland (PG), as evidenced by the presence of chief and oxyphil cells (×132).

throughout the cytoplasm, are believed to contain thyroglobulin that was packaged in the Golgi complex and is destined for exocytosis into the follicle lumen. **Iodide** is essential for the synthesis of the thyroid hormones (T_3 and T_4); iodination of tyrosine residues occurs in the follicles at the colloid–follicular cell interface.

During great demand for thyroid hormone, follicular cells extend pseudopods into the follicles to envelop and absorb the colloid. When demand for the hormone declines, the amount of colloid in the follicle lumen increases.

Synthesis of Thyroid Hormones (T_3 and T_4)

Thyroid hormone synthesis is regulated by iodide levels and by TSH binding to TSH receptors of follicular cells.

The synthesis of thyroid hormone is regulated by the iodide levels in the follicular cell as well as by the binding of TSH to TSH receptors of the follicular cells. The occupation of TSH receptors triggers cAMP production, resulting in protein kinase A activity and synthesis of T_3 and T_4.

Figure 13–9 outlines the pathway for the synthesis and release of thyroid hormones. Thyroglobulin is synthesized in the RER and subsequently glycosylated in both the RER and the Golgi apparatus. The modified protein is packaged in the *trans* Golgi network. The vesicles containing thyroglobulin are transported to the

Figure 13–8 Electron micrograph of a thyroid follicular cell bordering the colloid *(black area, upper left hand corner)* (×10700). (From Mestdagh C, Many MC, Haalpern S, et al. Correlated autoradiographic and ion-microscopic study of the role of iodine in the formation of "cold" follicles in young and old mice. *Cell Tissue Res*. 1990;260:449-457.)

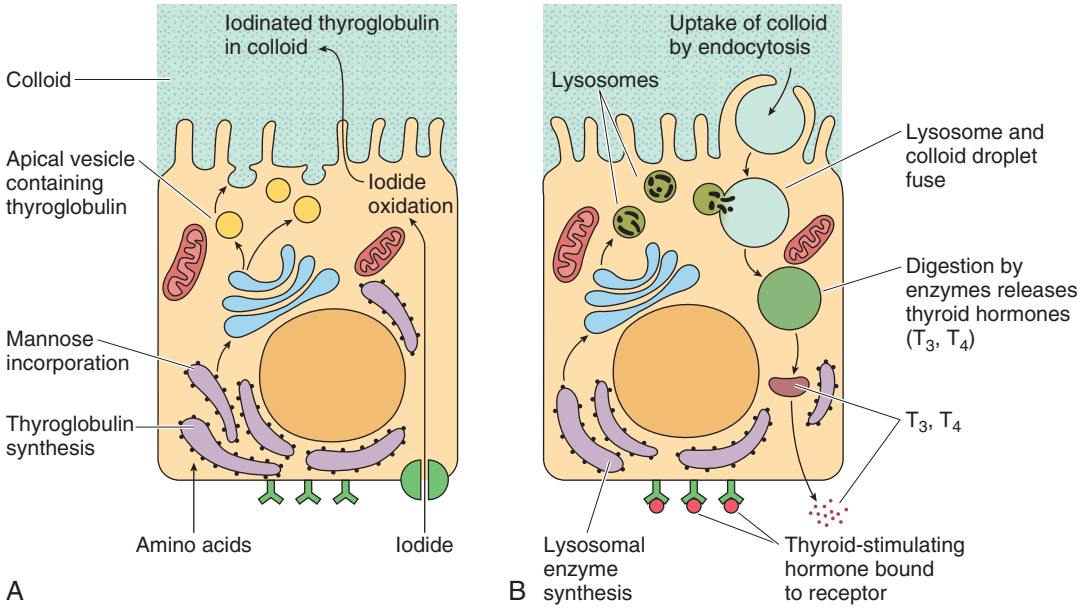


Figure 13–9 Schematic diagram of the synthesis and iodination of thyroglobulin **(A)** and release of thyroid hormone **(B)**.

apical plasmalemma, where their contents are released into the colloid and stored in the lumen of the follicle.

Iodine is reduced to iodide (I^-) within the alimentary canal and is preferentially absorbed and transported by the bloodstream to the thyroid gland. Iodide is actively transported via sodium/iodide symporters, which are located in the basal plasmalemma of the follicular cells, so that the intracellular iodide concentration is 20- to 40-fold that of plasma. Once in the cytosol, iodide is transferred out of the follicular cell to the colloid-cell membrane interface via an iodide/chloride transporter, known as ***pendrin***. While the iodide is being transferred, noniodinated **thyroglobulin**, along with **thyroid peroxidase**, also leaves the follicular cell to enter the colloid. At the colloid–follicular cell interface iodide is oxidized by thyroid peroxidase, a process that requires the presence of hydrogen peroxide (H_2O_2). The activated iodide enters the colloid and iodinates tyrosine residues of thyroglobulin still at the interface of the colloid and the apical plasmalemma of the thyroid follicular cell. Tyrosine residues of thyroglobulin are iodinated, forming **monoiodinated tyrosine** (MIT) and **diiodinated tyrosine** (DIT). Triiodinated and tetraiodinated tyrosines are then formed by the coupling of an MIT and a DIT or by the coupling of two DITs, respectively. Each thyroglobulin molecule has fewer than four T_4 molecules and fewer than 0.3 T_3 residues. The iodinated thyroglobulin is released by the follicular cells to be stored in the colloid.

Release of Thyroid Hormones (T_3 and T_4)

Thyroid-stimulating hormone stimulates follicular cells of the thyroid gland to release T_3 and T_4 into the bloodstream.

TSH, released from the basophils of the anterior pituitary, binds to TSH receptors on the basal plasmalemma of the follicular cells. Binding of TSH facilitates an alteration in the morphology of the follicular cells in that these cuboidal cells become tall columnar cells that form filopodia at their apical cell membrane, effecting endocytosis of aliquots of the colloid. Cytoplasmic vesicles containing colloid fuse with early (or late) endosomes. Within the endosomes, iodinated residues are cleaved from thyroglobulin by proteases and are transferred into the cytosol as free monoiodotyrosine, diiodotyrosine, T_3, and T_4.

Monoiodotyrosine and diiodotyrosine are stripped of their iodine by the enzyme **iodotyrosine dehalogenase**, and both the iodine and the amino acid tyrosine become part of their respective pools within the cytosol for later use.

T_3 and T_4 are released at the basal plasmalemma of the follicular cells, entering the connective tissue spaces of the thyroid for distribution by the bloodstream. Once in the bloodstream, both T_3 and T_4 bind to the carrier protein, thyroxine-binding globulin, to be transported to their target cells. It should be noted that both T_3 and

T_4 can bind to other carrier proteins, namely **transerythrin**, **albumin**, and some **lipoproteins**. Transerythrin is responsible for ferrying T_3 and T_4 to the cerebrospinal fluid; albumin binds approximately 10% of the thyroid hormones, and lipoproteins bind approximately 5% of the thyroid hormones to transport them to their target cells. T_4 constitutes about 90% of the released hormone, and although it has a half-life of about 6 days, it is not as effective as T_3, which has a half-life of only 1 day.

CLINICAL CORRELATIONS

Graves disease is characterized by hyperplasia of the follicular cells, increasing the size of the thyroid gland two to three times above normal. Thyroid hormone production is also greatly increased from 5 to 15 times normal **(hyperthyroidism)**. Other symptoms include **exophthalmos**, or protrusion of the eyeballs. Although Graves disease may develop from several causes, the most common agent is the binding of autoimmune immunoglobulin G (IgG) antibodies to TSH receptors, which stimulates thyroid follicular cells.

Insufficient dietary intake of iodine causes the thyroid gland to enlarge, a condition called ***simple goiter***. Goiter usually is not associated with hyperthyroidism or hypothyroidism. This condition can be treated with iodine in the diet.

Hypothyroidism is characterized by such conditions as fatigue, sleeping for up to 14 to 16 hours a day, muscular sluggishness, slowed heart rate, decreased cardiac output and blood volume, mental sluggishness, failure of body functions, constipation, and loss of hair growth. Patients with severe hypothyroidism may develop **myxedema**, which is characterized by bagginess under the eyes and a swollen face that is due to nonpitting edema of the skin and infiltration of excess glycosaminoglycans and proteoglycans into the extracellular matrix. **Cretinism** is an extreme form of hypothyroidism in fetal life through childhood that is characterized by failure of growth and mental retardation due to a congenitally missing thyroid gland.

The nerves supplying the laryngeal musculature (i.e., external laryngeal and recurrent laryngeal nerves) are closely applied to the thyroid gland and must be isolated and protected during **thyroidectomy**. Damage to either of these two nerves results in hoarseness and, possibly, loss of speech.

Physiological Effects of Triiodothyronine and Thyroxine

As indicated previously, once they enter the bloodstream **T_3** and **T_4** bind to plasma-binding proteins and are slowly released to the tissues and contact their target cells and pass through the cell membranes of these cells. Transport across the target cell membrane is effected by a number of transporter proteins, including **monocarboxylate transporter 8 (MTC8)** in the body and **organic anion transporter polypeptide 1C1 (OATP1C1)** in the brain. As they enter the target cell cytoplasm, they are bound to intracellular proteins and slowly used over a period of several days to weeks. Because only the free hormone has the ability to enter the cell and because T_3 is bound less avidly to transerythrin than does T_4, more T_3 gains entry into the cytoplasm than does T_4. Once in the target cell, both T_3 and T_4 bind to **nuclear thyroid hormone receptor proteins**, but T_3 binds with a much greater affinity than does T_4, which also accounts for the greater biological activity of T_3. The bound T_3 and T_4 enter the nucleus, where they stimulate transcription of many genes that encode various types of proteins (see Table 13–2), resulting in a generalized increase in cellular metabolism that may be as great as twice the resting rate. T_3 and T_4 also increase the growth rate in the young, facilitate mental processes, and stimulate endocrine gland activity.

Generally, thyroid hormones stimulate carbohydrate metabolism. They decrease synthesis of cholesterol, phospholipids, and triglycerides but increase synthesis of fatty acids and the uptake of various vitamins. Increased thyroid hormone production also decreases body weight and increases heart rate, metabolism, respiration, muscle function, and appetite. Excessive amounts of thyroid hormone cause muscle tremor, tiredness, impotence in men, and reduced or absence of menstrual bleeding in women.

CLINICAL CORRELATIONS

Individuals possessing a mutated form of the monocarboxylate transporter 8 (MTC8) have an X-linked hereditary condition known as the **Allen-Herndon-Dudley syndrome**. This rare condition, affecting almost entirely males, is distinguished by high T_3 serum levels as well as by a number of developmentally caused neurological conditions, including severe intellectual disability, spastic movement, weak muscle tone, joint malformations, and speech impairment.

Parafollicular Cells (Clear Cells, C Cells)

Parafollicular cells secrete calcitonin; they are found individually or may form small clusters of cells at the periphery of the follicle.

The pale-staining **parafollicular cells (clear cells; C cells)** lie singly or in clusters among the follicular cells, but they do not reach the lumen of the follicle. Although these cells are two to three times larger than follicular cells, they account for only about 0.1% of the follicular

epithelium. Electron micrographs display a round nucleus; moderate RER; elongated mitochondria; a well-developed Golgi complex; and small, dense secretory granules (0.1 to 0.4 μm in diameter) located in the basal cytoplasm. These secretory granules contain **calcitonin (thyrocalcitonin)**, a peptide hormone that inhibits bone resorption by osteoclasts, thereby lowering calcium concentrations in blood. When the circulating level of calcium is high, release of calcitonin is stimulated (see Chapter 7).

PARATHYROID GLANDS

The absence of parathyroid glands is incompatible with life because parathyroid hormone (PTH) regulates blood calcium levels.

The **parathyroid glands**, usually four in number, are located on the posterior surface of the thyroid gland; each gland is enveloped in its own thin, collagenous connective tissue capsule (see Fig. 13–6). The glands function in producing **PTH (parathormone)**, which acts on bone, kidneys, and intestines to increase blood calcium levels (see below, the section on parathyroid hormone).

Normally, one parathyroid gland is located on both poles (superior and inferior) of the right and left lobes of the thyroid gland. Because of their embryological origin and descent in the neck with the primordium of the thymus and thyroid tissues, the parathyroid glands may be located anywhere along the pathway of descent, even in the thorax, and there may also be supernumerary glands.

The parathyroid glands develop from the third and fourth pharyngeal pouches during embryogenesis. The parathyroid glands that develop in the third pharyngeal pouches descend with the thymus (also developing in the third pouches) to become the **inferior parathyroid glands**. The parathyroid glands that develop in the fourth pharyngeal pouches descend only a short distance to become the **superior parathyroid glands**. The glands grow slowly, reaching the adult size at about 20 years of age.

Parathyroid Cellular Organization

The parenchyma of the parathyroid glands consists of two cell types: chief cells and oxyphil cells.

Each parathyroid gland is a small, ovoid structure about 5 mm in length, 4 mm wide, and 2 mm in thickness and weighs about 25 to 50 mg. Extensions of the connective tissue capsule enter the gland as septa, accompanied by blood vessels, lymphatics, and nerves. The septa serve mainly to support the parenchyma but also to support a rich capillary network for the parenchymal cells that are arranged in cords or clusters of cells. The connective tissue stroma in older adults often contains several to many adipose cells, which may occupy up to 60% of the gland. The parenchyma of the parathyroid glands is composed of two cell types: **chief cells** and **oxyphil cells** (see Fig. 13–7).

Chief Cells

Chief cells synthesize parathyroid hormone (PTH).

The major functional parenchymal cells of the parathyroid glands are the slightly eosinophilic-staining **chief cells** (5 to 8 μm in diameter), which contain granules of lipofuscin pigment that is scattered throughout the cytoplasm. Smaller, dense granules, 200 to 400 nm in diameter, arising from the Golgi complex and moving to the cell periphery, represent the secretory granules and contain **PTH**. Electron micrographs also reveal a juxtanuclear Golgi complex, elongated mitochondria, and abundant RER. Occasionally, desmosomes join adjacent chief cells to each other. A single primary cilium may extend into the intercellular space. Some chief cells have a smaller Golgi complex, scant secretory granules, and large amounts of glycogen; these cells are thought to be in an inactive phase.

Chief cells function in manufacturing, storing, and rapidly releasing PTH in response to alterations of blood calcium levels. When the need arises, these cells can enter the cell cycle and increase their population. These properties of chief cells ensure that the parathyroid gland can respond to immediate, intermediate, and long-term adjustments to blood calcium level alterations and calcium homeostasis.

The precursor, **preproparathyroid hormone**, is synthesized by chief cells on ribosomes of the RER and rapidly cleaved as it is transported to the lumen of the RER to form **proparathyroid hormone** and a polypeptide. On reaching the Golgi complex, proparathyroid hormone is cleaved again into PTH and a small polypeptide. The hormone is packaged into secretory granules and is released from the cell surface by exocytosis. The release of PTH is dependent on a cell membrane calcium receptor, known as the ***transmembrane calcium receptor (CaSR)***, which is bound to a G protein. If CaSR has calcium bound to it, indicating that the blood calcium level is adequate, CaSR compels the G protein to inhibit PTH release. However, if calcium is not bound to CaSR, indicating an inadequate blood calcium levels, G protein becomes activated, and PTH is released by the chief cells (see Table 13–2).

Oxyphil Cells

Oxyphil cells are believed to be the inactive phase of chief cells.

The second cell type located in the parathyroid glands is the **oxyphil cell**. Its function is unknown, although

it is believed that oxyphil cells and a third cell, described as an **intermediate cell**, probably represent inactive phases of a single cell type, with chief cells being the actively secreting phase.

Oxyphil cells are less numerous, larger (6 to 10 μm in diameter), and more deeply stained with eosin than chief cells; they appear in groups and as isolated cells. They have more abundant mitochondria than do chief cells, but their Golgi apparatus is small, and there is little RER. Glycogen is also located in the cytosol and is surrounded by mitochondria.

Physiological Effect of Parathyroid Hormone

PTH helps maintain the proper extracellular fluid as well as the plasma concentration of calcium ions (8.5 to 10.5 mg/dL). This hormone acts on cells of the bones; the kidneys; and, indirectly, the intestines, leading to an increased calcium ion concentration in body fluids (see Table 13–2). When calcium ion concentration in body fluids falls below normal, the chief cells increase their production and release of PTH, quickly increasing their normal secretion rate 10-fold. This rapid response is especially important because of the many functions of calcium in homeostasis, including its role in stabilizing ion gradients across the plasmalemmae of muscle and nerve cells and its role in the release of neurotransmitter at axon terminals.

The interplay of PTH and calcitonin represents a dual mechanism for regulating calcium levels in the blood: PTH acts to increase serum calcium levels, and calcitonin has the opposite effect.

In bone, PTH binds to receptors on osteoblasts, signaling the cells to increase their secretion of **osteoclast-stimulating factor**. This factor induces activation of osteoclasts, thereby increasing bone resorption and the ultimate release of calcium ions into the blood (see Chapter 7). In the kidneys, PTH prevents loss of calcium in the urine and prompts the proximal tubule cells to manufacture and release **calcitriol** (the physiologically active form of **vitamin D**). Finally, PTH controls the rate of calcium uptake in the gastrointestinal tract because vitamin D is necessary for intestinal uptake of calcium. Vitamin D functions to stimulate the intestinal mucosa to reabsorb calcium by inducing epithelial cells of the intestinal villus to form calcium-binding protein that becomes localized on the microvilli that facilitates the transport of calcium into the epithelial cells.

CLINICAL CORRELATIONS

1. A condition called ***primary hyperparathyroidism***, which may be caused by a tumor in one of the parathyroid glands, is marked by high blood calcium levels, low blood phosphate levels, loss of bone mineral, and sometimes kidney stones. **Secondary hyperparathyroidism** may develop in patients with **rickets**, because calcium cannot be absorbed from the intestines due to vitamin D deficiency; therefore, calcium ion concentrations in the blood are low.
2. **Hypoparathyroidism** results from deficiency in secretion of PTH, commonly caused by injury of the parathyroid glands or by their removal during thyroid gland surgery. This condition is marked by low blood calcium levels, retention of bone calcium, and increased phosphate resorption in the kidney. The main symptoms are numbness, tingling, **carpopedal spasms** (muscle cramps) in the hands and feet, **muscle tetany** (tremors) in the facial and laryngeal muscles, mental confusion, and memory loss. The only treatment for survival is large intravenous doses of calcium gluconate, vitamin D, and oral calcium.
3. Care must be taken during surgical removal of the thyroid gland to save the parathyroid glands and to maintain their blood supply because absence of the parathyroid glands is incompatible with life.

SUPRARENAL (ADRENAL) GLANDS

Suprarenal glands produce two different groups of hormones: steroids and catecholamines.

The **suprarenal (adrenal) glands** are located at the superior poles of the kidneys and are embedded in adipose tissue. The right and left suprarenal glands are not mirror images of each other; rather, the right suprarenal gland is pyramid shaped and sits directly on top of the right kidney, and the left suprarenal gland is more crescent shaped and lies along the medial border of the left kidney from the hilus to its superior pole.

Both glands are about 1 cm in thickness, 2 cm in width at the apex, and up to 5 cm at the base; each weighs 7 to 10 g. The parenchyma of the gland is divided into two histologically and functionally different regions: an outer yellowish portion, accounting for about 80% to 90% of the organ, called the ***suprarenal cortex*** and a small, dark, inner portion called the ***suprarenal medulla*** (Fig. 13–10). Although both entities are endocrine in function, each develops from a different embryological origin and performs a different role. The **suprarenal cortex**, arising from mesoderm, produces a group of hormones called ***corticosteroids***, which are synthesized from **cholesterol**. Secretion of these hormones, including **cortisol** and **corticosterone**, is regulated by **ACTH**, a hormone secreted by the anterior pituitary gland. The **suprarenal medulla**, arising from neural crest, is functionally related to and regulated by the sympathetic nervous system; it produces the hormones **epinephrine** and **norepinephrine** (see Table 13–2).

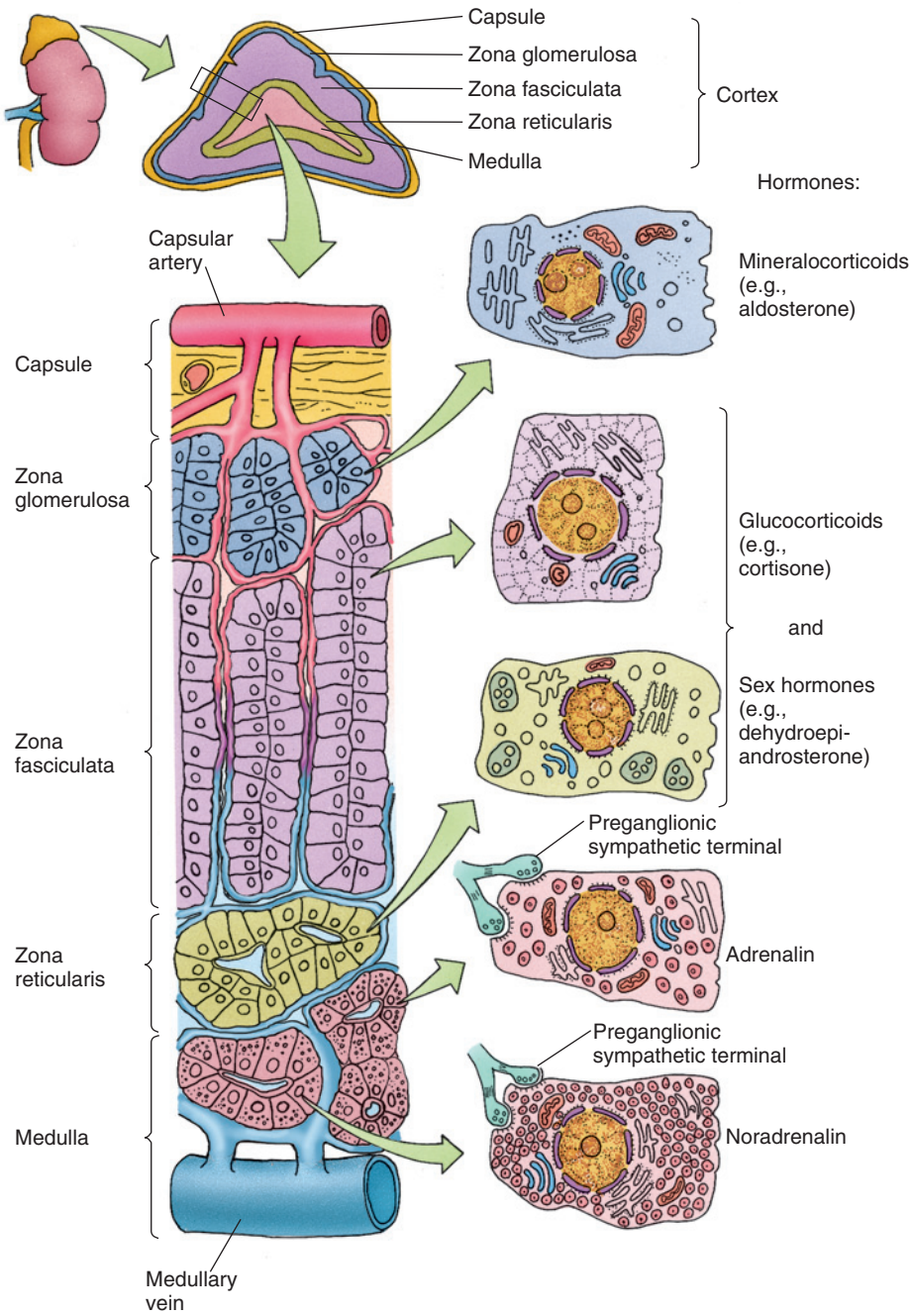


Figure 13–10 Schematic diagram of the suprarenal gland and its cell types.

The suprarenal glands are retroperitoneal, located behind the peritoneum, and surrounded by a connective tissue capsule that contains large amounts of adipose tissue. Each gland has a thick capsule of connective tissue that sends septa into the parenchyma of the gland, accompanied by blood vessels and nerves.

Blood Supply to the Suprarenal Gland

Arteries from three separate sources provide the suprarenal glands with an abundant blood supply.

The suprarenal glands have one of the richest blood supplies in the body (Fig. 13–11). Each suprarenal

Figure 13–11 Scanning electron micrograph of the rat adrenal gland demonstrating microcirculation in the cortex and medulla (×80). (From Kikuta A, Murakami T. Microcirculation of the rat adrenal gland: a scanning electron microscope study of vascular casts. *Am J Anat*. 1982;164:19-28. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

gland is served by three separate arteries that arise from three separate sources:

- The inferior phrenic arteries, from which the superior suprarenal arteries originate
- The aorta, from which the middle suprarenal arteries originate
- The renal arteries, from which the inferior suprarenal arteries originate

These branches pass over the capsule, penetrate it, and form a **subcapsular plexus**.

Arising from the plexus are **short cortical arteries**, which, in the cortical parenchyma, form a network of sinusoidal fenestrated capillaries (with diaphragms). The pore diameters of the fenestrated endothelial walls of the capillaries increase from 100 nm at the outer cortex to 250 nm in the deep cortex, where the sinusoidal capillaries become confluent with a venous plexus. Small venules arising from this area pass through the suprarenal medulla and drain into a suprarenal vein, emerging from the hilus. The right suprarenal vein joins the inferior vena cava, and the left suprarenal vein drains into the left renal vein.

Additional **long cortical arteries** pass unbranched through the cortex and into the medulla, where they form networks of capillaries. Thus, the medulla receives a dual blood supply (1) an arterial supply from the long cortical arteries and (2) numerous vessels from the cortical capillary beds.

Suprarenal Cortex

The suprarenal cortex is subdivided into three zones that produce three classes of steroids.

The **suprarenal cortex** contains parenchymal cells that synthesize and secrete several steroid **hormones** without storing them. The cortex is subdivided histologically into three concentric zones named, from the capsule inward, the **zona glomerulosa**, the **zona fasciculata**, and the **zona reticularis** (Fig. 13–12; see Fig. 13–10). Although each of the three identified zones of the suprarenal

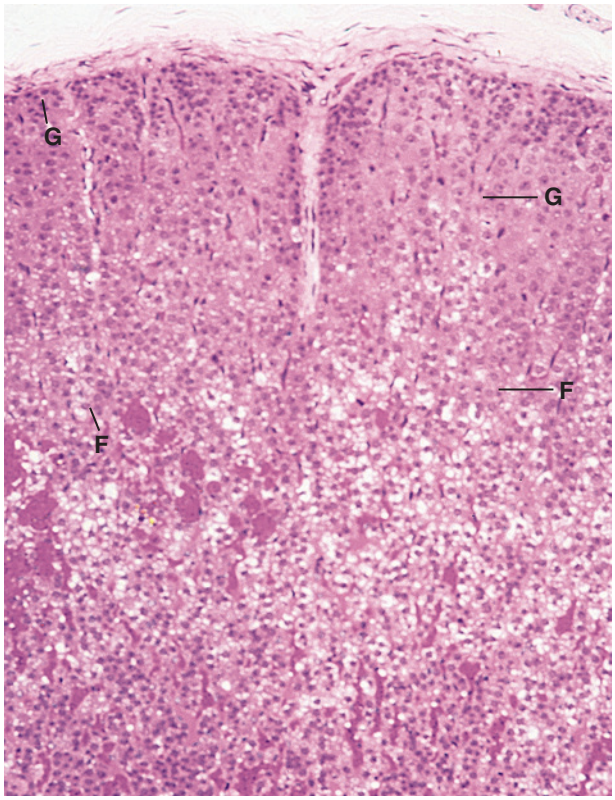


Figure 13–12 Light micrograph of the cortex of the suprarenal gland (×132). Observe the zona glomerulosa (G) and the zona fasciculata (F).

cortex is said to secrete specific hormones, it should be remembered that the boundaries between these zones overlap, thus it is better to remember the cortex as a secreting unit for the three classes of adrenocortical hormones (of course, the student's instructor may deem otherwise, in which case it is in the student's best interest to follow the instructor's point of view).

The three classes of adrenocortical hormones–**mineralocorticoids**, **glucocorticoids**, and **androgens**—are all synthesized from **cholesterol**, the major component of **low-density lipoprotein**. Cholesterol is taken up from the blood and stored esterified in lipid droplets within the cytoplasm of the cortical cells. When these cells are stimulated, cholesterol is freed and used in hormone synthesis in the **smooth endoplasmic reticulum (SER)** by enzymes that are located there and in the mitochondria. Cholesterol and the intermediate products of the hormone that is being synthesized are transferred between the SER and the mitochondria until the final hormone is produced. Therefore, the synthesis of these hormones depends on the ability of cholesterol and the intermediate products to enter the mitochondria. For this to occur, a regulatory protein, **steroidogenic acute regulatory protein** has to be present to transport these molecules to the inner mitochondrial membrane.

Zona Glomerulosa

Parenchymal cells of the zona glomerulosa, when stimulated by angiotensin II and ACTH, synthesize and release the hormones aldosterone and deoxycorticosterone.

The outer concentric ring of capsular parenchymal cells, located just beneath the suprarenal capsule, is the **zona glomerulosa**, which constitutes approximately 13% of the total adrenal volume (see Fig. 13–10). The small columnar cells composing this zone are arranged in cords and clusters. Their small, dark-staining nuclei contain one or two nucleoli, and their acidophilic cytoplasm contains an abundant and extensive SER, short **mitochondria** with **shelf-like cristae**, a well-developed Golgi complex, abundant RER, and free ribosomes. Some lipid droplets also are dispersed in the cytoplasm. Occasional desmosomes and small gap junctions join cells to each other, and some cells have short microvilli.

The parenchymal cells of the zona glomerulosa synthesize and secrete the **mineralocorticoid hormones**, principally **aldosterone** and some **deoxycorticosterone**. Synthesis of these hormones is stimulated by **angiotensin II** and **ACTH**, both required for normal existence of glomerulosa cells. The mineralocorticoid hormones function in controlling fluid and electrolyte balance in the body by affecting the function of the renal tubules, specifically the distal convoluted tubules (see Chapter 19).

Progenitor cells located at the interface of the zona glomerulosa and zona fasciculata sustain the size and cell population of the adrenal cortex by entering the cell cycle and produce more parenchymal cells of the adrenal cortex to replenish senescent and dying cells.

Zona Fasciculata

Parenchymal cells of the zona fasciculata (spongiocytes), when stimulated by ACTH, synthesize and release the hormones cortisol and corticosterone.

The intermediate concentric layer of cells in the suprarenal cortex is the **zona fasciculata**, the largest layer of the cortex, which accounts for up to 80% of the total volume of the gland. This zone contains sinusoidal capillaries that are arranged longitudinally between the columns of parenchymal cells. The polyhedral cells in this layer are larger than the cells of the zona glomerulosa and are arranged in radial columns, one to two layers thick, and stain lightly acidophilic. Because they have many lipid droplets in their cytoplasm, which are extracted during histological processing, these cells appear vacuolated and are called ***spongiocytes***. These cells have **spherical mitochondria** with **tubular** and **vesicular cristae**, extensive networks of SER, some RER, lysosomes, and granules of lipofuscin pigment.

Cells of the zona fasciculata synthesize and secrete the **glucocorticoid hormones**, **cortisol** and **corticosterone**. The synthesis of these hormones is stimulated by ACTH. Glucocorticoids function in the control of carbohydrate, fat, and protein metabolism.

Zona Reticularis

The cells of the zona reticularis, when stimulated by ACTH, synthesize and release dehydroepiandrosterone, androstenedione, and some glucocorticoids.

The innermost layer of the suprarenal cortex is the **zona reticularis**, constituting about 7% of gland volume. The darkly staining acidophilic cells in this layer are arranged in anastomosing cords. They are similar to the spongiocytes of the zona fasciculata but are smaller with fewer lipid droplets. They frequently contain large amounts of lipofuscin pigment granules. Several cells near the suprarenal medulla are dark with electron-dense cytoplasm and pyknotic nuclei, which suggests that this zone contains degenerating parenchymal cells.

Cells of the zona reticularis synthesize and secrete **androgens**, principally **dehydroepiandrosterone (DHEA)** and some **androstenedione**. Additionally, cells of the zona reticularis may synthesize and secrete small amounts of glucocorticoids. The secretion of these hormones is stimulated by ACTH. Both DHEA and androstenedione are weak, masculinizing hormones with negligible effects under normal conditions.

Histophysiology of the Suprarenal Cortex

As indicated previously, the three classes of hormones that are secreted by the suprarenal cortex are the steroids: (1) mineralocorticoids, (2) glucocorticoids, and (3) weak androgens. ACTH from the pars distalis of the pituitary is the trophic hormone that stimulates secretion of the suprarenal cortex hormones along with angiotensin II for the secretion of mineralocorticoids.

Mineralocorticoids

The **mineralocorticoids**, secreted by the zona glomerulosa, include **aldosterone** predominantly and some deoxycorticosterone. The targets of these hormones include the gastric mucosa, salivary glands, and sweat glands, where they stimulate absorption of sodium. However, the main target is the cells of the distal convoluted tubules of the kidney, where they function to stimulate the regulation of water balance and homeostasis of sodium and potassium by absorbing sodium and excreting potassium, a function regulated mostly by angiotensin II (see Chapter 19 on the urinary system).

Glucocorticoids

Glucocorticoids, secreted by the zona fasciculata, include **hydrocortisone (cortisol)** and **corticosterone**. These steroid hormones have a wide range of functions that affect most tissues of the body as well as control general metabolism. This group of hormones exerts an **anabolic** effect in the liver that promotes the uptake of fatty acids, amino acids, and carbohydrates for glucose synthesis and glycogen polymerization; in other tissues, however, the effect is **catabolic**. For example, in adipocytes, glucocorticoids stimulate **lipolysis**, and in muscle, these hormones stimulate **proteolysis**. Glucocorticoids, when circulating at above normal levels, also influence anti-inflammatory responses by inhibiting macrophage and leukocyte infiltration at sites of inflammation. These hormones also suppress the immune response by inducing atrophy of the lymphatic system, thereby reducing the circulating lymphocyte population. Table 13–3 lists some of the deleterious effects of excess blood levels of glucocorticoids.

TABLE 13–3 Deleterious Effects of Excess Blood Glucocorticoid Levels

Body/System/Organ/Tissue/Activity	Effect
Brain	Psychosis and depression
Immune system	Suppression of immune system; anti-inflammatory response
Stature	Decrease in height
Endocrine system	Depressed levels of luteinizing hormone, follicle-stimulating hormone, growth hormone, and thyroid-stimulating hormone
Gastrointestinal tract	Formation of peptic ulcers
Adipose tissue	Induces fat deposition in the viscera
Eye	Induces glaucoma
Calcium metabolism	Decreases bone formation and bone mass
Skin and muscle	Induces collagen proteolysis in skin; induces muscle atrophy
Cardiovascular	Hypertension
Kidney	Induces salt and water preservation
Carbohydrate metabolism	Increases gluconeogenesis in liver; increases peripheral insulin resistance, thereby promoting diabetes

The **negative feedback** mechanism for the glucocorticoids is partially controlled by their plasma concentration. When blood glucocorticoid levels are high, **CRH (corticotropin releasing hormone)** cells of the hypothalamus are inhibited, which in turn inhibits **corticotrophs** of the pars distalis of the pituitary gland from releasing ACTH.

Weak Androgens

Androgens secreted by the zona reticularis include **dehydroepiandrosterone** and **androstenedione**, both weak, masculinizing sex hormones that are only a small fraction as effective as the androgens that are produced in the testes. Under normal conditions, the influence of these hormones is insignificant in males but could have a masculinizing effect in females.

CLINICAL CORRELATIONS

1. **Addison disease** is characterized by decreased secretion of the adrenocortical hormones as a result of destruction of the suprarenal cortex. This disease is most often caused by an autoimmune process; it also can develop as a sequela of tuberculosis or from some other infectious diseases. Death occurs if steroid treatment is not provided.
2. **Cushing disease (hyperadrenocorticism)** is caused by small tumors in the basophils of the anterior pituitary gland that lead to an increase in the output of **ACTH**. The excess ACTH causes enlargement of the suprarenal glands and hypertrophy of the suprarenal cortex, resulting in overproduction of cortisol. Patients are obese, predominantly in the face, neck, and trunk, and exhibit osteoporosis and muscle wasting. Men become impotent, and women have amenorrhea.
3. Primary adrenal carcinoma, a very rare disease affects more than twice as many women than men. Usually the cancer arises in individuals at least 40 years of age, and the cancerous cells are hormone producing: about 45% synthesize only glucocorticoids, 45% percent form both glucocorticoids and androgens, and 10% release androgens only. Only approximately 1% of the cancerous cells release aldosterone. Unfortunately, by the time the cancer is recognized metastasis has occurred, and even with surgery and subsequent chemotherapy, the 5-year mortality rate is 80%.

Suprarenal Medulla

Chromaffin cells of the suprarenal medulla are modified postganglionic neurons that have a secretory function.

The central portion of the suprarenal gland, the **suprarenal medulla**, is completely invested by the suprarenal cortex and forms only 10% of the volume of the suprarenal gland. The suprarenal medulla, which develops from ectodermal neural crest cells, comprises two populations of parenchymal cells: **chromaffin cells** (Fig. 13–13), which produce the **catecholamines** (**epinephrine** and **norepinephrine**), and **sympathetic ganglion cells**, which are scattered throughout the connective tissue (see Table 13–2).

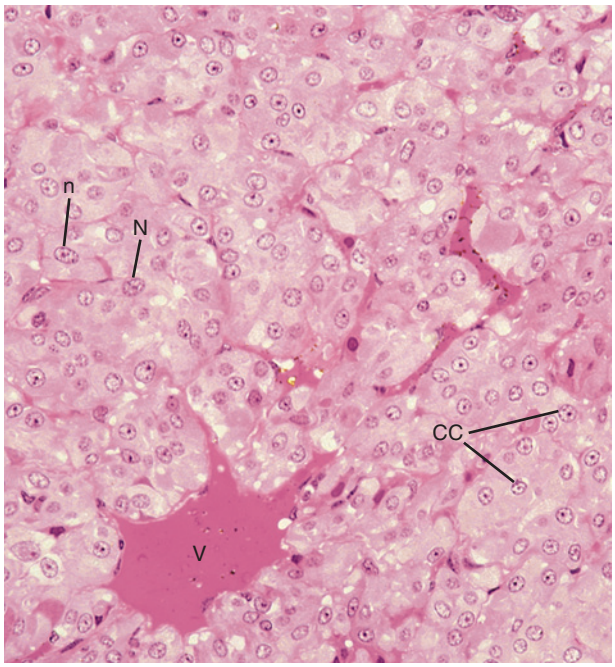


Figure 13–13 Light micrograph of the medulla of the suprarenal gland. Note the chromaffin cells (CC) whose nucleus (N) houses a single nucleolus (n). Observe the rich arterial supply and venous drainage (V) of the suprarenal medulla (×270).

Chromaffin Cells

The suprarenal medulla functions as a modified sympathetic ganglion, housing postganglionic sympathetic cells that lack dendrites and axons.

Chromaffin cells of the suprarenal medulla are large epithelioid cells, arranged in clusters or short cords; they contain granules that stain intensely with chromaffin salts. The reaction of the granules, which turn deep brown when exposed to chromaffin salts, indicates that the cells contain **catecholamines**, transmitters produced by postganglionic cells of the sympathetic nervous system. Thus, the suprarenal medulla functions as a modified sympathetic ganglion, housing postganglionic sympathetic cells that lack dendrites and axons. The catecholamines synthesized by the chromaffin cells are the sympathetic transmitters **epinephrine (adrenaline)** and **norepinephrine (noradrenaline)**

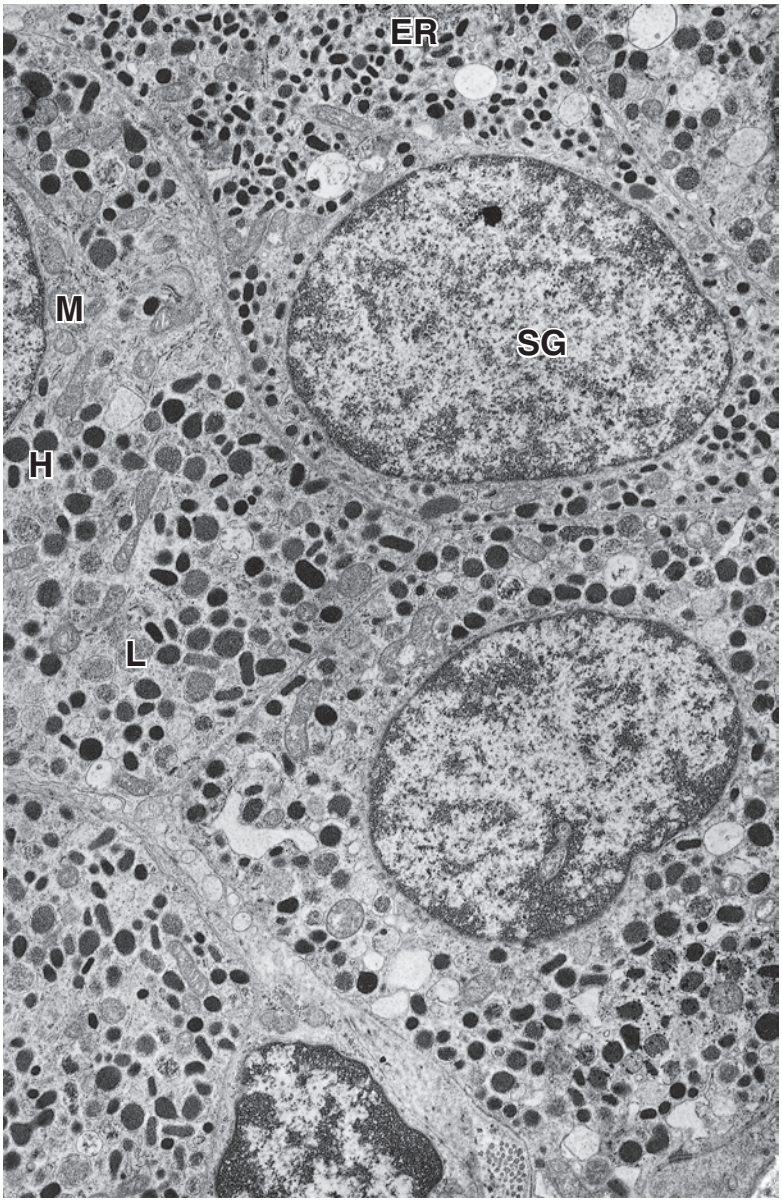


Figure 13–14 Electron micrograph of baboon adrenal medulla (×14000). The different osmiophilic densities of the vesicles may be a reflection of their maturational phases. ER, Endoplasmic reticulum; H, high-electron-density vesicle; L, low-electron-density vesicle; M, mitochondrion; SG, small granule cell. (From Al-Lami F, Carmichael SW. Microscopic anatomy of the baboon *(Papio hamadryas)* adrenal medulla. *J Anat*. 1991;178:213-221. Reprinted with permission from Cambridge University Press.)

(Fig. 13–14). Approximately 80% of the chromaffin cells produce epinephrine and 20% manufacture norepinephrine. These transmitters are secreted by the chromaffin cells in response to stimulation by **preganglionic sympathetic (cholinergic) splanchnic nerves**.

In some animals, but not in primates (or humans), two types of chromaffin cells have been identified via histochemical staining: those producing and storing **norepinephrine** and those producing and storing **epinephrine**. The granules of the norepinephrine-storing cells have an eccentric, electron-dense core within the limiting membrane of the granule, whereas the granules of chromaffin cells storing epinephrine are more homogeneous and less dense. Primate chromaffin cells have a well-developed juxtanuclear Golgi complex, some RER, and numerous mitochondria. The identifying

characteristic of the chromaffin cells is the 30,000 or so small, membrane-bound, dense granules in the cytoplasm; approximately 20% of these granules contain either epinephrine or norepinephrine. The remaining granules are composed of **ATP**, **enkephalins**, and soluble proteins called ***chromogranins***. Chromogranins are proteins that are believed to bind epinephrine and norepinephrine.

Histophysiology of the Suprarenal Medulla

The secretory activity of the suprarenal medulla is controlled by the splanchnic nerves. Release of the aggregated **catecholamines** from chromaffin cells is induced by stimulation of the sympathetic ganglion cells in the suprarenal medulla. Release of **acetylcholine** from these preganglionic sympathetic nerve endings depolarizes the chromaffin cell membranes, leading to an influx of calcium ions. The rise in intracellular calcium then induces release of **epinephrine** or **norepinephrine** via exocytosis.

When the stimulus is derived from an emotional source, secretion of norepinephrine predominates; when the stimulus is physiological (e.g., pain), secretion of epinephrine predominates. Catecholamines released by the suprarenal medulla exhibit a more generalized overall effect than do the catecholamines released by sympathetic neurons. However, these effects are not uniform for all tissues. For example, they increase oxygen consumption, increase heat production, and mobilize fat for energy; in the cardiovascular system, they function in controlling the heart rate and the arterial smooth muscles, thus increasing blood pressure. Additionally, catecholamines regulate muscle contractions in some tissues (e.g., bladder sphincters); in other organs, they influence muscle relaxation (e.g., intestinal smooth muscle).

In severe fear or stress, increased epinephrine is released to prepare the body for "fight, flight, or freeze." The resulting plasma levels of epinephrine, up to 300 times the normal level, increase alertness, cardiac output, and heart rate, as well as increase the release of glucose from the liver.

Epinephrine is most effective in controlling cardiac output and heart rate and increasing blood flow through organs. Norepinephrine has little effect on these but brings about an elevation in blood pressure by vasoconstriction.

Norepinephrine is produced also in the brain and in peripheral nerves, functioning as a neurotransmitter; however, norepinephrine produced in the suprarenal medulla has a short half-life because it is destroyed in the liver shortly after its release.

PINEAL GLAND

The pineal gland is responsive to diurnal light and dark periods and is thought to influence gonadal activity.

The **pineal gland** (or **pineal body**), one of the circumventricular organs, is an endocrine gland whose secretions are influenced by the light and dark periods of the day. It is a cone-shaped, midline projection from the roof of the diencephalon, within a recess of the third ventricle extending into the stalk that is attached to it. It is 5 to 8 mm long and 3 to 5 mm wide; it weighs approximately 120 mg. The gland is covered by pia mater, forming a capsule from which septa extend dividing the pineal gland into incomplete lobules. Blood vessels enter the gland via the connective tissue septa. The parenchymal cells of the gland are composed primarily of **pinealocytes** and **interstitial cells** (Fig. 13–15) The pineal gland produces **melatonin**, the hormone that controls the various circadian rhythms of the individual.

Pinealocytes

Pinealocytes are the parenchymal cells of the pineal gland that are responsible for secreting melatonin.

Pinealocytes constitute approximately 95% of the cell population of the pineal gland. These are slightly basophilic cells with one or two long processes whose terminal dilatations approximate capillaries and, occasionally, other parenchymal cells. Their spherical nuclei have a single prominent nucleolus. The cytoplasm contains SER and RER; a small Golgi apparatus; numerous mitochondria; and small secretory vesicles, some with electron-dense cores. Pinealocytes also contain a well-developed cytoskeleton composed of microtubules, microfilaments, and dense tubular structures invested by spherical vesicular elements. These unusual structures, known as ***synaptic ribbons*** (also observed in the retina and inner ear), increase in number during the dark period of the diurnal cycle, but the function of these synaptic ribbons is not understood.

The hormone **melatonin** is synthesized from tryptophan, through the serotonin pathway catalyzed by the rate limiting enzyme **arylalkylamine *N*-acetyltransferase (AANAT)**, by pinealocytes and is released almost exclusively at night. Melatonin is not stored in pinealocytes but is released almost immediately after being synthesized. This hormone inhibits the release of growth hormone and gonadotropin by the hypophysis and hypothalamus, respectively. It should be noted that melatonin is not synthesized or released during daylight because the action of the enzyme AANAT is inhibited during the day.

Interstitial Cells

Interstitial cells of the pineal gland are believed to be astroglia-like cells.

Interstitial cells, believed to be astrocyte-like neuroglia cells, are scattered throughout the pineal gland and

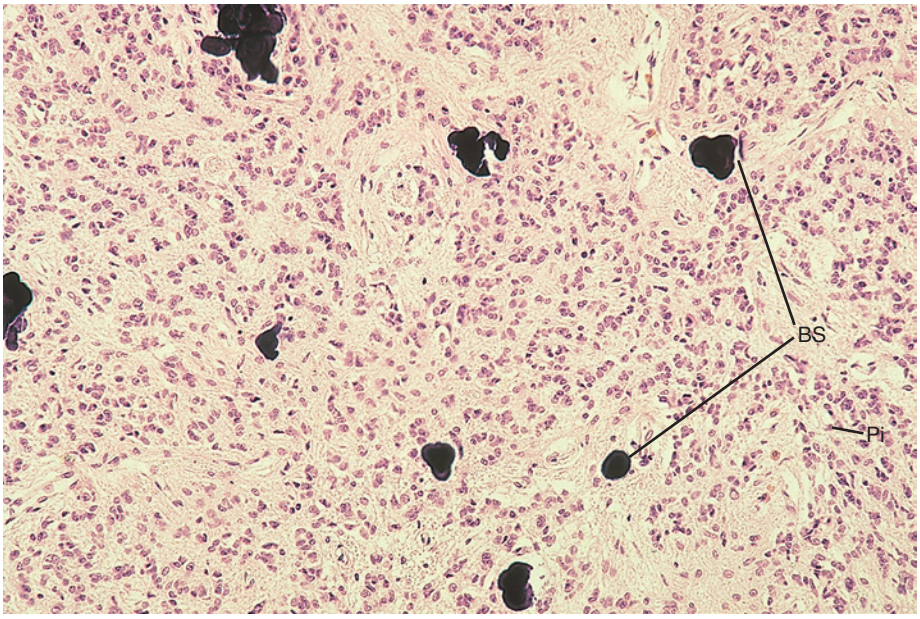


Figure 13–15 Pineal gland. The large dark-staining structures are brain sand (BS) scattered among the pinealocytes (Pi). Neuroglial cells are present but difficult to distinguish at this magnification (×132).

CLINICAL CORRELATIONS

1. Melatonin induces the feeling of sleepiness; therefore, some individuals use it as supplements to combat sleep disorders, mood disorders, and depression.
2. It has been suggested that **melatonin** may act to protect the central nervous system by its ability to scavenge and eliminate free radicals that are produced during oxidative stress. Additional suggestions include that melatonin may alter human moods, causing depression during shortened daylight hours of winter months. It has been reported that exposure to bright artificial light may reduce the secretion of melatonin and may result in alleviation of depression.

are particularly abundant in the pineal stalk that leads to the diencephalon. These cells constitute approximately 5% of the cell population of the pineal gland. They have deeply staining, elongated nuclei and well-developed RER; some have deposits of glycogen. Their long cellular processes are rich in intermediate filaments, microtubules, and microfilaments.

Corpora Arenacea ("Brain Sand")

The pineal gland also contains concretions of calcium phosphates and carbonates, which are deposited in concentric rings around an organic matrix. These structures, called ***corpora arenacea ("brain sand")***, appear in early childhood and increase in size throughout life. Although it is unclear how they are formed or function, they increase during short photoperiods and are reduced as the pineal gland is actively secreting.

Histophysiology of the Pineal Gland

It has been know for a long time that the pineal gland controls the various circadian cycles, but its location so far removed from access to daylight has intrigued researchers. It was recently discovered that approximately 5% of the **ganglion cells of the retina** of higher vertebrates, even those totally blind, react to light and *indirectly* relay this information to the pineal gland. The axons of these specialized ganglion cells join the retinohypothalamic tract to synapse with neurons located in the **suprachiasmatic nucleus (SCN)** of the hypothalamus. The neurons located in the SCN indirectly reach the preganglionic sympathetic neurons of the intermediolateral cell column. Axons of these preganglionic neurons synapse with postganglionic sympathetic neurons in the cervical chain ganglia whose postganglionic axons reach the pineal gland and induce the pinealocytes to manufacture and release melatonin.

Melatonin regulates not only the daily circadian rhythms but also controls the **reproductive axis** by probably inhibiting FSH release by somehow acting on the release of GnRH from the hypothalamus. The role of melatonin in human reproduction is not understood, but in animals with seasonal reproductive strategies, melatonin inhibits the reproductive cycle until the length of the gestational period coincides with the most favorable time for the birth of the newborn animal.

PATHOLOGICAL CONSIDERATIONS

See Figures 13–16 through 13–18.

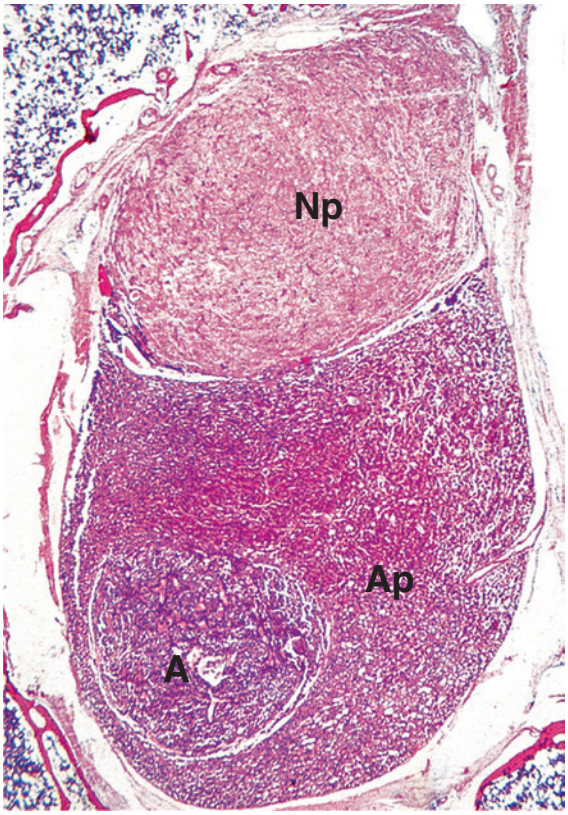


Figure 13–16 This photomicrograph of the pituitary gland is from an individual with a benign tumor pituitary adenoma (A) present in the adenohypophysis (AP). The fact that the tumor is well defined and circumscribed by a connective tissue capsule indicates its nonmalignant nature. Unfortunately, the patient died shortly after the discovery of the tumor because the cells of the tumor produced so much adrenocorticotropic hormone that the patient developed a very aggressive form of Cushing disease that produced very strenuous metabolic and cardiac complications. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:254.)

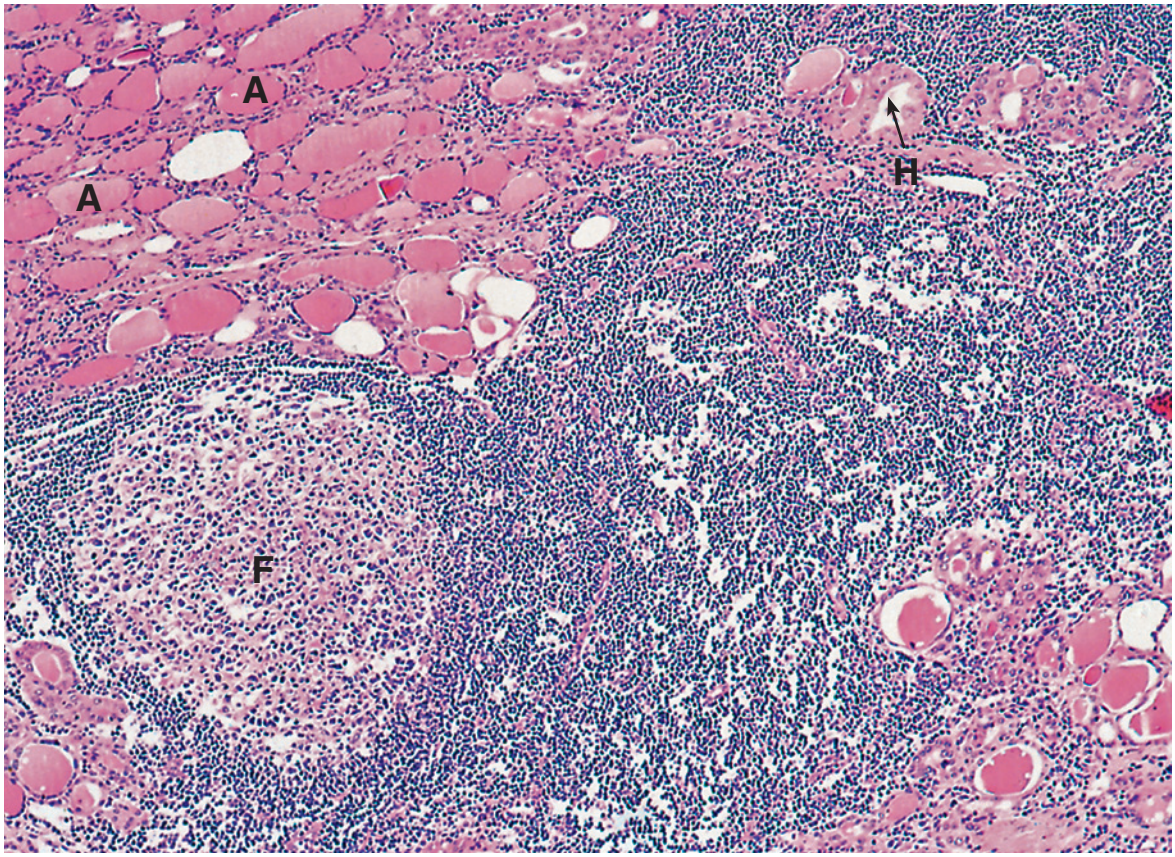


Figure 13–17 This photomicrograph of the thyroid gland is from an individual with Hashimoto disease, an autoimmune disease in which the individual develops autoantibodies against his or her own thyroid gland, destroying the thyroid follicles (A). The gland becomes infiltrated with lymphocytes, which even form lymphoid nodules (F). As the disease progresses, the follicular cells of the thyroid become transformed into Hurthle cells (H) that possess an eosinophilic granular cytoplasm but are not functional in the production of thyroid hormones. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:256.)

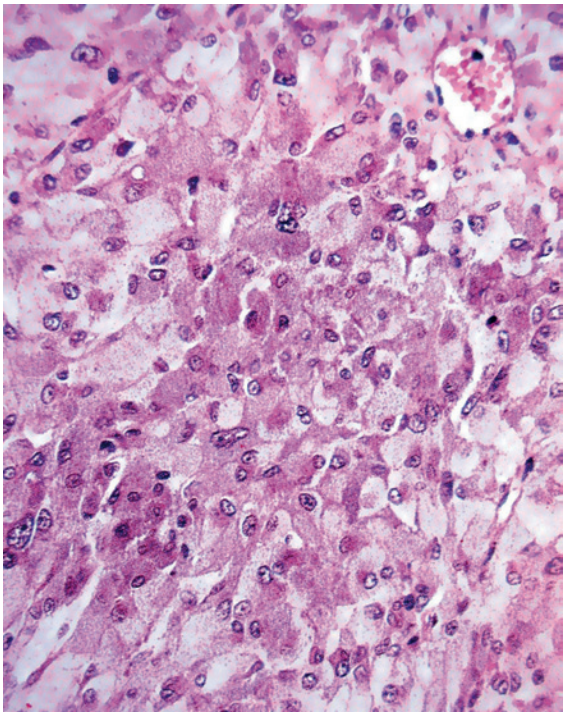
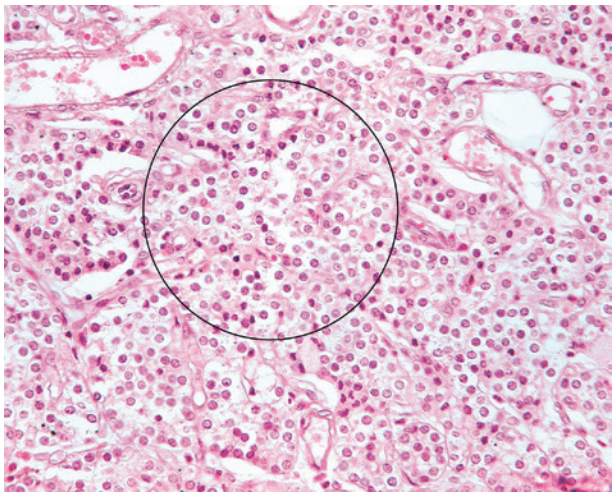


Figure 13–18 This photomicrograph is from an individual presenting with pheochromocytoma, a tumor of the chromaffin cells of the suprarenal medulla. Although the tumor is benign, the patient may display symptoms that are caused by excess production of catecholamines, namely heart palpitations, profuse sweating, and severe hypertensive disease that may lead to death. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:263.)

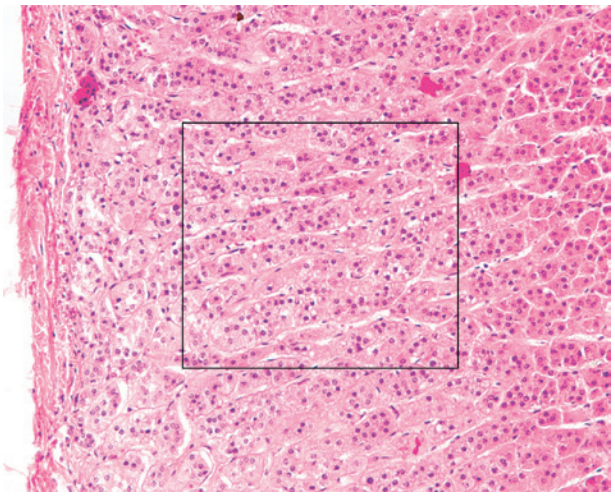
REVIEW QUESTIONS

1. The hormone that generally elevates the metabolic rates of cells is known as
   a. insulin-like growth factor I.
   b. insulin-like growth factor II.
   c. somatotropin.
   d. somatostatin.
   e. somatotropin-releasing hormone.
2. A male patient sees his physician because he noted that he has developed breast-like swellings and that he is producing a white-colored liquid from his nipples. The physician should suspect
   a. an underactive pituitary.
   b. empty sella syndrome.
   c. acromegaly.
   d. prolactinoma.
   e. diabetes insipidus.
3. Which of the following hormones is not manufactured by the pituitary gland but is stored in the pituitary gland?
   a. Oxytocin
   b. Follicle-stimulating hormone (FSH)
   c. Thyroid-stimulating hormone (TSH)
   d. Prolactin
   e. Somatotropin
4. Which of the following hormones resides in the Herring bodies of the pituitary gland?
   a. Prolactin
   b. Interstitial cell–stimulating hormone (ICSH)
   c. Luteinizing hormone (LH)
   d. Adrenocorticotropic hormone (ACTH)
   e. Antidiuretic hormone (ADH)
5. Which of the following hormones (factors) is secreted into the primary capillary plexus of the pituitary gland?
   a. Somatotropin
   b. Adrenocorticotropic hormone
   c. Follicle-stimulating hormone
   d. Prolactin inhibitory factor
   e. Oxytocin
6. A patient with simple goiter has
   a. too much iodine intake.
   b. too little iodine intake.
   c. hypothyroidism.
   d. hyperthyroidism.
   e. Graves disease.
7. A patient who has a tumor of the parathyroid gland also presents with
   a. low blood calcium levels.
   b. high blood phosphate levels.
   c. high blood calcium levels.
   d. muscle cramps in the feet.
   e. muscle cramps in the hands.
8. Patients with Cushing disease are usually obese and exhibit osteoporosis and muscle wasting. Male patients become impotent, and female patients have amenorrhea. Which of the following is the correct reason for the disease?
   a. Tumor of the suprarenal medulla
   b. Tumor of the thyroid gland
   c. Tumor of the pituitary gland
   d. Reduced ACTH production
   e. Reduced cortisol production

9. The cells located within the circle produce which of the following?
   a. Antibodies
   b. Calcitonin
   c. Parathyroid hormone
   d. Osteoclast-stimulating factor
   e. Norepinephrine



10. The region of the organ enclosed by the rectangle produce
    a. androgens.
    b. aldosterone.
    c. melatonin.
    d. adrenocorticotropic hormone.
    e. angiotensin II.



ANSWERS

1. C. Somatotropin, released by the acidophils, somatotrophs, from the adenohypophysis (anterior pituitary) function in increasing the metabolic rates of cells and induce the liver to release insulin-like growth factor I and II, which increases the mitotic rates of the chondrocytes of epiphyseal plates, thus assisting in the lengthening of long bones.
2. D. The physician should suspect prolactinoma due to a tumor of the cells of the anterior pituitary that produce the hormone prolactin. The condition, known as *galactorrhea*, also causes headaches, vision problems, and reduced or absent libido in men. A patient with an underactive pituitary would have reduced pituitary hormone output. Empty sella syndrome is most common in overweight women with high blood pressure. The sella turcica is enlarged, but the pituitary is of normal size. Acromegaly is caused by the overproduction of growth hormone in people between 30 and 50 years of age and is characterized by thickening of the bones—especially those of the face. Diabetes insipidus is usually caused by the decreased blood levels of antidiuretic hormone.
3. A. Oxytocin is manufactured in the paraventricular nuclei of the hypothalamus and is stored in Herring bodies within the pars nervosa of the pituitary gland. FSH and TSH are both produced in the basophils of the anterior pituitary, whereas prolactin and somatotropin are both produced in the acidophils of the anterior pituitary.
4. E. Antidiuretic hormone (ADH, vasopressin) is manufactured in the supraoptic nuclei of the hypothalamus and is stored in Herring bodies of the posterior pituitary (pars nervosa). Prolactin is manufactured and stored in the acidophils of the anterior pituitary (adenohypophysis), and ICSH, LH, and ACTH are all produced in the basophils of the anterior pituitary.
5. D. Prolactin inhibitory factor is manufactured by neurons in the hypothalamus, and the axons of these neurons terminate around the primary capillary plexus of the pituitary. The hypophysial portal system then delivers it to the second capillary plexus in the adenohypophysis, where it leaves the vascular system to activate acidophils, known as *mammotrophs*, to release prolactin. None of the other hormones listed enter the primary capillary plexus.
6. B. Simple goiter, a condition caused by insufficient iodine intake that is treated by the addition of iodine to the diet, is neither hypothyroidism or hyperthyroidism. Graves disease is usually caused by an autoimmune response, in which IgG binds to the TSH receptors, which stimulates thyroid follicular cells to overproduce thyroxine and triiodothyronine.
7. C. Parathyroid tumors usually lead to high parathyroid hormone secretion, resulting in high osteoclastic activity and increase in blood calcium levels and low blood phosphate levels. Muscle cramps in the feet and hands are usually caused by low blood calcium levels.
8. C. Patients with Cushing disease usually have tumors of the basophils of the anterior pituitary, which leads to an increase rather than a decrease in the production of ACTH and an overproduction of cortisol. The suprarenal medulla is not affected, but the cortex becomes hypertrophied. The thyroid gland is not involved.
9. C. The cells enclosed by the circle are chief cells of the parathyroid gland, and they produce parathyroid hormone. Antibodies are produced by plasma cells, and they have an acentric nucleus and a basophilic cytoplasm. Calcitonin is produced by parafollicular cells of the thyroid gland. Osteoclast-stimulating factor is produced by osteoblasts. Norepinephrine is produced by the chromophil cells of the suprarenal medulla.
10. B. The zona glomerulosa of the suprarenal cortex produces aldosterone and deoxycorticosterone. Androgens are produced by the zona reticularis of the suprarenal gland. Melatonin is manufactured by the pinealocytes of the pineal body. Adrenocorticotropic hormone is a product of the pituitary gland and induces the release of hormones from the adrenal cortex. Angiotensin II is a second-generation conversion product of angiotensinogen (a decapeptide present in the bloodstream), that induces the release of aldosterone from the zona glomerulosa of the suprarenal cortex.

Integument

14

The **integument**, composed of **skin** and its appendages, **sweat glands**, **sebaceous glands**, **hair**, and **nails**, is the largest organ, constituting 16% of the body weight. It invests the entire body, becoming continuous with the mucous membranes of the digestive system at the lips and the anus, the respiratory system in the nose, and the urogenital systems where they surface. Additionally, the skin of the eyelids becomes continuous with the conjunctiva lining the anterior portion of the eye. Skin also lines the external auditory meatus and covers the external surface of the tympanic membrane. The mammary glands are also derivatives of the epidermis, but their histology is discussed in Chapter 20.

SKIN

Skin, the largest organ of the body, is composed of an epidermis and the underlying dermis.

Besides providing a cover for the underlying soft tissues, skin performs many additional functions, including (1) **protection** against injury, bacterial invasion, and desiccation; (2) **regulation of body temperature**; (3) **reception** of continual sensations from the environment (e.g., touch, temperature, and pain); (4) **excretion** from sebaceous glands as well as from apocrine and eccrine sweat glands; and (5) **absorption** of ultraviolet (UV) radiation from the sun, a requirement for vitamin D synthesis.

Skin consists of two layers: an outer epidermis and a deeper connective tissue layer, the dermis (Fig. 14–1). The **epidermis** is composed of an **ectodermally** derived stratified squamous keratinized epithelium. Lying directly below and interdigitating with the epidermis is the **dermis**, derived from **mesoderm** and composed of dense, irregular collagenous connective tissue. The interface between the epidermis and dermis is formed by raised ridges of the dermis, the **dermal ridges (papillae)**, which interdigitate with invaginations of the epidermis called ***epidermal ridges***. Collectively, the two types of ridges are known as the ***rete apparatus***. Additional downgrowths of the epidermal derivatives (i.e., hair follicles, sweat glands, and sebaceous glands) that come to lie in the dermis cause the interface to have an irregular contour.

The **hypodermis**, a loose connective tissue containing varying amounts of fat, underlies the skin. The hypodermis is not part of the skin but is the **superficial fascia** of gross anatomical dissection that covers the entire body, immediately deep to the skin. Individuals who are overnourished or who live in cold climates possess a large amount of fat deposited in this layer, which then is named **panniculus adiposus**.

In certain regions of the body, the skin displays different textures and thicknesses. For example, skin of the eyelid is soft, fine, and thin and has fine hairs, whereas only a short distance away, on the eyebrow, the skin is thicker and manifests coarse hair. Skin of the forehead produces oily secretions; the skin on the chin lacks oily secretions but develops much hair.

The palms of the hands and soles of the feet are thick and do not produce hair but contain many sweat glands. In addition, finger and toe pad surfaces have well-defined, alternating ridges and grooves that form patterns of loops, curves, arches, and whorls called ***dermatoglyphs*** (fingerprints), which develop in the fetus and remain unchanged throughout life. Dermatoglyphs are so individualized that they are used for identification purposes in forensic medicine and in criminal investigations. Although fingerprints are determined genetically, perhaps by multiple genes, other grooves and flexure lines about the knees, elbows, and hands are, for the most part, related to habitual use and physical stresses in one's environment.

Epidermis

Epidermis, the surface layer of skin, is derived from ectoderm and is composed of stratified squamous keratinized epithelium.

The **epidermis** is 0.07 to 0.12 mm in thickness over most of the body, with localized thickening on the palms of the hands and the soles of the feet (where it may be as much as 0.8 mm and 1.4 mm in thickness, respectively). Thicker skin on the palms and soles is evident in fetuses, but use, applied pressure, and friction result in continued increases in skin thickness in these areas over time.

The stratified squamous keratinized epithelium of skin is composed of four populations of cells:

- Keratinocytes
- Melanocytes
- Langerhans cells
- Merkel cells

SUMMARY

The **integument** is composed of the **skin** and its appendages, **sweat glands**, **sebaceous glands**, **hair**, and **nails**. Skin, the largest organ, is the first defense of the body against pathogenic invasion. It is composed of an outer **epidermis** and an inner **dermis**. The epidermis is a stratified squamous keratinized epithelium, whereas the dermis is a dense irregular **collagenous connective tissue**, which is amply supplied by **elastic fibers**. The epidermis constantly renews itself by mitotic activity of the cells of its inner layers, a process that takes 2 to 3 weeks. The skin is separated from the deeper tissues of the body by a connective tissue layer, known as the ***superficial fascia***, also referred to as the ***hypodermis***. Sweat glands release **sweat**, a watery secretion, and function in **thermoregulation**. Sebaceous glands manufacture **sebum**, an oily substance that maintain the suppleness of skin and hair. Hairs are filamentous, keratinized structures that, in humans, act as **tactile sensors**; in other mammals, they serve as **thermal insulators**. Nails are formed of keratinized epithelial cells arranged in plates of keratin. They function in protecting the distal phalanx of fingers and toes.

KEY WORDS

Dermis; epidermis; glands; hair; hypodermis; integument; sebaceous nail; sebum; skin; superficial fascia; sweat; sweat glands; tactile sensors; thermal insulator; thermoregulation

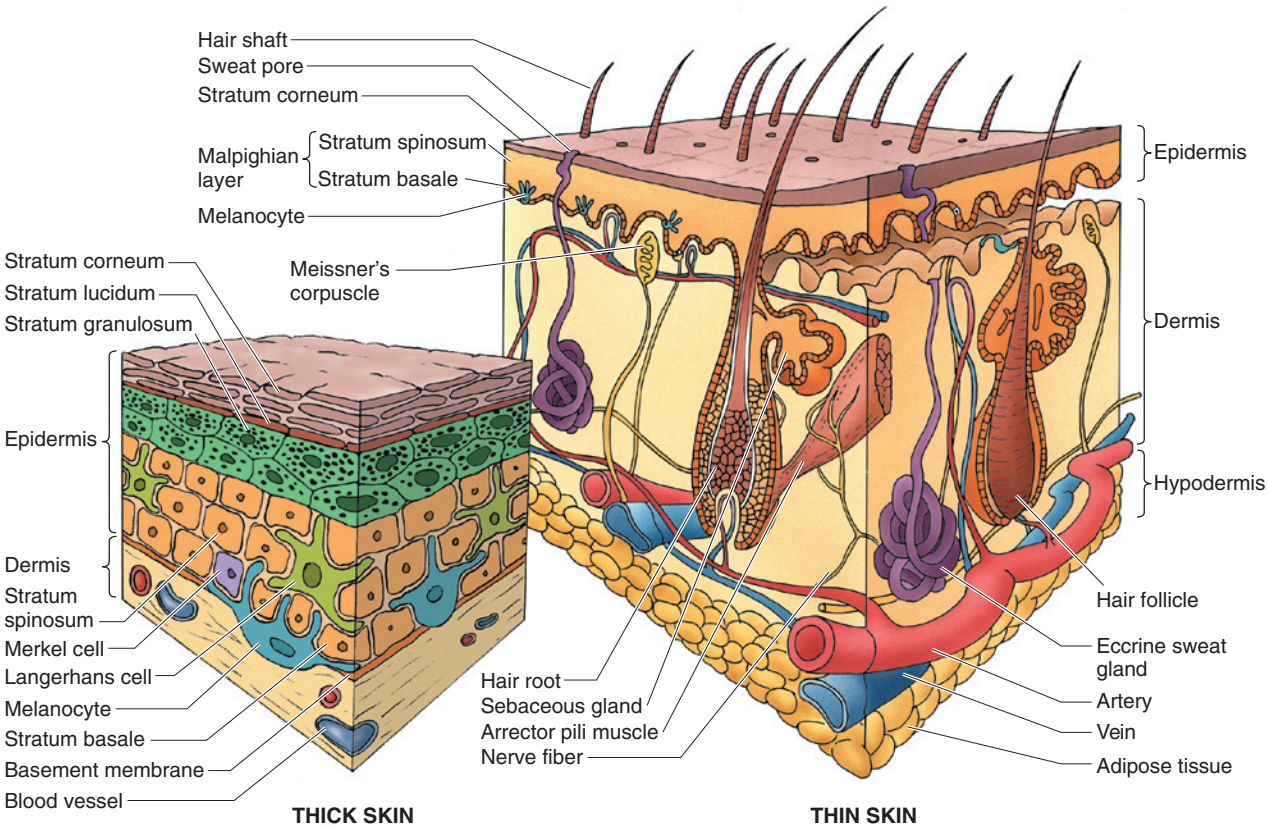


Figure 14–1 Comparison of thick skin and thin skin.

Keratinocytes of the Epidermis

Keratinocytes, which form the largest population of cells, are arranged in five recognizable layers; the remaining three cell types are interspersed among keratinocytes in specific locations. Because keratinocytes are being continually sloughed from the surface of the epidermis, this cell population must continually be renewed. Renewal is accomplished through mitotic activity of the keratinocytes in the basal layers of the epidermis. Keratinocytes undergo mitosis at night, and as the new cells are forming, the cells above continue to be pushed toward the surface. Along their way to the surface, the cells differentiate and begin to accumulate **keratin filaments** in their cytoplasm. Eventually, as they near the surface, the cells die and are sloughed off, a process that takes 25 to 30 days.

Because of the **cytomorphosis** of keratinocytes during their migration from the basal layer of the epidermis to its surface, five morphologically distinct zones of the epidermis can be identified. From the inner to the outer layer, these are the (1) **stratum basale** (previously known as the ***stratum germinativum***), (2) **stratum spinosum**, (3) **stratum granulosum**, (4) **stratum lucidum**, and (5) **stratum corneum**. Skin is classified as **thick** or **thin** according to the thickness of the epidermis. However, these two types of skin also are distinguished by the presence or absence of certain epidermal layers and the presence or absence of hair. Recently, it has been reported that keratinocytes synthesize and secrete signaling molecules, such as tumor necrosis factor, interleukins, colony-stimulating factors, and interferons, all of which contribute to the functioning of the immune system.

Thick skin covers the palms and soles (Table 14–1). The epidermis of thick skin, which is 400 to 600 μm thick, is characterized by the presence of all five layers. Thick skin lacks hair follicles, arrector pili muscles, and sebaceous glands but does possess sweat glands (Fig. 14–2).

Thin skin covers most of the remainder of the body. The epidermis of thin skin, which ranges from 75 to 150 μm in thickness, has a thin stratum corneum and lacks a definite stratum lucidum and stratum granulosum, although individual cells of these two layers are present in their proper locations. Thin skin has **hair follicles**, **arrector pili muscles**, **sebaceous glands**, and **sweat glands**.

TABLE 14–1 Strata and Histological Features of Thick Skin

Layer	Histological Features
Epidermis	Derived from ectoderm; composed of stratified squamous keratinized epithelium (keratinocytes).
Stratum corneum	Numerous layers of dead flattened keratinized cells, keratinocytes, without nuclei or organelles (squames or horny cells) that will be sloughed off.
Stratum lucidum*	Lightly stained thin layer of keratinocytes without nuclei or organelles; cells contain densely packed keratin filaments and eleidin.
Stratum granulosum*	A layer three to five cell layers thick. These keratinocytes still retain nuclei; cells contain large, coarse keratohyalin granules as well as membrane-coating granules.
Stratum spinosum	Thickest layer of epidermis, whose keratinocytes, known as *prickle cells*, interdigitate with one another by forming intercellular bridges and a large number of desmosomes; prickle cells have numerous tonofilaments and membrane-coating granules and are mitotically active; this layer also houses Langerhans cells.
Stratum basale (germinativum)	This single layer of cuboidal to low columnar, mitotically active cells is separated from the papillary layer of the dermis by a well-developed basement membrane; Merkel cells and melanocytes are also present in this layer.
Dermis	Derived from mesoderm; composed mostly of type I collagen and elastic fibers, the dermis is subdivided into two regions: the papillary layer and the reticular layer, a dense, irregular collagenous connective tissue.
Papillary layer	Interdigitates with epidermis, forming the dermal papilla component of the rete apparatus; type III collagen and elastic fibers in loose arrangement and anchoring fibrils (type VII collagen); abundant capillary beds, connective tissue cells, and mechanoreceptors are located in this layer; occasionally, melanocytes are also present in the papillary layer.
Reticular layer	Deepest layer of skin; type I collagen, thick elastic fibers, and connective tissue cells; contains sweat glands and their ducts, hair follicles and arrector pili muscles, and sebaceous glands as well as mechanoreceptors (e.g., pacinian corpuscles).

*Present in thick skin only. All layers are usually thinner in thin skin.

Stratum Basale (Stratum Germinativum)

Stratum basale, the germinal layer that undergoes mitosis, forms interdigitations with the dermis and is separated from it by a basement membrane.

The deepest layer of the epidermis, the **stratum basale**, is supported by a **basement membrane** and sits on the dermis, forming an irregular interface. The stratum basale consists of a single layer of mitotically active, cuboidal to low columnar cells containing basophilic cytoplasm and a large nucleus (Fig. 14–3). Many desmosomes are located on the lateral cell membrane attaching stratum basale cells to each other and to cells of the stratum spinosum. Basally located hemidesmosomes attach the cells to the basal lamina. Electron micrographs reveal a few mitochondria, a small Golgi complex, a few rough endoplasmic reticulum (RER) profiles, and abundant free ribosomes. Numerous bundles and single (10-nm) **intermediate filaments (tonofilaments)**, composed of **keratin 5** and **keratin 14**, course through the plaques of the laterally placed desmosomes and end in plaques of hemidesmosomes.

Mitotic figures should be common in the stratum basale because this layer is partially responsible for cell renewal in the epithelium. However, mitosis occurs mostly during the night, and histological specimens are procured during the day; thus, mitotic figures are rarely seen in histological slides of skin. When new cells are formed via mitosis, the previous layer of cells is pushed surfaceward to join the next layer of the epidermis, the stratum spinosum. Melanocytes and Merkel cells are dispersed among the keratinocytes of the stratum basale.

Stratum Spinosum

The stratum spinosum is composed of several layers of mitotically active polymorphous cells whose numerous processes give this layer a prickly appearance.

The thickest layer of the epidermis, the **stratum spinosum**, is composed of polyhedral to flattened cells. The basally located keratinocytes in the stratum spinosum also are mitotically active, and the two strata together, frequently referred to as the ***Malpighian layer***, are responsible for the turnover of epidermal keratinocytes. Cellular proliferation in the Malpighian layer is dependent on the presence of **epidermal growth factor (EGF)** and **interleukin-1 (IL-1)**, whereas, in the presence of **transforming growth factor (TGF)**, mitotic activity is curbed. Keratinocytes

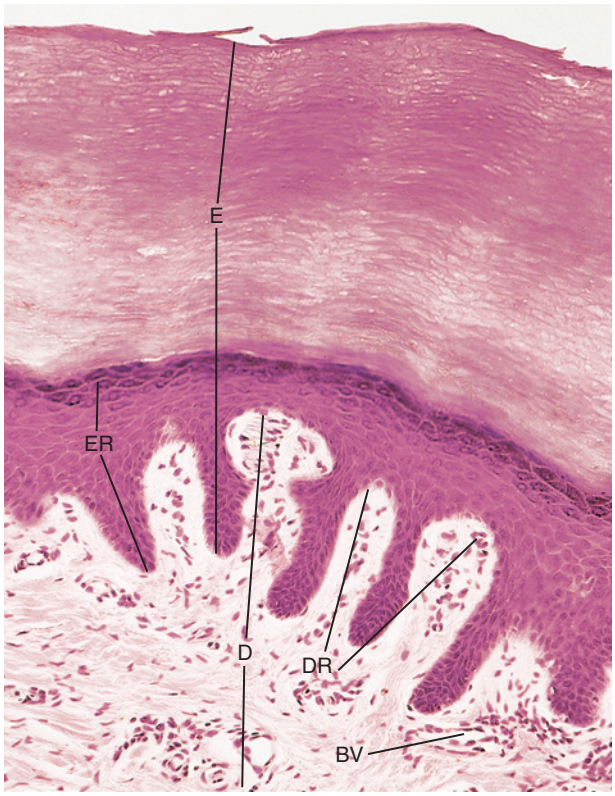


Figure 14–2 Light micrograph of thick skin (×132). Observe the epidermis (E) and dermis (D) as well as the dermal ridges (DR) that are interdigitating with epidermal ridges (ER). Several blood vessels (BV) are present.

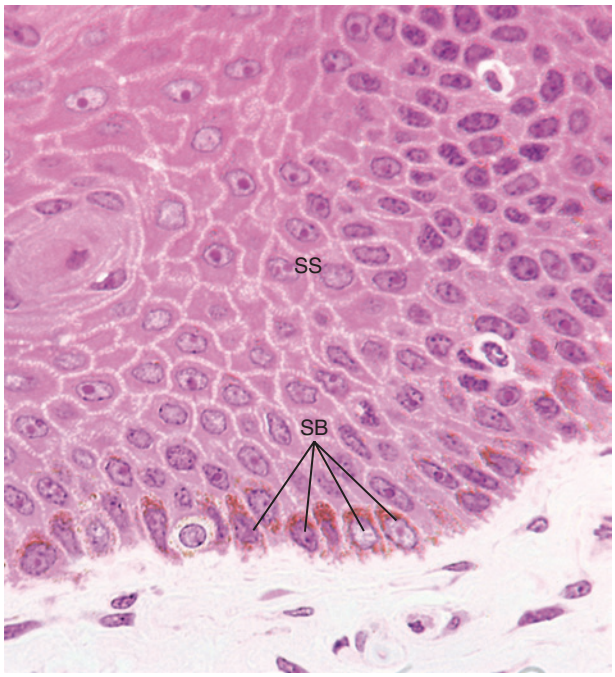


Figure 14–3 Light micrograph of thick skin demonstrating the stratum basale (SB) and stratum spinosum (SS) (×540).

of the stratum spinosum have the same organelle population as described for the stratum basale. However, the cells in the stratum spinosum are richer in thin bundles of intermediate **(keratin)** filaments (referred to as ***tonofilaments***) than are the cells of the stratum basale; moreover, instead of keratins 5 and 14, these cells synthesize **keratin 1** and **keratin 10**. In the cells of the stratum spinosum, these tonofilament bundles radiate outward from the perinuclear region toward highly interdigitated cellular processes, which attach adjacent cells to each other by desmosomes. These processes, called *intercellular bridges* by early histologists, give cells of the stratum spinosum a "prickle cell" appearance (see Fig. 14–3). As keratinocytes move toward the surface through the stratum spinosum, they continue to produce tonofilaments, which become enveloped by **keratohyalin**, a substance whose major constituents are trichohyalin and filaggrin. The combination of keratohyalin and tonofilaments creates groups of thickened bundles called ***tonofibrils***, causing the cytoplasm to become eosinophilic (Fig. 14–4). Cells of the stratum spinosum also contain cytoplasmic secretory granules (0.1 to 0.4 μm in diameter) called ***membrane-coating granules (lamellar bodies, Odland bodies)***. These flattened vesicles house lipid substances, composed mostly of phospholipids, glycosphingolipids, and ceramides, arranged in a closely packed, lamellar configuration. Some of these granules release their contents into the extracellular space, forming a boundary that is impermeable to aqueous substances.

Stratum Granulosum

The stratum granulosum is composed of three to five layers of cells housing keratohyalin granules.

The **stratum granulosum**, consisting of three to five layers of flattened keratinocytes, is the most superficial layer of the epidermis in which cells still possess nuclei. The cytoplasm of these keratinocytes contains large, irregularly shaped, coarse, basophilic **keratohyalin granules**, which are *not* membrane-bound. Bundles of keratin filaments pass through these granules.

Cells of the stratum granulosum also contain **membrane-coating granules**. The contents of these granules are released by exocytosis into the extracellular space, forming sheets of lipid-rich substance that acts as a waterproof barrier, one of the functions of skin. This impermeable layer prevents cells lying superficial to this region from being bathed in the nutrient-filled aqueous extracellular fluid. Consequently, the cells enter the

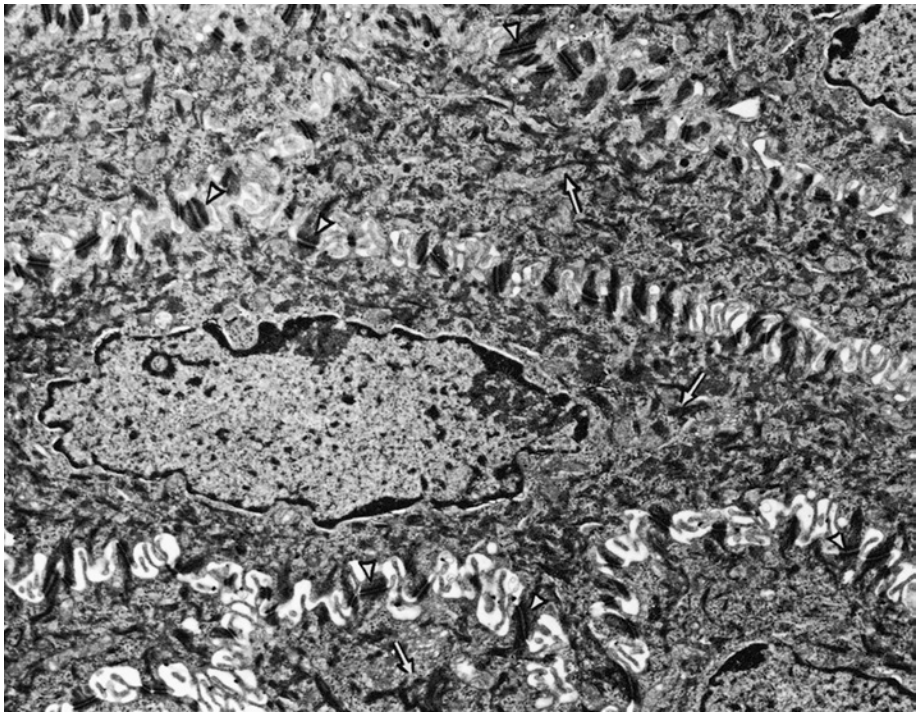


Figure 14–4 Electron micrograph of the stratum spinosum (×6800). The tonofibrils *(arrows)* and the cytoplasmic processes are bridging the intercellular spaces. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

apoptosis pathway, their organelles self-destruct, and the cells are filled with **keratohyalin-embedded keratin-based tonofibril complex**. The internal aspect of their cell membrane becomes coated with a 10 to 12 nm thick reinforcing layer of dense material and the cells of the stratum granulosum make numerous **claudin-rich** tight junctions with each other.

Stratum Lucidum

Present only in thick skin, cells of the stratum lucidum are devoid of nuclei and organelles but contain eleidin.

The clear, homogeneous, lightly staining, thin layer of cells immediately superficial to the stratum granulosum is the **stratum lucidum**. This layer is present only in thick skin (i.e., palms of the hands and soles of the feet). Although the flattened cells of the stratum lucidum lack organelles and nuclei, they are filled with densely packed **keratohyalin-embedded keratin based tonofibril complex** known as ***eleidin***. The cytoplasmic aspect of the plasma membrane of these cells has a thickened appearance because of the deposition of a nonkeratin protein, known as ***involucrin***, which provides support for the cell membrane.

Stratum Corneum

The stratum corneum is composed of several layers of flattened, keratin-containing dead cells known as squames.

The most superficial layer of skin, the **stratum corneum**, is composed of numerous layers of flattened, keratinized cells with a thickened plasmalemma. These cells also lack nuclei and organelles but are filled with **keratohyalin-embedded keratin-based tonofibril complex**. Those cells farther away from the skin surface display desmosomes and tight junctions, assume the shape of 14-sided polygons, and are called ***squames*** or ***horny cells***. The plasmalemmae of these cells are lined by a thickened, dense material composed of **small proline-rich protein**, **involucrin**, and **loricrin**, the three proteins that reinforce the cell membrane; this reinforced cell membrane is referred to as the ***cornified cell envelope***. The extracellular surface of the stratum corneum cell membrane is embedded in the **lipid material** that was released from the membrane-coating granules in the stratum spinosum and the stratum granulosum and the lipid coat. The combination of the lipid coat and the cornified cell envelope together form a

very strong impermeable barrier known as the ***compound cornified cell envelope***. The outermost squames lose their contact with each other and become **desquamated** (sloughed off). The pace at which the squames are desquamated from the stratum corneum equals the rapidity of new cell formation in the Malpighian layer; therefore, the epidermis retains its characteristic thickness.

Nonkeratinocytes in the Epidermis

In addition to keratinocytes, the epidermis contains three other cell types, Langerhans cells, Merkel cells, and melanocytes.

Langerhans Cells

Langerhans cells are antigen-presenting cells located among the cells of the stratum spinosum.

Although they are scattered throughout the epidermis where they normally represent 2% to 4% of the epidermal cell population, **Langerhans cells**, sometimes called ***dendritic cells*** because of their numerous long processes, are located primarily in the stratum spinosum. These cells also may be found in the dermis as well as in the stratified squamous epithelia of the oral cavity, esophagus, and vagina. However, they are most prevalent in the epidermis, where their numbers may reach 800 per square millimeter (mm^2).

Viewed with light microscopy, Langerhans cells display a dense nucleus, pale cytoplasm, and long slender processes that radiate out from the cell body into the intercellular spaces between keratinocytes. Electron micrographs reveal the nucleus to be polymorphous; the electron-lucent cytoplasm houses few mitochondria, sparse RER, and no intermediate filaments but contains lysosomes, multivesicular bodies, and small vesicles. Although the irregularly contoured nucleus and the absence of tonofilaments distinguish Langerhans cells from surrounding keratinocytes, the most unique feature of Langerhans cells are the membrane-bound **Birbeck granules (vermiform granules)**, which in section resemble ping pong paddles (15 to 50 nm in length, 4 nm thick).

Langerhans cells, once thought to be derived from neural crest cells, are now known to originate from precursors in the bone marrow and are a part of the mononuclear phagocyte system. Although they are capable of mitosis, this activity is restricted; thus, they are continually replaced by precursor cells leaving the bloodstream to migrate into the epidermis and differentiate into Langerhans cells. These cells function in the immune response and have cell-surface Fc (antibody) and C3 (complement) as well as other receptors. Birbeck granules of Langerhans cells contain **langerin**, an integral protein that is a lectin receptor that assists the engulfing of antigens by Birbeck granules so that they can degrade antigens into epitopes. Once the antigens are processed in the Birbeck granules, Langerhans cells migrate to lymph nodes in the vicinity, where they present epitopes of processed foreign antigens to T lymphocytes; thus, Langerhans cells are **antigen-presenting cells**.

Merkel Cells

Merkel cells, scattered among cells of the stratum basale, may serve as mechanoreceptors.

Merkel cells, which are interspersed among the keratinocytes of the stratum basale of the epidermis, are especially abundant in the fingertips and oral mucosa and at the base of hair follicles. These cells are derived from neural crest and are usually found as single cells oriented parallel to the basal lamina; however, they may extend their processes between keratinocytes, to which they are attached by desmosomes (Fig. 14–5). Merkel cell nuclei are deeply indented, and three types of cytokeratins within the cytoplasm make up the cytoskeletal filaments. Dense-cored granules are located in the perinuclear zone and in the processes and are considered to be the distinguishing feature of Merkel cells.

Myelinated sensory nerves traverse the basal lamina to approximate the Merkel cells, thus forming **Merkel cell–neurite complexes**. These complexes may function as **mechanoreceptors**. These cells exhibit a synaptophysin-like immunoreactivity, indicating that Merkel cells may release neurocrine-like substances, suggesting that Merkel cells display diffuse neuroendocrine system-related activity.

Melanocytes

Melanocytes, derived from neural crest cells, produce melanin pigment that imparts a brown coloration to skin.

Melanoblasts, derived from neural crest, migrate to the epidermis where they differentiate into **premelanocytes** and continue their migration into the stratum basale, where they take up residence, although some melanoblasts may continue, enter the superficial portions of the dermis to remain there (Fig. 14–6). Premelanocytes form adhesive junctions with the basal lamina but never with the keratinocytes surrounding them. **Stem cell factor**, permeating throughout the dermis and the extracellular spaces of the stratum basale and stratum spinosum, bind to **stem cell factor receptors** on the premelanocytes, inducing these cells to differentiate into **melanocytes**, cells that form processes known as ***dendrites***. These processes extend into the stratum spinosum and contact a number of keratinocytes; each melanocyte and the keratinocytes its processes contact is referred to as an ***epidermal melanin unit***.

Tyrosinase produced by the RER of the melanocyte is packaged by its Golgi apparatus into oval granules,

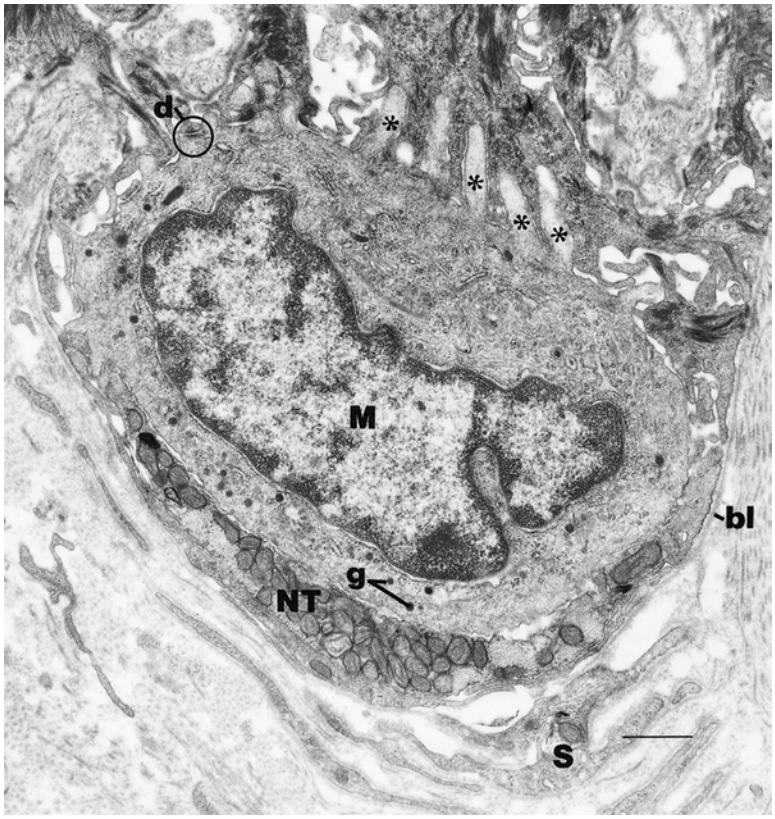


Figure 14–5 Electron micrograph of a Merkel cell (M) and its nerve terminal (NT) from an adult rat. (Scale bar = 0.5 µm.) Note the spine-like processes *(asterisks)* that project into the intercellular spaces of the stratum spinosum. Merkel cells form desmosomes (d) with cells of the stratum spinosum and share the basal lamina (bl) of cells of the stratum basale. (From English KB, Wang ZZ, Stayner N, et al. Serotonin-like immunoreactivity in Merkel cells and their afferent neurons in touch domes from hairy skin of rats. *Anat Rec*. 1991;232:112-120. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

known as ***melanosomes*** (although the melanosomes of red-haired individuals are spherical instead of oval). The amino acid tyrosine is preferentially transported into melanosomes. Under the influence of **melanocyte-stimulating hormone**, melanocytes express **microphthalmia-associated transcription factor**. This factor triggers the UV light–sensitive **tyrosinase** to convert **tyrosine**, through a series of reactions progressing through 3,4-dihydroxyphenylalanine (dopa, methyldopa) and dopaquinone, into **melanin** within the **melanosomes**. As the melanin content of melanosomes increases in quantity, the melanosome is said to be maturing. Mature melanosomes are conveyed along microtubules into the melanocytic dendrites to be released into the extracellular spaces of the stratum spinosum. These melanosomes become phagocytosed by keratinocytes of the stratum spinosum. Once engulfed, the melanosomes are ferried to the nuclear region, where they accumulate between the nucleus and the sun's UV radiation that strikes the skin, in that manner shielding the keratinocyte's chromosomes from radiation-induced injury. Eventually, the melanin pigment is attacked and degraded by lysosomes of the keratinocyte. This process occurs over a period of several days.

CLINICAL CORRELATIONS

Ultraviolet light darkens the melanin and speeds tyrosinase synthesis thus increasing melanin production. Also, pituitary adrenocorticotropic hormone (ACTH) influences pigmentation. In **Addison disease** there is insufficient production of cortisol by the adrenal cortex, so excess ACTH is produced, which leads to hyperpigmentation.

Albinism is the absence of melanin production resulting from a genetic defect in **tyrosinase synthesis**. Melanosomes are present, but the melanocytes fail to produce tyrosinase.

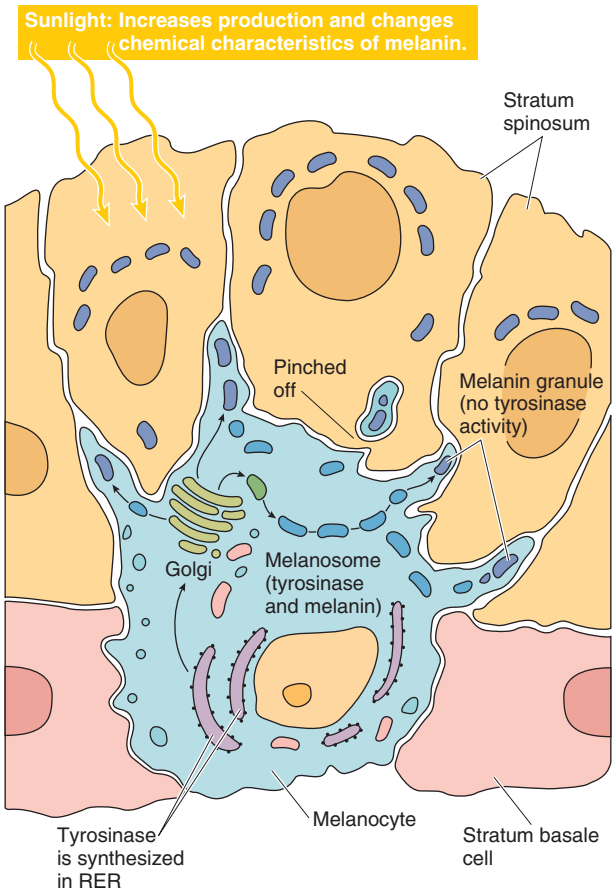


Figure 14–6 Diagram of melanocytes and their function. RER, Rough endoplasmic reticulum.

Melanocytes constitute approximately 3% of the epidermal cell population. The number of melanocytes per square millimeter varies in different regions of skin of an individual, ranging from 800 to 2300/mm^2. For example, there are much fewer melanocytes on the insides of the arms and thighs than on the face. The difference in skin pigmentation is related more to location of the melanin than to the total number of melanocytes in the skin, which is nearly the same for all races. For instance, there are more melanocytes in the skin on the dorsum than on the palmar surface of the hand; however, these numbers are very similar among the various races. The reason for the darker pigmentation is due not to the effective number of melanocytes but to an increase in their tyrosinase activity.

Although exposures to UV radiation increases the size and functional activity of the melanocytes, their population remains the same. After continued exposure to UV radiation, however, there is also an increase in the melanocyte population. In pale-skinned individuals, the melanosomes are smaller and fewer and congregate in the vicinity of the nucleus; in dark-skinned persons, melanosomes are large, more numerous, and dispersed throughout the cytoplasm of the keratinocytes. Also, melanosomes are degraded and removed more rapidly in the pale-skinned population than in the dark-skinned population.

CLINICAL CORRELATIONS

1. **Ultraviolet rays** exist as two types. Ultraviolet B (UVB) is the component in sunlight that produces sunburn, whereas ultraviolet A (UVA) is responsible for tanning. Until recently, it was believed that UVA was relatively safe, but it appears that UVA radiation penetrates the skin deeper and damages the deep layers of the skin, producing mutations that are implicated in tumor progression.
2. **Xeroderma pigmentosum (XP)** is an uncommon autosomal recessive genetic defect affecting 1 in 1 million individuals in the United States and Europe, but it is present with greater frequency in Morocco, the Middle East, and Japan. Individuals with XP are very sensitive to UV radiation and may get a severe sunburn after being exposed just a few minutes to sunlight. The condition expressed very early in childhood, and these children present with skin cancer, usually on the face and scalp, within the first decade of life. In some regions of the world, the average lifespan of patients with XP is less than 30 years. Approximately one-third of XP patients present not only with cloudy corneas and eye cancers but also with neurological conditions involving balance and movement disorders, seizures, and cognitive function disorders. There are a number of different forms of XP and, fortunately, not all of them lead to neurological problems.

Dermis (Corium)

The dermis, the layer of skin immediately deep to the epidermis, is derived from mesoderm and is composed of a loose papillary layer and a deeper, denser reticular layer.

The region of the skin lying directly beneath the epidermis, called the ***dermis***, is derived from the mesoderm and is divided into two layers: the superficial, loosely woven **papillary layer** and the deeper, much denser **reticular layer**. The dermis is composed of dense, irregular collagenous connective tissue, containing mostly **type I collagen fibers**, some **type III collagen fibers**, and broad bands of **elastic fibers**, all of which support the epidermis and bind the skin to the underlying **hypodermis** (superficial fascia). The dermis

ranges in thickness from 0.6 mm in the eyelids to 3 mm or so on the palm of the hand and the sole of the foot. However, there is not a sharp line of demarcation at its interface with the underlying connective tissue of the superficial fascia. Normally, the dermis is thicker in men than in women and on the dorsal rather than on the ventral surfaces of the body.

Papillary Layer of the Dermis

The most superficial layer of the dermis, the papillary layer, interdigitates directly with the epidermis but is separated from it by the basement membrane.

The superficial papillary layer of the dermis is uneven where it interdigitates with the epidermis, forming the dermal ridges **(papillae)** (see Fig. 14–2). It is composed of a loose connective tissue whose thin **type III collagen fibers** (reticular fibers) and slender **elastic fibers** are arranged in loose networks. **Anchoring fibrils**, composed of type VII collagen, extend from the basal lamina into the papillary layer, binding the epidermis to the dermis (see Chapter 4, Figs. 4–13 and 4–14). The papillary layer contains fibroblasts, macrophages, plasma cells, mast cells, and other cells common to connective tissue.

The papillary layer also possesses many capillary loops, which extend to the epidermis–dermis interface. These capillaries regulate body temperature and nourish the cells of the avascular epidermis. Located in some dermal papillae are pear-shaped encapsulated **Meissner corpuscles**, mechanoreceptors specialized to respond to slight deformations of the epidermis. These receptors are most common in areas of the skin that are especially sensitive to tactile stimulation (e.g., lips, external genitalia, and nipples). Another encapsulated mechanoreceptor present in the papillary layer is the **Krause end bulb**. Although this receptor was once thought to respond to cold, its function is currently unclear. Additionally, unmyelinated pain fibers also course through the papillary layer to enter the epidermis, where they remain as free nerve endings that respond to pain sensations.

CLINICAL CORRELATIONS

Itching (pruriception) is a somatic sensation that may be due to local causes such as a tiny insect walking on one's arm or to generalized and systemic causes such as a form of dermatitis or even to cancer and organ failure. The mechanism of an itch is better known in mice than in humans, but it is assumed that there may be a close correlation in the two species. It has been demonstrated in mice that itching sensation is conveyed by C fibers to the murine spinal cord, where secondary neurons reside that are capable of expressing gastrin-releasing peptide receptors (GRPR). Apparently, there are a number of GRPR types of neurons, and they are activated by various types of G protein–coupled receptors that respond to a particular cause of the itching sensation. Moreover, there seems to be a relationship between pain and itching sensations because they share similar but not identical pathways; they both possess receptors in the skin, spinal cord, and brain.

Reticular Layer of the Dermis

The reticular layer of the dermis also contains epidermally derived structures, including sweat glands, hair follicles, and sebaceous glands.

The interface between the papillary layer and **reticular layer** of the dermis is indistinguishable because the two layers are continuous with each other. Characteristically, the reticular layer is composed of dense, irregular collagenous connective tissue, displaying thick **type I collagen fibers**, which are closely packed into large bundles lying mostly parallel to the skin surface. Networks of **thick elastic fibers** are intermingled with the collagen fibers, appearing especially abundant near sebaceous and sweat glands. Proteoglycans, rich in **dermatan sulfate**, fill the interstices of the reticular layer. Cells are more sparse in this layer than in the papillary layer. They include fibroblasts, mast cells, lymphocytes, macrophages, and, frequently, fat cells in the deeper aspects of the reticular layer.

Sweat glands, **sebaceous glands**, and **hair follicles**, all derived from the epidermis, invade the dermis and hypodermis during embryogenesis, where they remain permanently (see Fig. 14–1). Groups of **smooth muscle cells** are located in the deeper regions of the reticular layer at particular sites, such as the skin of the penis and scrotum and the areola around the nipples; contractions of these muscle groups wrinkle the skin in these regions. Other smooth muscle fibers, called ***arrector pili muscles***, are inserted into the hair follicles; contractions of these muscles erect the hairs when the body is cold or suddenly exposed to a cold environment, giving the skin "goose bumps." Additionally, a particular group of striated muscles located in the face, parts of the anterior neck, and scalp **(muscles of facial expression)** originate in the superficial fascia and insert into the dermis.

At least three types of encapsulated mechanoreceptors are located in the deeper portions of the dermis: (1) **pacinian corpuscles**, which respond to pressure and vibrations; (2) **Ruffini corpuscles**, which respond to tensile forces; and (3) Krause end bulbs, whose function is not known but is assumed to be mechanoreception rather than the earlier belief that they were cold receptors. Ruffini corpuscles are most abundant in the dermis of the soles of the feet.

Epidermis–Dermis Interface

The interdigitation of the epidermal ridges with the dermal ridges is known as the rete apparatus.

The interdigitations of the epidermal and dermal layers are translated through the epidermis and are apparent on the surface of the skin, especially of the palms and soles, where they are represented by whorls, arches, and loops **(dermatoglyphs)**. Because these interdigitations are not easily visualized from two-dimensional histological sections, ethylenediaminetetraacetic acid can be used to chelate calcium ions (Ca^{2+}) of hemidesmosomes, permitting the dissociation of the epidermis from the dermis. Once the epidermis and dermis are dissociated, the three-dimensional surface of the papillary layer of the dermis may be examined with scanning electron microscopy.

The papillary layer presents parallel **primary dermal ridges** on its surface separated by **primary grooves**, which house projections of the epidermis (see Fig. 14–2). Also in the center of each primary dermal ridge is a **secondary groove**, which receives a downgrowth of the epidermis known as an ***interpapillary peg***. Along this and other adjacent ridges are rows of round-topped **dermal papillae** that project into concavities in the epidermis, thus firmly interlocking the epidermis and dermis at the interface. The epidermis–dermis interface in thin skin is much less complex, lacking such deep and widespread interlocking.

Histophysiology of Skin

The structural protein produced by the keratinocytes is **keratin**, which are 10-nm-wide intermediate filaments within the cytoplasm of keratinocytes. Approximately 20 different species of keratin have been identified, and four of these are present within the epidermis. Keratins may be classified as acidic or neutral-basic. Interestingly, the genes that code for acidic keratins are located on chromosome 17, whereas the genes that code for neutral-basic keratins are located on chromosome 12.

Stratum basale cells synthesize two of the four keratins, whereas the cells of the stratum spinosum synthesize the other two, which tend to form coarser bundles of filaments. Cells of the stratum spinosum also produce and deposit the protein **involucrin** on the cytoplasmic aspect of their plasmalemma. Moreover, cells of the stratum spinosum also form the **membrane-coating granules**, which later release their lipid-rich contents into the intercellular spaces, forming a permeability barrier.

The keratin-synthesizing machinery shuts down after keratinocytes enter the stratum granulosum. The cells in this layer produce **filaggrin**, a protein thought to help assemble keratin filaments into still coarser bundles. Once keratinocytes reach this stratum, they also become permeable to calcium ions, which assist in cross-linking involucrin with other proteins, thereby forming a tough layer beneath the plasmalemma. As keratinocytes move through the stratum granulosum into the stratum lucidum, enzymes released from lysosomes digest the organelles and the nucleus. When the cells finally enter the stratum corneum, they are nonliving, organelle-free, tough shells filled with bundles of keratin filaments.

EGF and **interleukin 1 alpha (IL-1 α)** influence the growth and development of keratinocytes, at least in tissue culture. In contrast, **TGF** suppresses keratinocyte proliferation and differentiation.

Glands of the Skin

The glands of the skin include eccrine sweat glands, apocrine sweat glands, sebaceous glands, and the mammary gland (a modified and highly specialized type of sweat gland). The mammary gland is described in Chapter 20.

Eccrine Sweat Glands

Eccrine sweat glands are abundant throughout the skin and release their secretory product, sweat, via the merocrine method of secretion.

Eccrine sweat glands are about 0.4 mm in diameter and are located in the skin throughout most of the body. Numbering as many as 3 to 4 million, they are important organs of thermoregulation. Eccrine sweat glands develop as invaginations of the epithelium of the dermal ridge that grows down into the dermis, with its deep aspect becoming the glandular portion of the sweat gland. These glands, which begin to function soon after birth, excrete about 1 L of sweat per day but may form as much as 10 L of sweat a day under extreme conditions in highly active people engaged in vigorous exercise in hot weather. These glands are innervated by the sympathetic nervous system, but the nerve fibers innervating the secretory units are mostly **cholinergic**, that is, they release **acetylcholine**, and only some of the nerve fibers are adrenergic.

Eccrine sweat glands are **simple coiled tubular glands** located deep in the dermis or in the underlying hypodermis (Figs. 14–7 and 14–8). Passing from the secretory portion of each gland is a slender, coiled **duct** that traverses the dermis and epidermis to open on the surface of the skin at a **sweat pore**. These glands are merocrine in their method of releasing their secretory product. They are innervated by postganglionic fibers of the sympathetic nervous system.

Secretory Unit

The secretory portion of the gland is said to be a simple cuboidal to low columnar epithelium composed of dark cells and clear cells. Additionally, the secretory portion of eccrine sweat glands possesses myoepithelial cells.

CLINICAL CORRELATIONS

Freckles are hyperpigmented spots located on sun-exposed areas of the skin, especially in fair-skinned individuals who sunburn easily. Freckles are usually exhibited by 3 years of age and are the result of increased melanin production and accumulation in the basal area of the epidermis without an increase in melanocytes. They tend to fade in the winter and darken with exposure to ultraviolet light.

Psoriasis is a disease characterized by patchy lesions caused by greater keratinocyte proliferation in the stratum basale and stratum spinosum and an accelerated cell cycle (turnover is increased as much as seven times), resulting in accumulations of keratinocytes and stratum corneum. The lesions are common on the scalp, elbows, and knees, but they may occur almost anywhere on the body. In some cases, the nails may also be involved. Psoriasis is an incurable but manageable chronic condition whose symptoms periodically escalate and then diminish with no apparent cause.

Warts are benign epidermal growths caused by infection of the keratinocytes with **papillomaviruses**. The resulting epidermal hyperplasia thickens the epidermis with scaling. Deeper ingrowth of the dermis brings capillaries closer to the surface. Warts are common in children, young adults, and immunosuppressed patients.

Basal cell carcinoma, the most common human malignancy, arises in the **stratum basale cells** of the epidermis and usually is caused by exposure to ultraviolet radiation. Although basal cell carcinomas do not usually metastasize, they are destructive to local tissue. Of the several types of lesions that occur, the most common is the nodular variety, characterized by a papule or nodule with a central depressed "crater" that eventually ulcerates and crusts. These lesions are most common on the face, especially the nose. Surgery is the usual treatment, and up to 90% of patients recover with no additional sequelae.

Squamous cell carcinoma, the second most common skin cancer, arises in the keratinocytes of the epidermis. It is locally invasive and may metastasize. It is characterized by a hyperkeratotic scaly plaque or nodule that often bleeds or ulcerates. It invades deeply, resulting in fixation to the underlying tissues. Several factors may cause this disease, including ultraviolet radiation, x-irradiation, soot, chemical carcinogens, and arsenic. The lesions are most common on the head and neck. Surgery is the usual treatment of choice.

Malignant melanoma, a skin cancer, is increasing in incidence, being most prevalent in fair–skinned individuals The malignant cells originate from transformed melanocytes and usually are associated with excessive exposure to the sun. Malignant melanoma is very invasive because the melanocytes penetrate the dermis and enter lymphatic vessels as well as the bloodstream to gain wide distribution throughout the body. Interestingly, even thought the incidence of melanoma is much greater in Caucasians than in individuals of African and Hispanic descent, metastasis occurs in a much greater percentage of Hispanic (18%) and African (26%) patients than in Caucasian (12%) patients. It is believed that the discrepancy is caused by the difference in the level of medical care in these populations.

Some investigators consider the secretory portion to be pseudostratified.

Dark Cells (Mucoid Cells)

Dark cells line the lumen of the secretory unit and secrete a mucus-rich substance.

Dark cells resemble an inverted cone, with the broad end lining the lumen. The narrowed ends, which seldom reach the basal lamina, conform to fit between adjacent clear cells. Electron micrographs reveal some RER, numerous free ribosomes, elongated mitochondria, and a well-developed Golgi complex. Moderately dense glycoprotein-containing secretory granules are located in the apical cytoplasm of the dark cells, and the secretion released by these cells is **mucous** in nature.

Clear Cells

Clear cells do not possess secretory granules; they release a watery secretion.

Clear cells have a narrow apical area and a broader base that extends to the basal lamina. Unlike dark cells, clear cells do not contain secretory granules but do contain accumulations of **glycogen**; their organelles are similar to those of dark cells, except that they have little RER. The bases of the clear cells are tortuously infolded, similar to those of other cell types involved in transepithelial transport. Clear cells have limited access to the lumen of the gland because of the dark cells; therefore, their **watery**, **electrolyte-rich secretion** enters **intercellular canaliculi** interposed between adjacent clear cells, where it mixes with the mucous secretion of the dark cells.

Myoepithelial Cells

Myoepithelial cells surrounding the secretory portion of the gland contain actin and myosin, imparting a contractile ability to these cells.

Myoepithelial cells surrounding the secretory portion of the eccrine sweat glands are enveloped by the basal

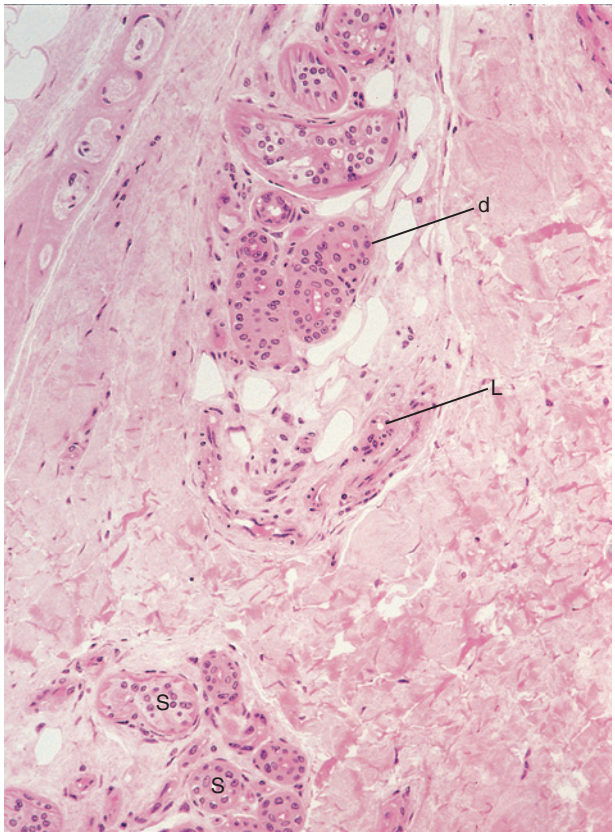


Figure 14–7 Light micrograph of sweat gland showing secretory units (S) and ducts (d), some displaying a lumen (L) (×132).

lamina of the secretory cells. The cytoplasm of myoepithelial cells has **myosin filaments** as well as many deeply acidophil-staining **actin** filaments, which give the cell contractile capability. Contractions of the myoepithelial cells assist in expressing the fluid from the secretory portion of the gland.

Duct

The duct of eccrine sweat glands, composed of basal and luminal cells, is highly coiled and traverses the dermis and epidermis on its way to opening on the skin surface.

The **duct** of an eccrine sweat gland is continuous with the secretory unit at its base but narrows as it passes through the dermis on its way to the epidermal surface. The duct is composed of a stratified cuboidal epithelium made up of two layers (see Figs. 14–7 and 14–8). The **cells of the basal layer** have a large, heterochromatic nucleus and abundant mitochondria. The **cells of the luminal layer** have an irregularly shaped nucleus, little cytoplasm, only a few organelles, and a terminal web immediately deep to the apical plasma membrane.

The ducts follow a helical path through the dermis. As a duct reaches the epidermis, keratinocytes envelop the duct on its way to the sweat pore. The fluid secreted by the secretory portion of the gland is similar to blood plasma in regard to electrolyte balance, including potassium and sodium chloride as well as ammonia and urea. However, most of the potassium, sodium, and chloride ions are reabsorbed by cells of the duct as the secretion travels through its lumen. Duct cells excrete ions, urea, and lactic acid, as well as certain drugs into the lumen.

Apocrine Sweat Glands

Apocrine sweat glands are found only in the axilla, areola of the nipple, and anal region and may represent vestigial scent glands.

Apocrine sweat glands are found only in certain locations: the axilla (arm pit), the areola of the nipple, and the anal region. Modified apocrine sweat glands constitute the **ceruminous (wax) glands** of the external auditory canal and the **glands of Moll** in the eyelids. Apocrine sweat glands are much larger than eccrine sweat glands, up to 3 mm in diameter. These glands are embedded in the deeper portions of the dermis and hypodermis. Unlike the ducts of eccrine sweat glands, which open onto the skin surface, the ducts of apocrine sweat glands open into canals of the hair follicles just superficial to the entry of the sebaceous gland ducts.

The secretory cells of apocrine glands are simple cuboidal to low columnar in profile. When the lumen of the gland is filled with secretory product, these cells may become squamous. The lumina of these glands are much larger than those of eccrine glands, and the secretory cells contain granules that are isolated from the apical membrane by the presence of a prominent terminal web. The viscous secretory product of apocrine glands is odorless upon secretion, but when metabolized by bacteria, it presents a distinctive odor. Myoepithelial cells surround the secretory portion of the apocrine sweat glands and assist in expressing the secretory product into the duct of the gland.

An apocrine sweat gland arises from the epithelium of the hair follicles as an epithelial bud that develops into a gland. Secretion by apocrine glands is under the influence of hormones and does not begin until puberty. Their innervation is provided by fibers of the postganglionic sympathetic nervous system. Because of the similarity of their location, their histology, and the fact that the odor is most likely due to the bacterial metabolism of 3-methyl-1-2 hexanoic acid (a volatile acid similar to pheromone signals), it is speculated that apocrine sweat glands evolved from glands that secrete sex attractants in lower animals. As an interesting note, apocrine sweat glands in women undergo cyclical changes that seem to be related to the menstrual cycle, so that

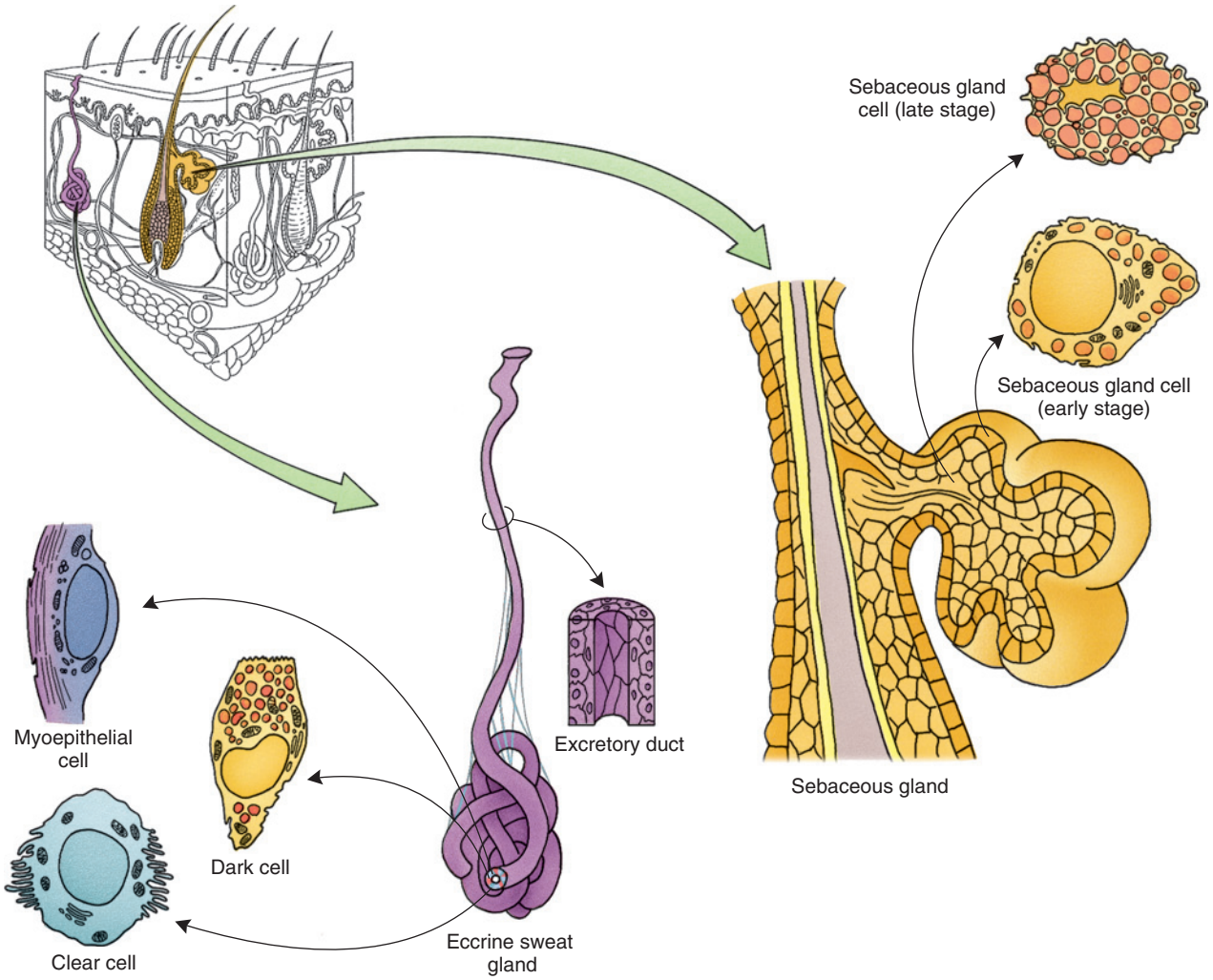


Figure 14–8 Diagram of an eccrine sweat gland, a sebaceous gland, and their constituent cells.

the secretory cells and lumina enlarge before the premenstrual period and diminish during menstruation.

The name given to these special sweat glands, apocrine sweat glands, implies that the secretion contains a portion of the cytoplasm of the secreting cells. Although some researchers suggest that these cells release their secretion via the apocrine method, most investigators report that, despite their name, apocrine sweat glands release their secretory product via the merocrine mode of secretion.

Sebaceous Glands

Sebaceous glands secrete an oily substance known as sebum, *which maintains the suppleness of the skin.*

Except for the palms of the hands, soles of the feet, and sides of the feet inferior to the hairline, **sebaceous glands** are found throughout the body, embedded in the dermis and hypodermis. These glands are most abundant on the face, scalp, and forehead. The secretory product of the sebaceous glands, **sebum**, is a wax-like, oily mixture of cholesterol, triglycerides, and secretory cellular debris. Sebum is believed to facilitate the maintenance of proper skin texture and hair flexibility; additionally, sebum is antimicrobial and assists in preventing watery fluids from entering or leaving skin.

Similar to apocrine sweat glands, sebaceous glands are appendages of hair follicles. The ducts of the sebaceous glands open into the upper third of the follicular canal, where they discharge their secretory product to coat the hair shaft and, eventually, coat the skin surface (see Fig. 14–8). The ducts of sebaceous glands in certain regions of the body lacking hair follicles (i.e., the lips, glans penis, areola of the nipples, labia minora, and

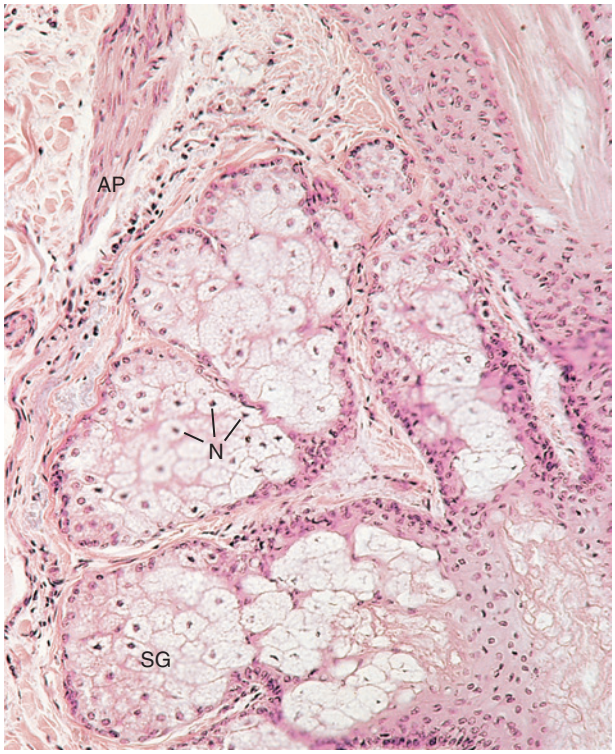


Figure 14–9 Light micrograph of a human sebaceous gland (sG) whose cells are displaying nuclei (N) and the arrector pili muscle (AP) (×132).

mucous surface of the prepuce) open onto the surface of the skin to empty their secretions. Sebaceous glands are under the influence of sex hormones and increase their activity greatly after puberty.

Sebaceous glands are lobular with clusters of acini opening into single short ducts. Each acinus is composed of peripherally located small basal cells (resting on the basal lamina), which surround larger round cells that fill the remainder of the acinus (Fig. 14–9). The basal cells have a spherical nucleus, both smooth and RER, glycogen, and lipid droplets. These cells undergo cell division to form more basal cells and larger round cells. The larger cells have abundant smooth ER and cytoplasm filled with lipid droplets. The central region of the acinus is filled with cells in different stages of degeneration. These pale-staining cells display only strands of cytoplasm; deeply staining, pyknotic nuclei; ruptured plasmalemmae; and coalescing lipid droplets. Lipid synthesis continues for a short time followed by necrosis of the cells and the ultimate release of lipid and cellular debris, which form the secretory product (i.e., **holocrine secretion**). The secretory product is released into a duct lined with a stratified squamous epithelium that is continuous with the follicular canal at the hair follicle.

CLINICAL CORRELATIONS

Acne, the most common disease seen by dermatologists, is a chronic inflammatory disease involving the sebaceous glands and hair follicles. Obstructions resulting from impaction of sebum and keratinous debris within hair follicles is one cause of acne lesions. Anaerobic bacteria near these obstructions may contribute to development of acne, although the role of bacteria is not clear. However, the efficacy of antibiotic treatment for acne supports the idea of bacterial involvement in its pathogenesis. The disease is most severe in boys, with onset commonly from age 9 to 11 years, when increasing levels of sex hormones begin to stimulate the sebaceous glands. Acne usually subsides through the later teen years, but it may not resolve until the fourth decade of life. In some people, acne does not begin until adulthood.

Hair

Hairs are filamentous, keratinized structures that project from the epidermal surface of the skin (see Fig. 14–1). Hair grows over most of the body except on the vermilion zone of the lips, palms and sides of the palms, soles and sides of the feet, dorsum of the distal phalanges of the fingers and toes, glans penis, glans clitoris, labia minora, and vestibular aspect of the labia majora.

Two types of hairs are present on the human body. Hairs that are soft, fine, short, and pale (e.g., those covering the eyelids) are called ***vellus hairs***; the hard, large, coarse, long, and dark hairs (e.g., those of the scalp and eyebrows) are called ***terminal hairs***. Additionally, very fine hair, called ***lanugo***, is present on fetuses.

The number of hairs on humans is essentially the same as on other primates, but most human hair is of the vellus type, whereas terminal hairs predominate on other primates. Human hair does not provide thermal insulation as does the fur of animals. Instead, human hairs serve in tactile sensation, such that any stimulus that deforms a hair is translated down the shaft to sensory nerves that surround the hair follicle.

Hair growth is optimal from about 16 to 46 years of age; after age 50 years, hair growth begins to diminish. During pregnancy, hair growth is normal; after delivery, the cycle of hair growth subsides, and hair loss is temporarily increased.

Hair Follicles

Hair follicles develop from the epidermis and invade the dermis and hypodermis.

Hair follicles, the organs from which hairs develop, arise from invaginations of the epidermis that invade the dermis, hypodermis, or both. Hair follicles are

Figure 14–10 Light micrograph of a longitudinal section of a hair follicle with its hair root (HR) and papilla (P). The dark structures at the tip of the arrow is pigment (×122).

Figure 14–11 Light micrograph of hair follicles in cross section (×132). Observe the external root sheath (E), the internal root sheath (I), and the cortex (C).

surrounded by dense accumulations of fibrous connective tissue belonging to the dermis (Fig. 14–10). A thickened basement membrane, the **glassy membrane**, separates the dermis from the epithelium of the hair follicle. The expanded terminus of the hair follicle, the **hair root**, is indented, and the concavity conforms to the shape of the **dermal papilla** occupying it. The hair root and the dermal papilla together are known as the ***hair bulb***. The dermal papilla contains a rich supply of capillaries that provide nutrients and oxygen for the cells of the hair follicle. Also, the dermal papilla acts as an inductive force controlling the physiological activities of the hair follicle.

The bulk of the cells composing the hair root is called the ***matrix***. Proliferation of these matrix cells **(stem cells)** accounts for the growth of hair; thus, they are homologous to the stratum basale of the epidermis. These stem cells are present even in bald individuals, but the hair follicle cells produce prostaglandin D2, which, acting through the G protein-coupled receptors, prevents the stem cells from entering the cell cycle to form a new hair follicle. The outer layers of follicular epithelium form the **external root sheath**, which is composed of a single layer of cells at the hair bulb and several layers of cells near the surface of the skin (Figs. 14–11 and 14–12).

The external root sheath surrounds several layers of epidermally derived cells, the **internal root sheath**, which consists of three components: (1) an outer single row of cuboidal cells, **Henle layer**, which contacts the innermost layer of cells of the external root sheath; (2) one or two layers of flattened cells forming **Huxley layer**; and (3) the **cuticle of the internal root sheath**, formed by overlapping scale-like cells whose free ends project toward the base of the hair follicle. The internal root sheath ends where the duct of the sebaceous gland attaches to the hair follicle (see Fig. 14–12).

The hair shaft is the long slender filament that extends to and through the surface of the epidermis (Fig. 14–13). It consists of three regions: **medulla**, **cortex**, and the **cuticle of the hair**. As the cells of the matrix within the hair root proliferate and differentiate, they move toward the surface of the skin, eventually developing into the hair shaft. The cells in the center of the matrix are closest to the underlying dermal papilla and thus are most influenced by it; cells lying more and more peripheral to the matrix center are progressively less influenced by the dermal papilla. The distinctive layers of the follicle develop from different matrix cells as follows:

- The *most central* matrix cells give rise to large vacuolated cells that form the core of the hair shaft (the **medulla**). This layer is present in thick hair only.

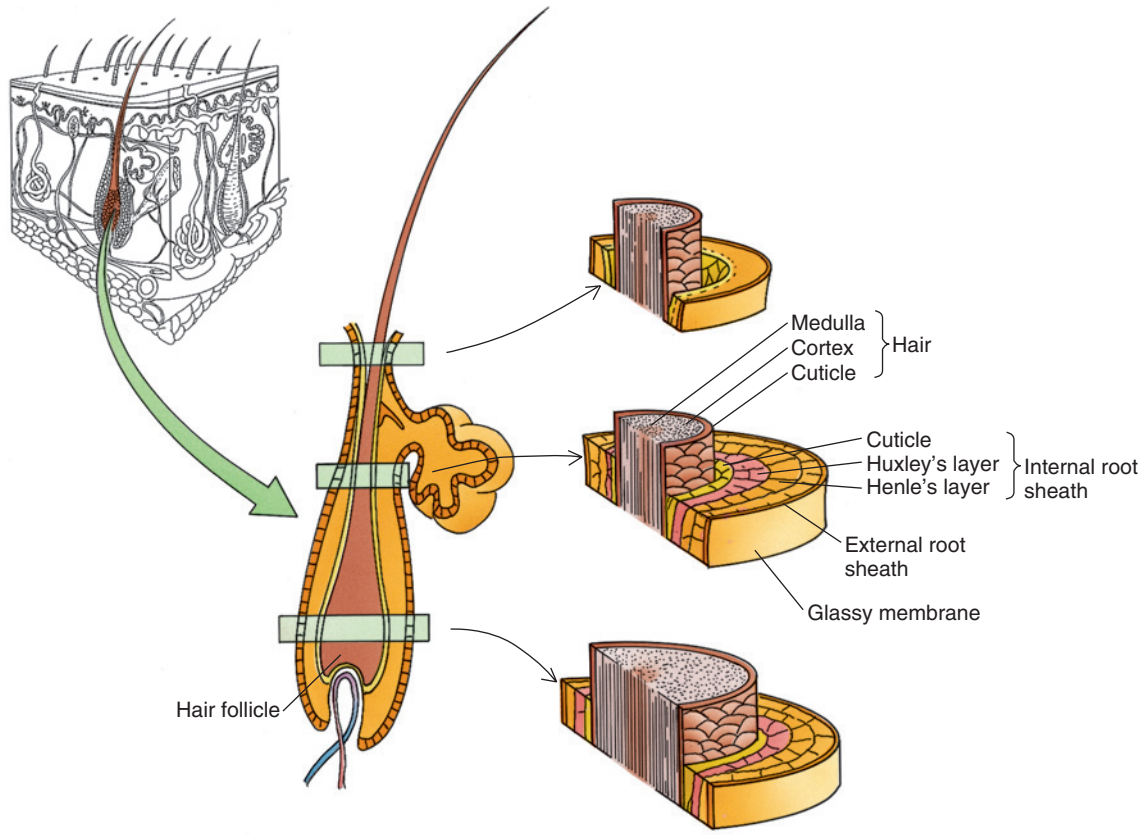


Figure 14–12 Schematic diagram of the hair follicle.

- Matrix cells *slightly peripheral* to the center become the **cortex** of the hair shaft.
- *More peripheral* matrix cells become the **cuticle** of the hair.
- The *most peripheral* matrix cells develop into the cells of the **internal root sheath**.

As the cells of the cortex are displaced surfaceward, they synthesize abundant **keratin filaments** and **trichohyalin granules** (resembling keratohyalin granules of the epidermis). These granules coalesce, forming an amorphous substance in which the keratin filaments are embedded. Scattered among the cells of the matrix nearest to the dermal papilla are large **melanocytes**, with long dendritic processes that transfer **melanosomes** to the cells of the cortex. The melanosomes remain in these cells, imparting to the hair a color based on the amount of melanin present. With age, the melanocytes gradually lose their ability to produce **tyrosinase**, which is essential for the production of melanin. Additionally, the production of the enzyme catalase is diminished, which increases hydrogen peroxide levels in these cells, bleaching the hair. Consequently, the reduction in the amount of these two enzymes results in the hair turning gray.

Arrector Pili Muscles

Arrector pili muscles are smooth muscle cells extending from midshaft of the hair follicle to the papillary layer of the dermis.

Attached to the connective tissue sheath surrounding the hair follicles and to the papillary layer of the dermis are the **arrector pili muscles** (see Fig. 14–1). These smooth muscles attach to the hair follicle above its middle at an oblique angle. Contractions of these muscles *depress* the skin over their attachment at the papillary layer of the dermis and elevate the hair shaft and *elevate* the skin around the hair shaft, forming tiny "goose bumps" on the surface of the skin. These are easily observed when one is chilled or suddenly frightened.

Histophysiology of Hair

Hair grows at an average rate of about 1 to 1.3 cm/month, but hair growth is not continuous. The hair growth cycle consists of three successive phases: (1) the growth period, the **anagen phase**; (2) a brief period of involution, the **catagen phase**; and (3) the final phase of rest, the **telogen phase**, in which the mature, aged

Figure 14–13 Scanning electron micrograph of monkey scalp that shows three hair shafts and their sebaceous glands surrounded by the dense, irregular, collagenous connective tissue of the dermis (×235). (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1998.)

hair is shed (falls out or is pulled out). Hairs shed in this fashion are called ***club hairs*** because they retain their club-shaped root. Soon afterward, a new hair is formed by the hair follicle, and the hair growth cycle begins again.

The duration of the hair growth cycle varies in different areas of the body. For example, the life span of an axillary hair is roughly 4 months, whereas scalp hair may remain in the anagen phase for as long as 6 years and in the telogen phase for 4 months.

Hair follicles in certain regions of the body respond to male sex hormones. For this reason, men begin to develop more dark-pigmented terminal hairs about the chin, cheeks, and upper lip at puberty. Although women possess the same number of hair follicles in these regions, these hairs on women remain of the fine, pale, vellus type. In both sexes at puberty, however, heavily pigmented, coarse terminal hairs begin to grow in the axillary and pubic regions.

The keratinization processes in hair and in skin, although generally similar, differ in some respects. The superficial cell layers of the epidermis of the skin form a **soft keratin**, consisting of keratin filaments embedded in trichohyalin and filaggrin; the keratinized cells are sloughed continuously. In contrast, not only does keratinization of hair form a **hard keratin**, consisting of keratin filaments embedded in trichohyalin but not filaggrin, but the keratinizing cells are not shed; instead, they accumulate, becoming compressed and hard.

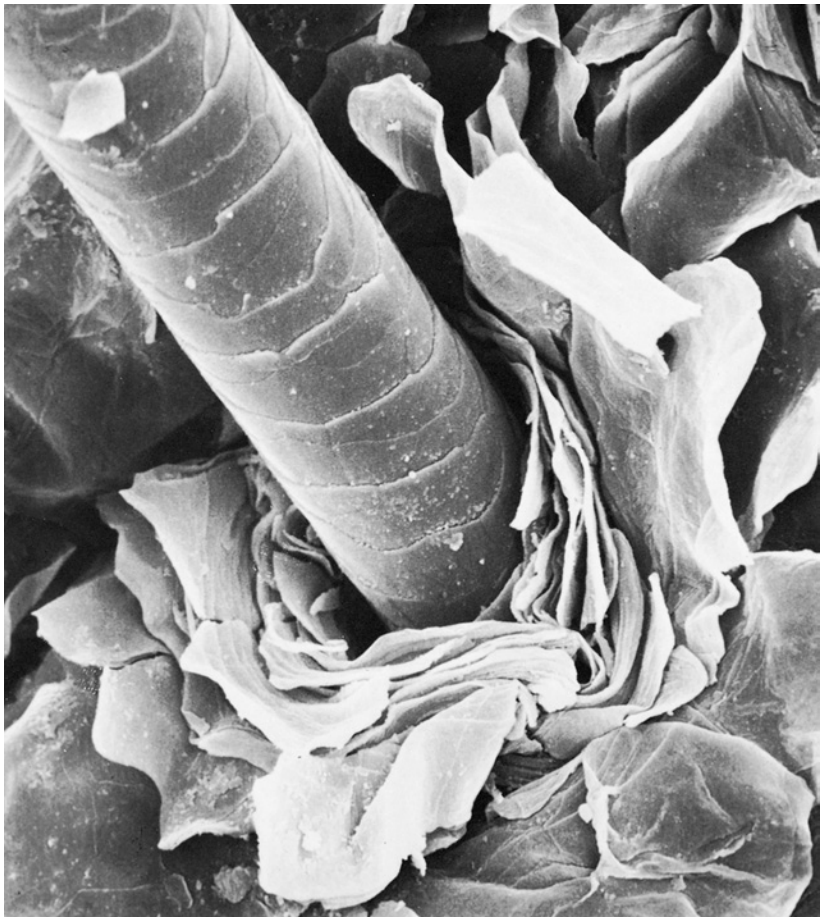


Figure 14–14 Scanning electron micrograph of a hair from a monkey's scalp (×1115). (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

The arrangement of cells composing the cuticle of the hair and cuticle of the internal root sheath interlocks the opposing free edges of these cells, making it difficult to pull the hair shaft out of its follicle (Fig. 14–14).

Nails

Nails represent keratinized epithelial cells arranged in plates of hard keratin.

Nails, located on the distal phalanx of each finger and toe, are composed of plates of heavily compacted, highly keratinized epithelial cells that form the **nail plate**, lying on the epidermis, known as the ***nail bed*** (Figs. 14–15 and 14–16). The nails develop from cells of the **nail matrix** that proliferate and become keratinized. The nail matrix, a region of the **nail root**, is located beneath the **proximal nail fold**. The stratum corneum of the proximal nail fold forms the **eponychium (cuticle)**, which extends from the proximal end up on the nail for about 0.5 to 1 mm. Laterally, the skin turns under as **lateral nail folds**, forming the **lateral nail**

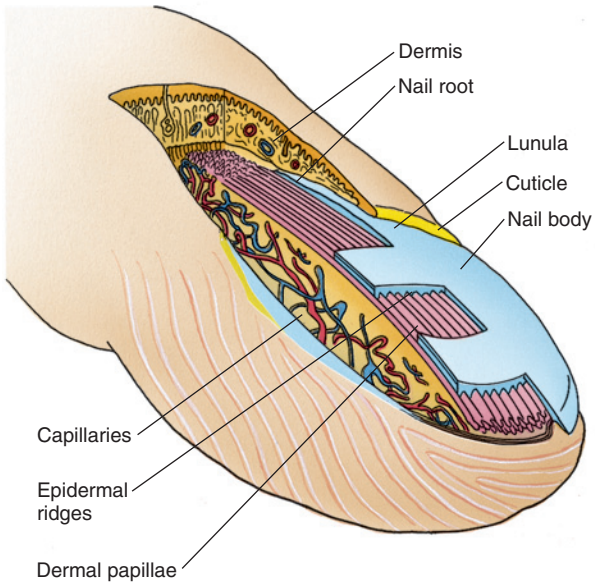


Figure 14–15 Diagram of the structure of the thumbnail.

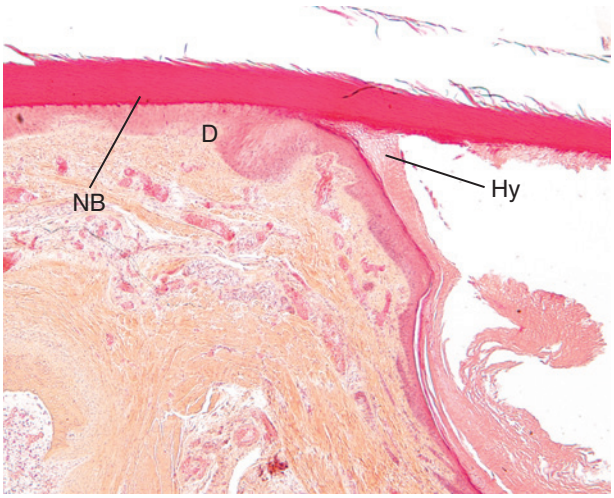


Figure 14–16 Longitudinal section through a fingernail. Observe the dermis (D), hyponychium (Hy), and the nail bed (NB) (×14).

grooves; the epidermis continues beneath the nail plate as the **nail bed**, and the nail plate occupies the position (and function) of the stratum corneum.

The **lunula**, the white crescent, is observed at the proximal end of the nail. The distal end of the nail plate is not attached to the nail bed, which becomes continuous with the skin of the finger (or toe) tip. Near this junction is an accumulation of stratum corneum called the ***hyponychium***. Fingernails grow continuously at the rate of about 0.5 mm/week; toenails grow somewhat more slowly. The translucency of the fingernails provides a quick indication of the health of an individual; pinkness indicates a well-oxygenated blood supply.

PATHOLOGICAL CONSIDERATIONS

See Figures 14–17 through 14–20.

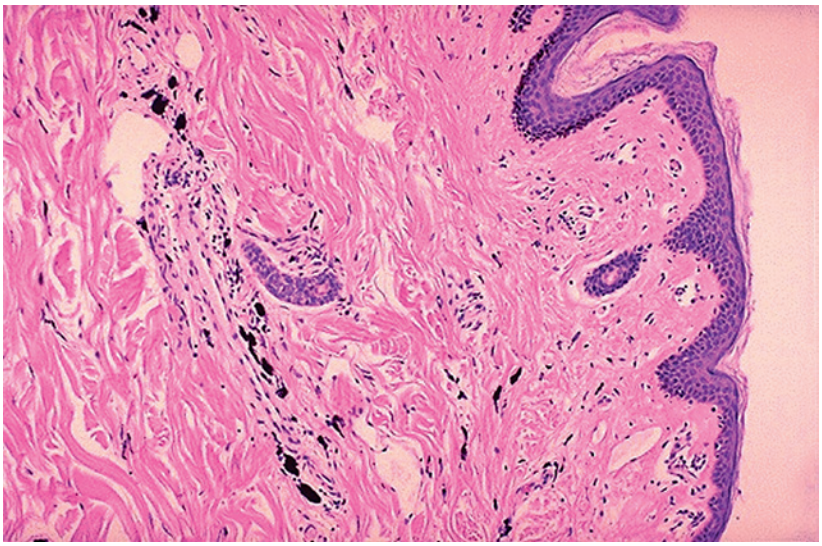


Figure 14–17 Photomicrograph of a lateral surface of a hand with a tattoo. Note that there are tattoo pigments in the dermis. Because the pigment is deposited rather deep in the dermis, it is very difficult to remove it at a later date. Also, unless the tattooing conditions are very clean, infections may be introduced at the same time. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:5.)

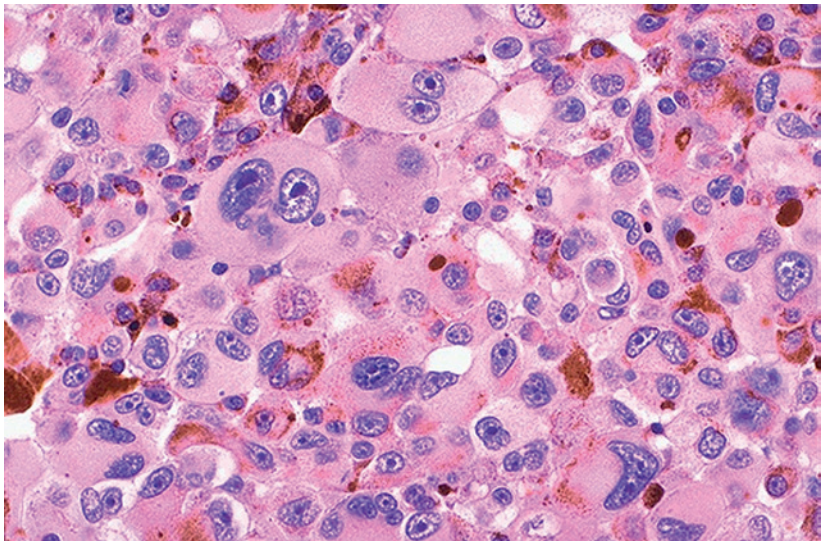


Figure 14–18 Photomicrograph of a malignant melanoma. Note the large polygonal cells with pleomorphic nuclei and clearly evident nucleoli. The amount of pigment in melanoma cells is inconstant, and this variability in pigment formation permits the differentiation of melanoma from a benign nevus. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:406.)

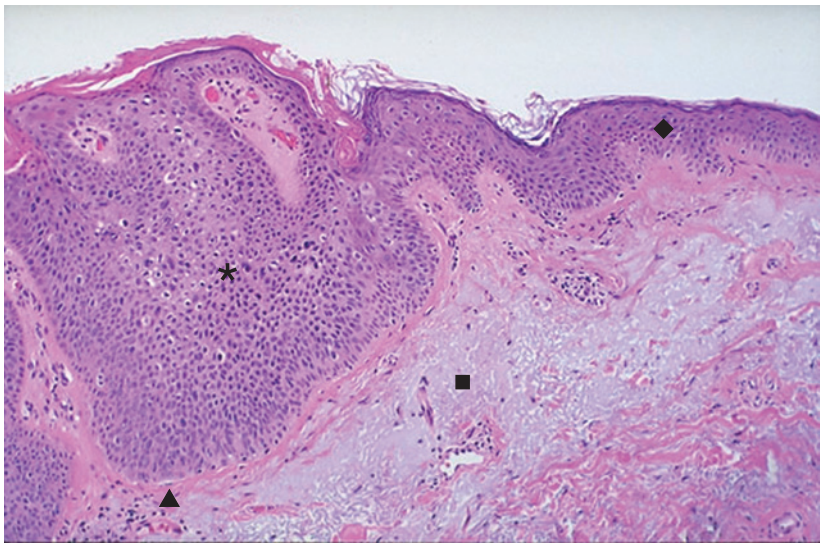


Figure 14–19 Photomicrograph of a squamous cell carcinoma in situ. The reason for the term *in situ* is that the lesion did not pass through the basement membrane (triangle). The right side of the photomicrograph presents healthy, normal skin (diamond) whereas the cancerous epidermis is very thick (*). Observe the ultraviolet ray–damaged dermis, where the collagen fibers are pale, referred to as *solar elastosis*. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:412.)

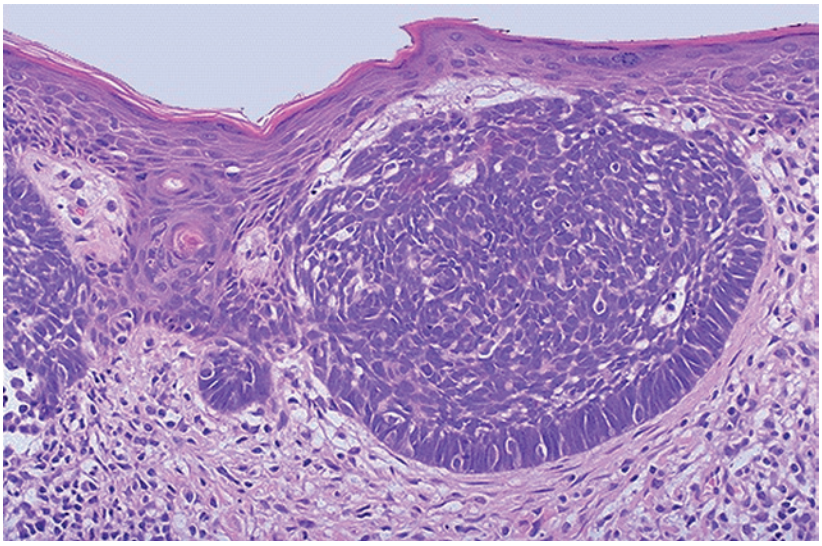
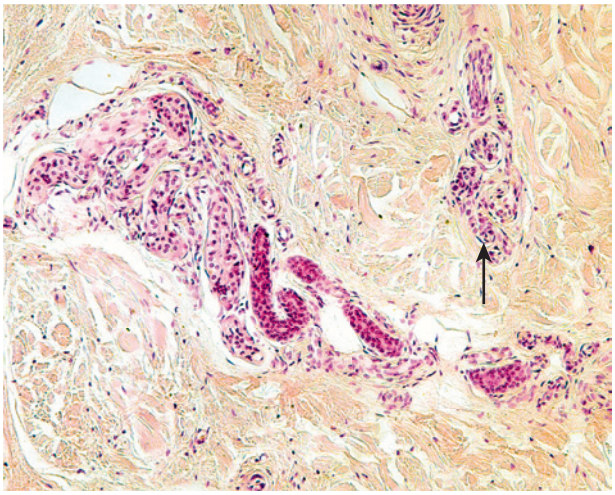


Figure 14–20 Photomicrograph of a basal cell carcinoma. The dark blue malignant cells are more or less elliptical in shape with very little cytoplasm. Most of the cells are similar to cells of the stratum basale of the normal epidermis. The tumor cells are compacted into islands or peninsulas that infiltrate the dermis and are surrounded by a fibrous stroma that displays variable degrees of inflammatory cells. Patients who have been chronically exposed to the sun and those who are immunosuppressed with xeroderma pigmentosum frequently develop basal cell carcinoma. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:413.)

REVIEW QUESTIONS

1. Which of the following is correct about freckles? They
   a. are hyperpigmented spots on the skin.
   b. are due to an increased number of melanocytes in the dermis.
   c. are due to an increased number of melanocytes in the epidermis.
   d. fade in the summer.
   e. darken in the winter.
2. A 45-year-old patient sees his dermatologist presenting with a skin condition. The patient was bitten on the leg by a dog and then developed a red, warm, painful, swollen area in the region of the bite. The physician suspects
   a. boils.
   b. impetigo.
   c. rosacea.
   d. sebaceous cyst.
   e. cellulitis.
3. Which of the following statements is not correct concerning skin?
   a. Melanocytes possess tyrosinase, a substance located in the endosomes.
   b. Merkel cells are mechanoreceptors.
   c. Langerhans cells are derived from the bone marrow.
   d. Cells of the stratum granulosum possess keratohyalin.
   e. Cells of the stratum spinosum possess membrane-coating granules.
4. Sebaceous glands are classified as
   a. apocrine glands.
   b. compound glands.
   c. holocrine glands.
   d. simple coiled tubular glands.
   e. mixed glands.
5. A medical student playing softball chased the ball through a field populated with patches of poison ivy. The student's skin came in contact with the poisonous plant. Which of the following cells processes and presents the plant antigen to initiate the immune response of the skin?
   a. T helper cell
   b. Cytotoxic T lymphocyte
   c. Plasma cell
   d. Langerhans cell
   e. T reg cell
6. A young African American child is taken to his dermatologist by his parents, who are concerned about a lack of pigmentation in some regions of his body. The dermatologist should suspect
   a. albinism.
   b. tinea versicolor.
   c. vitiligo.
   d. squamous cell carcinoma.
   e. melanoma.
7. A 30-year-old healthy patient is seen by his family physician with a scaly, red, crusty area on his forehead that does not appear to heal. The physician determines that the lesion has increased in size. The physician should suspect
   a. sunburn.
   b. rosacea.
   c. melanoma.
   d. squamous cell carcinoma.
   e. Kaposi sarcoma.
8. The structure at the tip of the arrow
   a. is a mechanoreceptor.
   b. manufactures sebum.
   c. manufactures a mucous secretion.
   d. manufactures sweat.
   e. is the duct of a sweat gland.



9. The encircled structure
   a. is a mechanoreceptor.
   b. manufactures sebum.
   c. manufactures a mucous secretion.
   d. manufactures sweat.
   e. is the duct of a sweat gland.

10. Melanocytes are located in which of the following layers of the epidermis?
    a. Stratum corneum
    b. Stratum lucidum
    c. Stratum granulosum
    d. Stratum spinosum
    e. Stratum basale

ANSWERS

1. Freckles are hyperpigmented areas that are located on the sun-exposed areas of the skin, especially in fair-skinned individuals who burn easily. Freckles are the result of excess melanin production and not to the presence of more melanocytes. They fade in the winter and darken in the summer because of ultraviolet light exposure.
2. E. The physician should suspect cellulitis, a condition common after an animal bite and is usually caused by staphylococcal or streptococcal infection. Boils usually form around hair follicles and contain pus or a bloody discharge. Impetigo is usually present in children who present pus-filled blisters. Rosacea usually appears on the face and presents red areas that frequently display the presence of small pimples. Sebaceous cysts are keratin and sebum-containing cysts located usually on the face, head, and back.
3. A. Melanocytes possess tyrosinase, but the enzyme is located in melanosomes not endosomes. All of the other statements are correct.
4. C. Sebaceous glands are classified as holocrine gland because their cells die and become the secretion. Apocrine glands are classified as such because a portion of their cytoplasm becomes part of the secretion. The ducts of compound glands branch, whereas the ducts of sebaceous glands do not branch. Simple coiled tubular glands are cork-screw shaped, such as sweat glands. Mixed glands produce a secretion that contains both serous and mucous components.
5. C. Langerhans cells are antigen-presenting cells that are derived from monocyte precursors in the bone marrow and migrate into the stratum spinosum of the epidermis. Langerhans cells pick up and process antigens and migrate to the nearest lymph node to present their MHC II–epitope complex to T cells to initiate an immune response to the antigen. T helper cells, cytotoxic T lymphocytes, plasma cells, and T reg cells do not reside in the epidermis.
6. C. Vitiligo is a condition in which patches of skin lose their melanocytes and appear to be whitish and smooth. This condition is most apparent in individuals with dark pigmentation. Albinism is lack of pigment over the entire body and the iris. Tinea versicolor is a fungal infection that may resemble vitiligo, but often the involved region appears darker than the surrounding skin. Squamous cell carcinoma appears scaly and reddened with ulcerations being common. Melanoma is usually present in fair-skinned people and is related to excess exposure to sunlight.
7. D. Squamous cell carcinoma originates in regions of the skin that are usually exposed to the sun. It can spread to other areas of the body and then can be fatal, but usually, minor surgical procedures will cure the patient. Sunburn is evidenced by a reddish coloration and, depending on the intensity of the sunburn, a great deal of pain and blistering. Rosacea usually appears on the face and presents red areas that frequently display the presence of small pimples. Melanoma frequently develops from preexisting moles and is characterized by pigmentation. Frequently, melanomas metastasize, with death ensuing within months or, at most, a few years. Kaposi sarcoma is a carcinoma that originates in the vascular supply of the dermis. It is a disease of older people of Mediterranean background or immunocompromised younger patients who have AIDS.
8. D. The structure at the tip of the arrow is the secretory portion of an eccrine sweat gland and manufactures sweat; it is composed of a simple cuboidal epithelium. The duct of the sweat gland is to the right of the arrow, evident as a darker staining structure composed of a stratified cuboidal epithelium (two layers of cuboidal cells).
9. B. The structure located within the circle is a part of a sebaceous gland that manufactures sebum. Sebaceous glands are classified as holocrine glands because the secretory cells accumulate sebum and die to become a part of the secretion.
10. E. Melanocytes, derived from cells of the neural crest, are located in the stratum basale (stratum germinativum). Occasionally, they may also be present in the dermis–epidermis interface. The processes of melanocytes extend into the stratum spinosum, but the cell bodies of melanocytes do not enter the spinosum layer.

This page intentionally left blank

Respiratory System

15

The **respiratory system**, comprising the lungs and a sequence of airways leading to the external environment, functions in providing oxygen (O_2) to and eliminating carbon dioxide (CO_2) from the cells of the body. The realization of this goal requires the fulfillment of the following four discrete events, collectively known as *respiration*:

- Movement of air in and out of the lungs **(breathing** or **ventilation)**
- Exchange of O_2 in the inspired air for carbon dioxide in the blood **(external respiration)**
- Conveyance of O_2 and CO_2 to and from the cells **(transport of gases)**
- Exchange of CO_2 for O_2 in the vicinity of the cells **(internal respiration)**

The first two of these events, ventilation and external respiration, occur within the confines of the respiratory system, whereas the transport of gases is performed by the circulatory system, and internal respiration occurs in the cells throughout the body.

The respiratory system is subdivided into two major components: the conducting portion and the respiratory portion. The **conducting portion**, situated both outside and within the lungs, conveys air from the external milieu to the lungs. The **respiratory portion**, located strictly within the lungs, functions in the actual exchange of oxygen for carbon dioxide (external respiration). Characteristic features of these two portions are listed in Table 15–1.

CONDUCTING PORTION OF THE RESPIRATORY SYSTEM

The conducting portion of the respiratory system conveys air to and from the respiratory portion of the respiratory system.

The **conducting portion** of the respiratory system, listed in order from the exterior to the inside of the lung, is composed of the nasal cavity, mouth, nasopharynx, pharynx, larynx, trachea, primary bronchi, secondary bronchi (lobar bronchi), tertiary bronchi (segmental bronchi), bronchioles, and terminal bronchioles. These structures not only transport but also filter, moisten, and warm the inspired air before it reaches the respiratory portion of the lungs.

The patency of the conducting airways is maintained by a combination of bone, cartilage, and fibrous elements. As the air progresses along the airway during inspiration, it encounters a branching system of tubules. Although the luminal diameter of each succeeding tubule continues to decrease, the total cross-sectional diameter of the various branches increases at each level of branching. As a result, the velocity of air flow for a given volume of inhaled air decreases as the air proceeds toward the respiratory portion.

Nasal Cavity

The **nasal cavity** is divided into right and left halves by the cartilaginous and bony nasal septum. Each half of the nasal cavity is bounded laterally by a bony wall and a cartilaginous ala (wing) of the nose; it communicates with the outside, anteriorly, via the **naris** (nostril) and with the nasopharynx by way of the **choana**. Projecting from the bony lateral wall are three thin, scroll-like bony shelves, situated one above the other: the superior, middle, and inferior **nasal conchae**.

Anterior Portion of the Nasal Cavity (Vestibule)

The anterior portion of the nasal cavity, in the vicinity of the nares, is dilated and is known as the ***vestibule***. This region is lined with thin skin and has **vibrissae**—short, stiff hairs that prevent larger dust particles from entering the nasal cavity. The dermis of the vestibule houses numerous sebaceous and sweat glands. The dermis is anchored by numerous collagen bundles to the perichondria of the hyaline cartilage segments that form the supporting skeleton of the ala.

Posterior Aspect of the Nasal Cavity

Except for the vestibule and the olfactory region, the nasal cavity is lined by pseudostratified ciliated columnar epithelium, frequently called the ***respiratory epithelium*** (see the discussion on the trachea), which is well-endowed with goblet cells in the more posterior regions of the nasal cavity. The subepithelial connective tissue **(lamina propria)** is richly vascularized, especially in the region of the conchae and the anterior aspect of the nasal septum, housing large arterial plexuses and venous sinuses. The lamina propria has many seromucous glands and abundant lymphoid elements, including occasional lymphoid nodules, mast cells, and plasma cells. Antibodies produced by plasma cells

SUMMARY

The **respiratory system** consists of the lungs and the system of ducts **(nasal cavity**, **trachea**, **primary bronchi**, **secondary bronchi**, **bronchioles**, **terminal bronchioles)** that bring oxygen-rich air to and carbon dioxide-rich air from the **lungs**. The movement of air into and out of the lungs is known as ***ventilation***, and the exchange of oxygen in the inspired air for carbon dioxide in the blood is known as ***external respiration***. These two events occur in the respiratory system. The **transport of gases** to and from the cells of the body and the exchange of carbon dioxide for oxygen in the vicinity of the cells is known as ***internal respiration***. These two events occur outside of the respiratory system. External respiration occurs across the **blood–gas barriers** of small air-filled spaces of the lungs, known as ***alveoli***, ***alveolar sacs***, ***alveolar ducts***, and **respiratory bronchioles**; the walls of these spaces, composed of **type I and type II pneumocytes**, are lined on the air side with **pulmonary surfactant**, a substance that reduces surface tension and helps in maintaining the patency of the air spaces and, on the capillary side, by the fused **basal laminae** of the capillary endothelium and type I pneumocytes. The **blood–gas barrier** separates blood of the capillaries from the air of these spaces.

KEY WORDS

Alveolar ducts; alveolar sacs; alveoli; basal lamina; blood–gas barrier; bronchioles; external respiration; internal respiration; lungs; nasal cavity; primary bronchi; pulmonary surfactant; respiratory bronchioles; respiratory system; secondary bronchi; terminal bronchioles; trachea; transport of gases; type I pneumocytes; type II pneumocytes; ventilation

TABLE 15–1 Characteristic Features of the Respiratory System

Division	Region	Support	Glands	Epithelium	Cell Types	Additional Features
Extrapulmonary conducting	Nasal vestibule	Hyaline cartilage	Sebaceous and sweat glands	Stratified squamous keratinized	Epidermal	Vibrissae
	Nasal cavity: respiratory	Hyaline cartilage and bone	Seromucous glands	Respiratory	Basal, goblet, ciliated, brush, serous, and DNES	Erectile-like tissue
	Nasal cavity: olfactory	Bone	Bowman glands (serous)	Olfactory	Olfactory, sustentacular, and basal	Olfactory vesicle
Extrapulmonary conducting	Nasopharynx	Skeletal muscle	Seromucous glands	Respiratory	Basal, goblet, ciliated, brush, serous, and DNES	Pharyngeal tonsils and eustachian tubes
	Larynx	Hyaline and elastic cartilages	Mucous and seromucous glands	Respiratory and stratified squamous nonkeratinized	Basal, goblet, ciliated, brush, serous, and DNES	Epiglottis, vocal folds, and vestibular folds
	Trachea and primary bronchi	Hyaline cartilage and dense, irregular collagenous connective tissue	Mucous and seromucous glands	Respiratory	Basal, goblet, ciliated, brush, serous, and DNES	C-rings and trachealis muscle (smooth muscle) in adventitia
Intrapulmonary conducting	Secondary (intrapulmonary) bronchi	Hyaline cartilage and smooth muscle	Seromucous glands	Respiratory	Basal, goblet, ciliated, brush, serous, and DNES	Plates of hyaline cartilage and two ribbons of helically oriented smooth muscle
	(Primary) bronchioles	Smooth muscle	No glands	Simple columnar to simple cuboidal	Ciliated cells and club cells (and occasional goblet cells in larger bronchioles)	Less than 1 mm in diameter; supply air to lobules; two ribbons of helically oriented smooth muscle
	Terminal bronchioles	Smooth muscle	No glands	Simple cuboidal	Some ciliated cells and many club cells (no goblet cells)	Less than 0.5 mm in diameter; supply air to lung acini; some smooth muscle
Respiratory	Respiratory bronchioles	Some smooth muscle and collagen fibers	No glands	Simple cuboidal and highly attenuated simple squamous	Some ciliated cuboidal cells, club cells, and types I and II pneumocytes	Alveoli in their walls; alveoli have smooth muscle sphincters in their opening
	Alveolar ducts	Type III collagen (reticular) fibers and smooth muscle sphincters of alveoli	No glands	Highly attenuated simple squamous	Type I and type II pneumocytes of alveoli	No walls of their own, only a linear sequence of alveoli
	Alveolar sacs	Type III collagen and elastic fibers	No glands	Highly attenuated simple squamous	Type I and type II pneumocytes	Clusters of alveoli
	Alveoli	Type III collagen and elastic fibers	No glands	Highly attenuated simple squamous	Type I and type II pneumocytes	200 μm in diameter; have alveolar macrophages

DNES, Diffuse neuroendocrine system.

(immunoglobulins IgA, IgE, and IgG) protect the nasal mucosa against inhaled antigens as well as against microbial invasion.

CLINICAL CORRELATIONS

Nasal bleeding usually occurs from **Kiesselbach area**, the anteroinferior region of the nasal septum, which is the site of anastomosis of the arterial supply of the nasal mucosa. The bleeding may be arrested by applying pressure on the region or by packing the nasal cavity with cotton.

Olfactory Region of the Nasal Cavity

The olfactory region comprises the olfactory epithelium and the underlying lamina propria that houses Bowman glands and a rich vascular plexus.

The roof of the nasal cavity, the superior aspect of the nasal septum, and the superior concha are covered by an olfactory epithelium 60 µm thick. The underlying lamina propria houses the serous fluid–secreting Bowman glands, a rich vascular plexus, and collections of axons that arise from the olfactory cells of the **olfactory epithelium**. The olfactory epithelium, which has a yellow color in the living person, is composed of three types of cells: olfactory, sustentacular, and basal (Fig. 15–1).

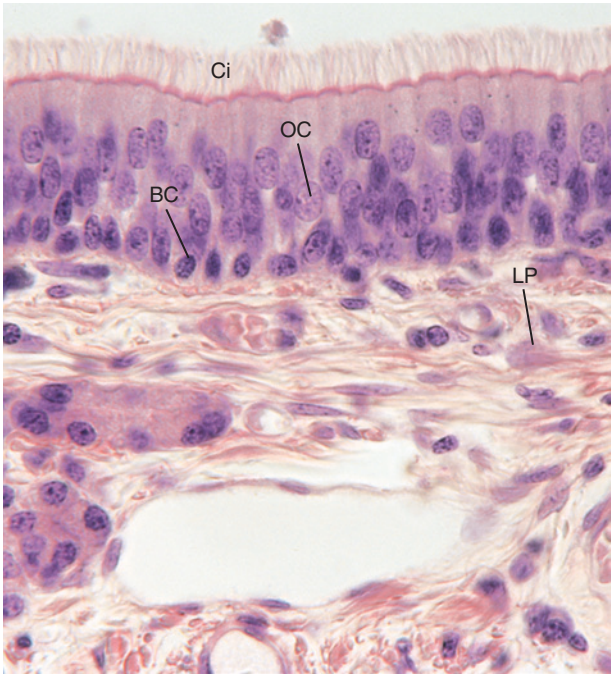


Figure 15–1 Photomicrograph of the human olfactory mucosa. Observe that the olfactory cilia (Ci) are well represented and that the connective tissue displays the presence of Bowman glands (×540). BC, Basal cell; LP, lamina propria; OC, olfactory cell.

Olfactory Cells

Olfactory cells are bipolar neurons whose peripheral (distal) aspect is modified to form the olfactory vesicle and olfactory cilia, whereas its central aspect forms an axon that joins other axons to synapse in the olfactory bulb.

Olfactory cells are bipolar neurons whose apical aspect, the distal terminus of its slender dendrite, is modified to form a bulb, the **olfactory vesicle**, which projects above the surface of the sustentacular cells (Figs. 15–2 and 15–3). The nucleus of the olfactory cell is spherical and is closer to the basal lamina than to the olfactory vesicle. Most of the organelles of the cell are in the vicinity of the nucleus.

Scanning electron micrographs demonstrate that six to eight long, nonmotile olfactory cilia extend from the olfactory vesicle and lie on the free surface of the epithelium. Transmission electron micrographs of these cilia display an unusual axoneme pattern that begins as a typical peripheral ring of nine **doublet** microtubules surrounding two central **singlets** (9 + 2 configuration) but without the characteristic dynein arms. The axoneme changes distally so that it is composed of nine **singlets** surrounding the two central singlets, and, near the end of the cilium, only the central singlets are present.

The basal region of the olfactory cell is its **axon**, which penetrates the basal lamina and joins similar axons to form bundles of nerve fibers. Each axon, although unmyelinated, has a sheath composed of Schwann cell-like olfactory ensheathing (glial) cells. The nerve fibers pass through foramina of the cribriform plate in the roof of the nasal cavity to synapse with secondary neurons in the olfactory bulb.

CLINICAL CORRELATIONS

Human herpes virus 6 (HHV-6) is responsible for a very common mild infection, known as ***roseola***, in infants and toddlers. In fact, most adults possess the HHV-6 that they acquired as an infant. During an HHV-6 infection, the virus can sometimes invade the nasal cavity and establish residence in the mucus and, from there, follow the olfactory nerves through the cribriform plate and infect the olfactory bulb of the brain.

Sustentacular and Basal Cells

Sustentacular cells are 50- to 60-µm-tall columnar cells whose apical aspects have a striated border composed of microvilli. Their oval nuclei are in the apical third of the cell, somewhat superficial to the location of the olfactory cell nuclei. The apical cytoplasm of these cells displays the presence of secretory granules housing a yellow pigment that provides the characteristic color of the olfactory mucosa. Electron micrographs of sustentacular cells demonstrate that they form junctional

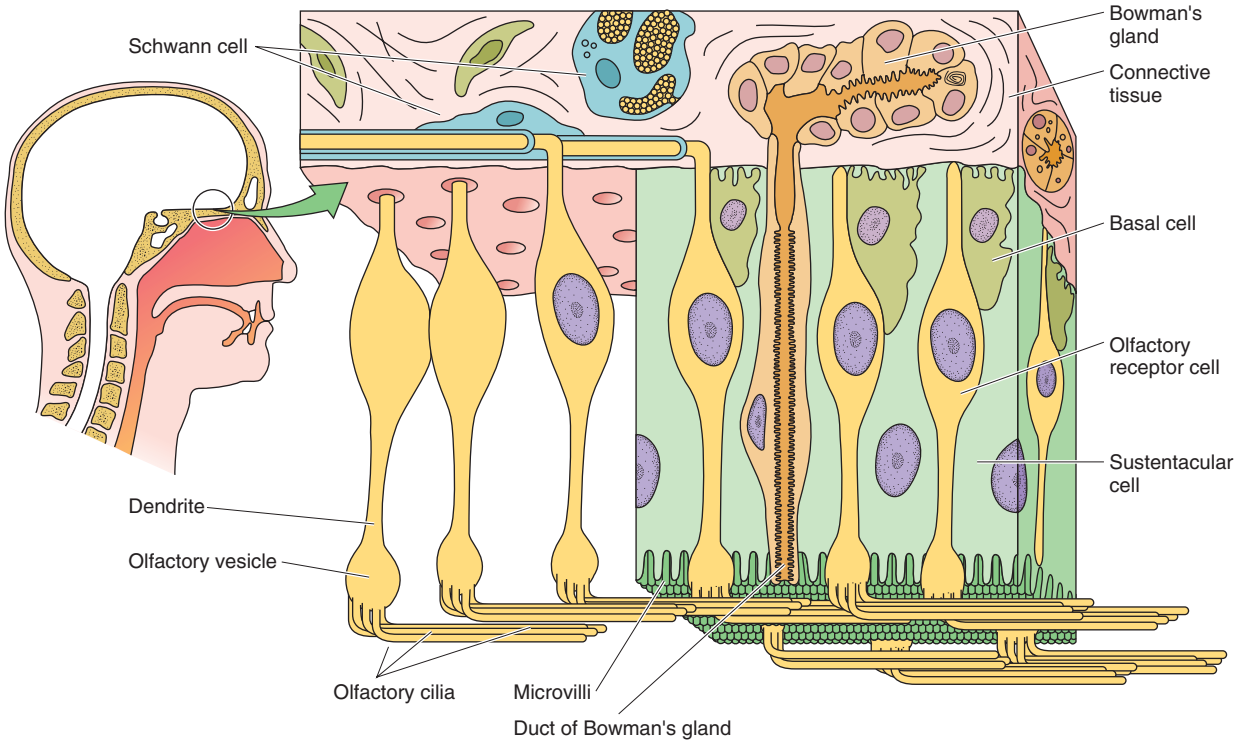


Figure 15–2 Schematic diagram of the olfactory epithelium showing basal, olfactory, and sustentacular cells. Compare this diagram with the actual photomicrograph of the olfactory mucosa presented in the previous figure.

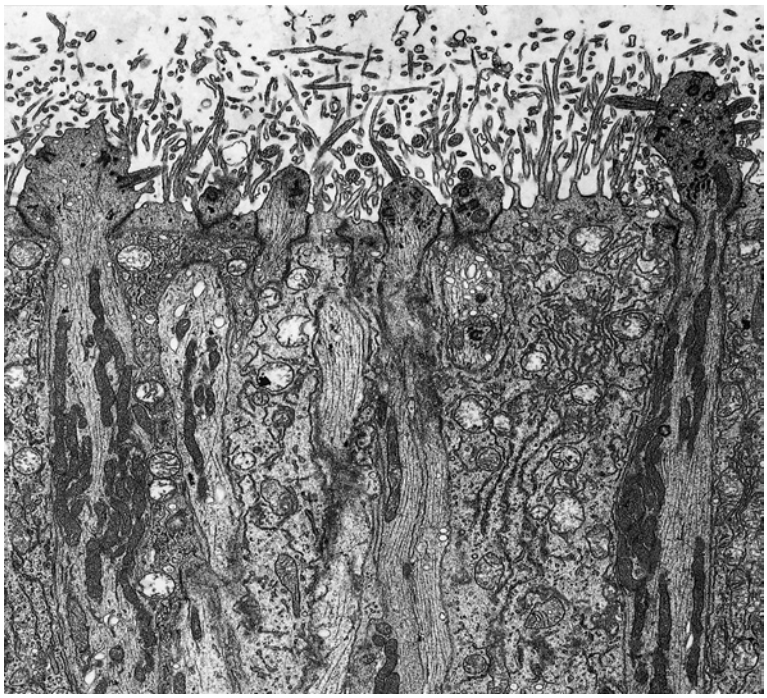


Figure 15–3 Transmission electron micrograph of the apical region of the rat olfactory epithelium. Note the olfactory vesicles and the cilia projecting from them. Compare this electron micrograph with the previous two figures (×8260). (From Mendoza AS, Kühnel W. Postnatal changes in the ultrastructure of the rat olfactory epithelium: the supranuclear region of supporting cells. *Cell Tissue Res*. 1991;265:193-196. Copyright by Springer-Verlag.)

complexes with the olfactory vesicle regions of olfactory cells as well as with other contiguous sustentacular cells. The morphology of sustentacular cells is not remarkable, although they do display a prominent terminal web of actin microfilaments. These cells are believed to provide physical support, nourishment, and electrical insulation for the olfactory cells.

Basal cells are of two types: horizontal and globose. Horizontal cells are flat and lie against the basement membrane, whereas globose cells are round, short, basophilic, pyramid-shaped cells; the apical aspects of the basal cells do not reach the epithelial free surface. Their nuclei are centrally located, but because these are short cells, the nuclei occupy the basal third of the epithelium. The globose type of basal cells have considerable proliferative capacity and can replace both sustentacular and olfactory cells. In a healthy person, the olfactory cells live for less than 3 months, and sustentacular cells have a life span of less than 1 year. The horizontal basal cells replicate to replace the globose basal cells.

Lamina Propria

The lamina propria of the olfactory mucosa is composed of a richly vascularized, loose to dense, irregular collagenous connective tissue that is firmly attached to the underlying periosteum. It houses numerous lymphoid elements as well as the collection of axons of the olfactory cells, which form fascicles of unmyelinated nerve fibers. **Bowman glands (olfactory glands)**, which produce a *serous* secretory product, are also present and are indicative of the olfactory mucosa. These glands release IgA, lactoferrin, lysozyme, and odorant binding protein, a molecule that prevents the odorant from leaving the region of the olfactory epithelium, thus enhancing the individual's ability to detect odors.

Histophysiology of the Nasal Cavity

The nasal mucosa filters, warms, and humidifies the inhaled air and is also responsible for the perception of odors.

The moist nasal mucosa filters inhaled air. Particulate matter, such as dust, is trapped by the mucus produced by the goblet cells of the epithelium and the seromucous glands of the lamina propria. The serous fluid, produced by the seromucous glands, is situated between the mucus and the apical plasmalemmae of the respiratory epithelial cells. Because the cilia of the ciliated columnar cells do not reach the mucous layer, their movement is restricted to the serous fluid layer. As the cilia move within that watery fluid, the mucus is swept along *(hydroplaned)* at the interface of the two fluids. The particulate matter trapped in mucus is thus delivered, by ciliary action, to the pharynx to be swallowed or expectorated.

In addition to being filtered, the air is also warmed and humidified by being passed over the mucosa, which is kept warm and moist by its rich blood supply. Warming of the inspired air is facilitated by the presence of an extensive network of rows of arched vessels grouped in an anteroposterior arrangement. Capillary beds arising from these vessels lie just beneath the epithelium and the flow of blood into this vascular network is directed from posterior to anterior, opposite to the flow of air; thus, heat is continuously being transferred to the inspired air by a countercurrent mechanism.

Antigens and allergens carried by the air are combated by lymphoid elements of the lamina propria. Secretory immunoglobulin (IgA), produced by plasma cells, is transported across the epithelium into the nasal cavity by ciliated columnar cells and by the acinar cells of the seromucous glands. IgE, which is also produced by plasma cells, binds to IgE receptors (FcεRI receptors) of mast cell and basophil plasmalemmae. Subsequent binding of a specific antigen or allergen to the bound IgE causes the mast cell (and basophil) to release various mediators of inflammation. These, in turn, act on the nasal mucosa, inducing the symptoms associated with colds and hay fever.

CLINICAL CORRELATIONS

1. The nasal mucosa is protected from dehydration by alternating blood flow to the venous sinuses of the lamina propria overlying the conchae of the right and left nasal cavities. The erectile tissue–like region **(swell bodies)** of one side expands when its venous sinuses become engorged with blood, reducing the flow of air through that side. Seepage of plasma from the sinuses and seromucous secretions from the glands thus rehydrate the mucosa approximately every half hour.
2. Chemical irritants and particulate matter are removed from the nasal cavity by the **sneeze reflex**. The sudden explosive expulsion of air usually clears the nasal passage of the irritant.

The olfactory epithelium is responsible for the perception of odors, which also makes a major contribution to the sense of taste discrimination. The mechanism of odor discernment is poorly understood, although it is known that the plasmalemma of the olfactory cilia of a particular olfactory cell has numerous copies of one particular **odor receptor molecule**. Molecules of an odoriferous substance dissolved in the serous fluid bind to its specific receptor. When a threshold number of odor receptors are occupied, the olfactory cell becomes stimulated, an action potential is generated, and the information is transmitted via its axon to the olfactory bulb, a projection of the central nervous system, for processing. Axons of olfactory cells synapse with the

dendrites of 1 of 30 mitral cells within small spherical regions of the olfactory bulb, known as ***glomeruli***. If a threshold level of impulses reaches a mitral cell, it becomes depolarized and relays the signal to the olfactory cortex for further processing.

Each glomerulus receives input (information) from approximately 2000 olfactory neurons, each specific for the same odoriferous substance. Similar to antigens, which may have several epitopes, each of which binds a specific antibody, odoriferous substances possess several small regions, each of which binds to a specific odor receptor molecule. Thus, a particular odoriferous substance may bind to several odor receptor molecules, activating a number of olfactory neurons and providing input to several glomeruli. To ensure that a single stimulus does not produce repeated responses, the continuous flow of serous fluid from Bowman glands provides a constant refreshing of the odorants impinging on the olfactory cilia.

CLINICAL CORRELATIONS

1. Although there are only about 1000 glomeruli, each receiving information concerning a single odor receptor molecule, the human olfactory cortex has the ability to distinguish about 1 trillion different scents. It does so by recognizing information arising from a particular combination of glomeruli as a single scent. Thus, a particular glomerulus may be active in the recognition of several odors.
2. It appears that individuals who experience fear express that sense of fear as a specific scent in their sweat. Certain individuals have the ability to smell that fear and may react to it by also experiencing fear, suggesting that certain emotions, such as fear, may be transmitted from one person to another by the secretion and olfactory recognition of chemical signals.

Paranasal Sinuses

The ethmoid, sphenoid, frontal, and maxilla bones of the skull house large, mucoperiosteum-lined spaces, the **paranasal sinuses** (named after their location), which communicate with the nasal cavity. The mucosa of each sinus is composed of a vascular connective tissue lamina propria fused with the periosteum. The thin lamina propria resembles that of the nasal cavity, in that it houses seromucous glands as well as lymphoid elements. The respiratory epithelial lining of the paranasal sinuses, similar to that of the nasal cavity, has numerous ciliated columnar cells whose cilia sweep the mucus layer toward the nasal cavity.

Nasopharynx

The pharynx begins at the choana and extends to the opening of the larynx. This continuous cavity is subdivided into three regions: (1) the superior **nasopharynx**, (2) the middle **oral pharynx**, and (3) the inferior **laryngeal pharynx**. The nasopharynx is lined by a respiratory epithelium, whereas the oral and laryngeal pharynges are lined by a stratified squamous epithelium. The lamina propria is composed of a loose to dense, irregular type of vascularized connective tissue housing seromucous glands and lymphoid elements. It is fused with the epimysium of the skeletal muscle components of the pharynx. The lamina propria of the posterior aspect of the nasopharynx houses the **pharyngeal tonsil**, an unencapsulated collection of lymphoid tissue described in Chapter 12.

CLINICAL CORRELATIONS

The lateral walls of the right and left nasopharynx display the openings of the **auditory tubes (eustachian tubes)**, which connect the middle ear cavities of the respective side to the nasopharynx. Occasionally, the opening in the nasopharynx becomes plugged with mucus, and when atmospheric pressure differences occur between the middle ear and the nasopharynx, as during the landing or take-off of an airplane, the individual experiences an earache that can be alleviated by swallowing saliva or blowing one's nose.

Larynx

The larynx, or voice box, is responsible for phonation and for preventing the entry of food and fluids into the respiratory system.

The **larynx**, situated between the pharynx and the trachea, is a rigid, short, cylindrical tube 4 cm in length and approximately 4 cm in diameter. It is responsible for phonation and prevents the entry of solids or liquids into the respiratory system during swallowing. The wall of the larynx is reinforced by several hyaline cartilages (the unpaired thyroid and cricoid cartilages, and the inferior aspect of the paired arytenoids) and elastic cartilages (the unpaired epiglottis, the paired corniculate and cuneiform cartilages, and the superior aspect of the arytenoids). These cartilages are connected to each other by ligaments, and their movements with respect to one another are controlled by **intrinsic** and **extrinsic skeletal muscles**.

The thyroid and cricoid cartilages form the cylindrical support for the larynx, whereas the epiglottis provides a cover over the laryngeal **aditus** (opening). During respiration, the epiglottis is in the vertical position, permitting the flow of air. During swallowing of food, fluids, or saliva, however, it is positioned horizontally, closing the laryngeal aditus. Yet, normally, even in the absence of an epiglottis, swallowed material bypasses the laryngeal opening. The arytenoid and corniculate cartilages are occasionally fused to each other, and most

of the intrinsic muscles of the larynx move the two arytenoids with respect to each other and to the cricoid cartilage.

The lumen of the larynx is characterized by two pairs of shelf-like folds: the superiorly positioned vestibular folds and the inferiorly placed vocal folds. The **vestibular folds** are immovable. Their lamina propria, composed of loose connective tissue, houses seromucous glands, adipose cells, and lymphoid elements. The free edge of each **vocal fold** is reinforced by dense, regular elastic connective tissue, called the ***vocal ligament***. The vocalis muscle, attached to the vocal ligament, assists the other intrinsic muscles of the larynx in altering the tension on the vocal folds. These muscles also regulate the width of the space between the vocal folds (the **rima glottidis**), thus permitting precisely regulated vibrations of their free edges by the exhaled air.

During silent respiration, the vocal folds are partially *abducted* (pulled apart), and during forced inspiration, they are fully abducted. During phonation, however, the vocal folds are strongly *adducted*, forming a narrow interval between them. The movement of air against the edges of the strongly adducted vocal folds produces and modulates sound (but not speech, which is formed by movements of the pharynx, soft palate, tongue, and lips). The longer and more relaxed the vocal folds, the deeper the **pitch** of the sound. Because the larynx of a postpubescent male is larger than that of a female, men tend to have deeper voices than women.

The larynx is lined by **pseudostratified ciliated columnar epithelium**, except on the superior surfaces of the epiglottis and vocal folds, which are covered by stratified squamous nonkeratinized epithelium. The cilia of the larynx beat toward the pharynx, transporting mucus and trapped particulate matter toward the mouth to be expectorated or swallowed.

CLINICAL CORRELATIONS

1. **Laryngitis**, inflammation of the laryngeal tissues, including the vocal folds, prevents the vocal folds from vibrating freely. Persons with laryngitis sound hoarse or can only whisper.
2. The presence of chemical irritants or particulate matter in the upper air passages, including the trachea or bronchi, elicits the **cough reflex**, producing an explosive rush of air that removes the irritant. The cough reflex begins with the inhalation of a large volume of air and the closure of the epiglottis and glottis (adduction of the vocal folds), followed by a powerful contraction of the muscles responsible for forced expiration (intercostal and abdominal muscles). The sudden opening of the glottis and epiglottis generates a rush of air whose velocity can exceed 100 miles per hour, removing the irritant with an enormous force.

Trachea

The trachea has three layers: mucosa, submucosa, and adventitia; the hyaline cartilaginous support of the trachea is performed by a dozen or so C-shaped structures, known as C-rings, that are located in the adventitia.

The **trachea** is a tube, 12 cm long and 2 cm in diameter, that begins at the cricoid cartilage of the larynx and ends when it bifurcates to form the primary bronchi. The wall of the trachea is reinforced by 10 to 12 horseshoe-shaped hyaline cartilage rings **(C-rings)**. The open ends of these rings face posteriorly and are connected to each other by smooth muscle: the trachealis muscle. Because of this arrangement of the C-rings, the trachea is rounded anteriorly but flattened posteriorly. The perichondrium of each C-ring is connected to the perichondria of other C-rings lying directly above and below it by fibroelastic connective tissue, which provides flexibility to the trachea and permits its elongation during inspiration. Contraction of the trachealis muscle decreases the diameter of the tracheal lumen, resulting in faster air flow, which assists in the dislodging of foreign material (or mucus or other irritants) from the larynx by coughing.

The trachea has three layers: mucosa, submucosa, and adventitia (Fig. 15–4).

Mucosa

The **mucosal lining** of the trachea is composed of pseudostratified ciliated columnar (respiratory) epithelium, the subepithelial connective tissue (lamina propria), and a relatively thick bundle of elastic fibers separating the mucosa from the submucosa.

Respiratory Epithelium

The respiratory epithelium is a pseudostratified ciliated columnar epithelium composed of six cell types; three of these, goblet cells, ciliated columnar cells, and basal cells constitute 90% of the cell population.

The **respiratory epithelium**, a pseudostratified ciliated columnar epithelium, is separated from the lamina propria by a **thick basement membrane**. The epithelium is composed of six cell types: goblet cells, ciliated columnar cells, basal cells, brush cells, serous cells, and cells of the diffuse neuroendocrine system (DNES). All of these cells come into contact with the basement membrane, but all do not reach the lumen (Fig. 15–5).

Goblet cells constitute about 30% of the total cell population of the respiratory epithelium. They produce **mucinogen**, which becomes hydrated and is known as ***mucin*** when released into an aqueous environment; once the mucin is mixed with other material in the watery environment, it is known as ***mucus***. Just as goblet cells elsewhere, goblet cells in the respiratory epithelium

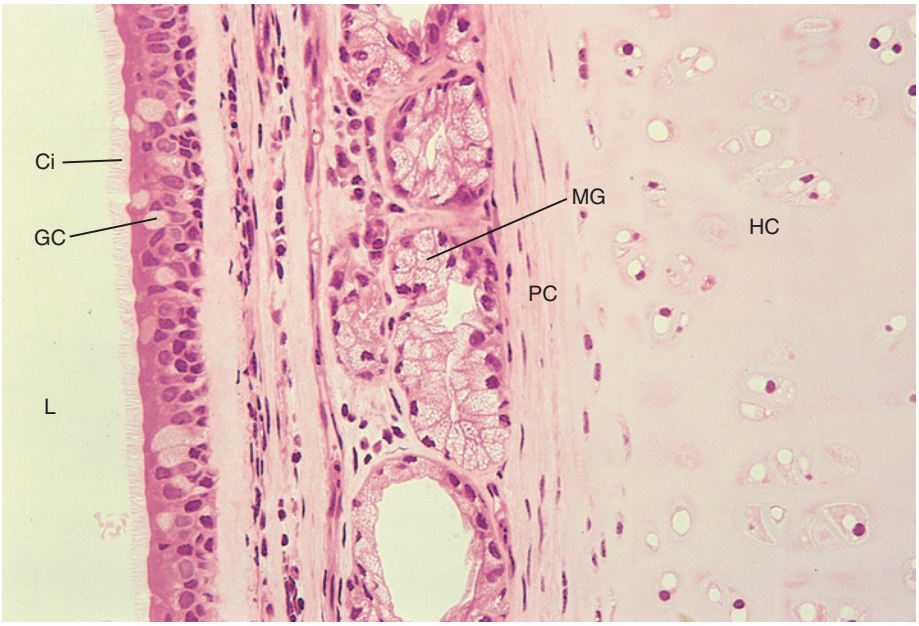


Figure 15–4 Light photomicrograph of the trachea in a monkey. There are numerous cilia (Ci) as well as goblet cells (GC) in the epithelium. Also observe the mucous glands (MG) in the subepithelial connective tissue as well as the presence of the hyaline cartilage (HC) C-ring in the adventitia (×270). L, Lumen; PC, perichondrium.

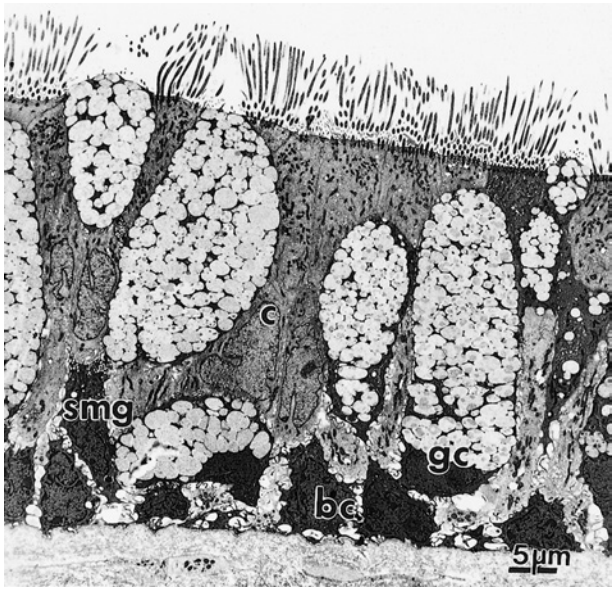


Figure 15–5 Transmission electron micrograph of the monkey respiratory epithelium from the anterior nasal septum. Note the presence of goblet cells (gc), ciliated cells (c), basal cells (bc), and small granule mucous cells (smg). (From Harkema JR, Plopper CG, Hyde DM, et al. Nonolfactory surface epithelium of the nasal cavity of the bonnet monkey: a morphologic and morphometric study of the transitional and respiratory epithelium. *Am J Anat*. 1987;180:266-279. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

have a narrow, basally positioned **stem** and an expanded **theca** containing secretory granules. Electron micrography demonstrates that the nucleus and most organelles are located in the stem. This region displays a rich network of rough endoplasmic reticulum (RER), a well-developed Golgi complex, numerous mitochondria, and an abundance of ribosomes. The theca is filled with numerous mucinogen-containing secretory granules of varied diameters. The apical plasmalemma has a few short, blunt microvilli (see Fig. 15–5).

Ciliated columnar cells constitute approximately 30% of the total respiratory epithelial cell population. These tall, slender cells have a basally located nucleus and possess cilia and microvilli on their apical cell membrane (Fig. 15–6). The cytoplasm just below these structures is rich in mitochondria and has a Golgi complex. The remainder of the cytoplasm possesses some RER and a few ribosomes. The cilia of these cells move the mucus and its trapped particulate matter, via ciliary action, toward the nasopharynx for elimination.

The short **basal cells** compose about 30% of the total respiratory epithelial cell population. They are located on the basement membrane, but their apical surfaces do not reach the lumen (see Fig. 15–5). These relatively undifferentiated cells are considered to be stem cells that proliferate to replace defunct goblet, ciliated columnar, and brush cells.

Brush cells (small-granule mucous cells) constitute about 3% of the total respiratory epithelial cell

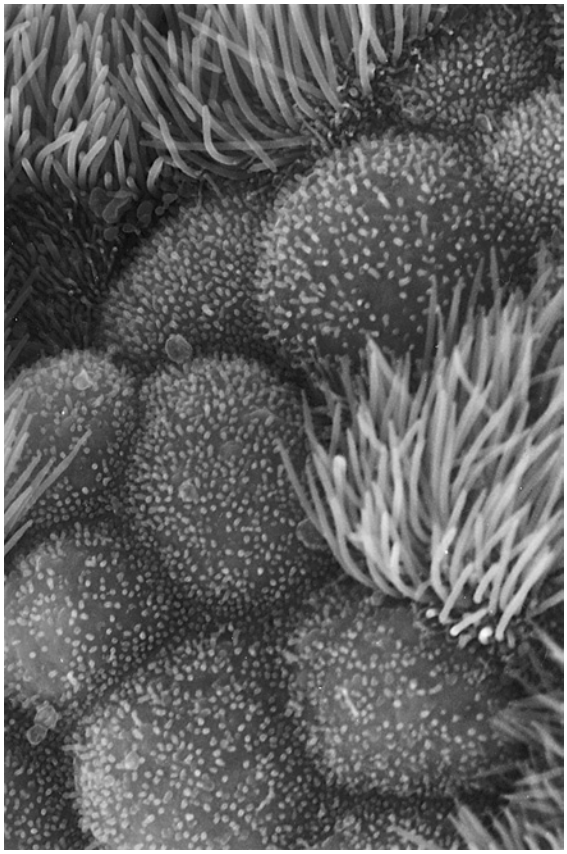


Figure 15–6 Scanning electron micrograph of the human fetal trachea displaying ciliated and nonciliated cells (×5500). (From Montgomery PQ, Stafford ND, Stolinski C. Ultrastructure of the human fetal trachea: a morphologic study of the luminal and glandular epithelia at the mid-trimester. *J Anat*. 1990;173:43-59. Reprinted with permission from Cambridge University Press.)

population. They are narrow, columnar cells with tall microvilli. Their function is unknown, but they have been associated with nerve endings; thus, some investigators suggest that they may have a sensory role. Other investigators believe that brush cells are merely goblet cells that have released their mucinogen.

Serous cells, which make up about 3% of the total cell population of the respiratory epithelium, are columnar cells. They have apical microvilli and apical granules containing an electron-dense secretory product, a serous fluid of unknown composition.

DNES cells, also known as ***small-granule cells*** or Kulchitsky cells, constitute about 3% to 4% of the total cell population. Many of these cells possess long, slender processes that extend into the lumen, and it is believed that they have the ability to monitor the oxygen and carbon dioxide levels in the lumen of the airway. These cells are closely associated with naked sensory nerve endings with which they make synaptic contact, and, together with these nerve fibers, they are referred to as ***pulmonary neuroepithelial bodies***. DNES cells contain numerous granules in their basal cytoplasm, which house pharmacological agents such as amines, polypeptides, acetylcholine, serotonin, and adenosine triphosphate. Under hypoxic conditions, these agents are released not only into the synaptic clefts but also into the connective tissue spaces of the lamina propria, where they act as paracrine hormones or may enter the vascular supply to act as hormones. Therefore, it has been suggested that these pulmonary neuroepithelial bodies can exert local effects to alleviate localized hypoxic conditions by regulating perfusion and ventilation in their vicinity, or they may have generalized effects via the efferent nerve fibers that relay information about hypoxic conditions to the **respiratory regulators** located in the medulla oblongata.

Lamina Propria and Elastic Fibers

The **lamina propria** of the trachea is composed of a loose, fibroelastic connective tissue. It contains lymphoid elements (e.g., lymphoid nodules, lymphocytes, and neutrophils) as well as mucous and seromucous glands, whose ducts open onto the epithelial surface. A dense layer of elastic fibers, the **elastic lamina**, separates the lamina propria from the underlying submucosa.

Submucosa

The tracheal **submucosa** is composed of a dense, irregular fibroelastic connective tissue housing numerous **mucous** and **seromucous glands**. The short ducts of these glands pierce the elastic lamina and the lamina propria to open onto the epithelial surface. Lymphoid elements are also present in the submucosa. Moreover, this region has a rich blood and lymph supply, the smaller branches of which reach the lamina propria.

Adventitia

The adventitia of the trachea houses C-rings of hyaline cartilage.

The **adventitia** of the trachea is composed of a fibroelastic connective tissue (see Fig. 15–4). The most prominent features of the adventitia are the hyaline cartilage C-rings and the intervening fibrous connective tissue. The adventitia also is responsible for anchoring the trachea to the adjacent structures (i.e., esophagus and connective tissues of the neck).

Bronchial Tree

The **bronchial tree** begins at the bifurcation of the trachea, as the **right** and left **primary bronchi**, which *arborize* (form branches that gradually decrease in size). The bronchial tree is composed of airways located outside of the lungs (the primary bronchi,

CLINICAL CORRELATIONS

The respiratory epithelium of people chronically exposed to irritants, such as cigarette smoke and coal dust, undergoes reversible alterations, known as ***metaplasia***, associated with an increase in the number of goblet cells relative to ciliated cells. The increased number of goblet cells produces a thicker layer of mucus to remove the irritants, but the reduced number of cilia retards the rate of mucus elimination, resulting in congestion. Moreover, the seromucous glands of the lamina propria and submucosa increase in size, forming a more copious secretion. A few months after elimination of the pollutants, the cell ratio returns to normal (1 : 1), and the seromucous glands revert to their previous size.

extrapulmonary bronchi) and airways located inside of the lungs: the intrapulmonary bronchi (lobar [secondary] and segmental [tertiary] bronchi), bronchioles, terminal bronchioles, and respiratory bronchioles (Fig. 15–7). The bronchial tree divides 15 to 20 times before reaching the level of terminal bronchioles. As the airways progressively decrease in size, several trends are observed, including a *decrease* in all of the following: the amount of cartilage, the numbers of glands and goblet cells, and the height of epithelial cells. Also, there is an *increase* in smooth muscle and elastic tissue (but only with respect to the thickness of the wall).

Primary (Extrapulmonary) Bronchi

The structure of the **primary bronchi** is identical to that of the trachea, except that bronchi are smaller in diameter, and their walls are thinner. Each primary bronchus, accompanied by the pulmonary arteries, veins, and lymph vessels, pierces the **root (hilum)** of the lung. The right bronchus is straighter than the left bronchus. The right bronchus trifurcates to lead to the three lobes of the right lung, and the left bronchus bifurcates, sending branches to the two lobes of the left lung. These branches then enter the substance of the lungs as intrapulmonary bronchi.

Intrapulmonary (Secondary [Lobar] and Tertiary [Segmental]) Bronchi

Each intrapulmonary bronchus serves a lobe of the lung; tertiary bronchi serve bronchopulmonary segments.

Each ***secondary (lobar) bronchus*** is the airway to a lobe of the lung. The left lung has two lobes and thus has two secondary bronchi; the right lung has three lobes and thus has three secondary bronchi. These airways are similar to primary bronchi, with the following exceptions. The cartilage C-rings are replaced by irregular plates of hyaline cartilage that completely surround the lumina of the secondary bronchi; thus, these airways do not have a flattened region but are completely round. The smooth muscle is located at the interface of the fibroelastic lamina propria and submucosa as two distinct smooth muscle layers spiraling in opposite directions. Elastic fibers, which radiate from the adventitia, connect to elastic fibers arising from the adventitia of other parts of the bronchial tree.

As in the primary bronchi and in the trachea, seromucous glands and lymphoid elements are present in the lamina propria and the submucosa of the secondary bronchi. Ducts of these glands deliver their secretory products onto the surface of the pseudostratified ciliated epithelial lining of the lumen.

As secondary bronchi enter the lobes of the lung, they subdivide into smaller branches, **tertiary (segmental) bronchi**. Each tertiary bronchus arborizes but leads to a discrete section of lung tissue, known as a ***bronchopulmonary segment***. Each lung has 10 bronchopulmonary segments that are completely separated from one another by connective tissue elements and are clinically important in surgical procedures involving the lungs. As the arborized branches of intrapulmonary bronchi decrease in diameter, they eventually lead to bronchioles. Lymphoid nodules are particularly evident where these airways branch to form increasingly smaller intrapulmonary bronchi. The smaller intrapulmonary bronchi have thinner walls, decreasing amounts of hyaline cartilage plates, and shorter epithelial-lining cells.

Bronchioles

Bronchioles possess no cartilage in their walls, are smaller than 1 mm in diameter, and have club cells (Clara cells) in their epithelial lining.

Each **bronchiole** (or **primary bronchiole**) supplies air to a pulmonary lobule. Bronchioles are considered the 10th to 15th generation of dichotomous branching of the bronchial tree. Their diameter commonly is described as less than 1 mm, although this number varies among authors from 5 mm to 0.3 mm. This disagreement concerning the diameter of bronchioles may lead to confusion in the descriptions of their structure (but should not be regarded as a purposeful attempt to complicate the life of the student).

The epithelial lining of bronchioles ranges from ciliated simple columnar with occasional goblet cells in larger bronchioles to simple cuboidal (many with cilia) with occasional club cells (also known as *Clara cells*) and no goblet cells in smaller bronchioles.

Club cells (Clara cells) are columnar with dome-shaped apices that have short, blunt microvilli (Fig. 15–8). Their apical cytoplasm houses numerous secretory granules containing glycoproteins manufactured on their abundant RER. Club cells are believed to protect the bronchiolar epithelium by lining it with their secretory product, known as ***club cell secretory***

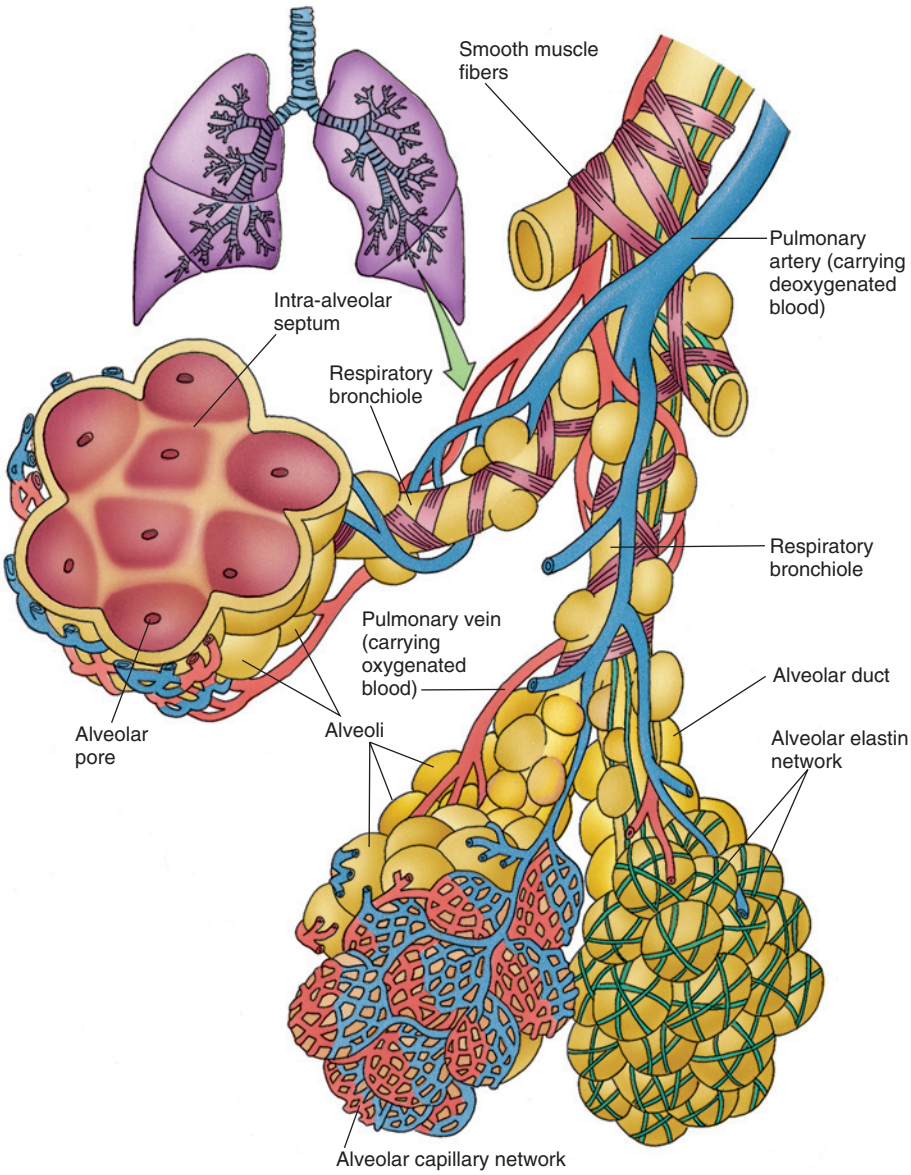


Figure 15–7 Schematic diagram of the respiratory system, displaying bronchioles, terminal bronchioles, respiratory bronchioles, alveolar ducts, sacs, and alveoli.

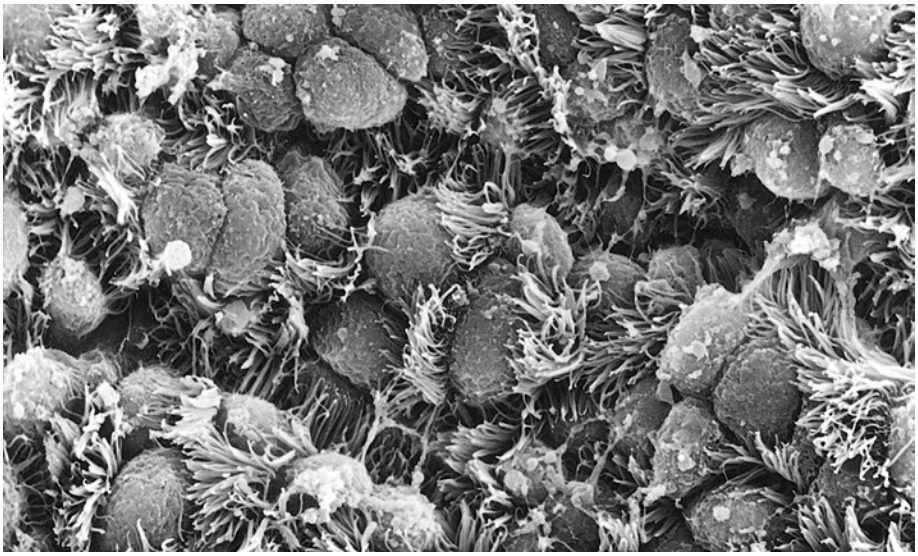


Figure 15–8 Scanning electron micrograph of club cells and ciliated cuboidal cells of rat terminal bronchioles (×1817). (From Peao MND, Aguas AP, De Sa CM, Grande NR. Anatomy of Clara cell secretion: surface changes observed by scanning electron microscopy. *J Anat*. 1993;183:377-388. Reprinted with permission from Cambridge University Press.)

CLINICAL CORRELATIONS

Cystic fibrosis (CF) is a recessive hereditary disease caused by one of a number of mutations in the gene that codes for the protein known as **cystic fibrosis transmembrane conductance regulator (CFTR)**. This protein is inserted into the parenchymal cell membranes of glands as a **chloride channel** and regulates the transport of Cl^- ions across the cell membrane. Although the disease affects most components of the digestive system and the conducting portion of the respiratory system, it is the effects on the bronchioles of the respiratory system that are the most problematic, causing bronchiolar infections and, eventually, the inability to breathe. The mechanism of the pathology involves problems with the transport of Cl^- ions out of the cell. Mucous glands of the bronchi and bronchioles release mucinogen into the lumen of their ducts. Normally, as Cl^- ions leave the secretory cells of the mucous acinus through the CFTR channel to enter the duct portion of the acinus, H_2O molecules also leave the cell, thereby hydrating and diluting the mucinogen, forming mucin that is sufficiently thin to flow through the ducts and away from the site of mucin production. However, in individuals with mutated CFTR, the Cl^- ions and water molecules are unable to leave the cell. The abnormally thick mucinogen that is released is unable to flow out of the duct and is trapped not only in the lumina of the acini but also in the ducts of the mucous glands. This rich, thick mucin becomes invaded by bacteria, usually *Pseudonomar aeruginosa*, which thrives in this nutrient-laden environment. Eventually, the blocked conducting system of the respiratory system becomes too inefficient and can no longer conduct enough air to the alveoli of the lungs and the individual dies. Before the advent of antibiotics, CF patients died at a very early age; currently, they can live to 35 years of age. Almost every gland of the digestive system is affected by this disease, including the pancreas, but it is the respiratory effects of CF that are responsible for the death of the individual.

protein. Additionally, these cells degrade toxins in the inhaled air via **cytochrome P-450 enzymes** in their smooth endoplasmic reticulum. Some investigators also suggest that club cells produce a **surfactant-like material** that reduces the surface tension of bronchioles and facilitates the maintenance of their patency. Moreover, club cells divide to regenerate the bronchiolar epithelium.

The lamina propria of bronchioles has no glands; it is surrounded by a loose meshwork of helically oriented smooth muscle layers (Fig. 15–9). The walls of bronchioles and their branches have no cartilage. Elastic fibers radiate from the fibroelastic connective tissue that surrounds the smooth muscle coats of bronchioles. These elastic fibers connect to elastic fibers ramifying from other branches of the bronchial tree. During inhalation, as the lung expands in volume, the elastic fibers exert tension on the bronchiolar walls; by pulling uniformly in all directions, the elastic fibers help maintain the patency of the bronchioles.

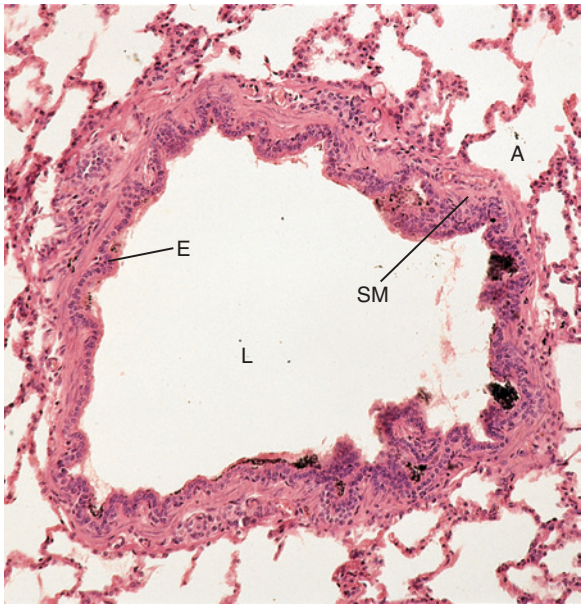


Figure 15–9 Light photomicrograph of a bronchiole. Note the presence of smooth muscle (SM) and the absence of cartilage in its wall. Observe that the entire structure is intrapulmonary and is surrounded by lung tissue. A, Alveolus; E, epithelium; L, lumen. (×117)

CLINICAL CORRELATIONS

1. The smooth muscle layers of bronchioles are controlled by the parasympathetic nervous system. Normally, the smooth muscle coats contract at the end of expiration and relax during inspiration. In patients with **asthma**, the smooth muscle coat undergoes prolonged contraction during expiration; thus, they have difficulties in expelling air from their lungs. Steroids and β_2-agonists relax bronchiolar smooth muscle and are frequently used to relieve asthmatic attacks.
2. The risk of acquiring asthma by young children is decreased by consumption of milk and fruit as well as by a diet rich in eggs, vegetables, and cereals. However, consumption of fast foods and "junk food," especially those rich in trans fats, saturated fats, carbohydrates, and sugar, increases the incidence of asthma in children.
3. It has been reported recently that bronchiolar smooth muscle cells possess taste receptors on their sarcolemmae that recognize bitter tastants. When exposed to bitter taste, these smooth muscle cells relax, permitting the bronchioles to open to 90% of their widest diameter. Current research is attempting to take advantage of this situation by developing inhalants that incorporate bitter tasting substances in their composition to assist asthmatic patients in relieving breathing problems without having to resort to steroids and β_2-agonists.

Terminal Bronchioles

Terminal bronchioles form the smallest and most distal region of the conducting portion of the respiratory system.

Each bronchiole subdivides to form several smaller **terminal bronchioles**, which are smaller than 0.5 mm in diameter and constitute the terminus of the conducting portion of the respiratory system. These structures supply air to lung acini, subdivisions of the lung lobule. The epithelium of terminal bronchioles is composed of club cells and cuboidal cells, some with cilia. The narrow lamina propria consists of fibroelastic connective tissue and is surrounded by one or two layers of smooth muscle cells. Elastic fibers radiate from the adventitia and, as with the bronchioles, bind to elastic fibers radiating from other members of the bronchial tree. Terminal bronchioles branch to give rise to respiratory bronchioles.

RESPIRATORY PORTION OF THE RESPIRATORY SYSTEM

The respiratory portion of the respiratory system is composed of respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli.

Respiratory Bronchioles

Respiratory bronchioles are the first region of the respiratory system where exchange of gases can occur.

Respiratory bronchioles are similar in structure to terminal bronchioles, in that their epithelium is a simple cuboidal epithelium rich in club cells and some ciliated cells, but this epithelium is broken up by the presence of thin-walled, pouch-like structures, known as ***alveoli***, composed of an attenuated simple squamous epithelium, where gaseous exchange (O_2 for CO_2) can occur. As respiratory bronchioles branch, they become narrower in diameter, and their population of alveoli increases. Subsequent to several branchings, each respiratory bronchiole terminates in an alveolar duct (Fig. 15–10).

Alveolar Duct, Atrium, and Alveolar Sac

Alveolar ducts, atria, and alveoli are supplied by a rich capillary network.

Alveolar ducts do not have walls of their own; they are merely a continuous sequence of alveoli (Figs. 15–11 and 15–12). An alveolar duct that arises from a respiratory bronchiole forms branches, and each of the resultant alveolar ducts usually ends as a blind outpouching composed of two or more small clusters of alveoli, in which each cluster is known as an ***alveolar sac***. These alveolar sacs thus open into a common space, which some investigators call the ***atrium***. The cells lining the

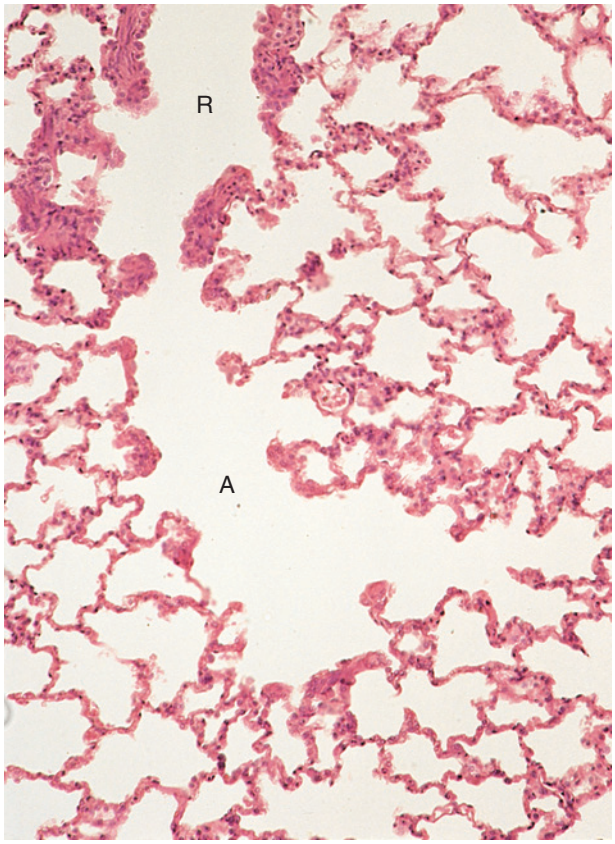


Figure 15–10 Photomicrograph of a human respiratory bronchiole (R) giving rise to an alveolar duct (A). The respiratory bronchiole has a definite wall with alveoli interjected along its wall, whereas the alveolar duct has no wall of its own; instead, it is composed of alveoli.

alveolar ducts are of two types: **type I** and **type II pneumocytes** (described in later sections on pneumocytes).

Slender connective tissue elements between alveoli, the **interalveolar septa**, reinforce the alveolar duct, stabilizing it somewhat. Additionally, the opening of each alveolus to the alveolar duct is controlled by a single smooth muscle cell (viewed with the light microscope, this smooth muscle cell has a knob-like appearance), embedded in type III collagen, which forms a delicate sphincter regulating the diameter of the opening.

Fine elastic fibers ramify from the periphery of alveolar ducts and sacs to intermingle with elastic fibers radiating from other intrapulmonary elements. This network of elastic fibers not only maintains the patency of these delicate structures during inhalation, but also protects them against damage during distention and is responsible for nonforced exhalation.

Alveolus

Alveoli are small air sacs composed of highly attenuated type I pneumocytes and larger type II pneumocytes.

Each **alveolus** is a small outpouching, about 200 µm in diameter, of respiratory bronchioles, alveolar ducts, and alveolar sacs (Fig. 15–13; see Figs. 15–11AB and 15–12). Alveoli form the primary structural and functional unit of the respiratory system because their thin walls permit the exchange of CO_2 for O_2 between the air in their lumina and blood in adjacent capillaries. Although each alveolus is a small structure, about 0.002 mm^3, their total number approximates 300 million, conferring on the lung its sponge-like consistency. It has been estimated that the total surface area of all alveoli available for gas exchange exceeds 140 m^2 (the approximate floor space of an average-sized two-bedroom apartment or the size of a singles tennis court).

Alveoli are separated from each other by **interalveolar septa** of various width containing diverse amounts of connective tissue elements. In fact, because of their large number, alveoli are frequently pressed against each other, and the connective tissue interstitium of the interalveolar septum is eliminated between them. In such areas of contact, the air spaces of the two alveoli may communicate with each other through an **alveolar pore (of Kohn)**, whose diameter varies from 8 to 60 µm (see Fig. 15–12). These pores presumably function to equilibrate air pressure within pulmonary segments. The region between adjacent alveoli, where the alveoli are not pressed against each other, interalveolar septum is wider and is occupied by an extensive capillary bed composed of **continuous capillaries**, supplied by branches of the pulmonary artery and drained by tributaries of the pulmonary vein. The connective tissue of such an interalveolar septum is rich in elastic fibers and type III collagen (reticular) fibers.

Because alveoli and capillaries are composed of epithelial cells, they are invested by a prominent basal lamina. The openings of alveoli associated with alveolar sacs, unlike those of respiratory bronchioles and alveolar ducts, *are devoid of smooth muscle cells.* Instead, their orifices are circumscribed by elastic and, especially, by reticular fibers. Walls of alveoli are composed of two types of cells: type I pneumocytes and type II pneumocytes.

Type I Pneumocytes

Approximately 95% of the alveolar surface is composed of simple squamous epithelium, whose cells are known as ***type I pneumocytes*** (also called ***type I alveolar cells*** or ***squamous alveolar cells***). Because the cells of this epithelium are highly attenuated, their cytoplasm may be as thin as 80 nm in width (Fig. 15–14; see Fig. 15–12). The region of the nucleus is, as expected, wider,

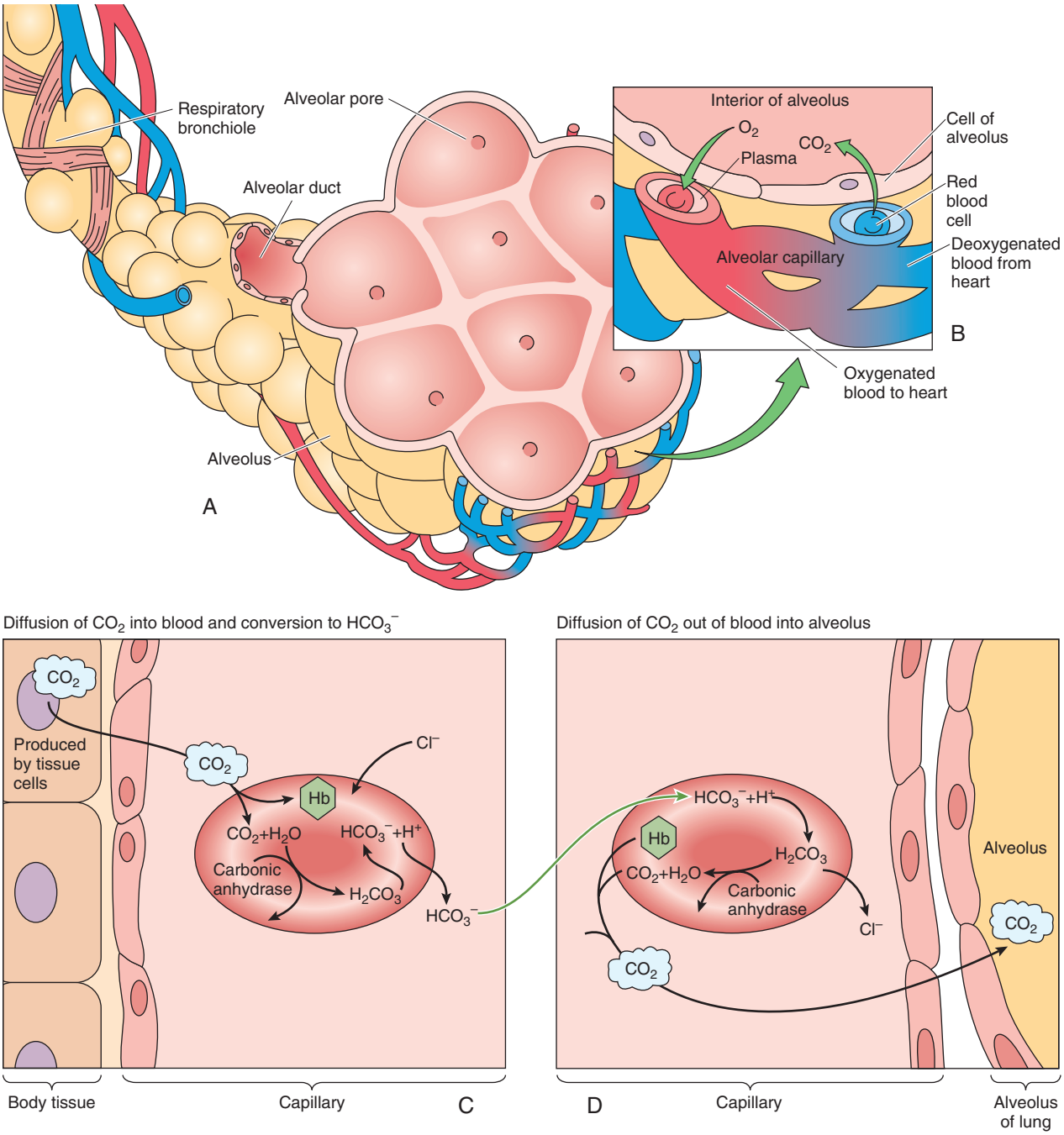


Figure 15–11 Schematic diagram. **A,** A respiratory bronchiole, alveolar sac, alveolar pore, and alveoli. **B,** Interalveolar septum. **C,** Carbon dioxide uptake from body tissues by erythrocytes and plasma. **D,** Carbon dioxide release by erythrocytes and plasma in the lung. Compare part A with Figure 15–10 depicting a photomicrograph of the alveolar duct.

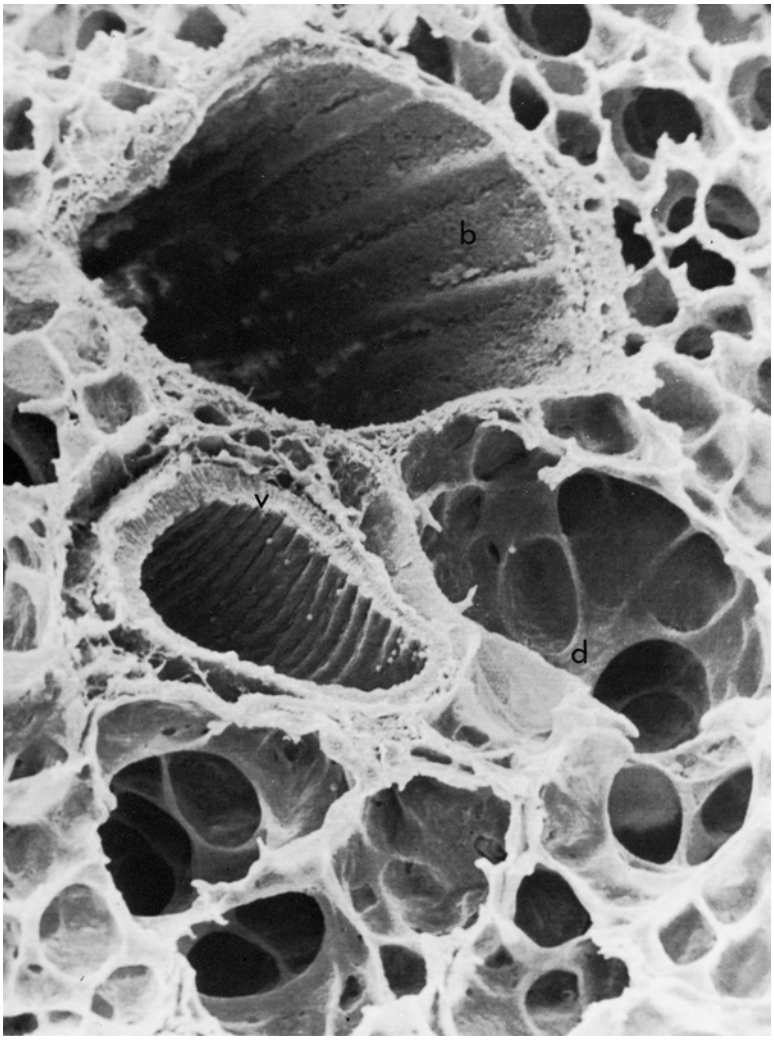


Figure 15–12 Scanning electron micrograph of a rat lung displaying a bronchiole (b), a small artery (v), and alveoli (d), some of which present alveolar pores. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

and it houses much of the cell's organelle population, composed of a small number of mitochondria, a few profiles of RER, and a modest Golgi apparatus.

Type I pneumocytes form occluding junctions with each other, thus preventing the seepage of extracellular fluid into the alveolar lumen. The adluminal aspect of these cells is covered by a well-developed basal lamina, which extends almost to the rim of the alveolar pores (of Kohn). The rim of each alveolar pore is formed by the fusion of the cell membranes of two closely apposed type I pneumocytes that belong to two discrete alveoli. The luminal aspect of type I pneumocytes is lined by surfactant as detailed in the following paragraphs.

Type II Pneumocytes

Although **type II pneumocytes** (also known as ***great alveolar cells***, ***septal cells***, or ***type II alveolar cells***) are more numerous than type I pneumocytes, they occupy only about 5% of the alveolar surface. These cuboidal cells are interspersed among type I pneumocytes and form occluding junctions with them. Their dome-shaped apical surface juts into the lumen of the alveolus (Figs. 15–15 and 15–16). Type II pneumocytes are usually located in regions where adjacent alveoli are separated from each other by a septum (hence the name *septal cells*), and their adluminal surface is covered by basal lamina.

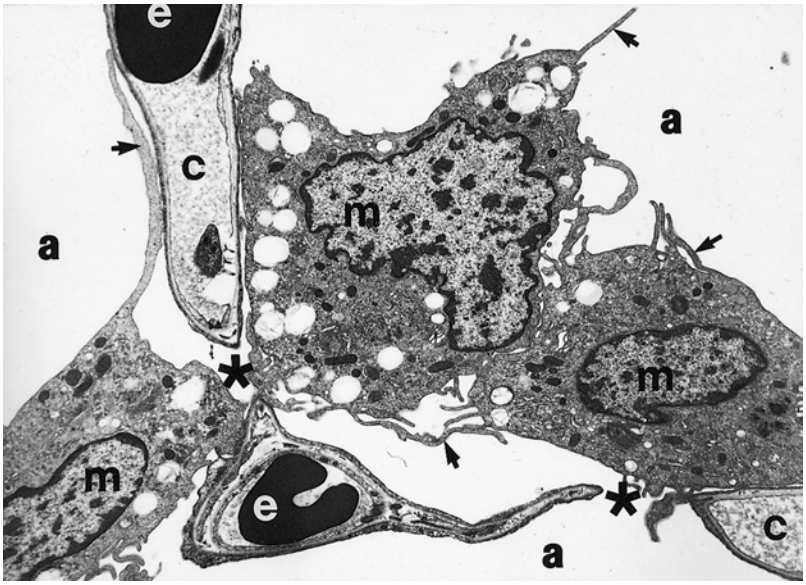


Figure 15–13 Transmission electron micrograph of the interalveolar septum in a monkey. Note the presence of alveoli (a), erythrocytes (e) within capillaries (c), and alveolar macrophages (m). The filopodia *(arrows)* are evident. *Asterisks* indicate the presence of alveolar pores. (From Maina JN. Morphology and morphometry of the normal lung of the adult vervet monkey *[Cercopithecus aethiops]*. *Am J Anat*. 1988;183:258-267. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Figure 15–14 Transmission electron micrograph of the blood–gas barrier (×71250). Note the presence of the alveolus (a), the attenuated type I pneumocytes (ep), the fused basal laminae (b), the attenuated endothelial cell of the capillary (en) with pinocytotic vesicles *(arrows)*, the plasma (p), and the erythrocyte (r) within the capillary lumen. (From Maina JN. Morphology and morphometry of the normal lung of the adult vervet monkey *[Cercopithecus aethiops]*. *Am J Anat*. 1988;183:258-267. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Electron micrographs of type II pneumocytes display short apical microvilli. They have a centrally placed nucleus, an abundance of RER profiles, a well-developed Golgi apparatus, and mitochondria. The most distinguishing feature of these cells is the presence of membrane-bound **lamellar bodies** that contain **pulmonary surfactant**, the secretory product of these cells.

Pulmonary surfactant, synthesized on the RER of type II pneumocytes, is composed primarily of two phospholipids—**dipalmitoyl phosphatidylcholine** and **phosphatidylglycerol**, **neutral lipid** and **cholesterol**—and four unique proteins, **surfactant apoproteins SP-A**, **SP-B**, **SP-C**, and **SP-D**. The surfactant is modified in the Golgi apparatus and is then released from the *trans*-Golgi network into secretory vesicles, known as ***composite bodies***, the immediate precursors of lamellar bodies.

The surfactant is released by exocytosis into the lumen of the alveolus. Here, it forms a broad,

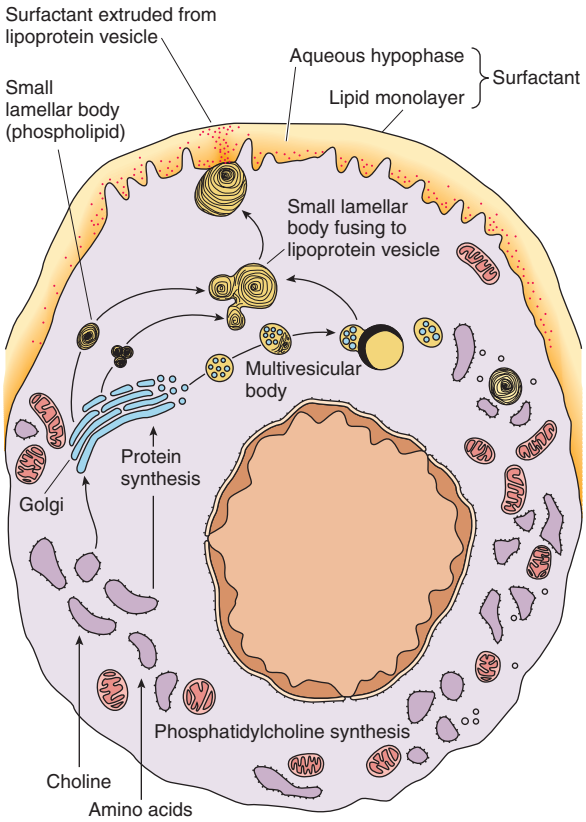


Figure 15–15 Schematic diagram of a type II pneumocyte. Compare this drawing with the transmission electron micrograph of the type II pneumocyte in Figure 15–16.

CLINICAL CORRELATIONS

At birth, an infant's lungs expand upon the first intake of breath, and the presence of pulmonary surfactant permits the alveoli to remain patent. Immature infants (those born before 7 months of gestation) who have not as yet produced surfactant (or who have produced an inadequate supply of surfactant) have potentially fatal **respiratory distress of the newborn**. These newborns are treated with a combination of synthetic surfactant and glucocorticoid therapy. The synthetic surfactant acts immediately to reduce surface tension, and glucocorticoids stimulate type II pneumocytes to produce surfactant.

lattice-like network, known as ***tubular myelin***, which becomes a mononuclear film separated into lipid and protein portions. The lipid is inserted into a monomolecular phospholipid film forming an interface with air (the **superficial lipid phase**), and the protein enters an aqueous layer, the **lower aqueous phase**, between the pneumocytes and the phospholipid film. The surfactant not only decreases surface tension, thus preventing atelectasis (the collapse of the alveolus), but also resists (not prevents) fluid entry into the alveolar air space. It is continuously manufactured by type II pneumocytes and is also phagocytosed and recycled by type II pneumocytes and, less frequently, by alveolar macrophages (dust cells). The apoproteins SPA-A and SP-D act as **opsonins** by bonding to microorganisms and making them more attractive for macrophages for phagocytosis.

In addition to producing and phagocytosing surfactant, type II pneumocytes undergo mitosis to regenerate themselves as well as type I pneumocytes.

Alveolar Macrophages (Dust Cells)

Alveolar macrophages phagocytose particulate matter in the lumen of the alveolus as well as in the interalveolar spaces.

Monocytes gain access to the pulmonary interstitium, become **alveolar macrophages (dust cells)**, migrate between type I pneumocytes, and enter the lumen of the alveolus. These cells phagocytose particulate matter, such as dust, other inhaled particulate matter, and microorganisms, and thus maintain a sterile environment within the lungs. However, they are not nearly as avid phagocytes as are most other macrophages (Fig. 15–17; see Fig. 15–13). Dust cells also assist type II pneumocytes in the uptake of surfactant. Interestingly, these cells are unusual in that, unlike macrophages in other regions of the body, they are programmed to be anti-inflammatory. Approximately 100 million macrophages migrate to the bronchi each day and are transported from there by ciliary action to the pharynx to be eliminated by being swallowed or by being expectorated. Some alveolar macrophages, however, reenter the pulmonary interstitium and migrate into lymph vessels to exit the lungs.

CLINICAL CORRELATIONS

Alveolar macrophages of patients with pulmonary congestion and congestive heart failure contain phagocytosed, extravasated red blood cells. These macrophages are frequently called ***heart failure cells***.

Emphysema is a disease usually associated with the sequelae of long-term exposure to cigarette smoke and/or other inhibitors of the protein α_1-antitrypsin. This protein safeguards the lungs against the destruction of elastic fibers by elastase synthesized by dust cells. In such patients, elasticity of the lung tissue is reduced, and large, fluid-filled sacs are present that decrease the gas-exchange capability of the respiratory portion of the respiratory system.

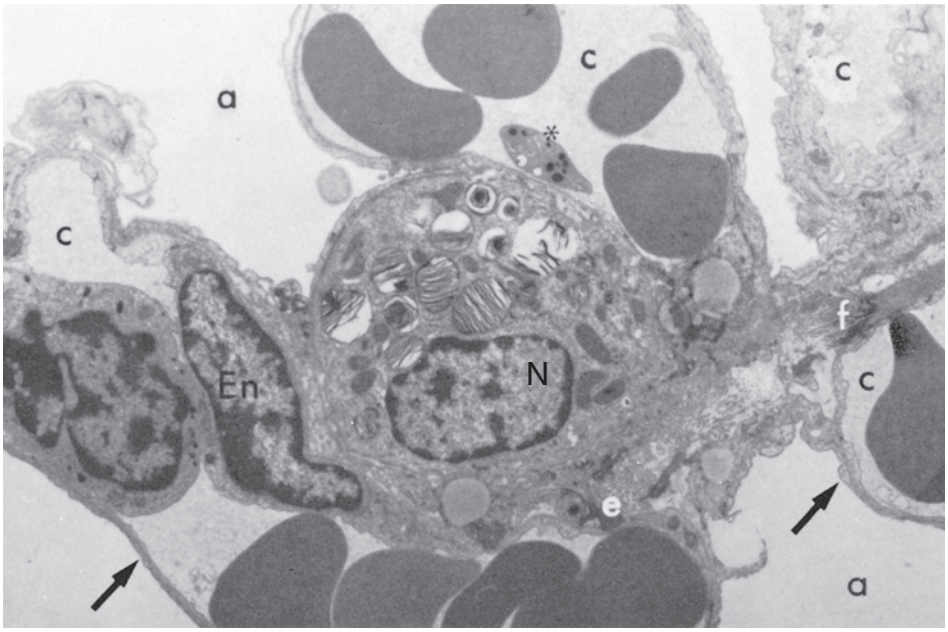


Figure 15–16 Transmission electron micrograph of a type II pneumocyte. Observe the centrally placed nucleus (N) flanked by several lamellar bodies. a, Alveolus; c, capillaries; e, elastic fibers; En, nucleus of endothelial cell; f, collagen fibers. *Arrows* represent the blood–gas barrier; *asterisk* represents a platelet. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

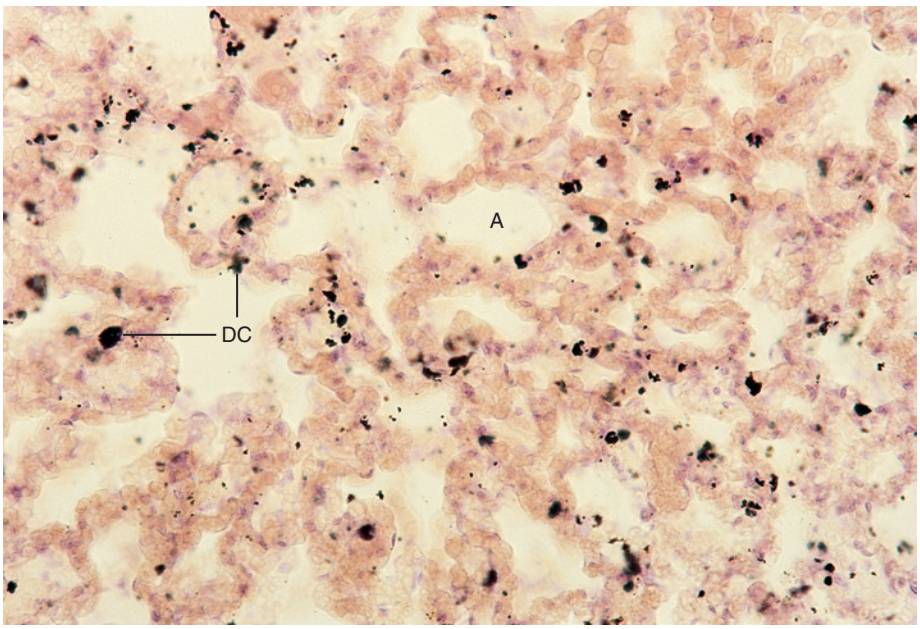


Figure 15–17 Alveolar macrophages (dust cells) in the human lung. Dust cells (DC) appear as black spots on the image because they have phagocytosed dust particles that were present in the air spaces of the lung. A, Alveolus. (×270)

Interalveolar Septum

As indicated previously, the region between two adjacent alveoli is known as an ***interalveolar septum***, which is lined on both sides by alveolar epithelium (see Fig. 15–13). The interalveolar septum may be extremely narrow, housing nothing or only a **continuous capillary** and its basal lamina, or it may be somewhat wider, including connective tissue elements, such as type III collagen and elastic fibers, macrophages, fibroblasts (and myofibroblasts), mast cells, and lymphoid elements.

Blood–Gas Barrier

The blood–gas barrier is that region of the interalveolar septum that is traversed by O_2 and CO_2; these gases go from the lumen of the blood vessel to the lumen of the alveolus and vice versa.

The thinnest regions of the interalveolar septum where gases can be exchanged are called the ***blood–gas barriers*** (see Fig. 15–14). The narrowest blood–gas barrier, where the type I pneumocyte is in intimate contact with the endothelial lining of the capillary and the basal laminae of the two epithelia become fused, is the most efficient site for the exchange of O_2 (in the alveolar lumen) for CO_2 (in the blood). These regions are composed of the following three structures:

- Surfactant and type I pneumocytes
- Fused basal laminae of type I pneumocytes and endothelial cells of the capillary
- Endothelial cells of the continuous capillary

Exchange of Gases between the Tissues and Lungs

In the lungs, O_2 is exchanged for CO_2 carried by blood; in the tissues of the body, CO_2 is exchanged for O_2 carried by blood.

During inspiration, oxygen-containing air enters the alveolar spaces of the lung. Because the total surface area of all alveoli exceeds 140 m^2 and the total blood volume in all of the capillaries in the lungs at any one time is no more than 140 mL, the space available for diffusion of gases is enormous. Moreover, the diameter of the capillaries is small enough so that red blood cells may travel in a single file only, thus oxygen can reach each erythrocyte from all around, using all of the red blood cell surface area available for gas exchange. Oxygen diffuses through the blood–gas barrier to enter the lumina of the capillaries and binds to the **heme** portion of the erythrocyte hemoglobin, forming **oxyhemoglobin**. CO_2 leaves the blood, diffuses through the blood–gas barrier into the lumina of the alveolus, and exits the alveolar spaces as the CO_2-rich air is exhaled. The passage of O_2 and CO_2 across the blood–gas barrier is due to passive diffusion in response to the partial pressures of these gases within the blood and alveolar lumina (see also Chapter 10 in the section on hemoglobin).

Approximately 200 mL of CO_2 is formed by the cells of the body per minute. CO_2 enters the bloodstream and is transported in three forms: as a dissolved gas in plasma (20 mL), bound to hemoglobin (40 mL), and as a plasma bicarbonate ion (140 mL). The following sequence of events occurs (see Fig. 15–11C):

1. Most of the CO_2 dissolved in the plasma diffuses into the cytosol of the erythrocytes.
2. Some of the CO_2 binds to the globin moiety of hemoglobin. Although CO_2 is carried in a different region of the hemoglobin molecule, its binding capacity is greater in the absence than in the presence of O_2 in the heme portion.
3. Within the cytosol of the erythrocyte, most of the CO_2 combines with water, a reaction catalyzed by the enzyme **carbonic anhydrase**, to form carbonic acid (H_2CO_3), which dissociates into hydrogen ion (H^+) and bicarbonate ion (HCO_3^-). The hydrogen ion binds to hemoglobin, and the bicarbonate ion leaves the erythrocyte to enter the plasma. To maintain ionic equilibrium, chloride ion (Cl^-) enters the erythrocyte from the plasma; this exchange of bicarbonate for chloride ions is known as the ***chloride shift***.

The bicarbonate-rich blood is delivered to the lungs by the pulmonary arteries. Because the level of CO_2 is greater in the blood than in the lumina of the alveoli, CO_2 is released (following the concentration gradient). The *mechanism of release* is the reverse of the previous reactions. The following sequence of events occurs (see Fig. 15–11D):

1. Bicarbonate ions enter the erythrocytes (with a consequent release of Cl^- from the red blood cells into the plasma, also known as the ***chloride shift***).
2. Bicarbonate ions and hydrogen ions within the erythrocyte cytosol combine to form carbonic acid (H_2CO_3).
3. In the lung, the combining of O_2 with hemoglobin makes the hemoglobin more acidic and reduces its ability to bind CO_2. Additionally, the excess hydrogen ions released because of the greater acidity of hemoglobin become bound to bicarbonate ions, forming carbonic acid.
4. **Carbonic anhydrase** catalyzes the cleavage of carbonic acid to form water and CO_2.
5. CO_2 dissolved in the plasma, bound to hemoglobin, and cleaved from carbonic acid follows the concentration gradient to diffuse across the blood–gas barrier to enter the lumina of the alveoli.

Hemoglobin also has two types of binding sites for **nitric oxide (NO)**, a neurotransmitter substance that, when released by endothelial cells of blood vessels, causes relaxation of the vascular smooth muscle cells with a resultant dilation of the blood vessels.

Hemoglobin, *S*-nitrosylated (binding site 1) by NO manufactured by blood vessels of the lung, ferries bound NO to arterioles and metarterioles of the tissues, where NO is released and causes vasodilation. In this fashion, hemoglobin not only contributes to the modulation of blood pressure but also facilitates the more efficient exchange of O_2 for CO_2. Moreover, once O_2 leaves the heme portion of hemoglobin to oxygenate the tissues, NO takes its place on the iron atoms (binding site 2) and is transported into the lungs, where it is released into the alveoli to be exhaled along with CO_2.

Pleural Cavities and the Mechanism of Ventilation

Alteration of the volume of the pleural cavities by muscle action is responsible for the movement of gases into and out of the respiratory system.

The thoracic cage is separated into three regions: the left and right thoracic cavities and the centrally located mediastinum. Each thoracic cavity is lined by a serous membrane, the **pleura**, composed of simple squamous epithelium and subserous connective tissue. The pleura may be imagined as an inflated balloon; as the lung develops, it pushes against this serous membrane, as if a fist were pushing against the outer surface of a balloon. In this fashion, a portion of the pleura, the **visceral pleura**, covers and adheres to the lung, and the remainder of the pleura, the **parietal pleura**, lines and adheres to the walls of the thoracic cavity.

The space between the visceral and parietal pleura (inside the balloon) is known as the ***pleural cavity***. This space contains a slight amount of serous fluid (produced by the serous membranes) that permits a nearly frictionless movement of the lungs during **ventilation** (breathing), which involves air moving into the lungs (inhalation) and out of the lungs (exhalation).

Inhalation is an energy-requiring process because it involves contraction of the diaphragm, intercostal, scalenus, and other accessory respiratory muscles. As these muscles contract, the volume of the thoracic cage expands. Because the parietal pleura is firmly attached to the walls of the thoracic cage, the pleural cavities also increase in volume, and, consequently, the pressure within the pleural cavities decreases. The pressure differential between the atmospheric pressure outside of the body and the pressure within the pleural cavities drives air into the lungs. With the influx of air, the lungs expand, stretching the elastic fiber network of the pleural interstitium, and the visceral pleura is brought closer to the parietal pleura, reducing the volume of the pleural cavities and thus increasing the pressure inside of the pleural cavities.

For **exhalation** to occur, the respiratory (and accessory respiratory) muscles relax, decreasing the volume of the pleural cavities, with a consequent increase in the pressure within the pleural cavities. Additionally, the stretched elastic fibers return to their resting length, driving air out of the lungs. Thus, normal expiration does not require energy. In forced expiration, the internal intercostal and abdominal muscles also contract further, decreasing the volume of the pleural cavity, forcing additional air to leave the lungs.

CLINICAL CORRELATIONS

In patients afflicted with **poliomyelitis**, the muscles of respiration may become so weakened that the accessory muscles hypertrophy because they become responsible for the elevation of the thoracic cage. In other diseases, such as **myasthenia gravis** and **Guillain-Barré syndrome**, the weakness of the respiratory and accessory respiratory muscles may lead to respiratory failure and consequent death even though the lungs function normally.

Gross Structure of the Lungs

The left lung has two lobes, and the right lung has three lobes.

The left lung is subdivided into two lobes, and the right lung is subdivided into three lobes. Each lung has a medial indentation, the **hilum**, where the primary bronchi, bronchiolar arteries, and pulmonary arteries enter and the bronchiolar veins, pulmonary veins, and lymph vessels leave the lung. This group of vessels and the airway that enter the hilus make up the **root** of the lung.

Each lobe is subdivided into several **bronchopulmonary segments** supplied by a tertiary intrapulmonary (segmental) bronchus. In turn, bronchopulmonary segments are subdivided into many **lobules**, each served by a bronchiole. Lobules are separated from one another by connective tissue septa, in which lymph vessels and tributaries of pulmonary veins travel. Branches of bronchial and pulmonary arteries follow bronchioles in their passage through the center of the lobule.

Pulmonary Vascular and Lymphatic Supply

The pulmonary arteries supply deoxygenated blood to the lungs from the right side of the heart at a rate of 5 L per minute. Branches of these vessels follow the bronchial tubes into the lobules of the lung (see Fig. 15–7). When they reach the respiratory bronchioles, these vessels form an extensive pulmonary capillary network composed strictly of **continuous capillaries**. Because these capillaries are only 8 μm in diameter, erythrocytes, as indicated previously, follow each other in single file through them, reducing the space that

gases have to traverse and maximally exposing the erythrocytes to oxygen.

The blood in the capillary bed becomes oxygenated and then drains into veins of increasing diameter. These tributaries of the pulmonary vein carry oxygenated blood and travel in the septa between lobules of the lung. Thus, the veins follow a path that is different from that of the arteries, until they reach the apex of the lobule, where they accompany the bronchial tubes to the hilus of the lung to deliver oxygenated blood to the left side of the heart.

Bronchial arteries, which are branches of the thoracic aorta, bring nutrient-laden and oxygen-laden blood to the bronchial tree, interlobular septa, and pleura of the lungs. Many of the small branches anastomose with those of the pulmonary system. Others are drained by tributaries of the **bronchial veins**, which return the blood to the azygos system of veins.

The lung has a dual-lymph drainage, a superficial system of vessels in the visceral pleura and a deep network of vessels in the pulmonary interstitium, but these systems have numerous interconnections. The superficial system of lymph vessels forms several larger vessels, which drain into the hilar (bronchopulmonary) lymph nodes at the root of each lung. The deep network is organized into three groups following the pulmonary arteries, pulmonary veins, and bronchial tree down to the levels of the respiratory bronchioles. All of these networks drain into the hilar lymph nodes at the root of each lung. Efferent lymph vessels from these lymph nodes deliver their lymph to the thoracic duct or the right lymphatic duct, which return the lymph to the junction of the internal jugular and subclavian veins of the left and right sides, respectively.

Mucosal Immune Defense of the Respiratory System

The respiratory system possesses its own regional immune system, **bronchus-associated lymphoid tissue (BALT)**, that creates a barrier to the invasion of various pathogens in its immediate milieu while maintaining an equilibrium between mounting a defense against invading pathogens and "ignoring" the presence of various commensal and symbiotic organisms. The barrier that is established by the respiratory system involves both the innate and the adaptive immune systems. The components of these systems include the pharyngeal tonsil; bronchiolar lymph nodes and nodules; nasal/pharyngeal/tracheal/bronchial/bronchiolar epithelium and the **antimicrobial peptide**-containing mucus that lines its luminal surface; subepithelial lymphoid tissue and lymphoid cells (including dendritic cells); humoral antibodies (IgA, IgG, IgE); and the surfactant proteins SP-A and SP-D.

Although this particular aspect of the regional immune system is referred to as *bronchiolar*, it also includes the alveoli where **innate immune responses** are present but are finely modulated to ensure that inflammation, a process that would interfere with the exchange of gases, does not occur. Because there is no mucus production in the respiratory portion of the respiratory system, it is the presence of some of the surfactant proteins that act as components of the innate immune process. Some of these surfactant components bind to carbohydrate molecules, pathogen-associated molecular patterns, that are expressed on many pathogen surfaces, opsonizing these pathogens, thus marking them for phagocytosis by dust cells. Others, such as SP-A, prevents macrophage Toll-like receptors from activating dust cells, whereas SP-D diminishes the phagocytic activity of dust cells.

The **adaptive immunity** of the BALT depends on plasma cells and T cells. **Plasma cells** are recruited into the subepithelial connective tissue by chemokines released by the respiratory epithelium; the plasma cells secrete IgA, which passes through the epithelium into the lumina of the conducting portion of the respiratory system, where they bind to and inactivate antigens. Some IgE is also manufactured and released by plasma cells, but these remain in the connective tissue, where they bind to mast cells, thus eliciting inflammatory responses. T-cell responses are due to the activities of **dendritic cells** that protrude their processes into the lumina of the bronchiolar system of airways and sequester antigens. These dendritic cells then migrate to the pharyngeal tonsil, and the bronchiolar lymph nodes and nodules and present their epitopes to T cells that reside in those structures to elicit a cell-mediated immune response.

Pulmonary Nerve Supply

The thoracic sympathetic chain ganglia provide sympathetic fibers, and the vagus nerve supplies parasympathetic fibers to the smooth muscles of the bronchial tree. **Sympathetic fibers** (β-adrenergic) cause *relaxation* of bronchial smooth muscles and thus bronchodilation (while causing constriction of pulmonary blood vessels: "paradoxical response"). **Parasympathetic fibers** are cholinergic; they elicit *contraction* of bronchial smooth muscles, causing bronchoconstriction. Additionally nonadrenergic, noncholinergic fibers also travel with the vagus nerve, causing bronchodilation by releasing NO near bronchial smooth muscle effecting their relaxation.

Synapses occasionally involve type II pneumocytes, suggesting the possibility of some neural control over the production of pulmonary surfactant.

PATHOLOGICAL CONSIDERATIONS

See Figures 15–18 through 15–21.

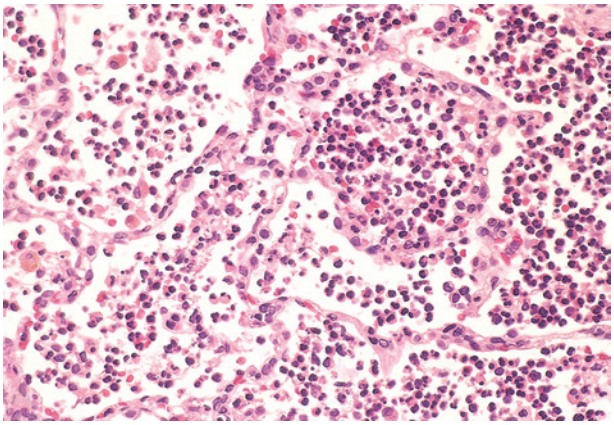


Figure 15–18 Photomicrograph of the lung of an individual with acute bacterial pneumonia. Observe the congested capillaries of the interalveolar septa as well as the large number of neutrophils occupying the alveolar air spaces. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:705.)

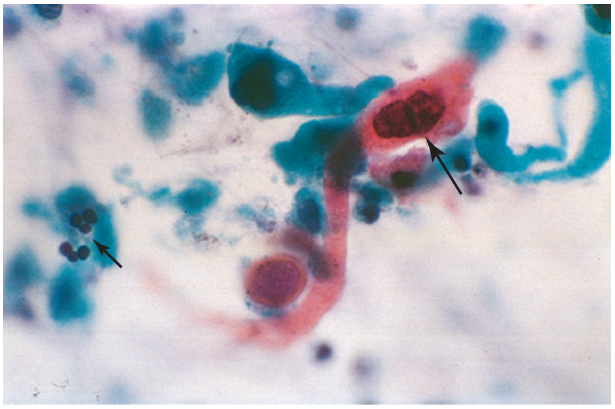


Figure 15–20 Photomicrograph of a sputum that is characteristic of an individual with cancer of the lung. Observe the characteristic large densely staining nucleus of keratinized squamous carcinoma cell *(large arrow)*. The size of the cancer cell is evident compared with the size of a normal neutrophil *(small arrow)*. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:717.)

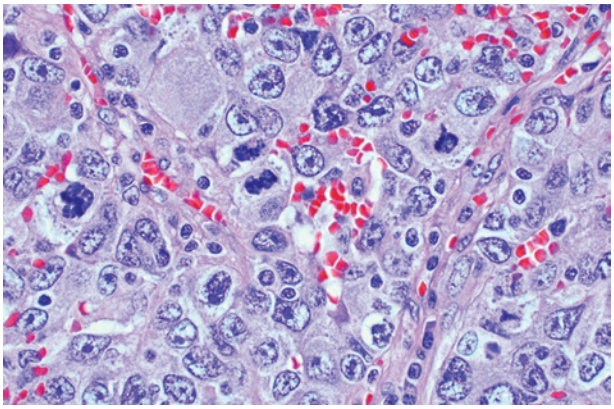


Figure 15–19 Photomicrograph of the lung of an individual with large cell carcinoma. Observe the large, pleomorphic tumor cells and the absence of glandular differentiation. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:715.)

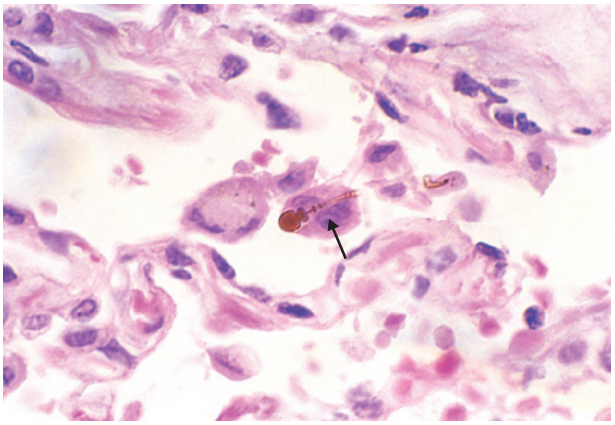
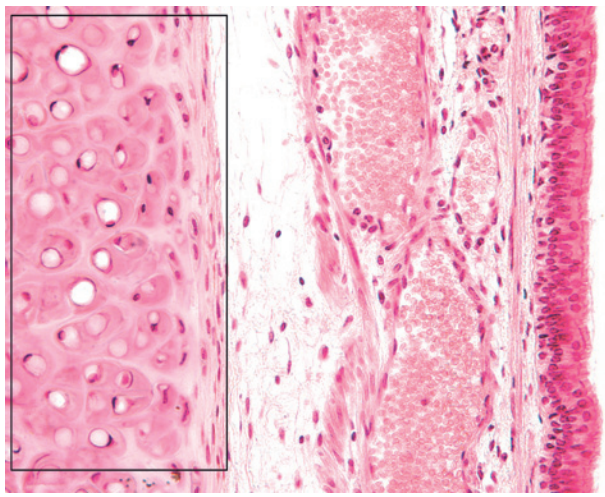


Figure 15–21 Photomicrograph of the lung of an individual with asbestosis. Observe the asbestos body *(arrow)* characterized by the beaded appearance with knob-shaped ends. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:691.)

REVIEW QUESTIONS

1. A patient is brought into the emergency department because she is bleeding from Kiesselbach area. Blood is issuing from the patient's
   a. external ear canal.
   b. lip.
   c. nostril.
   d. rectum.
   e. urethra.
2. The olfactory cells possess olfactory cilia whose terminal ends have
   a. dynein arms.
   b. nine peripheral doublets.
   c. two central doublets.
   d. nine peripheral singlets.
   e. two central singlets and nine peripheral doublets.
3. A patient goes to his allergist for the symptoms of headache, pain below the eyes, toothache affecting the upper teeth, and general malaise. The allergist determines that the patient has an infection of the
   a. frontal sinuses.
   b. maxillary sinuses.
   c. sphenoidal sinuses.
   d. ethmoidal sinuses.
   e. larynx.
4. Pneumocytes II (type II pneumocytes) are responsible for which of the following?
   a. Regeneration of the alveolar epithelium
   b. Phagocytosis of dust
   c. Formation of the blood–air barrier
   d. Synthesis of mucinogen
   e. Exchange of gases
5. The walls of bronchioles have
   a. a C-ring of cartilage in their submucosa.
   b. plates of cartilage in their adventitia.
   c. no cartilage in their wall.
   d. no elastic fibers associated with them.
   e. a pseudostratified epithelium.
6. Which of following structures belongs to the respiratory (as opposed to the conducting) portion of the respiratory system?
   a. Alveolar sac
   b. Primary bronchus
   c. Secondary bronchus
   d. Trachea
   e. Terminal bronchiole
7. Oxygen and carbon dioxide crossing between blood and air in the lungs must pass through cells of the following types:
   a. type I pneumocyte only.
   b. type I pneumocyte and endothelial cells.
   c. type I pneumocyte and type II pneumocyte.
   d. type II pneumocyte only.
   e. club (Clara) cells and endothelial cells.
8. A baby who was just born has respiratory distress of the newborn and is given glucocorticoids to stimulate
   a. bronchiolar smooth muscle contraction.
   b. contraction of the diaphragm.
   c. type I pneumocytes to function.
   d. type II pneumocytes to function.
   e. dust cells to function.
9. 

   Identify the structure enclosed in the rectangle.
   a. Elastic cartilage of the epiglottis
   b. Fibrocartilage of the intervertebral disk
   c. Cartilage plate of secondary bronchus
   d. C-ring of trachea
   e. Epiphyseal plate of a growing bone

ANSWERS

1. C. The patient has a nose bleed. Kiesselbach area is the anteroinferior region of the nasal septum; it is the region of anastomosis of the arterial supply of the nasal mucosa. The bleeding may be arrested by applying pressure on the region or by packing the nasal cavity with cotton.
2. D. The cilia of the olfactory cells are long and nonmotile and begin with a pair of central singlets surrounded by nine peripheral doublets but near the distal terminus; the cilia have only a pair of central singlets surrounded by nine peripheral singlets. Cilia of olfactory cells do not possess dynein arms.
3. B. The patient has maxillary sinusitis because the symptoms include pain beneath the eyes and affecting the upper teeth. A frontal sinus infection would involve a headache above the eyes. Sphenoidal sinus infections involve pain in the back of the head as well as in front of the head; an ethmoidal sinus infection would involve a "splitting headache" in the middle of the head and pain between the eyes. Laryngitis is recognized by a hoarse voice and red regions in the back of the throat.
4. A. Type II pneumocytes function in the regeneration of the alveolar epithelium as well as in the formation of pulmonary surfactant. Phagocytosis of dust is the function of dust cells, macrophages that reside in the lungs. The blood–air barrier is formed by the capillary endothelium, the fused basal laminae of the type I pneumocyte and the endothelium, attenuated cytoplasm of the type I pneumocyte, as well as the pulmonary surfactant. Mucinogen is synthesized by the goblet cells of the respiratory epithelium, and the exchange of gases occurs across the blood–air barrier.
5. C. Bronchioles are very small tubular structures that do not have cartilage in their walls. Their lumina remain patent because of the elastic fibers that are attached to their outer perimeter. They are lined by a simple cuboidal (and in the larger bronchioles simple columnar) epithelium.
6. A. The respiratory portion of the respiratory system is defined as the region where exchange of gases may occur, and it is composed of the respiratory bronchiole, alveolar duct, alveolar sac, and alveoli. The conducting portion of the respiratory system conducts air to the respiratory portion. There is no exchange of gases in the conducting portion of the respiratory system.
7. B. The narrowest blood–air barrier is that which is composed only of the highly attenuated endothelial cell, the fused basal laminae, the highly attenuated type I pneumocyte, and the pulmonary surfactant.
8. D. Newborns with respiratory distress of the newborn cannot produce enough surfactant to maintain patent alveoli and alveolar ducts. They receive glucocorticoids to stimulate their type II pneumocytes to produce surfactant. Their bronchiolar smooth muscles and their diaphragm contract normally. Their type I pneumocytes are attenuated and can permit exchange of gases to occur, and their dust cells function without any problems.
9. D. The hyaline cartilage enclosed in the rectangle is the C-ring of the trachea. This is not a photomicrograph of elastic or of fibrocartilage. Cartilage plates of a secondary bronchus are irregular in outline. Epiphyseal plates of a growing bone do not have a uniform appearance.

This page intentionally left blank

Digestive System: Oral Cavity

16

The digestive system, composed of the oral cavity, alimentary tract, and associated glands, functions in the ingestion, mastication, deglutition (swallowing), digestion, and absorption of food as well as in the elimination of its indigestible remnants. Regions of the digestive system are modified and have specialized structures in order to be able to perform these varied tasks.

This and the following two chapters detail the histology and functions of the component parts of the digestive system. This chapter discusses the contents of the oral cavity; Chapter 17 describes the alimentary tract (esophagus, stomach, small and large intestines, rectum, and anus); and Chapter 18 considers the glands of the digestive system (major salivary glands, pancreas, and liver and gallbladder).

ORAL MUCOSA: OVERVIEW

The oral mucosa, composed of a wet stratified squamous epithelium (nonkeratinized, parakeratinized, or orthokeratinized) and an underlying dense, irregular, collagenous connective tissue, may be divided into three classifications: lining mucosa, masticatory mucosa, and specialized mucosa.

The oral cavity is lined by the **oral mucosa**, composed of a wet **stratified squamous keratinized**, **nonkeratinized**, or **parakeratinized epithelium** and an underlying connective tissue (see Chapter 5). Regions of the oral cavity that are exposed to considerable frictional and shearing forces (gingiva, dorsal surface of the tongue, and hard palate) are lined or covered by a **masticatory mucosa** composed of parakeratinized to completely keratinized stratified squamous epithelium with an underlying dense, irregular, collagenous connective tissue. The remainder of the oral cavity is lined or covered by a **lining mucosa**, composed of a nonkeratinized epithelium overlying a looser type of dense, irregular, collagenous connective tissue (Table 16–1). Moreover, the aspects of the oral mucosa that bear taste buds (dorsal surface of the tongue and patches of the soft palate and pharynx) are covered by **specialized mucosa**, that is, they are specialized to perceive taste.

Ducts of the three pairs of major salivary glands (parotid, submandibular, and sublingual) open into the oral cavity, delivering saliva to moisten the mouth. These major salivary glands (discussed in Chapter 18 on the digestive system) also manufacture and release the enzyme **salivary amylase** to break down carbohydrates, **lactoferrin** and **lysozymes**, antibacterial agents, and **secretory immunoglobulin (IgA)**. In addition, **minor salivary glands**, located in the connective tissue elements of the oral mucosa, add to the flow of saliva into the oral cavity. It is in the oral cavity that food is moistened with saliva, chewed, and isolated by the tongue, ultimately forming spherical masses about 2 cm in diameter. These spherical masses, each known as a ***bolus***, are forced by the tongue into the pharynx to be swallowed.

The lips form the anterior boundary, and the palatoglossal folds form the posterior boundary of the oral cavity. The structures of interest in and about the oral cavity are the lips, the teeth and their associated structures, the palate, and the tongue.

CLINICAL CORRELATIONS

Occasionally, bad breath **(halitosis)** occurs in almost everyone, especially when the individual's mouth is dry; however, in approximately a quarter of all adults, halitosis is a constant phenomenon. This chronic condition is caused by anaerobic bacteria that reside in the crevices of the posterior aspect of the tongue and in the gingival sulcus (the shallow groove between the teeth and the gums). These bacteria emit noxious gases, such as mercaptans, hydrogen sulfide, and methyl and dimethyl sulfides, and as they digest decomposing food remnants, they form malodorous compounds such as indoles and skatoles. In most cases, gentle scraping of the tongue surface, meticulous dental hygiene, and the use of mouthwashes containing zinc and certain ions can alleviate the bad breath for a few hours. Unfortunately, as yet, there is no permanent solution to the problem of chronic bad breath. In fewer than 10% of individuals with chronic halitosis, other factors may be involved, such as deep cavities in the teeth, tonsillar crypts, sinuses, nasal cavity, and stomach.

Lips

The lip has three regions: the skin aspect, the vermilion zone, and the mucous (internal) aspect.

The upper and lower **lips** are usually in contact with one another and thus resemble a drawstring, in that they guard the entrance into the oral cavity. The core of the lips is composed of skeletal muscle fibers that are responsible for lip mobility. Each lip may be subdivided

SUMMARY

The **oral cavity**, a region extending from the **lips** to the **palatoglossal folds**, is a highly modified region of the digestive system, and it is responsible for the preparation of food (moistening, mastication, bolus formation, and deglutition) so that it can be delivered to the alimentary canal in a form that may be digested. The lining of the oral cavity is a **mucosa** (**oral mucosa**) composed of a **wet stratified squamous** (nonkeratinized, parakeratinized, or keratinized) **epithelium** and an underlying dense irregular collagenous **connective tissue**. There are three types of oral mucosae: **lining mucosa**, **masticatory mucosa**, and **specialized mucosa**. The structures of interest in the oral cavity are the lips, **teeth** (composed of **enamel**, **dentin**, **cementum**, and **pulp**), **gingiva**, **bony alveolus**, **hard palate**, **soft palate**, and **tongue** (with its **lingual papillae** and **taste buds**).

KEY WORDS

Bony alveolus; cementum; dentin; enamel; gingiva; hard palate; lingual papillae; lining mucosa; masticatory mucosa; oral cavity; oral mucosa; pulp; soft palate; specialized mucosa; taste buds; teeth; tongue

TABLE 16–1 Summary of the Histological Features of the Oral Mucosa

Mucosal Region	Type of Epithelium/Mucosa	Height of CT Papilla	Special Comments
Lip			
Skin aspect	Stratified squamous keratinized	Medium	Hair, sebaceous and sweat glands
Vermilion zone	Stratified squamous keratinized	High	Few sebaceous glands(?)
Mucosal aspect	Stratified squamous nonkeratinized	Medium	Mucous (mixed) minor salivary glands and Fordyce granules
Cheek			
Skin aspect	Stratified squamous keratinized	Medium	Hair, sebaceous and sweat glands
Mucosal aspect	Stratified squamous nonkeratinized	Medium	Mucous (mixed?) minor salivary glands and Fordyce granules
Gingiva			
Free and attached	Masticatory mucosa	High	Tightly bound to the periosteum
Sulcular	Lining mucosa	Low	Lines the gingival sulcus
Junctional epithelium	Stratified squamous nonkeratinized	None	Attached to tooth surface and to gingival CT by hemidesmosomes
Alveolar Mucosa	Lining mucosa	Low	Some minor mucous salivary glands
Hard Palate (oral surface)	Masticatory mucosa	High	Adipose tissue in the CT
Anterior lateral	Masticatory mucosa	High	Minor mucous salivary glands in the CT
Posterior lateral	Masticatory mucosa	High	Tightly bound to periosteum
Raphe	Masticatory mucosa	High	Tightly bound to periosteum
Hard Palate (nasal surface)	Respiratory epithelium	NA	Mucous glands in the CT
Soft Palate (oral surface)	Lining mucosa	Low	Elastic lamina; minor mucous salivary glands
Uvula	Lining mucosa	Low	Minor mucous salivary glands in the CT
Soft Palate (nasal surface)	Respiratory epithelium	NA	Mucous glands in the CT
Uvula	Lining mucosa	Low	Mucous glands in the CT
Floor of the Mouth	Lining mucosa	Low	Minor mucous salivary glands in the CT
Tongue			
Dorsal surface	Specialized mucosa embedded in masticatory mucosa	Low High	Taste buds; lingual papillae; serous, mucous, and mixed minor salivary glands
Ventral surface	Lining mucosa	Low	Sublingual folds

CT, Connective tissue; NA, not applicable.
Modified with permission from Gartner LP. *Oral Histology and Embryology*, 3rd Edition. Baltimore: Jen House Publishing; 2014 (Table 8-9).

into three regions: the external aspect, the vermilion zone, and the mucous (internal, wet) aspect.

The **external aspect** of the lip is covered with thin skin and is associated with sweat glands, hair follicles, and sebaceous glands. This region is continuous with the **vermilion zone**, the pink region of the lip, which is also covered by thin skin. However, the vermilion zone is devoid of sweat glands and hair follicles, although occasional, nonfunctional sebaceous glands are present there. The interdigitation between the epithelial and connective tissue components of the oral mucosa **(the rete apparatus)** is highly developed, so that the capillary loops of the dermal papillae are close to the surface of the skin, imparting a pink color to the vermilion zone. The absence of functional glands in this region necessitates the occasional moistening of the vermilion zone by the tongue.

The **mucous (internal) aspect** of the lip is always wet and is lined by stratified squamous nonkeratinized epithelium. The subepithelial connective tissue is of the dense, irregular collagenous type and houses numerous, mostly mucous, minor salivary glands.

Teeth

Each tooth, whether deciduous or permanent, has a crown, a cervix, and a root.

Humans have two sets of teeth: 20 **deciduous (milk) teeth**, which are replaced by 32 permanent (adult) teeth composed of 20 **succedaneous teeth** (that is teeth that succeed their deciduous forerunners) and 12 **molars**. Both the deciduous and permanent dentitions are evenly distributed between the maxillary and mandibular arches.

The various teeth have different morphological features; numbers of roots; and functions, such as seizing prey, cutting smaller pieces from large chunks, and macerating the chunks to form a bolus. Only the general structure of teeth is discussed here.

Each tooth is suspended in its bony socket, the **alveolus**, by a dense, irregular collagenous connective tissue, the **periodontal ligament (PDL)**. The gingiva also supports the tooth, and its epithelium seals the oral cavity from the subepithelial connective tissue spaces (Fig. 16–1).

The portion of the tooth that is visible in the oral cavity is called the ***clinical crown***, and the region housed within the alveolus is known as the ***root***. The portion between the crown and the root is the **cervix**.

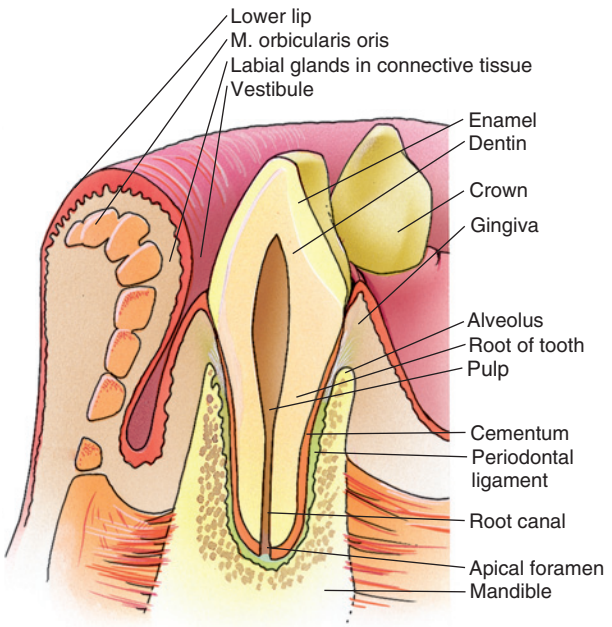


Figure 16–1 Schematic diagram of a tooth in the oral cavity. Note the location of the vestibule between the lip and the labial aspect of the enamel of the tooth and the gingiva as well as the oral cavity on the buccal aspect of the teeth and gingiva.

The entire tooth is composed of three calcified substances, which enclose a soft, gelatinous connective tissue, the **pulp**, located in a continuous space subdivided into the pulp chamber and root canal. The root canal communicates with the **PDL** space via a small opening, the apical foramen, at the tip of each root. It is through this opening that blood and lymph vessels as well as nerves enter and leave the pulp (Fig. 16–2).

Mineralized Components

The mineralized components of the tooth are enamel, dentin, and cementum.

The mineralized structures of the tooth are enamel, dentin, and cementum. Dentin surrounds the pulp chamber and root canal and is covered on the crown by enamel and on the root by cementum. Thus, the bulk of the hard substance of the tooth is composed of dentin. Enamel and cementum meet each other at the cervix of the tooth.

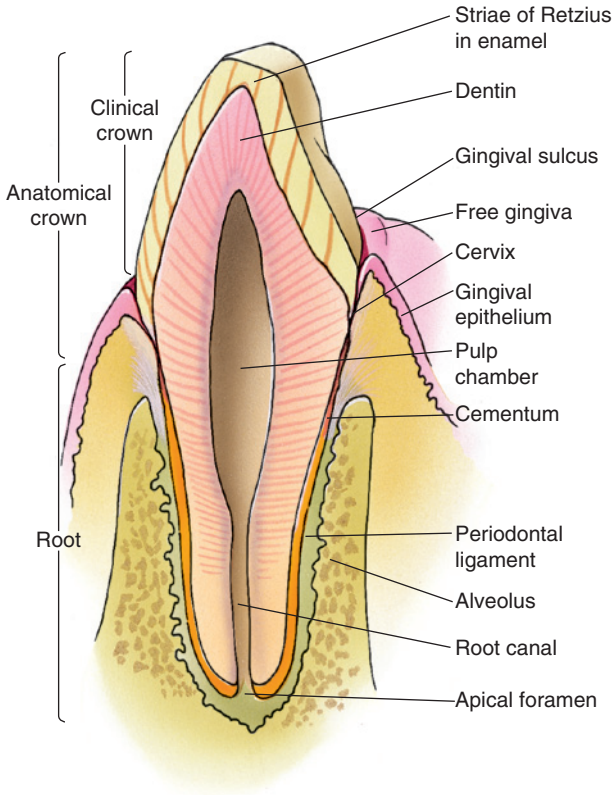


Figure 16–2 Schematic diagram of a tooth and its surrounding structures. Note that the clinical crown is the portion of the crown that is visible in the oral cavity, and the anatomical crown extends from the cementoenamel junction to the occlusal surface of the tooth.

Enamel

Enamel overlies dentin of the crown; it is composed of 96% calcium hydroxyapatite crystals and is the hardest substance in the body.

Enamel is the hardest substance in the body. It is translucent, and its coloration is due to the color of the underlying dentin. Enamel consists of 96% calcium hydroxyapatite and 4% organic material and water. The calcified portion of enamel is composed of large crystals, and each crystal is coated with a thin layer of organic matrix. The organic constituents of enamel are the keratin-like, high-molecular-weight glycoproteins, tyrosine-rich **enamelins** as well as related proteins, **amelogenins** and **ameloblastin**.

Enamel is produced by cells known as ***ameloblasts***, which elaborate enamel daily in 4- to 8-μm segments known as ***rod segments***. Successive rod segments adhere to one another, forming more or less cylindrical-shaped **enamel rods (prisms)**, which extend over the complete width of the enamel from the dentinoenamel junction (DEJ) to the enamel surface. The calcium hydroxyapatite crystal orientation within rods varies, permitting a subdivision of the enamel rod into a cylindrical head to which a tail (interrod enamel), in the shape of a rectangular solid, is attached. Enamel is a nonvital substance; because the ameloblasts die before the tooth erupts into the oral cavity, the body cannot repair enamel.

CLINICAL CORRELATIONS

Caries (cavities) usually result from the accumulation of microorganisms in and on slight defects of the enamel surface. As these bacteria metabolize nutrients in the saliva and on the tooth surface, they produce acids that begin to decalcify the enamel. As the bacteria proliferate in the cavity that they have "excavated," they and the toxins that they release enlarge the caries.

Fluoride increases the hardness of enamel, especially in young individuals, making the enamel more resistant to caries. The incidence of cavities has been greatly reduced by the addition of fluoride to the public water supply and to toothpastes and by its topical application in the dental office. As the individual ages, the enamel crystals enlarge in size, and there is less space available for the exchange of hydroxyl ions for fluoride ions. Therefore, the use of fluoride treatments in adults is not nearly as effective as for young children.

Because during its formation, enamel is elaborated in daily segments, the quality of the enamel produced varies with the health of the mother during prenatal stages or the health of the person after birth. The **enamel rod** then reflects the metabolic state of the mother during the time of enamel formation, resulting in successive rod segment sequences of hypocalcified and normally calcified enamel. These alternating sequences, analogous to growth rings in a tree trunk, are evident histologically and are called ***striae of Retzius***.

The free surface of a newly erupted tooth is covered by a basal lamina–like substance, the **primary enamel cuticle**, manufactured by the same ameloblasts that elaborated enamel. This cuticle wears away shortly after the tooth's emergence into the oral cavity.

Dentin

Dentin forms the bulk of the tooth; it is composed of 70% calcium hydroxyapatite and is the second hardest substance in the body.

Dentin is the second hardest tissue in the body (Fig. 16–3; see Fig. 16–2). It is yellowish in color, and its high degree of elasticity protects the overlying brittle enamel from becoming fractured. Dentin is composed of 65% to 70% calcium hydroxyapatite, 20% to 25% organic materials, and about 10% bound water. Most of the

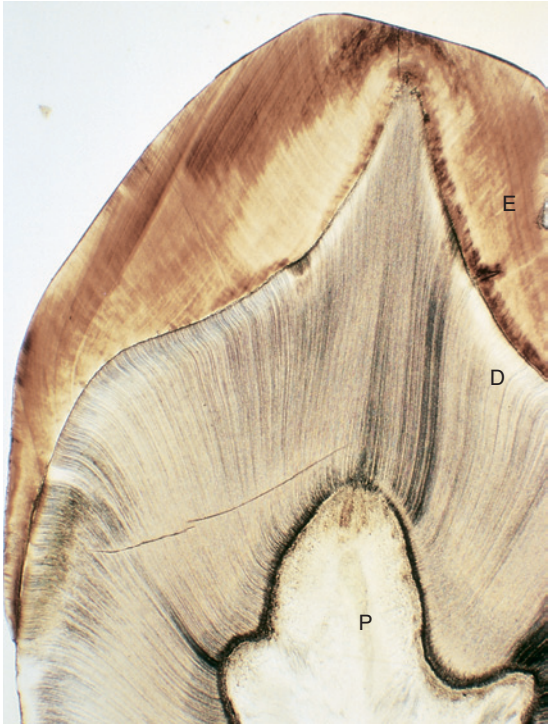


Figure 16–3 Photomicrograph of the crown and neck of a tooth. Observe that this is a ground section (nondecalcified) and that the enamel (E) appears brown, and the dentin (D) appears grayish in this preparation. The pulp (P) cavity occupies the center of the tooth (×14).

organic substance is **type I collagen** associated with proteoglycans and glycoproteins.

The cells that produce dentin are known as ***odontoblasts***. Unlike ameloblasts, they maintain their association with dentin for the life of the tooth. These cells are located at the periphery of the pulp, and their cytoplasmic extensions, **odontoblastic processes**, occupy tunnel-like spaces within dentin. These extracellular fluid–filled spaces, known as ***dentinal tubules***, extend from the pulp to the dentinoenamel (in the crown) or dentinocemental (in the root) junctions.

During dentinogenesis, odontoblasts manufacture about 4 to 8 µm of dentin every day. The quality of dentin, as of enamel, varies with the health of the mother prenatally or of the child postnatally. Thus, along the length of the dentinal tubule, dentin displays alternating regions of normal calcification and hypocalcification. These are recognizable histologically as **lines of Owen**, analogous to the striae of Retzius in enamel.

Because odontoblasts remain functional, dentin has the capability of self-repair, and **reparative dentin** is elaborated on the surface of preexisting dentin within the pulp chamber, thus reducing the size of the pulp chamber with age.

CLINICAL CORRELATIONS

Dentin sensitivity is mediated by sensory nerve fibers that are closely associated with odontoblasts, their processes, and the dentinal tubules. Disturbance of the tissue fluid within dentinal tubules is thought to depolarize the nerve fibers somehow, sending a signal to the brain, where the signal is interpreted as pain.

Cementum

Cementum overlies dentin of the roots; it is composed of about 50% calcium hydroxyapatite and approximately 50% organic matrix and bound water; therefore, it is approximately as hard as bone.

The third mineralized tissue of the tooth is **cementum**, a substance that is restricted to the root (see Figs. 16–2 and 16–3). Cementum is composed of 45% to 50% calcium hydroxyapatite and 50% to 55% organic material and bound water. Most of the organic material is composed of **types I**, **III**, **and XII collagen** with associated glycosaminoglycans, proteoglycans, and glycoproteins.

The apical region of cementum is similar to bone in that it houses cells, **cementocytes** within lenticular spaces, known as *lacunae*. Processes of cementocytes extend from lacunae within narrow **canaliculi** that extend toward the vascular PDL. Because of the presence of cementocytes, this type of cementum is called ***cellular cementum***. The coronal region of cementum is without cementocytes and is called ***acellular cementum***. Both cellular cementum and acellular cementum have **cementoblasts**, cells that are responsible for the formation of cementum. These cells cover cementum at its interface with the PDL and continue to elaborate cementum for the life of the tooth.

Type I collagen fibers of the PDL, known as ***Sharpey fibers***, are embedded in cementum and in the alveolus, and in this fashion, the PDL suspends the tooth in its bony socket.

Cementum can be resorbed by osteoclast-like cells known as ***odontoclasts (cementoclasts)***. During exfoliation, the replacement of deciduous teeth by their succedaneous counterparts, odontoclasts resorb cementum (and dentin) of the root.

CLINICAL CORRELATIONS

Cementum does not resorb as readily as does bone, a property that orthodontists use to their advantage in moving improperly positioned teeth. By placing the correct force on a tooth, the orthodontist reshapes the bony socket and, consequently, causes the tooth to be moved into its correct position.

Pulp

Pulp, a richly vascularized and innervated loose connective tissue, is surrounded by dentin and communicates with the periodontal ligament via the apical foramen.

The **pulp** of the tooth is composed of a loose, gelatinous connective tissue that is rich in proteoglycans and glycosaminoglycans, has an extensive vascular and nerve supply, and has some lymph circulatory elements (Fig. 16–4; see Fig. 16–2). The pulp communicates with the PDL through the apical foramen, a small opening at the tip of each root. Therefore, teeth with multiple roots have an apical foramen at the tip of each root. Vessels and nerves enter and leave the pulp through these openings. Anatomically, the pulp has two regions, coronal pulp (located in the crown) and radicular pulp (located in the roots). Naturally, the coronal and radicular pulps are continuous with each other.

It is customary to subdivide the pulp, histologically, into three concentric zones around a central **core**:

- The outermost, **odontoblastic zone** of the pulp is composed of a single layer of **odontoblasts**, whose processes extend into the adjacent dentinal tubules of dentin.
- The **cell-free zone** forms the layer deep to the odontoblastic zone, and as its name implies, it is mostly devoid of cells.
- The **cell-rich zone**, consisting of fibroblasts and mesenchymal cells, is the deepest zone of the pulp, immediately surrounding the **pulp core**.

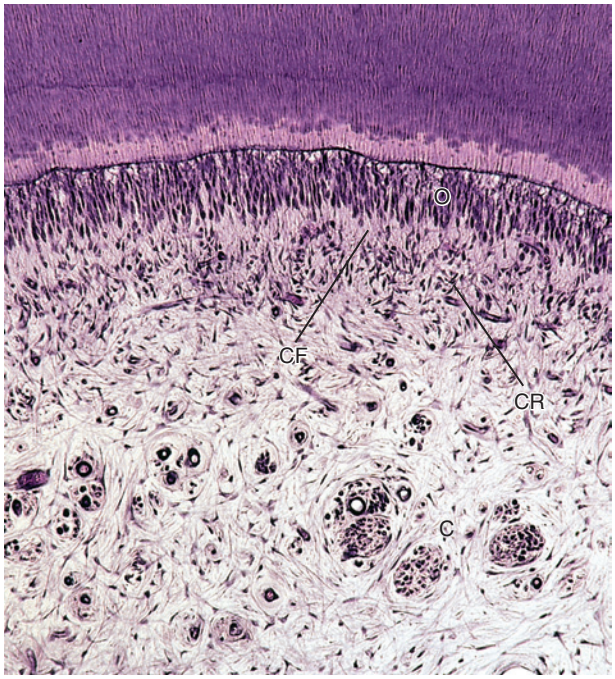


Figure 16–4 Photomicrograph of the pulp of a tooth. Note the three layers, odontoblastic zone (O), cell poor (cell free) zone (CF), cell rich zone, and the core of the pulp (C) (×132).

The core of the pulp resembles most other loose connective tissues but lacks adipose cells. Another notable difference is that the pulp core is highly vascularized, and occasionally, coronal pulp houses calcified elements called ***pulp stones (denticles)***.

The nerve fibers of the pulp are of two types: (1) **sympathetic** (vasomotor) fibers control the luminal diameters of blood vessels, and (2) **sensory** fibers are responsible for the transmission of pain sensation. Most of these **pain fibers** are thin myelinated fibers that form the **Raschkow plexus**, just deep to the cell-rich zone and are believed to be Aδ fibers that conduct sharp pain. Additionally, some nonmyelinated C fibers also enter Raschkow plexus and are responsible for the conveyance of dull pain. As nerve fibers continue through this plexus, the Aδ fibers lose their myelin sheath, pass through the cell-free zone, and penetrate the space between odontoblasts to enter the dentinal tubule. Some nerve fibers synapse on the odontoblasts or their processes instead of entering the dentinal tubules.

CLINICAL CORRELATIONS

Hemorrhage of the pulp is evident clinically as dark discoloration of the tooth. Because the pulp may recover, however, hemorrhage should not be the sole indicator for root canal treatment.

Odontogenesis

Odontogenesis begins with the appearance of the dental lamina.

The first sign of **odontogenesis** (tooth development) occurs between the sixth and seventh weeks of gestation, when the ectodermally derived **oral epithelium** proliferates (Fig. 16–5). The result of this mitotic activity is the formation of a horseshoe-shaped band of epithelial cells, the **dental lamina**, surrounded by **neural crest**–derived **ectomesenchyme** of the mandibular and maxillary arches. The dental lamina is separated from the ectomesenchyme by a well-defined basal lamina.

Bud Stage

Shortly after the appearance of the dental lamina, mitotic activity increases on the inferior aspect of this epithelial band of each arch. This activity is responsible for the formation of 10 discrete epithelial structures, known as ***buds***, initiating the **bud stage** of tooth development. These buds presage the 10 deciduous teeth of both the maxillary and the mandibular arches. At the inferior tip of each bud, ectomesenchymal cells congregate to form the presumptive **dental papilla**. Further development, although similar for each bud, is asynchronous, corresponding to the order of emergence of the various teeth of the child.

Cap Stage

The cap stage of tooth development is recognized by the three-layered enamel organ, composed of the outer enamel epithelium, stellate reticulum, and inner enamel epithelium.

As cells of the bud proliferate, this structure not only increases in size but also alters its shape to form a three-layered configuration, known as the ***cap***, initiating the **cap stage** of tooth development. Two of the three layers—the convex *simple squamous* **outer enamel epithelium (OEE)** and the concave *simple squamous* **inner enamel epithelium (IEE)**—are continuous with each other at a rim-like region, the **cervical loop**. They enclose a third layer, the **stellate reticulum (SR)**, whose cells have numerous processes that are in contact with one another. These epithelially derived layers, constituting the "plump" **enamel organ**, are separated from the surrounding ectomesenchyme by a basal lamina. The concavity of the cap is occupied by a congregation of ectomesenchymal cells, the **dental papilla**. The dental papilla becomes vascularized and innervated during the cap stage of tooth development. The dental papilla and the enamel organ, together, are known as the ***tooth germ***. One can imagine the enamel organ to resemble an insulated cup in which the handle

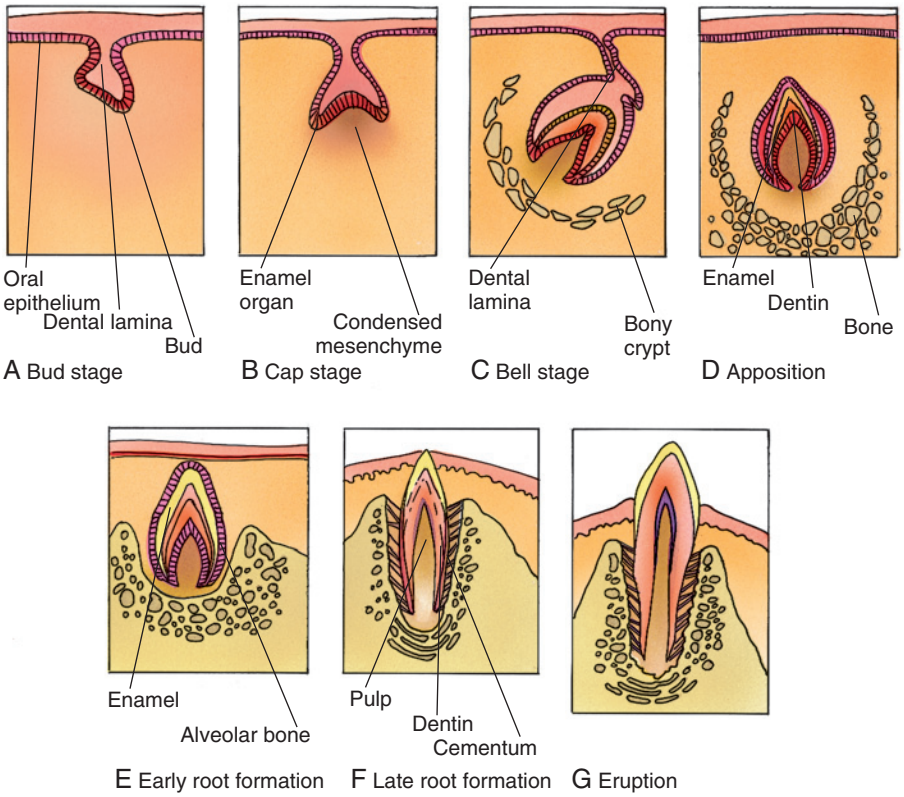


Figure 16–5 Schematic diagram of odontogenesis.

is the dental lamina that is connected to the oral epithelium; the outer surface of the cup is the OEE; the inner surface is the IEE; and the rim of the cup, where the inner and outer surface meet, is the cervical loop. The insulation located between the inner and outer surfaces is the SR, and the cup is filled with the dental papilla.

The process of morphodifferentiation is responsible for the establishment of the template of the presumptive tooth; that is, the enamel organ assumes the shape of an incisor (incisiform), canine (caniniform), or a molar (molariform). This event is controlled by the **primary enamel knot**, a dense clump of cells located adjacent to the IEE within the substance of the enamel organ. It appears that the ectomesenchymal cells of the dental papilla induce the cells of the enamel knot to begin to express signaling molecules, thus transforming the enamel knot into one of the principal signaling centers of tooth morphogenesis.

Cells of the primary enamel knot synthesize **bone morphogenic proteins BMP-2**, **BMP-4**, **sonic hedgehog**, and **fibroblast growth factor-4 (FGF-4)**, and proteins encoded by the **Wnt genes** in a particular sequential relationship that result in cusp formation in the developing tooth. To be able to establish this time-specific relationship, the ectomesenchymal cells of the dental papilla release **epidermal growth factor (EGF)** and **FGF-4**; without EGF and FGF-4, the primary enamel knot cells are driven into apoptosis, and cusps will not develop, which is exactly what happens in incisiform and caniniform tooth germs. Therefore, cusp formation is the responsibility of the primary enamel knot. As the cusps are being formed, cells from the primary enamel knot migrate to the regions of cusp formation and thereby seed the formation of the **secondary enamel knots** that preside over the development of the future cusps. Once the primary and secondary enamel knots have performed their functions, the ectomesenchymal cells cease their release of EGF and FGF-4, and the presence of BMP-4 drives the cells of the primary and secondary enamel knots into apoptosis.

The dental papilla, whose most peripheral layer of cells is separated from the IEE by the basal lamina, is responsible for the formation of the pulp and dentin of the tooth. Ectomesenchymal cells surrounding the tooth germ form a vascularized membranous capsule, the **dental sac (dental follicle)**, which gives rise to the cementum, PDL, and alveolus. Cells of the IEE differentiate into preameloblasts, which mature into

ameloblasts to form enamel. Therefore, except for enamel, the rest of the tooth and its associated structures are derived from cells of neural crest origin.

During the cap stage of tooth development, a solid cord of epithelial cells, the **succedaneous lamina**, derived from the dental lamina, grows deep into the ectomesenchyme. The cells at the tip of the succedaneous lamina proliferate to form a bud, the precursor of the **succedaneous tooth**, that eventually replaces the **deciduous tooth** being developed. Because there are only 20 deciduous teeth, only the same number of succedaneous teeth are formed. The remaining 12 permanent teeth, known as ***accessional teeth*** (three permanent molars in each quadrant), because they do not replace existing deciduous dentition; instead they arise from the posterior extensions of the maxillary and mandibular dental laminae. The formation of the posteriorly directed extension of the original dental laminae begins in the fifth month of gestation.

Bell Stage and Appositional Stage

The bell stage is recognized by the four-layered enamel organ, composed of the outer enamel epithelium, stellate reticulum, stratum intermedium, and inner enamel epithelium.

Proliferation of the cells of the tooth germ increases its size, and the accumulation of fluid within the enamel organ increases its plump appearance. In addition, its concavity deepens, and another layer of cells develops between the IEE and SR of the enamel organ. This new layer of cells is the **stratum intermedium**, and its appearance characterizes the **bell stage** of tooth development. Because of changes in the morphology of the enamel organ and changes in the shape of certain cells of the tooth germ, this stage of odontogenesis is also called the ***stage of morphodifferentiation*** **and** ***histodifferentiation***.

As most of the fluid within the enamel organ is resorbed, much of the OEE collapses over the stratum intermedium, bringing the vascularized dental sac close to that new layer. The proximity of blood vessels apparently causes the stratum intermedium to induce the simple squamous cells of the IEE to differentiate into preameloblasts that will mature into enamel-producing columnar cells, known as ***ameloblasts*** (Fig. 16–6). In response to the histodifferentiation of the inner enamel epithelial cells, the most peripheral cells of the dental papilla (those in contact with the basal lamina) also differentiate to become preodontoblasts that will mature into dentin-producing columnar cells, known as ***odontoblasts*** (Fig. 16–7; see Fig. 16–6).

Shortly after the odontoblasts begin to elaborate dentin matrix (a noncalcified material) into the basal lamina, the ameloblasts also begin to manufacture enamel matrix (also a noncalcified material). The dentin and enamel matrices adjoin each other, and the junction between them is the **dentinoenamel junction (DEJ)** (see Fig. 16–3). The tooth germ is now said to be in the **appositional stage** of odontogenesis. The process of dentin and enamel formation depends on the presence of various signaling molecules, such as FGF-8, bone morphogenetic protein-2, transforming growth factor-β, and insulin-like growth factors I and II.

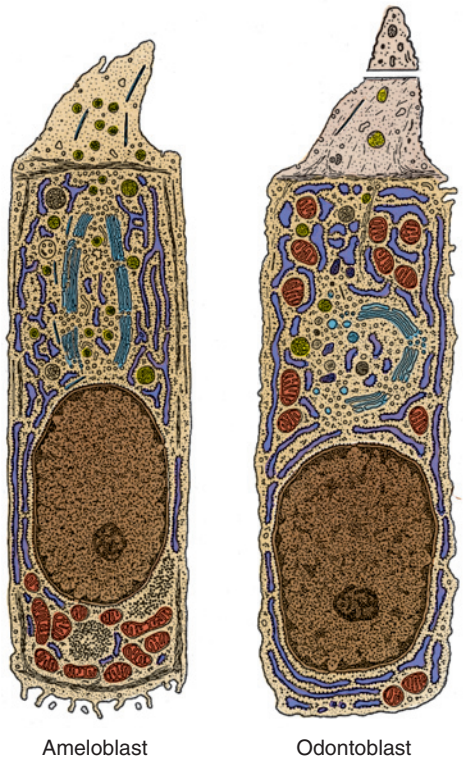


Figure 16–6 Diagram of an electron micrograph of an ameloblast and an odontoblast. Note that the odontoblastic process is very long, and a large section of it has been cut out—white space. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

During the formation of dentin, as the odontoblasts move away from the DEJ, the distal tip of their process remains at that junction, and the process continues to elongate. This cytoplasmic extension, known as the ***odontoblastic process***, is surrounded by dentin. The space occupied by the odontoblastic process is the dentinal tubule.

As the ameloblasts secrete enamel matrix, their apical region becomes constricted by the matrix, forming **Tomes process**. The ameloblasts then move away from the newly elaborated enamel, and the constricted region expands to its previous size. The cyclic nature of the Tomes process formation continues until enamel formation ceases. As dentin matrix becomes calcified to form

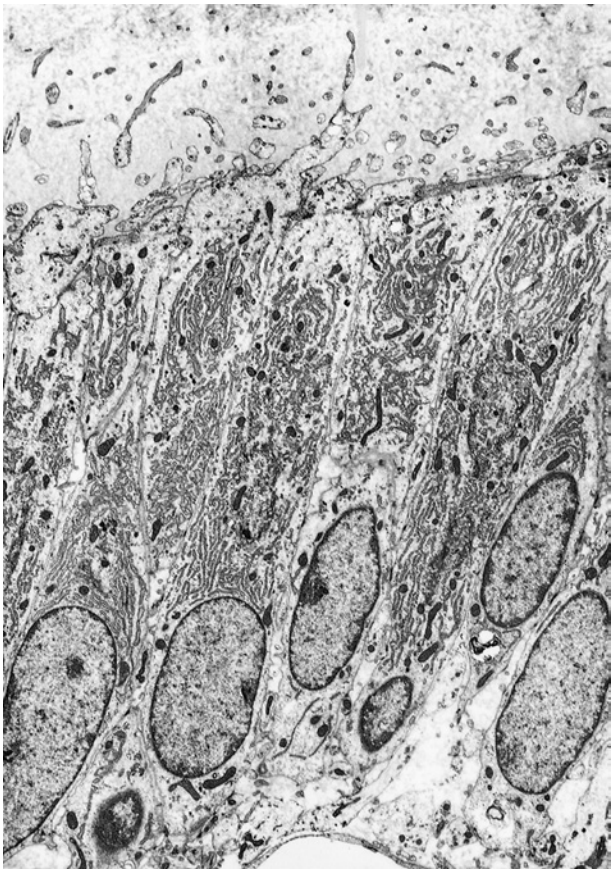


Figure 16–7 Electron micrograph of rat incisor odontoblasts (×3416). (From Ohshima H, Yoshida S. The relationship between odontoblasts and pulp capillaries in the process of enamel-related cementum-related dentin formation in rat incisors. *Cell Tissue Res*. 1992;268:51-63.)

dentin, the process of calcification spreads into the enamel matrix, which also calcifies and becomes known as ***enamel***.

Root Formation

Root formation begins after the completion of the crown and is organized by the Hertwig epithelial root sheath.

When all of the enamel and coronal dentin (dentin of the crown) have been manufactured, the tooth germ enters the next stage of odontogenesis, known as ***root formation***. The outer and inner enamel epithelia of the cervical loop elongate, forming a two-layered (OEE and IEE) sleeve-like structure known as the ***Hertwig epithelial root sheath (HERS)***, which encompasses ectomesenchymal cells located deep to the developing crown, forming an elongation of the dental papilla.

The absence of the stratum intermedium prevents the cells of the IEE from differentiating into ameloblasts; thus, enamel is not formed on the developing root surface. However, the most peripheral cells of the root dental papilla differentiate into preodontoblasts, which release **nuclear factor Ic**, a transcription factor that triggers the initiation of dentin formation and the differentiation of these cells into odontoblasts, cells that continue to elaborate root dentin. As the HERS elongates, more and more of the root continues to be manufactured, and the region of HERS closer to the cervical loop begins to disintegrate, forming perforations in this sleeve-like structure. Ectomesenchymal cells from the **dental sac** migrate through the openings in the HERS, approximate the newly formed dentin, and differentiate into **cementoblasts**. These newly differentiated cells manufacture cementum matrix, which subsequently calcifies and is referred to as ***cementum***. It has been suggested by some authors that, in addition to the ectomesenchymal cells, cementoblasts may also form from cells of the HERS.

The elongation of the root is a consequence of the lengthening of the HERS. As the root becomes longer, the crown approaches and eventually erupts into the oral cavity; however, the lengthening of the root is not responsible for tooth eruption; instead, the two processes merely happen to occur simultaneously.

Structures Associated with Teeth

The structures associated with teeth are the PDLs, alveolus, and gingiva.

Periodontal Ligament

The periodontal ligament is a dense, irregular collagenous connective tissue whose principal fiber groups, composed of type I collagen, suspend the tooth in its alveolus.

The **periodontal ligament (PDL)** is located in the PDL space, defined as the region between the cementum of the root and the bony alveolus (see Figs. 16–1 and 16–2). In a healthy mouth, the PDL space is less than 0.5 mm wide. Although this richly vascularized connective tissue is classified as dense irregular collagenous connective tissue, it has **principal fiber groups**, composed mostly of **type I collagen fibers**, that are arranged in specific, predetermined patterns to absorb and counteract masticatory forces. The ends of the principal fiber groups are embedded in the alveolus and cementum as **Sharpey fibers**, which permit the PDL to suspend the tooth in its socket (Fig. 16–8).

Fibroblasts are the most populous cells of the PDL. These cells not only manufacture the collagen and amorphous intercellular components of the PDL but also help **resorb** collagen fibers, thus being responsible for the **high turnover of collagen** in the PDL. In addition, mast cells, macrophages, plasma cells, and leukocytes are also present in the PDL.

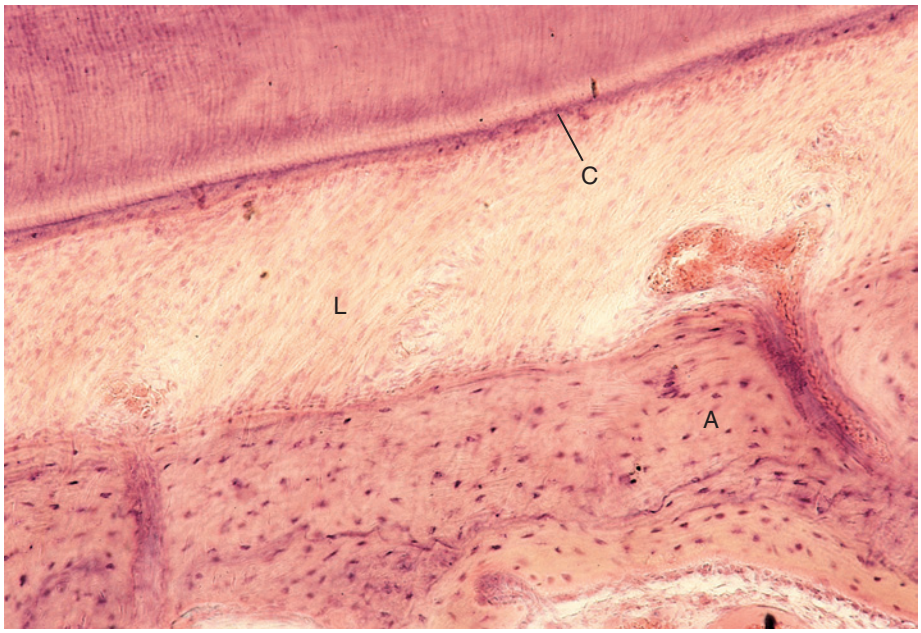


Figure 16–8 The periodontal ligament (L) is a dense, irregular, collagenous connective tissue located between the cementum (C) of the root and the bony alveolus (A) (×132).

Nerves of the PDL include (1) **autonomic fibers**, which regulate the luminal diameter of the arterioles; (2) **pain fibers**, which mediate pain sensation; and (3) **proprioceptive fibers**, which are responsible for the perception of spatial orientation.

CLINICAL CORRELATIONS

Proprioceptive fibers in the periodontal ligament are responsible for the **jaw-jerk reflex**, an involuntary opening of the jaw when one unexpectedly bites down on something hard. This reflex causes relaxation of the muscles of mastication and contraction of muscles responsible for opening the jaw, thus protecting the teeth from fracture.

Alveolus

The alveolus is the bony socket in which the tooth is suspended by fibers of the periodontal ligament.

The alveolar process, a bony continuation of the mandible and maxilla, is divided into compartments, each known as an ***alveolus***, that house the root or, in the case of multirooted teeth, roots of a tooth. Adjacent alveoli are separated from each other by a bony interalveolar septum. The alveolus has three regions (see Figs. 16–1 and 16–2). The **cortical plates**, disposed lingually and labially, form a firm supporting ledge of compact bone, lined by cancellous bone, the **spongiosa**. The spongiosa surrounds a thin layer of compact bone, the **alveolar bone proper**, whose shape mirrors that of the root suspended in it.

Nutrient arteries travel in canals (referred to as *nutrient canals*) within the spongiosa, supplying the bony alveolus. The alveolar bone proper, said to be supported by the cortical plate and the spongiosa, has numerous perforations. Branches of the nutrient artery, named *perforating arteries*, pass from the spongiosa into the PDL, contributing to its vascularization.

Gingiva (Gums)

The gingiva is attached to the enamel surface by a thin, wedge-shaped, stratified squamous nonkeratinized epithelium, known as the junctional epithelium.

Because the **gingiva** is exposed to strenuous frictional forces, its stratified squamous epithelium is either fully keratinized **(orthokeratinized)** or partially keratinized (parakeratinized) (see Figs. 16–1 and 16–2). Deep to the epithelium is a dense, irregular collagenous connective tissue whose type I collagen fibers form principal fiber groups that resemble those of the PDL.

As the epithelium of the gingiva approaches the tooth, it forms a hairpin turn, proceeds apically (toward the root tip) for 1 to 2 mm, and then attaches to the enamel surface by the formation of hemidesmosomes.

The 1- to 2-mm-deep space between the gingiva and the tooth is the **gingival sulcus**.

The region of the gingival epithelium that attaches to the enamel surface is known as the ***junctional epithelium***, which forms a collar around the neck of the tooth. The junctional epithelium forms a robust barrier between the bacteria-laden oral cavity and the sterile environment of the gingival connective tissue. The principal fiber groups of the gingiva assist in the adherence of the junctional epithelium to the tooth surface, maintaining the integrity of the epithelial barrier. This barrier is about 1 mm long, and is only about 35 to 50 cells wide coronally and 5 to 7 cells wide apically.

Palate

The palate, composed of the hard palate, the soft palate, and the uvula, separates the oral cavity from the nasal cavity.

The oral and nasal cavities are separated from each other by the **hard palate** and the **soft palate**. The hard palate, positioned anteriorly, is immovable and receives its name from the bony shelf contained within it. In contrast, the soft palate is movable, and its core is occupied by skeletal muscle responsible for its movements.

The **masticatory mucosa** on the oral aspect of the **hard palate** is composed of a wet stratified squamous keratinized (or parakeratinized) epithelium underlain by dense, irregular collagenous connective tissue. The connective tissue of the anterior lateral region of the hard palate displays clusters of adipose tissue, whereas its posterior lateral aspect exhibits acini of mucous minor salivary glands. This connective tissue is fused with and indistinguishable from the periosteum of the hard palate; therefore, the palatal epithelium and underlying connective tissue together are referred to as the ***mucoperiosteum***. The nasal aspect of the hard palate is covered by respiratory epithelium with occasional patches of stratified squamous nonkeratinized epithelium.

The oral surface of the **soft palate** is covered by a **lining mucosa**, composed of a wet stratified squamous nonkeratinized epithelium and a subjacent dense, irregular collagenous connective tissue housing mucous minor salivary glands that are continuous with glands of the hard palate. The epithelium of its nasal aspect, similar to that of the hard palate, is of the pseudostratified ciliated columnar type. The most posterior extension of the soft palate is the **uvula**, whose histological appearance is similar to that of the soft palate, but its epithelium is composed solely of stratified squamous nonkeratinized epithelium. The connective tissue of the uvula is also a dense irregular collagenous type and possesses mucous minor salivary glands, and its core is composed of skeletal muscle that is responsible for its movement.

Tongue

The tongue has three regions: the anterior two thirds, the posterior one third, and a root.

The **tongue** is the largest structure in the oral cavity. Its extreme mobility is due to the large intertwined mass of skeletal muscle fibers that compose its bulk (Fig. 16–9). The muscle fibers may be classified into two groups: those that originate outside of the tongue, the **extrinsic muscles**, and those that originate within and insert into the tongue, the **intrinsic muscles**. The extrinsic muscles are responsible for moving the tongue in and out of the mouth as well as from side to side, whereas the intrinsic muscles alter the shape of the tongue. The intrinsic muscles are arranged in four groups: superior and inferior longitudinal, vertical, and transverse.

Interspersed among the intrinsic muscles of the tongue are the minor salivary glands:

- Anterior lingual glands (Blandin-Nuhn glands) near the tip of the tongue
- Posterior mucous glands in the core of the posterior one third of the tongue
- Glands of von Ebner, located in the posterior aspect of the anterior one third of the tongue and whose ducts deliver their serous saliva into the epithelially lined groove of circumvallate papillae and the furrows of the foliate papillae

The tongue has a dorsal surface, a ventral surface, and two lateral surfaces. The dorsal surface is observed to have two unequal regions, the larger **anterior two thirds** and the smaller **posterior one third**. The two regions are separated from one another by a shallow, V-shaped groove, the **sulcus terminalis**, whose apex points posteriorly and contains a deep concavity, the **foramen cecum**.

The dorsal surface of the posterior one third of the tongue is uneven because of the presence of the **lingual tonsils** (see Chapter 12). The most posterior portion of the tongue is known as the ***root of the tongue***. Lingual papillae, most of which project above the surface, cover the anterior two thirds of the tongue's dorsal surface.

Lingual Papillae

There are four types of lingual papillae: filiform, fungiform, foliate, and circumvallate.

On the basis of their structure and function, the lingual papillae are of four types: filiform, fungiform, foliate, and circumvallate (Fig. 16–10; see Fig. 16–9). They are all located anterior to the sulcus terminalis on the dorsal or lateral aspect of the tongue.

The numerous **filiform papillae** are slender structures that impart a velvety appearance to the dorsal surface (see Figs. 16–9 and 16–10). These papillae are

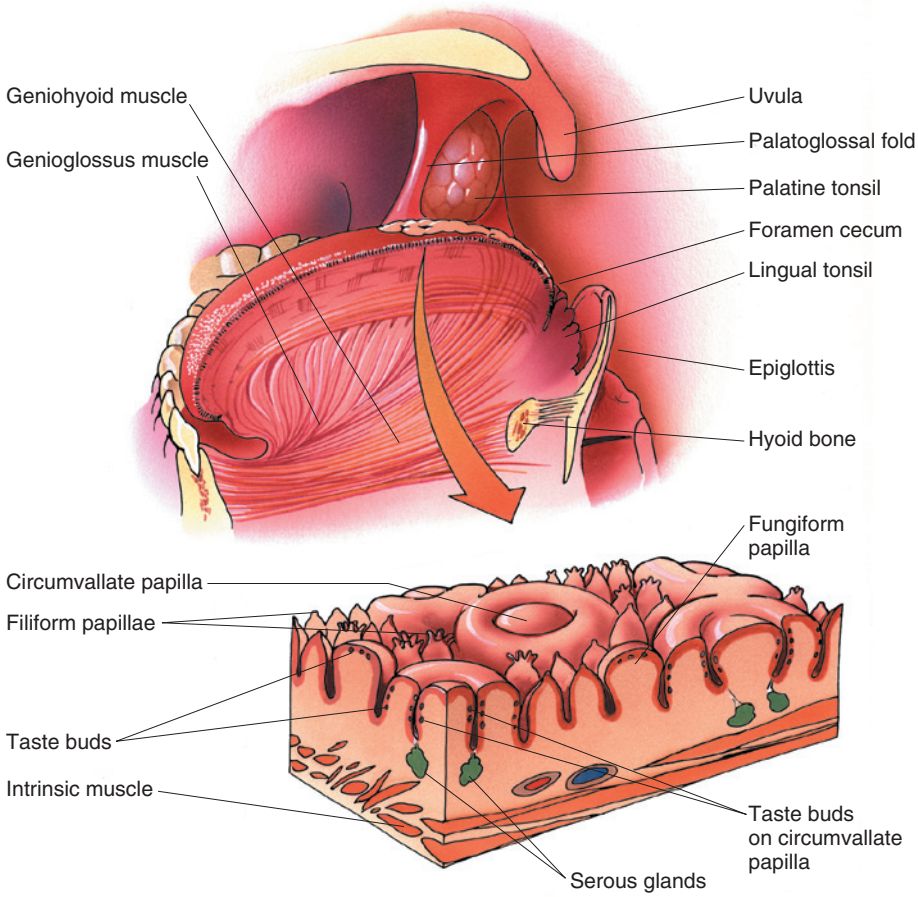


Figure 16–9 Schematic diagram of the tongue and its lingual papillae.

covered by stratified squamous keratinized epithelium and help scrape food off a surface. The high degree of keratinization is especially apparent in the sandpaper-like quality of a cat's tongue. Filiform papillae do not have taste buds.

Each **fungiform papilla** resembles a mushroom whose slender stalk connects a broad cap to the tongue surface (see Figs. 16–9 and 16–10). The epithelial covering of these papillae is stratified squamous nonkeratinized; thus, the blood coursing through the subepithelial capillary loops is evident as red dots distributed randomly among the filiform papillae on the dorsum of the tongue. Fungiform papillae have taste buds on the dorsal aspect of their cap.

Foliate papillae are located along the posterolateral aspect of the tongue. They appear as vertical furrows, reminiscent of pages of a book. These papillae have functional taste buds in the neonate, but these taste buds degenerate by the second or third year of life. As indicated previously, slender ducts of serous minor salivary **glands of von Ebner**, located in the core of the tongue, empty into the base of the furrows.

There are 8 to 12 large **circumvallate papillae** in a V-shaped arrangement just anterior to the sulcus terminalis. These papillae are submerged into the surface of the tongue so that they are surrounded by an epithelially lined groove, whose base is pierced by slender ducts of glands of von Ebner (Fig. 16–11; see Figs. 16–9 and 16–10). The epithelial lining of the groove and the side (but not the dorsum) of these papillae have taste buds.

Taste Buds

Taste buds are intraepithelial sensory organs that function in the perception of taste. The surface of the tongue and the posterior aspect of the oral cavity have approximately 2000 to 8000 taste buds. Each taste bud, composed of 60 to 80 spindle-shaped cells, is an oval structure, 70 to 80 μm long and 30 to 40 μm wide, and is distinctly paler than the epithelium surrounding it

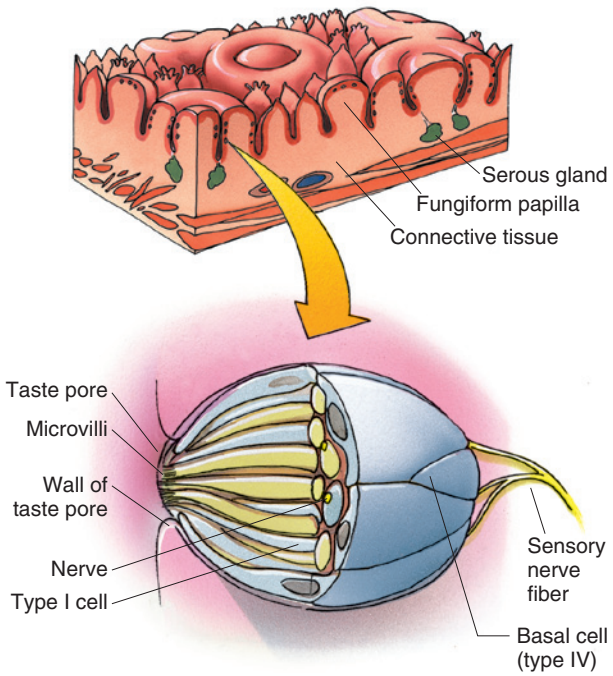


Figure 16–10 Schematic diagram of lingual papillae and a taste bud.

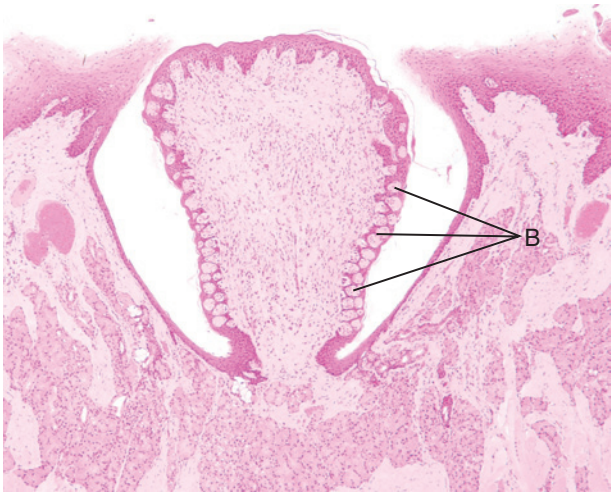


Figure 16–11 Low power photomicrograph of a monkey circumvallate papilla (×58).

(Fig. 16–12; see Figs. 16–10 and 16–11). The narrow end of the taste bud, located at the free surface of the epithelium, projects into an opening, the **taste pore**, formed by the squamous epithelial cells that overlie the taste bud (see Figs. 16–11 and 16-12).

Four types of cells constitute the taste bud:

- Dark cells (type I cells)
- Light cells (type II cells)
- Intermediate cells (type III cells)
- Basal cells (type IV cells)

The relationship among the various cell types is not clear, although researchers agree that basal cells function as reserve cells and regenerate the cells of the taste buds, which have an average life span of 10 days. Most investigators believe in the following progression: Basal cells give rise to dark cells, which mature into light cells, which become intermediate cells and die.

Nerve fibers enter the taste bud and form synaptic junctions with type I, type II, and type III cells, indicating that all three cell types probably function in the discernment of taste and are known as ***neuroepithelial cells***. Each of these cell types has long, slender microvilli that protrude from the taste pore. In the past, these microvilli were noted with light microscopes and were called ***taste hairs***.

Tastants, chemicals from food dissolved in saliva, interact either with ion channels or with receptors located on the microvilli of the taste cells, effecting electrical alterations in the resting potentials of these cells, resulting in depolarization of the cell and initiating an action potential that is transmitted to the brain where the signals are interpreted as specific taste sensations. There are five primary taste sensations: salty, sweet, sour, bitter, and umami (a savory taste sensed via glutamate receptors). It is believed that, although every taste bud can discern each of the five sensations, each taste bud specializes in two of the four tastes. The reaction to these taste modalities is due to the presence of specific ion channels (salty and sour) and G protein–coupled membrane receptors (bitter, sweet, and umami) in the plasmalemma of the cells of the taste bud. Recently, another receptor was localized on taste buds, CD36, a fatty acid transporter, that has the ability to detect fat, and individuals possessing these receptors prefer foods that are fatty.

It is now known that three of the five taste sensations, bitter, sweet, and umami, depend on the presence of **G protein–linked receptors** in the cell membranes of the cilia protruding through the taste pores. The taste receptors for sweet and umami are coded for by three genes: ***T1R1***, ***T1R2***, and ***T1R3***. Each receptor is coded for by **two** of these three genes. The receptors for bitter are coded for by at least 43 different genes, known as ***T2R#***; the best known is **T2R38** whose protein product recognizes a large number of different tastants that register as bitter. The receptors for sour and salt depend on the presence of hydrogen and sodium ion channels, respectively, and the sour taste also depends on the expression of the **PKD2L1 gene** (codes for **polycystic kidney disease 2-like 1 protein**). Lipid taste depends on the presence of a cluster of differentiation molecule (CD36) on the cell membrane of the cilia protruding from the taste pore (Table 16–2).

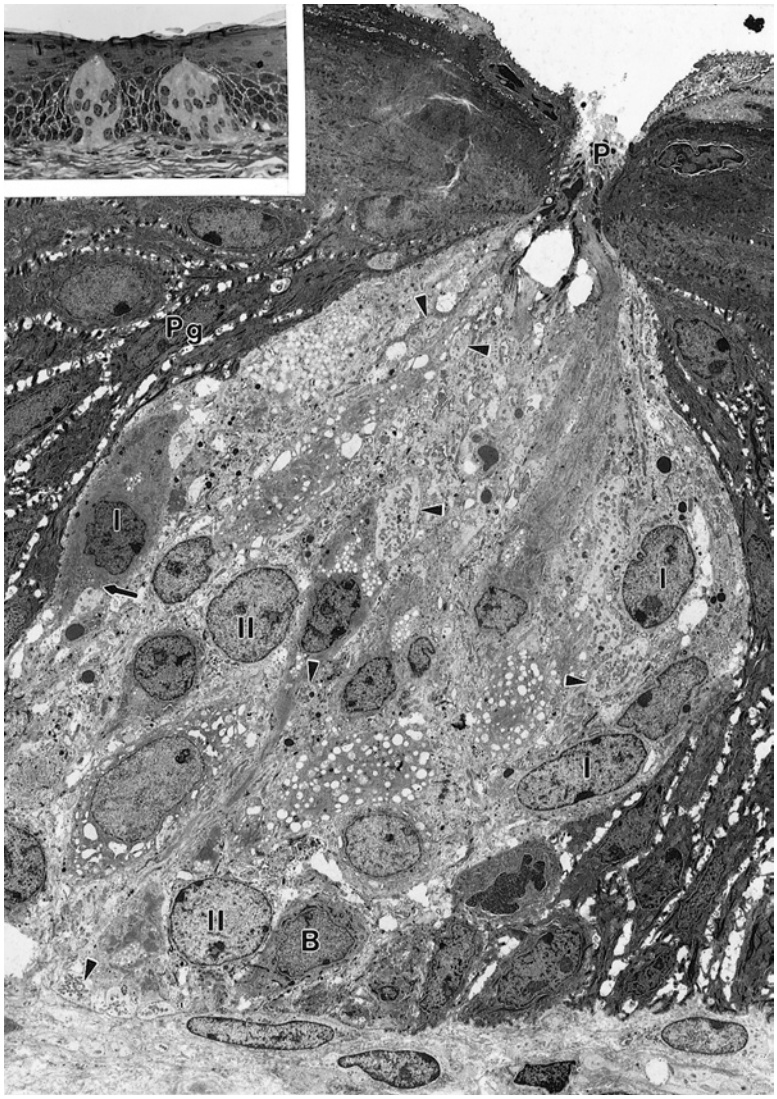


Figure 16–12 Low-power electron micrograph of a taste bud from the lamb epiglottis (×2353). B, Basal cell; I, type I cell; II, type II cell; P, taste pore; Pg, perigemmal cell. *Arrowheads* represent nerve fibers; *arrow* represents synapse-like structure between a type I cell and a nerve fiber. (From Sweazy RD, Edwards CA, Kapp BM. Fine structure of taste buds located on the lamb epiglottis. *Anat Rec*. 1994;238:517-527. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

TABLE 16–2 Properties of Taste Buds

Taste Sensation	Receptor or Protein Expressed	G Protein or Ion Channel
Bitter	T2R38	G protein–linked
Sweet	T1R2-T1R3	G protein–linked
Umami	T1R1-T1R3	G protein–linked (glutamate receptors)
Sour	PKD2L1	Hydrogen ion channels
Salt	?	Sodium ion channels
Fat	CD36	

CD36, cluster of differentiation-36 receptor; PKD2L1, polycystic kidney disease 2-like 1 protein; T2R, T1R, taste receptor gene product.

These five (six, including lipid) taste sensations are only part of what one considers to be the taste of food. The flavor perception also involves olfaction, temperature, as well as the consistency of the food being consumed. The process of complex taste perception is due more to the olfactory apparatus than to the taste buds, as evidenced by the decreased taste ability of people with nasal congestion from colds. Interestingly, visual cues also play a part in taste because when wine connoisseurs were asked to critique a white wine that, unknown to them, was dyed red, they used terminology specific to red wines to describe its characteristics.

PATHOLOGICAL CONSIDERATIONS

See Figures 16–13 and 16–14.

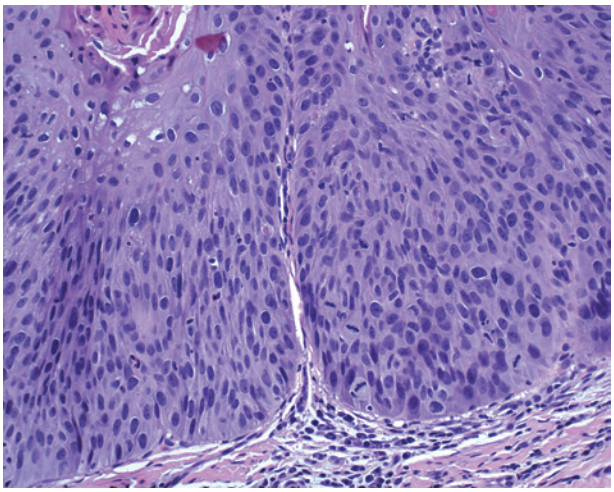


Figure 16–13 Photomicrograph of leukoplakia, a white patch that is premalignant in less than 25% of the cases and cannot be removed by gentle scraping, in the oral cavity. Note that the epithelium presents a severe dysplasia with a large population of immature epithelial cells and a higher than normal incidence of mitotic activity. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran*: *Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:732.)

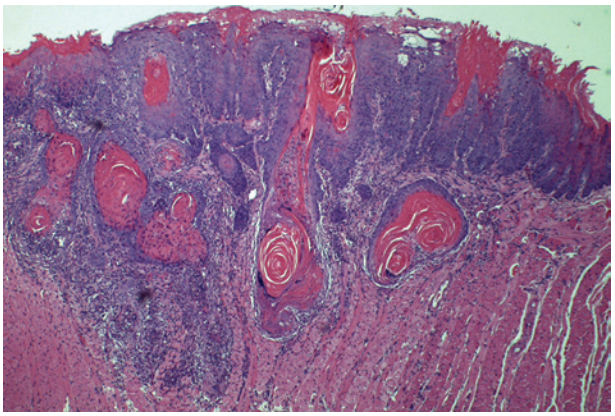
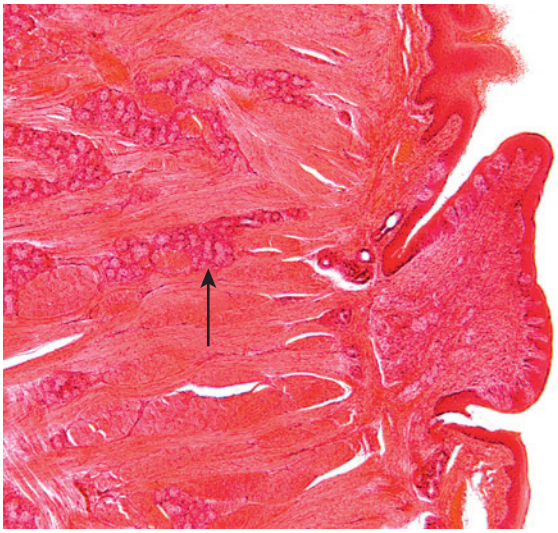
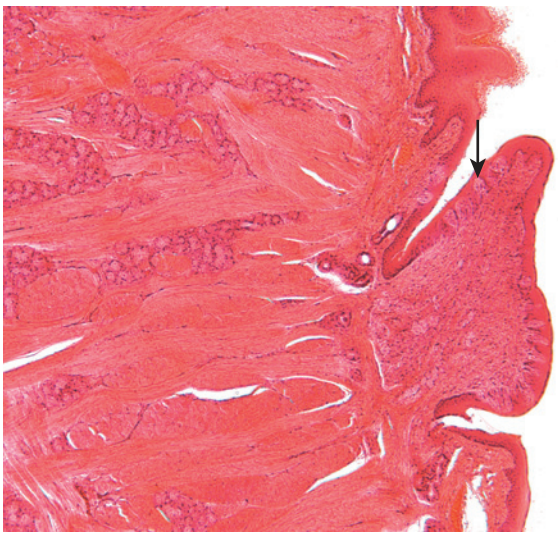


Figure 16–14 Photomicrograph of squamous cell carcinoma in the oral cavity. Observe the islands of keratinocytes that have invaded the subepithelial connective tissue and the skeletal muscle. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran*: *Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:733.)

REVIEW QUESTIONS

1. Which of the following possesses specialized mucosa?
   a. Ventral surface of the tongue
   b. Attached gingiva
   c. Buccal mucosa
   d. Dorsal surface of the tongue
   e. Free gingiva
2. Fordyce granules are nonfunctional
   a. mucous minor salivary glands.
   b. sebaceous glands.
   c. sweat glands.
   d. mixed minor salivary glands.
   e. serous minor salivary glands.
3. Which of the following principal fiber groups insert into the cementum only?
   a. Horizontal fibers
   b. Dentoperiosteal fibers
   c. Interradicular fibers
   d. Transseptal fibers
   e. Circular fibers
4. Which of the following regions of the oral cavity possesses all three types of minor salivary glands (serous only, mucous only, and mixed)?
   a. Cheek
   b. Hard palate
   c. Lip
   d. Tongue
   e. Uvula
5. Which of the following structures initiates cusp formation of a molar tooth?
   a. Enamel navel
   b. Enamel knot
   c. Enamel cord
   d. Enamel tuft
   e. Enamel lamella
6. A patient presents at the dentist with a tooth that has suddenly become discolored and looks dark. The dentist should
   a. extract the tooth.
   b. perform a root canal treatment.
   c. test the pulp for viability.
   d. place a crown on the tooth.
   e. bleach the tooth.
7. Which of the following principal fiber groups contact neither cementum nor bone?
   a. Horizontal fibers
   b. Dentoperiosteal fibers
   c. Interradicular fibers
   d. Transseptal fibers
   e. Circular fibers
8. 

   Identify the gland at the tip of the arrow
   a. Glands of Blandin-Nuhn
   b. Posterior mucous gland
   c. Glands of von Ebner
   d. Glands of Serres
   e. Glands of Littre
9. 

   Identify the structure at the tip of the arrow.
   a. Gland of von Ebner
   b. Fordyce granules
   c. Taste bud
   d. Sustentacular cell
   e. Basal cell

10. An elastic lamina is present in which of the following?
    a. Lip
    b. Soft palate
    c. Cheek
    d. Tongue
    e. Floor of the mouth

11. Which of the following is the hardest tissue in the oral cavity?
    a. Enamel
    b. Dentin
    c. Cementum
    d. Bone
    e. Enamel matrix

ANSWERS

1. D. Specialized mucosa possesses taste buds, structures that are located on the dorsal surface of the tongue. The ventral surface of the tongue, the buccal mucosa, and the gingiva (attached and free) do not possess taste buds.
2. B. Fordyce granules are nonfunctional sebaceous glands located on the inner lining of the cheek and on the lips. These yellowish structures are not salivary glands or sweat glands.
3. D. The transseptal fibers insert into the cementum of adjacent teeth. Horizontal, dentoperiosteal, and interradicular fibers extend between the cementum of the tooth and the bony alveolus (or its periosteum). Circular fibers do not contact either bone or cementum.
4. D. The tongue possesses all three types of minor salivary glands: serous only—glands of von Ebner; mucous only—posterior mucous glands; and mixed—glands of Blandin-Nuhn. The cheek has mixed, the hard palate has mucous only, the lip has mixed, and the uvula possesses only mucous minor salivary glands.
5. B. Cusp formation is initiated by the enamel knot (Ahren knot). If it disappears during the cap stage of tooth development, then an incisiform tooth is formed; if it remains in the bell stage, then a molariform tooth is formed. The enamel navel and enamel cord are transitory structures that have no known function and disappear during the cap stage of tooth development. Enamel tufts are located in the enamel, and they are hypocalcified enamel rods; enamel lamellae are defects in enamel. They are either cracks in enamel or developmental defects due to defective ameloblasts.
6. C. The dentist should test the pulp for viability because a sudden discoloration could be due to hemorrhage of the pulp that may clear up without the need for a root canal treatment. Extraction of a vital tooth is not beneficial for the patient. Placing a crown may not be necessary unless the tooth is not vital. Bleaching the tooth removes surface deposits but not the discoloration that is caused by the situation in the pulp.
7. E. Circular fibers, principal fiber groups of the gingiva, do not insert into the bony alveolus or into cementum; instead, they go around the circumference of the tooth and support the soft tissue as if they were the cord of a duffel bag. Horizontal, dentoperiosteal, and interradicular fibers contact both cementum and bone (or its periosteum), and transseptal fibers contact the cementum of adjacent teeth.
8. C. The gland at the tip of the arrow is the gland of von Ebner, as is evident from its position just deep to the circumvallate papilla. Glands of Blandin-Nuhn are located at the ventral aspect of the tongue. Posterior mucous glands are associated with the lingual tonsils. Glands of Serres are located in the developing oral region of the embryo, and their name is a misnomer, because they are epithelial rests that were the connection between the oral epithelium and the developing enamel organ. Glands of Littre are located in male and female urethras.
9. C. The structure at the tip of the arrow is a taste bud, as is evident from its position on the circumvallate papilla. Glands of von Ebner are located at the root of the circumvallate papilla. Fordyce granules are nonfunctional sebaceous glands located in the inner lining of the cheeks and lips. Sustentacular cells and basal cells are located in the taste bud but cannot be recognized at this magnification.
10. B. Of all of the regions listed, only the soft palate possesses an elastic lamina that separates the lamina propria from the submucosa. In the other regions, instead of a lamina propria and a submucosa, only a subepithelial connective tissue is recognized.
11. A. Of all of the tissues listed, enamel is the hardest because it is composed of 96% calcium hydroxyapatite and only 4% organic matrix. Dentin is 65% calcium hydroxyapatite, cementum is 45% to 50% calcium hydroxyapatite, and bone 65% calcium hydroxyapatite. Enamel matrix is not calcified.

This page intentionally left blank

Digestive System: Alimentary Canal

17

The **alimentary canal**, the continuation of the oral cavity, is the tubular portion of the digestive tract. It is here that food is churned, liquefied, and digested; its nutritional elements and water are absorbed; and its indigestible components are eliminated. The alimentary canal, which is about 9 m long, is subdivided into morphologically recognizable regions: the esophagus, stomach, small intestine (duodenum, jejunum, and ileum), and large intestine (cecum, colon, rectum, anal canal, and appendix). The time that is spent by the ingested food in the various regions of the alimentary canal depends on many factors, including its chemical composition; however, a "standard" meal spends in the

- Esophagus: 5 seconds
- Stomach: 3 to 5 hours
- Small intestine: 6 to 12 hours
- Large intestine: 30 to 40 hours

Before a discussion of the individual regions of the alimentary canal is presented, it is reasonable and preferable to describe the general plan of the digestive tract. Once the conceptual design of the alimentary canal is understood, variations on that common theme are easier to assimilate.

GENERAL PLAN OF THE ALIMENTARY CANAL

The alimentary canal is composed of the following concentric layers: mucosa, submucosa, muscularis externa, and serosa (adventitia).

The alimentary canal is composed of several histological layers, which are schematically illustrated in Figure 17–1. These layers are innervated by the enteric nervous system and modulated by parasympathetic and sympathetic nerves; they are also served by sensory fibers.

Histological Layers

The histology of the alimentary canal often is discussed in terms of four broad layers: the **mucosa**, **submucosa**, **muscularis externa**, and **serosa** (or **adventitia**). These layers are similar throughout the length of the digestive tract but display regional modifications and specializations.

Mucosa

The lumen of the alimentary canal is lined by an **epithelium**, deep to which is a loose connective tissue known as the ***lamina propria***. This richly vascularized connective tissue houses glands as well as lymph vessels and occasional lymphoid nodules, members of the gut-associated lymphoid system (GALT). Certain cells of the lamina propria are responsible for the synthesis and release of growth factors that control the cell cycle of the overlying epithelium. Surrounding this connective tissue coat is the **muscularis mucosae**, usually composed of an inner circular layer and an outer longitudinal layer of smooth muscle. The epithelium, lamina propria, and muscularis mucosae are collectively called the ***mucosa***.

Submucosa

The mucosa is surrounded by a dense, irregular fibroelastic connective tissue layer, the **submucosa** (see Fig. 17–1); this layer houses no glands except in the esophagus and duodenum. The submucosa also contains blood and lymph vessels as well as a component of the **enteric nervous system** known as the ***Meissner submucosal plexus (Meissner plexus)***. This plexus, which also houses postganglionic parasympathetic nerve cell bodies, controls the motility of the mucosa (and, to a limited extent, the motility of the submucosa) and the secretory activities of its glands.

Muscularis Externa

The muscularis externa is usually composed of inner circular and outer longitudinal smooth muscle layers.

The submucosa is invested by a thick muscular layer, the **muscularis externa**, responsible for **peristaltic activity**, which moves the contents of the lumen along the alimentary tract. The muscularis externa is composed of smooth muscle (except in the esophagus) and is usually organized in an inner circular layer and an outer longitudinal layer. Certain smooth muscle–like cells, the **interstitial cells of Cajal**, undergo rhythmic contractions and therefore are considered to be the pacemakers for the contraction of the muscularis externa. A second component of the enteric nervous system, known as the ***Auerbach myenteric plexus (Auerbach plexus)***, is situated between these two muscle layers and regulates the activity of the muscularis externa (and, to a limited extent, the activity of the mucosa). The Auerbach plexus also houses postganglionic parasympathetic nerve cell bodies. (See the following sections on the digestive tract and Chapter 9 for a description of the enteric nervous system.)

SUMMARY

The **alimentary canal**, the hollow tubular region of the digestive system, is a continuation of the oral cavity. It is composed of the **esophagus**, **stomach**, **small intestine (duodenum**, **jejunum**, and **ileum)** and **large intestine (cecum**, **ascending colon**, **transverse colon**, **descending colon**, **sigmoid colon**, **rectum**, **and anal canal)**. The **appendix** is a small diverticulum of the cecum. As the bolus of food leaves the oral cavity, it enters the esophagus, and **peristaltic action** delivers it to the stomach where it is churned, acidified, and liquefied to form **chyme**. Small aliquots of the acidic chyme enter the duodenum, become buffered, and it is here that **digestion** begins in earnest. As the food moves through the remainder of the small intestine, digestion proceeds and peristaltic action continues to move the food into the large intestine, where digestion is completed, fluids are resorbed, and the indigestible components of the food are compacted to be eliminated as **feces**. To accomplish its tasks, the alimentary canal is modified along its length; however, its general histological plan remains the same in that it has the following layers, beginning at the luminal aspect: **mucosa (epithelium**, **lamina propria**, **muscularis mucosae)**, **submucosa**, **muscularis externa**, **serosa/adventitia)**. The lamina propria houses **intramural glands** that aid in digestion. (The submucosa of the esophagus and duodenum also houses intramural glands.)

KEY WORDS

Adventitia; alimentary canal; anal canal; appendix; ascending colon; cecum; chyme; colon; descending colon; digestion; duodenum; epithelium; esophagus; ileum; intramural glands; jejunum; lamina propria; large intestine; mucosa; muscularis externa; muscularis mucosae; peristalsis; rectum; serosa; sigmoid colon; small intestine; stomach; submucosa; transverse colon

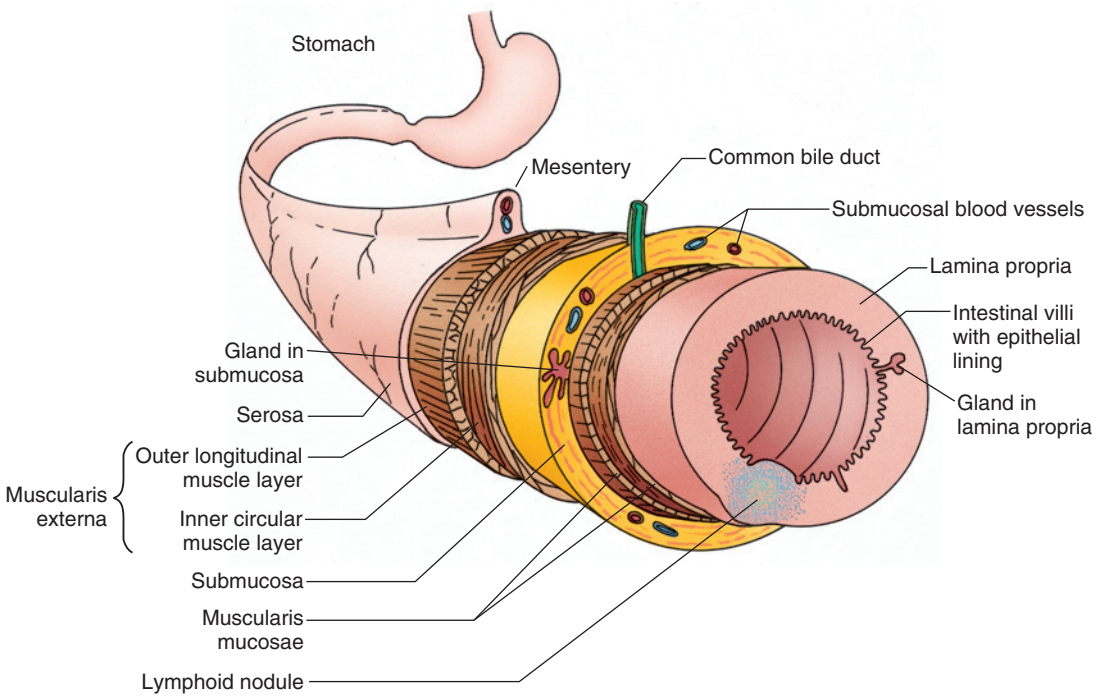


Figure 17–1 Schematic diagram of the alimentary tract displaying its various layers and the generalized contents of each layer.

Three-dimensional reconstruction of the muscularis mucosae and of the muscularis externa shows that both the inner circular layer and the outer longitudinal layer are arranged helically. The pitch of the helices differs, however; the inner circular layer displays a tight helix, whereas the outer longitudinal layer presents a loose helix in both muscular layers.

Serosa or Adventitia

The muscularis externa is enveloped by a thin connective tissue layer that may or may not be surrounded by the simple squamous epithelium of the visceral peritoneum. If the region of the alimentary canal is intraperitoneal, it is invested by peritoneum, and the covering is known as the ***serosa***. If the organ is retroperitoneal, it adheres to the body wall by its dense irregular connective tissue component and is known as the ***adventitia***.

Innervation of the Digestive Tract

The enteric nervous system, innervating the alimentary canal, is modulated by sympathetic and parasympathetic nervous systems.

The innervation of the alimentary canal is composed of two parts: the intrinsic element, the enteric nervous system, and the extrinsic constituent, namely the sympathetic and parasympathetic components. The major controlling factor resides in the enteric nervous system, which is self-sufficient; however, its functions are normally modified by the sympathetic and parasympathetic components. In fact, if the sympathetic and parasympathetic connections to the entire gut are completely severed, the alimentary canal can perform all of its functions without any major problems.

Enteric Nervous System

The enteric nervous system is a self-contained nervous system composed of numerous repeating ganglia known as the Meissner submucosal plexus *and* Auerbach myenteric plexus.

The digestive tract has its own self-contained nervous system (the **enteric nervous system**), which extends the entire length of the alimentary canal from the esophagus to the anus. The enteric nervous system is thus dedicated to controlling the secretory and motile functions of the alimentary canal. The 100 million or so neurons of the enteric nervous system are distributed in a large number of small clusters of nerve cell bodies and associated nerve fibers, in **Auerbach myenteric plexus (Auerbach plexus)** and in **Meissner submucosal plexus (Meissner plexus).** It is interesting that the number of neurons associated with the enteric nervous system approximates, or even exceeds, the total number of neurons contained within the spinal cord, suggesting that the enteric nervous system is an

exceptionally important entity; therefore, it is considered to be the third component of the autonomic nervous system (sympathetic, parasympathetic, and enteric nervous systems).

Although Auerbach and Meissner plexuses have numerous interconnections, they serve different functions. Generally speaking, the peristaltic motility of the digestive tract is under the direction of the myenteric plexus, whereas its secretory function and mucosal movement as well as the regulation of localized blood flow are governed by the submucosal plexus. Moreover, the myenteric plexus is concerned not only with local conditions but also with conditions along much of the digestive tract, whereas the submucosal plexus is attentive primarily to local conditions in the vicinity of the particular cluster of nerve cells in question. As with all generalizations, there are exceptions to these rules; therefore, it must be appreciated that there is a great deal of interaction between the two sets of plexuses, and the possibility of cross-controls has been suggested.

Sensory components have also been described in the wall of the alimentary canal. They convey information concerning the luminal contents, muscular status, and secretory status of the gut to the plexuses in the vicinity of the information as well as to plexuses at considerable distances from the location of the source of the information. In fact, some of the information is transmitted to sensory ganglia as well as to the central nervous system (CNS) by nerve fibers that accompany fibers of the sympathetic and parasympathetic nerve supplies of the gut.

Parasympathetic and Sympathetic Supply to the Gut

Parasympathetic innervation stimulates peristalsis, inhibits sphincter muscles, and triggers secretory activity; sympathetic nerves inhibit peristalsis and activate sphincter muscles.

The digestive tract receives its **parasympathetic nerve supply** from the vagus nerve (cranial nerve X), except for the descending colon and rectum, which are innervated by the **sacral spinal nerves (spinal outflow).** Most of the fibers of the vagus nerve are sensory and deliver information from receptors in the mucosa and muscularis of the alimentary canal to the CNS. Frequently, responses to the information are then conveyed by the vagal fibers to the alimentary canal. The parasympathetic fibers synapse with postganglionic parasympathetic nerve cell bodies as well as with nerve cell bodies of the enteric nervous system in both plexuses. The parasympathetic innervation is responsible for inducing secretions from the glands of the digestive tract as well as for smooth muscle contraction.

The **sympathetic innervation** is derived from the splanchnic nerves. Sympathetic fibers are vasomotor, controlling blood flow to the alimentary canal.

As a generalization, it may be stated that

- parasympathetic innervation stimulates peristalsis, inhibits sphincter muscles, and triggers secretory activity, whereas
- sympathetic innervation inhibits peristalsis and activates sphincter muscles.

The remainder of this chapter discusses the various regions of the alimentary canal and examines how they differ from the general plan.

ESOPHAGUS

The **esophagus** is a muscular tube, approximately 25 cm in length, that conveys the bolus (masticated food) from the oral pharynx to the stomach. Along its entire length, its mucosa presents numerous longitudinal folds with intervening grooves that cause the lumen to appear to be obstructed; however, when the esophagus is distended, the folds disappear, and the lumen becomes patent.

Esophageal Histology

Mucosa

The esophageal mucosa is composed of a stratified squamous epithelium, fibroelastic lamina propria, and a smooth muscle layer that is composed only of the longitudinally disposed muscularis mucosae.

The **mucosa** of the esophagus is composed of three layers: epithelium, lamina propria, and muscularis mucosae (Fig. 17–2).

The lumen of the esophagus, lined by a 0.5-mm-thick, **stratified squamous nonkeratinized epithelium**, presents a well-developed rete apparatus as it interdigitates with the underlying connective tissue. The epithelium is regenerated at a much slower rate than that of the remainder of the gastrointestinal (GI) tract; the newly formed cell in the basal layer of the epithelium reaches the free surface in about 3 weeks after formation. Interspersed within the keratinocytes of the epithelium are **antigen-presenting cells**, known as ***Langerhans cells***, which phagocytose and degrade antigens into small polypeptides known as *epitopes*. These cells also synthesize major histocompatibility complex (MHC) II molecules, attach the epitopes to these molecules, and place the MHC II–epitope complex on the external aspect of their plasmalemmae. Langerhans cells then migrate to neighboring lymph nodes, where they present the MHC II–epitope complex to lymphocytes (see Chapter 12 on the lymphoid [immune] system and Chapter 14 on integument).

The **lamina propria** is unremarkable. It houses **esophageal cardiac glands**, which are located in two regions of the esophagus, one cluster near the pharynx and the other near its juncture with the stomach. It also houses occasional lymphoid nodules, members of the

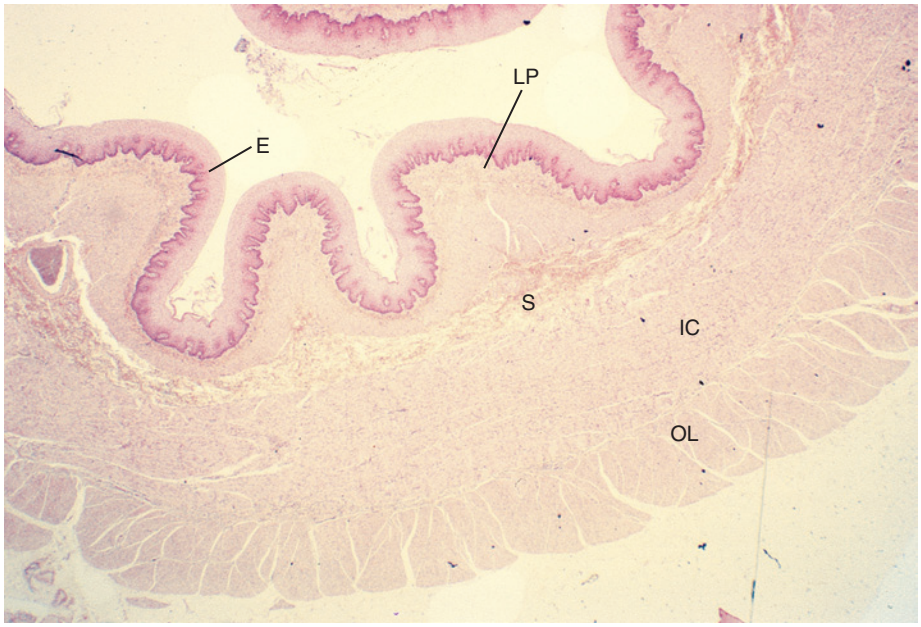


Figure 17–2 Esophagus. Note that its lumen is lined by a relatively thick stratified squamous epithelium (E) that forms a well-developed rete apparatus with the underlying lamina propria (LP). The submucosa (S) is surrounded by a thick muscularis externa composed of an inner circular (IC) and an outer longitudinal (OL) muscle layers (×17).

GALT (gut-associated lymphoid system). The **muscularis mucosae** is unusual in that it consists only of a *single layer* of longitudinally oriented smooth muscle fibers that become thicker in the vicinity of the stomach.

The **esophageal cardiac glands** produce a mucous secretion that coats the lining of the esophagus, lubricating it to protect the epithelium as the bolus is passed into the stomach. Because these glands resemble glands from the cardiac region of the stomach, some authors have suggested that they are ectopic patches of gastric tissue.

Submucosa

The submucosa of the esophagus houses mucous glands known as the esophageal glands proper.

The **submucosa** of the esophagus is composed of a dense, fibroelastic connective tissue, which houses the **esophageal glands proper**. The esophagus and the duodenum are the only two regions of the alimentary canal with glands in the submucosa. Electron micrographs of these tubuloacinar glands indicate that their secretory units are composed of two types of cells, mucous cells and serous cells.

Mucous cells have basally located, flattened nuclei and apical accumulations of mucinogen-filled secretory granules. The second cell type, known as ***serous cells***, possess round, centrally placed nuclei. The secretory granules of these cells contain the proenzyme **pepsinogen** and the antibacterial agent **lysozyme**. The ducts of these glands deliver their secretions into the lumen of the esophagus.

The **submucosal plexus** is in its customary location within the submucosa, in the vicinity of the inner circular layer of the muscularis externa.

Muscularis Externa and Adventitia

The muscularis externa of the esophagus is composed of both skeletal and smooth muscle cells.

The **muscularis externa** of the esophagus is arranged in the customary two layers, inner circular and outer longitudinal. However, these muscle layers are unusual in that they are composed of both skeletal and smooth muscle fibers. The muscularis externa of the upper third of the esophagus has mostly skeletal muscle and is served by the vagus nerve (cranial nerve X), the middle third has both skeletal and smooth muscle, and the lowest third has only smooth muscle fibers and are served by nerve fibers of the enteric nervous system. **Auerbach plexus** occupies its usual position between the inner circular and outer longitudinal smooth muscle layers of the muscularis externa.

The esophagus is covered by an **adventitia** until it pierces the diaphragm, after which it is covered by a **serosa**.

Histophysiology of the Esophagus

The esophagus does not have an anatomical sphincter but does have two physiological sphincters, the pharyngoesophageal sphincter (upper esophageal sphincter) and the internal and external gastroesophageal sphincters (lower esophageal sphincters), which normally prevent reflux into the pharynx from the esophagus and into the esophagus from the stomach, respectively. The internal gastroesophageal sphincter, composed of smooth muscle fibers, is located at the region where the esophagus pierces the diaphragm and joins the stomach. The muscle fibers of this sphincter are always in tonus except when a bolus is about to pass into the stomach or if the individual is vomiting. Additionally, skeletal muscle fibers from the diaphragm encircle the esophagus and close it during inspiration and during elevation of the intraabdominal pressure (as during defecation). A bolus entering the esophagus is conveyed, via peristaltic action of the muscularis externa, into the stomach at a rate of about 50 mm/sec.

STOMACH

The stomach is responsible for the formation and processing of the ingested food into an thick acidic fluid known as chyme.

The **stomach**, the most dilated region of the alimentary canal, is a sac-like structure that, in the resting state in the average adult, has a volume of only 50 mL; however, it can accommodate approximately 1500 mL of food and gastric juices at maximal distention. As the stomach expands, its intraluminal pressure remains relatively constant due to

- the hormone **ghrelin**, released by the diffuse neuroendocrine system (DNES) cells, known as *ghrelin cells*, not only induces the sensation of hunger but also modulates receptive relaxation of the smooth muscle fibers of the muscularis externa
- the **vagovagal reflex**, where the vagus nerve provides feedback information to the muscularis externa of the stomach to keep its relaxed condition. The vagus nerve also prompts three additional effects, namely, the stimulation of **hydrochloric acid (HCl)** production by parietal cells, histamine release by enterochromaffin-like (ECL) cells, and inhibition of delta cells whose function in to hinder the release of gastrin by G cells (see later section on HCl production).

The bolus passes from the esophagus through the gastroesophageal junction into the stomach, where it is churned into a viscous fluid known as ***chyme***. Intermittently, the stomach releases small aliquots of its contents through the **pyloric valve** into the duodenum. The stomach liquefies the food, continuing its digestion via the production of HCl and the enzymes **pepsin**, **rennin**, and **gastric lipase** and via production of paracrine hormones.

Anatomically, the stomach has a concave lesser curvature and a convex greater curvature. Gross observations disclose that the stomach has four regions:

CLINICAL CORRELATIONS

1. As the esophagus passes through the diaphragm, it is reinforced by fibers of that muscular structure. In some people, development is abnormal, causing a gap in the diaphragm around the wall of the esophagus that permits herniation of the stomach into the thoracic cage. This condition, known as ***hiatal hernia***, weakens the gastroesophageal sphincter, allowing reflux of the stomach contents into the esophagus.
2. Weakened or malfunctioning gastroesophageal sphincters permit the return of stomach content into the esophagus, a condition known as ***gastroesophageal reflux disease (GERD)***. Because of the acidic content of the stomach enters the lumen of the esophagus, it usually creates a burning feeling (heartburn) in the midsternum area of the chest occasionally accompanied by regurgitation. GERD affects approximately 15% to 20% of the population in the "developed" world. In most cases GERD can be treated by alteration of the patient's lifestyle, such as weight loss; restrained exercise; elevating of the head at night; and elimination of acidic foods, spicy foods, fatty foods, coffee, alcohol, and other foods that the patient notices aggravate the condition. If lifestyle changes do not control the condition then medications, such as **proton pump inhibitors (PPIs)**, and even surgery may be required. Interestingly, it has been reported that patients taking PPIs have a greater incidence of vitamin B_{12} deficiency than those who are not taking those drugs.
3. **Barrett syndrome** is a probably a premalignant condition initially caused by GERD. Part of the stratified squamous nonkeratinized epithelium of the esophagus, usually in the lowest region, is replaced by a simple columnar epithelium that resembles the lining of the stomach. Endoscopically, this metaplastic area is reddish in color, and at least 3 cm of the esophagus must be involved. If there are numerous red patches in the lower esophagus, esophageal resection may be necessary.

- **Cardia**: a narrow region at the gastroesophageal junction, 2 to 3 cm wide
- **Fundus**: a dome-shaped region to the left of the esophagus, frequently filled with gas
- **Body (corpus)**: the largest portion, responsible for the formation of chyme
- **Pylorus (pyloric antrum)**: a funnel-shaped, constricted portion equipped with a thick **pyloric sphincter** that controls the intermittent release of chyme into the duodenum

Histologically, the fundus and body are identical. All the gastric regions display **rugae**, longitudinal folds of the mucosa and submucosa (but transverse in the pyloric antrum), which disappear in the distended stomach. Rugae permit expansion of the stomach as it fills with food and gastric juices. Additionally, the epithelial lining of the stomach invaginates into the mucosa, forming **gastric pits (foveolae)**, which are shallowest in the cardiac region and deepest in the pyloric region. Gastric pits increase the surface area of the gastric lining. Five to seven **gastric glands** of the lamina propria empty into the bottom of each gastric pit.

Gastric Histology

The ensuing discussion of the stomach details the fundic region because the microscopic anatomy of each of the remaining regions is a variation of that of the fundic region. Figure 17–3 schematically depicts the major histological elements of the fundic region.

Fundic Mucosa

The **mucosa** of the fundic stomach is composed of the usual three components: (1) an epithelium lining the lumen; (2) an underlying connective tissue, the lamina propria; and (3) the smooth muscle layers forming the muscularis mucosae.

Epithelium

The epithelial lining of the stomach secretes a visible mucus that adheres to and protects the stomach lining.

The lumen of the fundic stomach is lined by a simple columnar epithelium composed of **surface-lining cells**, which manufacture a thick mucus layer, known as ***visible mucus*** (Fig. 17–4A), a gel-like substance that adheres to the lining of the stomach and protects it from autodigestion. Moreover, bicarbonate ions trapped in this mucus layer are able to maintain a relatively neutral pH at its interface with the cell membranes of the surface-lining cells despite the low (acidic) pH of the luminal contents. Surface-lining cells continue into the gastric pits, forming their epithelial lining. **Regenerative cells** are also present in the base of these pits, but because they are more numerous in the neck of the gastric glands, they are discussed along with the glands. A small number of **taste cells** that recognize sweet, bitter, and umami taste sensations are also present in the fundic epithelium (see the later section on the small intestine and Chapter 16, the section on taste buds).

Electron micrographs of surface-lining cells display glycocalyx-covered, short, stubby microvilli on their apical surfaces. Their apical cytoplasm houses secretory granules containing a homogeneous substance, the precursor of the visible mucus (Fig. 17–5). The lateral cell membranes of these surface-lining cells form intricate zonulae occludentes and zonulae adherentes with those of neighboring cells. The cytoplasm between their basally placed nuclei and apical secretory granules is occupied chiefly by mitochondria and the protein synthesis and packaging apparatus of the cell.

Lamina Propria

The loose, highly vascularized connective tissue, the lamina propria, has a rich population of plasma cells, lymphocytes, mast cells, fibroblasts, and occasional smooth muscle cells. Much of the lamina propria is occupied by the approximately 15 million closely packed gastric glands, known as ***fundic (oxyntic) glands*** in the fundic region (see Fig. 17–4B). Additionally, components of the GALT (gut-associated lymphoid tissue) are also present.

Fundic Glands

Each **fundic gland** extends from the muscularis mucosae to the base of the gastric pit and is subdivided into three regions: the (1) isthmus, (2) neck, and (3) base, of which the base is the longest (see Fig. 17–3). The simple columnar epithelium constituting the fundic gland is composed of six cell types: surface-lining cells, parietal (oxyntic) cells, regenerative (stem) cells, mucous neck cells, chief (zymogenic) cells, and DNES cells (previously known as *amine precursor uptake* and *decarboxylation* [APUD] cells, enteroendocrine, argentaffine, and argyrophilic cells). The distribution of these cells within the three regions of the gland is presented in Table 17–1.

The **surface-lining cells** in the isthmus region were described previously. The structure and function of the other five cell types are discussed in the following sections.

TABLE 17–1 Distribution of Cell Types in Fundic Glands

Region	Cell Types
Isthmus	Surface-lining cells and few DNES cells
Neck	Mucous neck cells, regenerative cells, parietal cells, and few DNES cells
Base	Chief cells, occasional parietal cells, and few DNES cells

DNES, Diffuse neuroendocrine system.

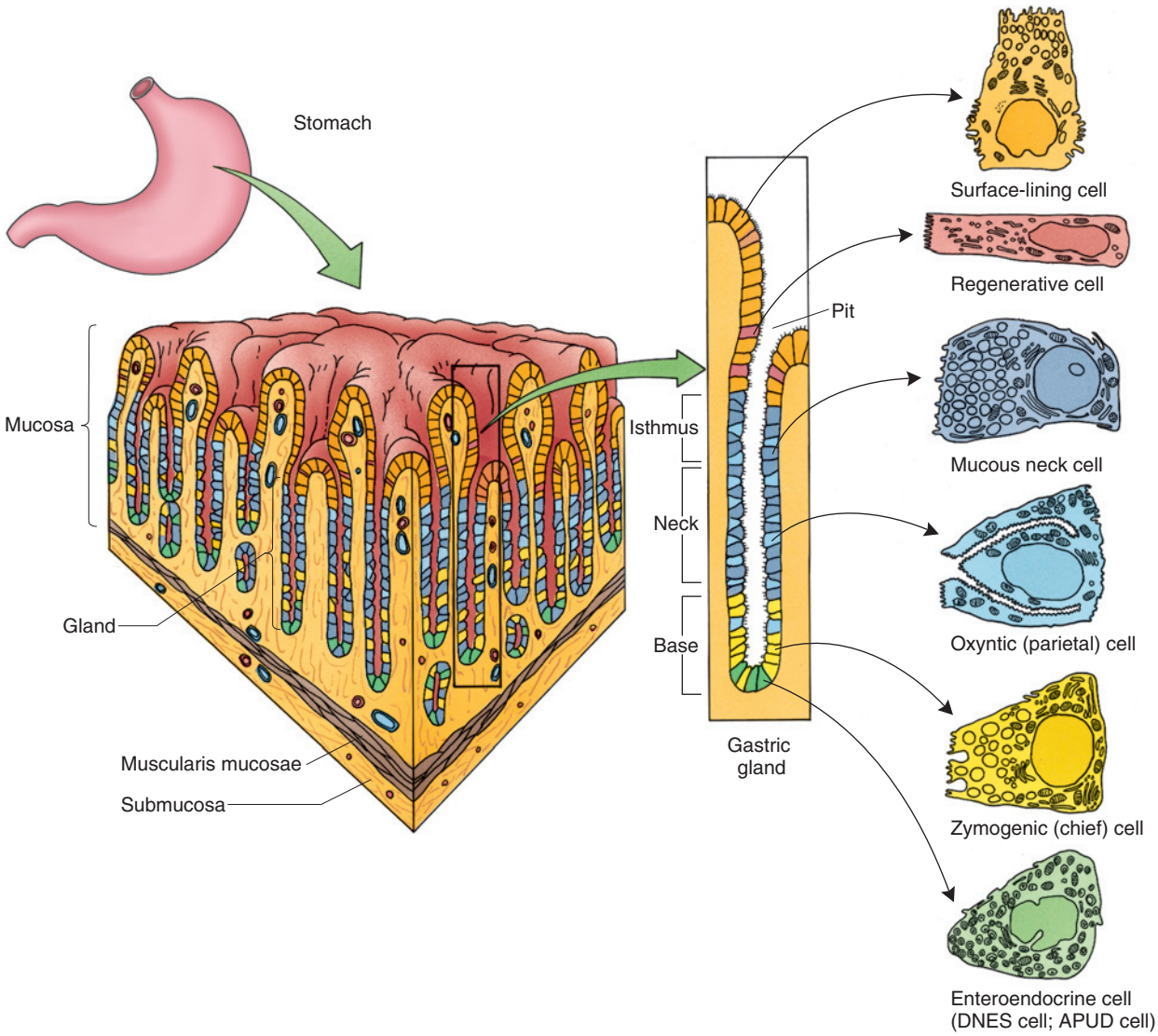


Figure 17–3 Schematic diagram of the fundic stomach and fundic gland and their cellular composition. The fundic glands open into the bottom of the gastric pits, and each gland is subdivided into an isthmus, a neck, and a base.

Mucous Neck Cells

Mucous neck cells produce a soluble mucus that is mixed with and lubricates the chyme, reducing friction as it moves along the digestive tract.

Mucous neck cells are columnar and resemble surface-lining cells, but they are distorted by pressures from neighboring cells. Thus, they also have short microvilli, basally located nuclei, and well-developed Golgi apparatus and rough endoplasmic reticulum (RER) (Fig. 17–6). Their mitochondria are located mainly in the basal region of the cell. The apical cytoplasm is filled with secretory granules containing a homogeneous secretory product, known as ***soluble mucus***, which differs from the visible mucus synthesized by surface-lining cells; as its name suggests, this mucus is soluble and functions to lubricate the lining of the stomach, thereby reducing frictional forces as the gastric contents is being churned. The lateral membranes of mucous neck cells form zonulae occludentes and zonulae adherentes with the adjacent cells.

Regenerative (Stem) Cells

A relatively few, thin **regenerative cells** are interspersed among the mucous neck cells of fundic glands (see Fig. 17–3). These columnar stem cells do not have

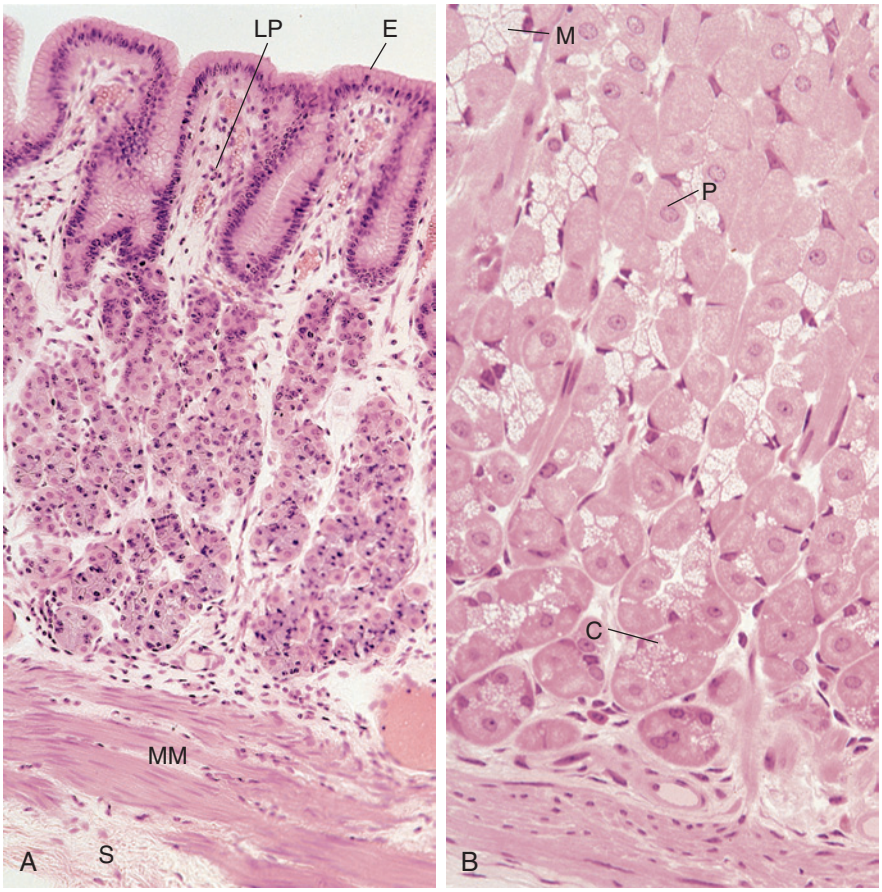


Figure 17–4 **A,** Photomicrograph of the mucosa of the fundic stomach. The mucosa is composed of the simple columnar epithelium (E), the connective tissue lamina propria (LP), and the muscularis mucosae (MM). A little section of the submucosa (S) is evident at the bottom left-hand corner of the photomicrograph (×132). **B,** Photomicrograph of fundic glands. Note that the glands are very tightly packed, and much of the connective tissue is compressed into thin wafers occupied by capillaries (×270). C, Chief cell; M, mucous neck cell; P, parietal cell.

many organelles but do have a rich supply of ribosomes. Their nuclei are basally located, have little heterochromatin, and display a large nucleolus. The lateral cell membranes of these cells also form zonulae occludentes and zonulae adherentes with those of adjacent cells.

Regenerative cells proliferate to replace all of the specialized cells lining the fundic glands, gastric pits, and luminal surface. Newly formed cells migrate to their new locations either deep into the gland or up into the gastric pit and gastric lining. Surface-lining cells, DNES cells, and mucous neck cells are replaced every 5 to 7 days; thus, regenerative cells have a high proliferative rate.

Parietal (Oxyntic) Cells

Parietal cells manufacture hydrochloric acid and gastric intrinsic factor; both products are released into the lumen of the stomach.

Large, round to pyramid-shaped **parietal cells** are located mainly in the upper half of the fundic glands and only occasionally in the base (see Figs. 17–3 and 17–4). They are about 20 to 25 µm in diameter and are situated at the periphery of the gland. These cells produce **HCl** and **gastric intrinsic factor**.

CLINICAL CORRELATIONS

Gastric intrinsic factor, a glycoprotein secreted into the lumen of the stomach, is necessary for vitamin B_{12} absorption from the ileum. Absence of this factor results in deficiency of vitamin B_{12} with the consequent development of **pernicious anemia**. Because the liver stores high quantities of vitamin B_{12}, a deficiency of this vitamin may take several months to develop after production of gastric intrinsic factor ceases.

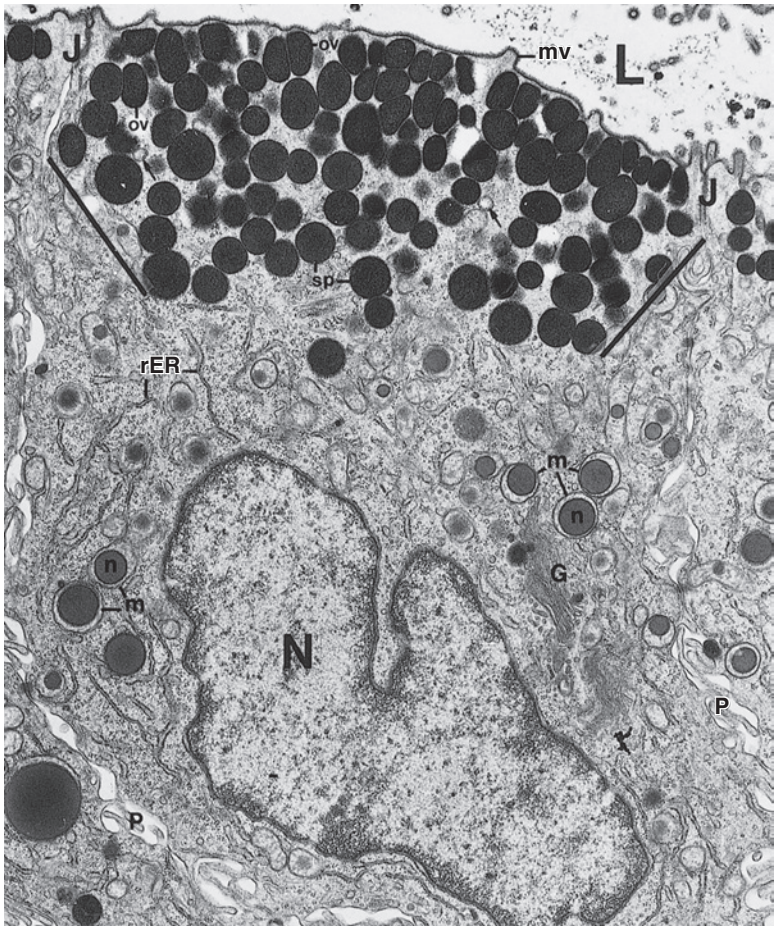


Figure 17–5 Electron micrograph of a surface-lining cell from the body of a mouse stomach (×11632). G, Golgi apparatus; J, junctional complex; L, lumen; m, mitochondria exhibiting large spherical densities known as *nodules* (n); mv, microvillus; N, nucleus; ov, oval secretory granules; P, intercellular projections; rER, rough endoplasmic reticulum; sp, spherical granules. (From Karam SF, Leblond CP. Identifying and counting epithelial cell types in the "corpus" of the mouse stomach. *Anat Rec*. 1992;232:231-246. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Parietal cells have round, basally located nuclei, and their cytoplasm is eosinophilic. Their most remarkable characteristic is the invaginations of their apical plasmalemma to form deep **intracellular canaliculi** lined by microvilli (Figs. 17–7 and 17–8). The cytoplasm bordering these canaliculi is richly endowed by round and tubular vesicles, the **tubulovesicular system**. Additionally, the cell is rich in mitochondria, whose combined volume constitutes almost half that of the cytoplasm. The protein synthetic apparatus, the RER and the Golgi, are present but only to a limited extent.

The number of microvilli and the abundance of vesicles of the tubulovesicular system are indirectly related to each other and vary with the HCl secretory activity of the cell. During active HCl production, the number of microvilli increases, and the tubulovesicular system decreases. Thus, the membrane, being stored as tubules and vesicles, is probably used for microvillar assembly, increasing the surface area of the cell by four to five times in preparation for HCl production.

The process of microvillus formation requires energy and involves polymerization of soluble forms of actin and myosin into filaments, which then interact to transport membranes from the tubulovesicular system to that of the intracellular canaliculus. The stored membranes have a high content of **H^+**, **K^+-ATPase** (a protein that pumps protons from the cytoplasm into the intracellular canaliculus). The formation of HCl is detailed below.

Chief (Zymogenic) Cells

Chief cells manufacture the enzymes pepsinogen, rennin, and gastric lipase and release them into the lumen of the stomach.

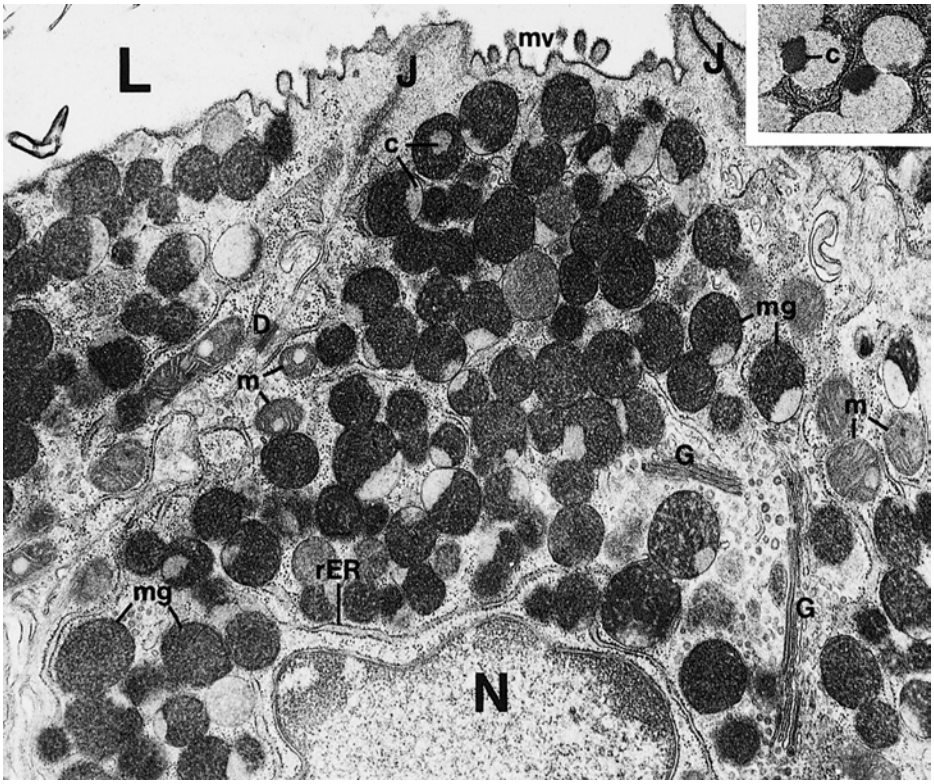


Figure 17–6 Electron micrograph of a mucous neck cell from the body of a mouse stomach. *Inset*: Secretory granule. c, Dense-cored granule; D, desmosome; G, Golgi apparatus; J, junctional complex; L, lumen; m, mitochondria; mg, mucous granules; mv, microvillus; N, nucleus; rER, rough endoplasmic reticulum. (From Karam SF, Leblond CP. Identifying and counting epithelial cell types in the "corpus" of the mouse stomach. *Anat Rec*. 1992;232:231-246. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Most of the cells in the base of fundic glands are **chief cells** (see Figs. 17–3 and 17–4). These columnar cells display a basophilic cytoplasm, basally located nuclei, and apically situated secretory granules that house the proenzyme **pepsinogen** (as well as rennin and gastric lipase). Electron micrographs of chief cells exhibit a rich supply of RER, an extensive Golgi apparatus, and numerous apical secretory granules interspersed with a few lysosomes (Fig. 17–9). Short, blunt, glycocalyx-covered microvilli project from the apical aspect of the cell into the lumen of the gland.

Exocytosis of pepsinogen from chief cells is induced by both neural and hormonal stimulation. Neural stimulation by the vagus nerve is the main contributor to pepsinogen release. Binding of the hormone **secretin** to receptors in the basal plasma membrane of chief cells triggers a second messenger system that also leads to exocytosis of pepsinogen.

Chief cells also manufacture **leptin**, a hormone produced predominantly by adipocytes, that acts on the arcuate nucleus of the hypothalamus to inhibit the sensation of hunger; thus, it is antagonistic to the hormone ghrelin.

DNES Cells (APUD or Enteroendocrine Cells)

DNES cells may be open or closed; they manufacture endocrine, paracrine, and neurocrine hormones.

A group of small cells that are individually dispersed among the other epithelial cells of the gastric mucosa are known collectively by several names:

- *Argentaffin* and *argyrophilic cells* because they stain with silver stains
- *APUD cells* because some of them can take up the precursors of amines and decarboxylate them
- *DNES cells* because they are members of the diffuse neuroendocrine system of cells
- *Enteroendocrine cells* because they secrete hormone-like substances and are located in the epithelium of the enteric (alimentary) canal

Some of these cells are individually designated according to the substance that they produce.

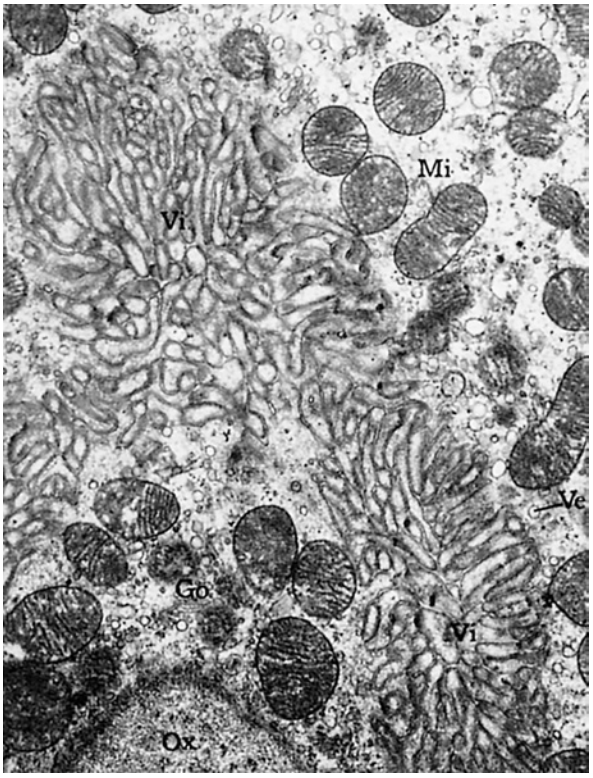


Figure 17–7 Electron micrograph of a parietal cell from the body of a mouse stomach (×14000). Go, Golgi apparatus; Mi, mitochondria; Ox, nucleus of oxyphil cell; Ve, tubulovesicular apparatus; Vi, microvilli. (From Rhodin JAG. *An Atlas of Ultrastructure*. Philadelphia: WB Saunders; 1963.)

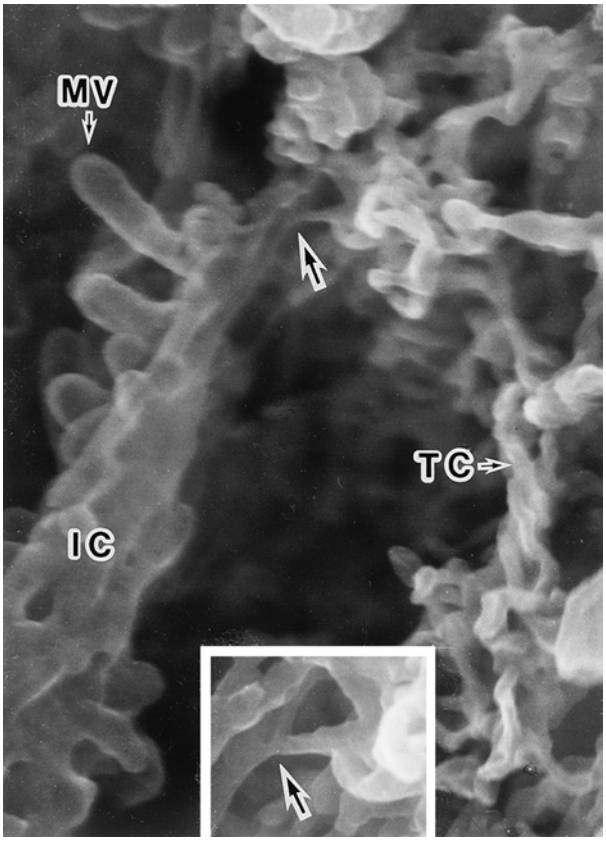


Figure 17–8 Scanning electron microscopy of the fractured surface of a resting parietal cell. The cytoplasmic matrix is removed by the aldehyde-osmium-DMSO-osmium method (or A-ODO method), exposing the cytoplasmic membranes. The tubulovesicular network (TC) is connected to the intracellular canaliculus (IC) lined with microvilli (MV) *(arrow)* (×50000). *Inset*: A higher magnification of the area indicated by the *arrow* in panel (×100000). (From Ogata T, Yamasaki Y. Scanning EM of the resting gastric parietal cells reveals a network of cytoplasmic tubules and cisternae connected to the intracellular canaliculus. *Anat Rec*. 2000;258:15-24. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Generally, a single type of DNES cell secretes only one hormone, although occasional cell types may secrete two different hormones. There are at least 13 different DNES cell types, only some of which are located in the mucosa of the stomach. Table 17–2 lists most of the better-known cells; their locations, granule size, and the substance being secreted; and the action of the released substances. Cells of the DNES have been localized not only in the digestive tract but also in the respiratory system and in the endocrine pancreas. Additionally, some of the secretory products synthesized and released by these DNES cells are identical with neurosecretions localized in the CNS. The significance of their diverse location and the substances they produce is only incompletely understood.

Electron micrographs of DNES cells reveal that these small cells, which sit on the basal lamina, are of two types: those that reach the lumen of the gut (the **open type**) and those that do not (the **closed type**). The open type DNES cells reach the lumen via long, thin apical processes with microvilli, which may serve to monitor the contents of the gastric lumen. The closed type DNES cells do not possess processes and, therefore, have no access to the gastric lumen. The cytoplasm of DNES cells has a well-developed RER and Golgi apparatus and numerous mitochondria. Additionally, small secretory granules are evident, disposed **basally** in most cells (Fig. 17–10).

All DNES cells release the contents of their granules basally into the lamina propria. The hormones that these cells release either travel short distances in the interstitial tissue to act on target cells in the immediate vicinity of the signaling cell (paracrine effect) or enter the circulation and travel a distance to reach their target cell (endocrine effect). Furthermore, the substance released may be identical with neurosecretions. Because of these three possibilities, some researchers have used

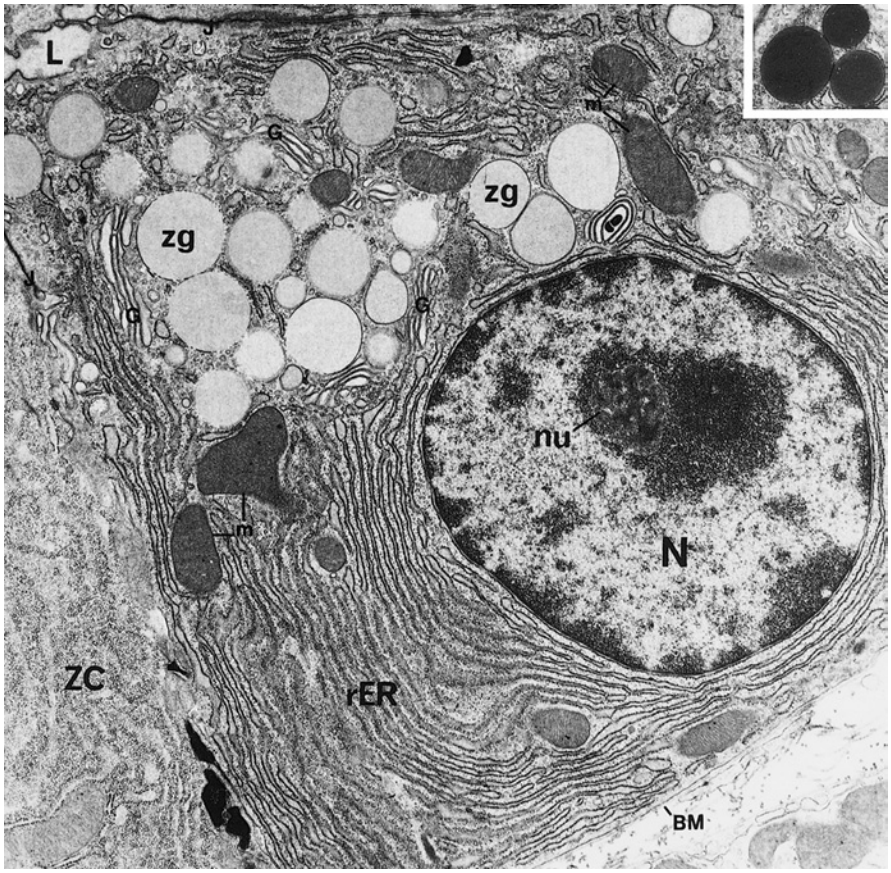


Figure 17–9 Electron micrograph of a chief cell from the fundus of a mouse stomach (×11837). BM, Basement membrane; G, Golgi apparatus; L, lumen; m, mitochondria; N, nucleus; nu, nucleolus; rER, rough endoplasmic reticulum; ZC, zymogenic (chief) cell; zg, zymogen granules. (From Karam SF, Leblond CP. Identifying and counting epithelial cell types in the "corpus" of the mouse stomach. *Anat Rec*. 1992;232:231-246. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

the terms ***endocrine***, ***paracrine***, and ***neurocrine*** to differentiate among the three variations of the secreted substances.

Muscularis Mucosae of the Stomach

The smooth muscle cells that compose the **muscularis mucosae** are arranged in three layers. The inner circular and outer longitudinal layers are well defined; however, an occasional third layer, whose fibers are disposed circularly **(outermost circular)**, is not always evident.

Differences in the Mucosa of the Cardiac and Pyloric Regions

The mucosa of the **cardiac region** of the stomach differs from that of the fundic region, in that the gastric pits are shallower, and the base of its glands is highly coiled. The cell population of these cardiac glands is composed mostly of surface-lining cells, some mucous neck cells, a few DNES cells and parietal cells, and *no chief cells* (Table 17–3).

The glands of the **pyloric region** contain the same cell types as those in the cardiac region, but the predominant cell type in the pylorus is the mucous neck cell. In addition to producing soluble mucus, these cells secrete **lysozyme**, a bactericidal enzyme. Pyloric glands are highly convoluted and tend to branch. Additionally, the gastric pits of the pyloric region are deeper than in either the cardiac and pyloric regions, extending approximately halfway down into the lamina propria (Fig. 17–11; see Table 17–3).

Submucosa of the Stomach

The dense, irregular collagenous connective tissue of the gastric **submucosa** has a rich vascular and lymphatic network that supplies and drains the vessels of

TABLE 17–2 Diffuse Neuroendocrine System Cells and Hormones of the Gastrointestinal Tract

Cell	Location	Hormone Produced	Granule Size (nm)	Hormonal Action
A	Stomach and small intestine	Glucagon (enteroglucagon)	250	Stimulates glycogenolysis by hepatocytes, thus elevating blood glucose levels
D	Stomach, small and large intestines	Somatostatin	350	Inhibits release of hormones by DNES cells in its vicinity
EC	Stomach, small and large intestines	Serotonin Substance P	300	Increases peristaltic movement
ECL	Stomach	Histamine	450	Stimulates HCl secretion
G	Stomach and small intestine	Gastrin	300	Stimulates HCl secretion, gastric motility (especially contraction of the pyloric region and relaxation of pyloric sphincter to regulate stomach emptying), and proliferation of regenerative cells in the body of the stomach
GL	Stomach, small and large intestines	Glicentin	400	Stimulates hepatocyte glycogenolysis, thus elevating blood glucose levels
Gr (P/D1 cell)	Stomach and small intestine as well as in Gr cells of the Islands of Langerhans in the pancreas (secreted mostly by adipocytes)	Ghrelin *and also* Leptin	?	Ghrelin induces the sensation of hunger and modulates receptive relaxation of the smooth muscle fibers of the muscularis externa Leptin inhibits hunger sensation
I	Small intestine	Cholecystokinin	250	Stimulates the release of pancreatic enzymes and contraction of the gallbladder; also reduces food intake and counteracts the effects of gastrin
K	Small intestine	Gastric inhibitory peptide (GIP)	350	Inhibits HCl secretion
L	Small and large intestines	PYY GLP-1 GLP-2 OXM	?	Reduces appetite, decreases gastric motility; boosts function of the colon Stimulates insulin and inhibits glucagon secretion; reduces appetite Encourages mitotic activity in the crypts of Lieberkuhn Reduces appetite and increases energy use
Mo	Small intestine	Motilin		Increases intestinal peristalsis
N	Small intestine	Neurotensin	300	Increases blood flow to ileum and decreases peristaltic action of small and large intestines
PP (F)	Stomach and large intestine (mostly by the Islets of Langerhans)	Pancreatic polypeptide	180	Reduces appetite
S	Small intestine	Secretin	200	Stimulates release of bicarbonate-rich fluid from pancreas
VIP	Stomach, small and large intestines	Vasoactive intestinal peptide		Increases peristaltic action of small and large intestines and stimulates elimination of water and ions by the GI tract

DNES, Diffuse neuroendocrine system; EC, enterochromaffin cell; ECL, enterochromaffin-like cell; G, gastrin-producing cell; GI, gastrointestinal; GL, glicentin-producing cell; GLP-1, glucagon-like peptide; Gr, ghrelin-producing cells; HCl, hydrochloric acid; MO, motilin-producing cell; N, neurotensin-producing cell; OXM, oxyntomodulin; PP, pancreatic polypeptide–producing cell; PYY, peptide YY; VIP, vasoactive intestinal peptide–producing cell.

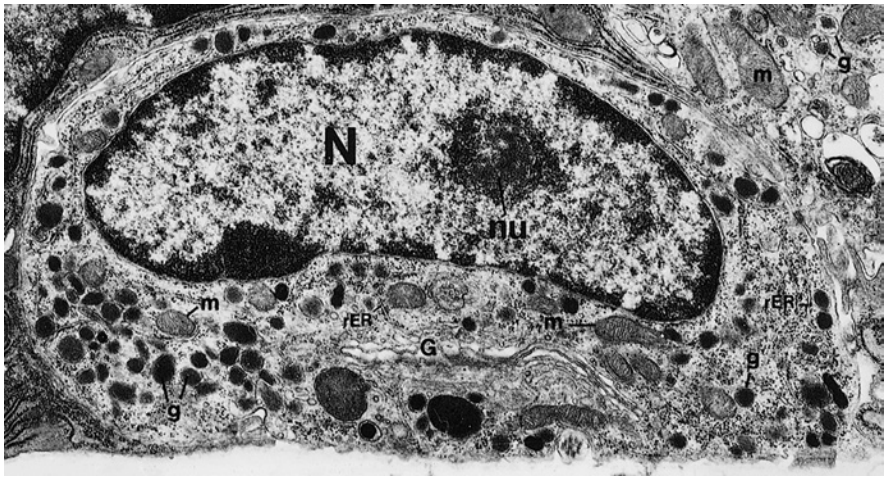


Figure 17–10 Electron micrograph of a diffuse neuroendocrine system cell from the body of a mouse stomach. g, Secretory granules; G, Golgi apparatus; N, nucleus; nu, nucleolus; m, mitochondria; rER, rough endoplasmic reticulum. (From Karam SF, Leblond CP. Identifying and counting epithelial cell types in the "corpus" of the mouse stomach. *Anat Rec*. 1992;232:231-246. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Figure 17–11 Photomicrograph of the pyloric stomach. The gastric pits are much deeper here than in the cardiac or fundic regions of the stomach (×132). P, Gastric pits; LP, lamina propria; MM, muscularis mucosae.

the lamina propria. The cell population of the submucosa resembles that of any connective tissue proper. **Meissner submucosal plexus** is in its accustomed location, within the submucosa in the vicinity of the muscularis externa.

CLINICAL CORRELATIONS

Infrequently, one of the arteries that serves the lesser curvature of the stomach, instead of arborizing as it enters the submucosa into capillaries 0.1 to 0.5 mm in diameter, remains as an arteriole between 1 and 5 mm in diameter. The pulsatility of this aberrant arteriole may slowly erode the submucosa and the vessel approaches the epithelial lining of the gastric mucosa This originally developmental defect may have a very serious complication, known as the ***Dieulafoy lesion***, characterized by erosion of the arterial wall and bleeding into the lamina propria and eventually into the lumen of the stomach. The presenting symptoms are **hematemesis** (vomiting of blood) and **melena** (black feces). If the condition is not diagnosed early and if the offending arteriole is large enough, the patient may die from sudden blood loss.

Muscularis Externa of the Stomach

The muscularis externa of the stomach is composed of three layers of smooth muscle: innermost oblique layer, middle circular layer, and outer longitudinal layer.

The smooth muscle cells of the gastric **muscularis externa** are arranged in three layers. The **innermost oblique layer** is not well defined, except in the cardiac region. The **middle circular layer** is clearly evident along the entire stomach and is especially pronounced in the pyloric region, where it forms the **pyloric sphincter**. The **outer longitudinal muscle layer** is most evident in the cardiac region and the body of the

TABLE 17–3 Histology of the Alimentary Canal

Organ	Epithelium	Cell Type of Epithelium	Lamina Propria	Cells of Glands	Muscularis Mucosae	Submucosa	Muscularis Externa	Serosa or Adventitia
Esophagus	Stratified squamous nonkeratinized		Esophageal cardiac glands	Mucus secreting	Longitudinal layer only	Esophageal glands proper	Inner circular and outer longitudinal	Adventitia (except serosa in abdominal cavity)
Cardiac stomach	Simple columnar	Surface-lining cells (no goblet cells)	Cardiac glands; shallow gastric pits	Surface-lining mucous neck cells, regenerative cells, DNES cells, parietal cells	Inner circular, outer longitudinal and, in places, outermost circular	No glands	Inner oblique, middle circular, outermost longitudinal	Serosa
Fundic stomach	Simple columnar	Surface-lining cells (no goblet cells)	Fundic glands	Surface-lining cells, mucous neck cells, parietal cells, regenerative cells, chief cells, DNES cells	Inner circular, outer longitudinal and, in places, outermost circular	No glands	Inner oblique, middle circular, outermost longitudinal	Serosa
Pyloric stomach	Simple columnar	Surface-lining cells (no goblet cells)	Pyloric glands; deep gastric pits	Mucous neck cells, surface-lining cells, parietal cells, regenerative cells, DNES cells	Inner circular, outer longitudinal and, in places, outermost circular	No glands	Inner oblique, middle circular (well developed to form pyloric sphincter), outermost longitudinal	Serosa
Duodenum	Simple columnar (goblet cells)	Surface absorptive cells, goblet cells, DNES cells, occasional M cells	Crypts of Lieberkühn	Surface absorptive cells, goblet cells, regenerative cells, DNES cells, Paneth cells	Inner circular, outer longitudinal	Brunner glands	Inner circular, outer longitudinal	Serosa and adventitia

Continued

TABLE 17–3 Histology of the Alimentary Canal—cont'd

Organ	Epithelium	Cell Type of Epithelium	Lamina Propria	Cells of Glands	Muscularis Mucosae	Submucosa	Muscularis Externa	Serosa or Adventitia
Jejunum	Simple columnar (goblet cells)	Surface absorptive cells, goblet cells, DNES cells, occasional M cells	Crypts of Lieberkühn	Surface absorptive cells, goblet cells, regenerative cells, DNES cells, Paneth cells	Inner circular, outer longitudinal	No glands	Inner circular, outer longitudinal	Serosa
Ileum	Simple columnar (goblet cells)	Surface absorptive cells, goblet cells, DNES cells, occasional M cells	Crypts of Lieberkühn; Peyer patches	Surface absorptive cells, goblet cells, regenerative cells, DNES cells, Paneth cells	Inner circular, outer longitudinal	No glands (Peyer patches may extend into this layer)	Inner circular, outer longitudinal	Serosa
Colon*	Simple columnar (goblet cells)	Surface absorptive cells, goblet cells, DNES cells	Crypts of Lieberkühn	Surface absorptive cells, goblet cells, regenerative cells, DNES cells,	Inner circular, outer longitudinal	No glands	Inner circular, outer longitudinal modified to form taeniae coli	Serosa and adventitia
Rectum	Simple columnar (goblet cells)	Surface absorptive cells, goblet cells, DNES cells	Shallow crypts of Lieberkühn	Surface absorptive cells, goblet cells, regenerative cells, DNES cells, Paneth cells	Inner circular, outer longitudinal	No glands	Inner circular, outer longitudinal	Adventitia
Anal canal	Simple cuboidal; stratified squamous nonkeratinized; stratified squamous keratinized		Rectal columns; circumanal glands; *at anus:* hair follicles and sebaceous glands		Inner circular, outer longitudinal	No glands; internal and external hemorrhoidal plexuses	Inner circular (forms internal and sphincter), outer longitudinal (becomes fibroelastic sheet)	Adventitia
Appendix	Simple columnar (goblet cells)	Surface absorptive cells, goblet cells, DNES cells, occasional M cells	Shallow crypts of Lieberkühn; lymphoid nodules	Surface absorptive cells, goblet cells, regenerative cells, DNES cells, Paneth cells	Inner circular, outer longitudinal	No glands; *occasional* lymphoid nodules; possible fatty infiltration	Inner circular, outer longitudinal	Serosa

*Includes cecum.
DNES, Diffuse neuroendocrine system.

stomach but is poorly developed in the pylorus. **Auerbach myenteric plexus** is located between the middle circular and outer longitudinal layers of smooth muscle.

The entire stomach is invested by a **serosa** composed of a thin, loose, subserous connective tissue covered by a smooth, wet, simple squamous epithelium. This external covering provides an almost friction-free environment during the churning movements of the stomach.

Histophysiology of the Stomach

The lining and glands of the stomach produce and release secretions into the lumen of the stomach; these secretions are composed of water, hydrochloric acid, gastric intrinsic factor, pepsinogen, rennin, gastric lipase, visible mucus, and soluble mucus.

The gastric glands of the stomach produce approximately 2 to 3 L of gastric juices a day. These secretions are composed of (1) **water** (derived from the extracellular fluid in the interstitial connective tissue and delivered via parietal cells); (2) **HCl** and **gastric intrinsic factor** (manufactured by parietal cells); (3) the enzymes **pepsinogen**, **rennin**, and **gastric lipase** (manufactured by chief cells); (4) a glycoprotein, **visible mucus** (manufactured by surface-lining cells) that forms a coat of mucus that lines and protects the epithelium of the stomach and serves as a favorable, mostly neutral pH, environment for the bacterium *Helicobacter pylori*; and (5) **soluble mucus** that becomes part of the gastric content (produced by mucous neck cells). Little absorption of food products occurs in the stomach, although some substances, such as alcohol, can be absorbed by the gastric mucosa.

The three muscle layers of the muscularis externa interact in such a fashion that during the contraction, the contents of the stomach are churned and the ingested food is liquefied to form **chyme**, a viscous fluid with the consistency of split pea soup. Independent contraction of the muscularis mucosae exposes the chyme to the entire surface area of the gastric mucosa.

Emptying of Gastric Contents

Interaction between neurons of the myenteric and submucosal plexuses, as well as due to the effect of the hormone **ghrelin**, a constant intraluminal pressure is maintained, irrespective of the degree of distention of the stomach. In an empty stomach, the pylorus is always open; however, during peristalsis, the pyloric sphincter is closed. Coordinated contraction of the muscularis externa and momentary relaxation of the pyloric sphincter permit emptying of the stomach by intermittently delivering small aliquots of the chyme into the duodenum. The rate at which the stomach releases its chyme into the duodenum is a function of the acidity, the caloric and fat content, and the osmolality of the chyme.

The production of the peristaltic waves occurs in a rhythmic fashion and is generated by the gastric pacemaker at a rate of about three per minute. Receptors in the duodenum, in response to the arrival of the chyme, cause a sudden closure of the pyloric sphincter and contraction of the muscularis externa of the pyloric antrum, driving the chyme back into the body of the stomach for a thorough mixing of the chyme with the digestive enzymes.

The factors that facilitate emptying of the stomach are the degree of its distention and the action of **gastrin**, a hormone that stimulates contraction of the muscularis externa of the pyloric region and relaxation of the pyloric sphincter. Factors that inhibit gastric emptying include distention of the duodenum; overabundance of fat, proteins, or carbohydrates; and increased osmolarity and excessive acidity of the chyme delivered into the duodenum. These factors activate a neural feedback mechanism by stimulating release of cholecystokinin, which counteracts gastrin, and by the release of gastric inhibitory peptide, which also inhibits gastric contractions.

Gastric Hydrochloric Acid Production

The three phases in the production of hydrochloric acid are cephalic, gastric, and intestinal.

HCl not only breaks down food material but also activates the proenzyme pepsinogen to become the active proteolytic enzyme **pepsin**. Because pepsin requires a low pH for its acidity, the presence of HCl also provides the necessary acidic conditions of **pH 2** or less.

HCl secretion occurs in three phases as a result of different stimuli:

- **Cephalic.** Secretion caused by psychological factors (e.g., the thought, smell, or sight of food; stress) is elicited by parasympathetic impulses from the vagus nerve, which cause the release of **acetylcholine**.
- **Gastric.** Secretion resulting from the presence of certain food substances in the stomach; also resulting from the stretching of the stomach wall, which is elicited by the paracrine hormones **gastrin** and **histamine** and by the neurocrine substance **acetylcholine**. Gastrin and histamine are released by the DNES cells (G cells and ECL cells, respectively) of the stomach, respectively, and acetylcholine is released by the vagus nerve.
- **Intestinal.** Secretion due to the presence of food in the small intestine is elicited by the endocrine hormone **gastrin**, released by G cells of the small intestine.

Mechanism of Gastric Hydrochloric Acid Production

HCl production is initiated when gastrin, histamine, and acetylcholine bind to the basal plasma membrane of parietal cells.

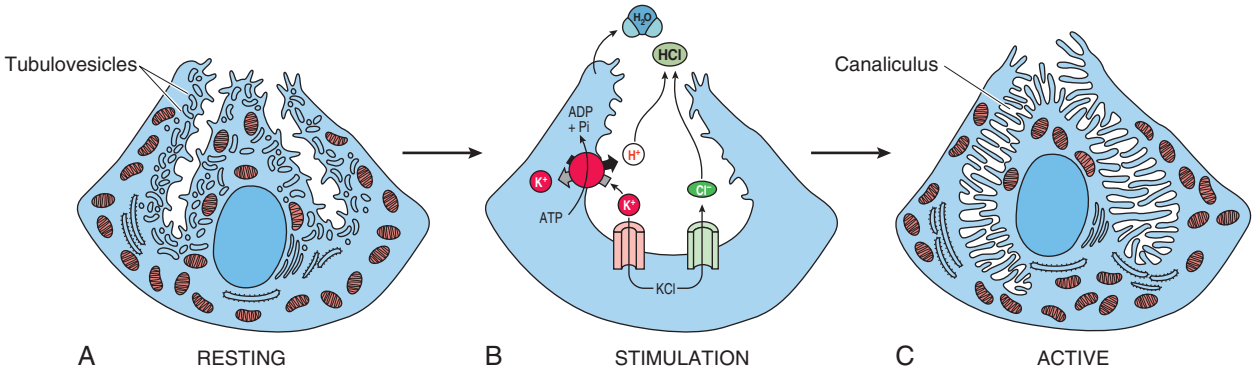


Figure 17–12 Schematic diagram of a parietal cell. Note the well-developed tubulovesicular apparatus in the resting cell **(A)** and the mechanism of hydrochloric acid release **(B)**. **C,** Numerous microvilli in the active cell.

Parietal cells have receptors for gastrin, histamine, and acetylcholine on their basal plasmalemma. Binding of these signaling molecules to the appropriate receptors causes the cells to manufacture and release HCl into the intracellular canaliculus. The process occurs as follows (Fig. 17–12):

1. The enzyme **carbonic anhydrase** facilitates the production of carbonic acid (H_2CO_3) from water (H_2O) and carbon dioxide (CO_2), which then dissociates into hydrogen ions (H^+) and bicarbonate (HCO_3^-) within the cytoplasm of the parietal cell.
2. An **H^+, K^+-ATPase**, using adenosine triphosphate (ATP) as an energy source, pumps intracellular H^+ ions out of the cell into the intracellular canaliculi and transfers extracellular potassium ions (K^+) into the cell.
3. Carrier proteins, using ATP as an energy source, pump K^+ and chloride ion (Cl^-) out of the cell and into the intracellular canaliculi. Thus, **Cl^-** and **H^+** ions enter the lumen of the intracellular canaliculi separately, there to combine into HCl.
4. K^+ is actively transported into the cell at the basal plasmalemma as well as at the microvilli jutting into the intracellular canaliculi, thus increasing the intracellular level of K^+. The high intracellular K^+ concentration forces K^+ to leave the cell via ion channels located in the basal plasmalemma and in the plasma membrane of the microvilli. Thus, K^+ is constantly recirculated in and out of the parietal cell.
5. Water, derived from the extracellular fluid, enters the parietal cell and then leaves the cytoplasm to enter the intracellular canaliculus as a consequence of the osmotic forces generated by the movement of ions just described. Because the intracellular canaliculus is continuous with the lumen of the stomach, HCl, manufactured by the parietal cells, enters the gastric lumen.

The lining of the stomach is protected from the acidic pH by the buffering activity of the HCO_3^- present in the layer of mucus manufactured by the surface-lining cells and, to a certain limited extent, by mucous neck cells. Additionally, the zonulae occludentes of the epithelial cells prevent the influx of HCl into the lamina propria, thus protecting the mucosa from damage. Moreover, evidence suggests that **prostaglandins**, released by DNES cells, protect the cells lining the gastric lumen and also increase local circulation, especially if the integrity of the epithelial barrier is compromised. This increased blood flow removes the accidentally escaped H^+ ions from the lamina propria.

Inhibition of Hydrochloric Acid Release

The hormones **somatostatin**, **prostaglandin**, and **gastric inhibitory peptide (GIP)** inhibit gastric HCl production. Somatostatin acts on G cells and ECL cells, inhibiting their release of gastrin and histamine, respectively. Prostaglandins and GIP act directly on parietal cells and inhibit their ability to produce HCl.

Additionally, **urogastrone (epidermal growth factor)**, produced by Brunner glands of the duodenum, acts directly on parietal cells to inhibit HCl production.

SMALL INTESTINE

The small intestine has three regions: duodenum, jejunum, and ileum.

Digestion begins in the oral cavity and continues in the stomach and in the **small intestine**, which, at 7 m of length, is the longest region of the alimentary tract. The small intestine is divided into three regions: duodenum, jejunum, and ileum. Although these regions are similar histologically, their minor differences permit their identification.

CLINICAL CORRELATIONS

1. Possibly the most common cause of **ulcers** in the United States is the prevalent use of the nonsteroidal anti-inflammatory drugs (NSAIDs) **ibuprofen** and **aspirin**. Both of these drugs inhibit the manufacture of prostaglandins, thus precluding their protective effects on the stomach lining.
2. The bacterium ***Helicobacter pylori***, which is localized in the mucus layer protecting the gastric epithelium, has also been implicated as a possible factor in ulcer formation.
3. Almost 12% of cancer-related fatalities are due to **gastric carcinoma**, one of the most common gastrointestinal (GI) malignancies. Although the cancer may be localized to any region of the stomach, usually the region of the lesser curvature and the pyloric antrum are the sites that are most generally involved.
4. Many individuals who had type 2 diabetes and, due to obesity or other conditions, underwent gastric bypass surgery, experienced a quick reduction in their blood glucose levels. Currently, there is no explanation for this unexpected result, although it has been suggested that hormones released by the DNES cells of the GI tract may play an important role in this outcome.

The small intestine digests food material and absorbs end products of the digestive process. To perform its digestive functions, the first region of the small intestine, the duodenum, receives enzymes and an alkaline buffer from the pancreas and bile from the liver. Additionally, epithelial cells and glands of the mucosa contribute buffers and enzymes to facilitate digestion.

Common Histological Features

Because the three regions of the small intestine are similar histologically, the common features are described first. Following this discussion, variations from this plan are described for each segment (see Table 17–3), and then the functional aspects are considered.

Modifications of the Luminal Surface

The surface area of the intestinal lumen is enlarged by the formation of plicae circulares, villi, microvilli, and crypts of Lieberkühn.

The luminal surface of the small intestine is modified to increase its surface area. Three types of modifications have been noted:

- **Plicae circulares (valves of Kerckring)** are transverse folds of the submucosa and mucosa that form semicircular to helical elevations, some as large as 8 mm in height and 5 cm in length. Unlike rugae of the stomach, these are permanent fixtures of the duodenum and jejunum and end in the proximal half of the ileum. They not only increase the surface area by a factor of 2 to 3 but also decrease the velocity of the movement of chyme along the alimentary canal.
- **Villi** are epithelially covered, finger-like or oak leaf–like protrusions of the lamina propria. The core of each villus **(lamina propria)** contains capillary loops, a blindly ending lymphatic channel **(lacteal)**, and a few smooth muscle fibers, embedded in loose connective tissue that is rich in lymphoid cells. Villi are permanent structures ranging in number from 10 to 40 per mm^2 (Figs. 17–13 to 17–15). Their numbers are greater in the duodenum than in the jejunum or the ileum, and their height decreases from 1.5 mm in the duodenum to about 0.5 mm in the ileum. These delicate structures confer a velvety appearance to the lining of the living organ. Villi increase the surface area of the small intestine by a factor of 10.
- **Microvilli**, modifications of the apical plasmalemma of the epithelial cells covering the intestinal villi, increase the surface area of the small intestine by a factor of 20.

Thus, the three types of intestinal surface modifications increase the total surface area available for absorption of nutrients by a factor of 400 to 600.

Invaginations of the epithelium into the lamina propria between the villi form intestinal glands, **crypts of Lieberkühn**, which also augment the surface area of the small intestine.

Intestinal Mucosa

The mucosa of the small intestine is composed of the usual three layers: a simple columnar epithelium, the lamina propria, and the muscularis mucosae.

Epithelium

The simple columnar epithelium covering the villi and the surface of the intervillar spaces is composed of surface absorptive cells, goblet cells, and DNES cells.

Surface Absorptive Cells

Surface absorptive cells are tall columnar cells that function in terminal digestion and absorption of water and nutrients.

The most numerous cells of the epithelium are **surface absorptive cells** (Fig. 17–16; see Figs. 17–13 and 17–15). They are tall cells, about 25 µm in length, with basally located oval nuclei. Their apical surface presents a **striated border**, and in good tissue preparations, the presence of terminal bars is also evident. The principal functions of these cells are terminal digestion and absorption of nutrients and water. Additionally, these cells reesterify fatty acids into triglycerides, form chylomicrons, and transport them as well as the bulk of the

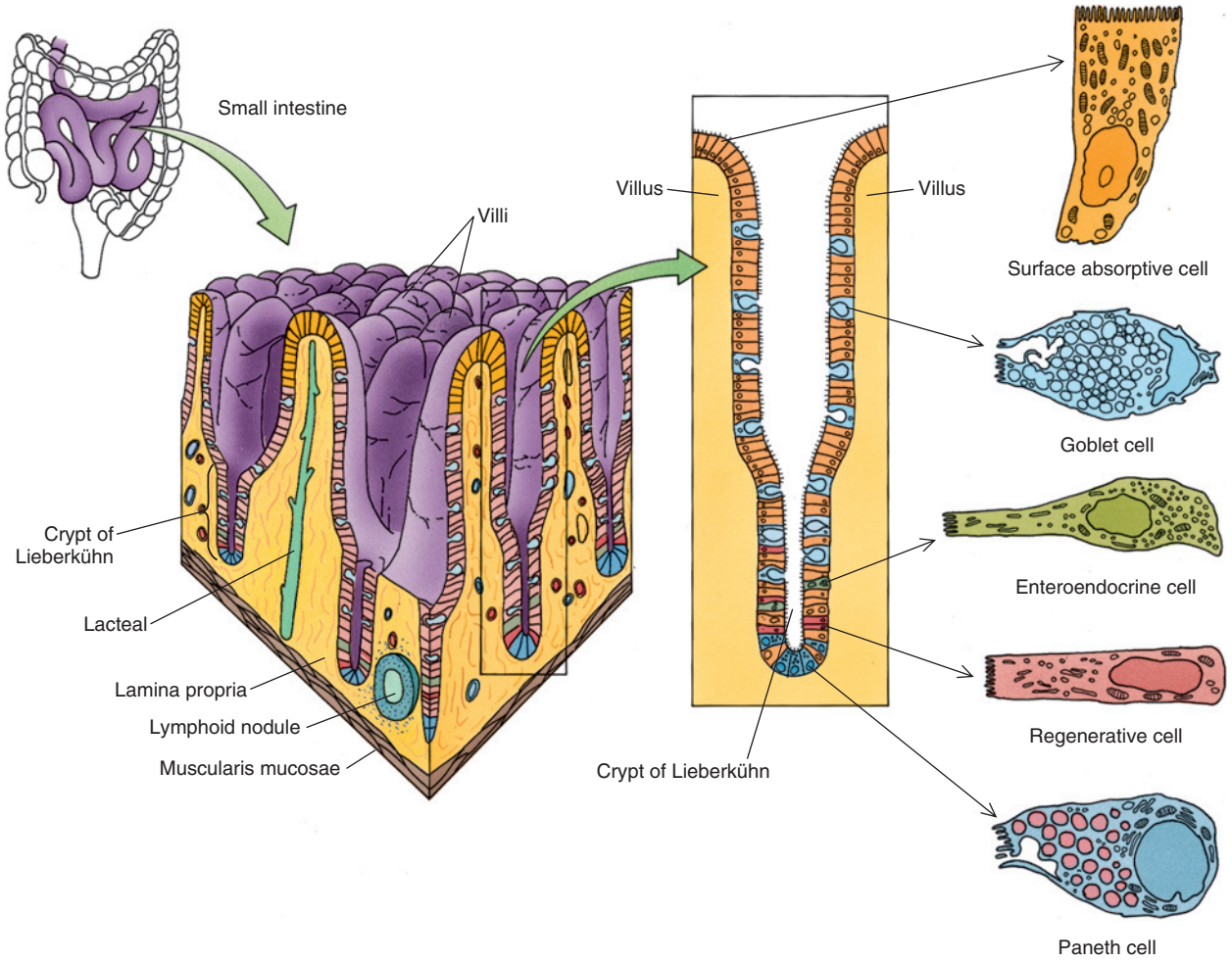


Figure 17–13 Schematic diagram of the mucosa, villi, crypts of Lieberkühn, and component cells of the small intestine. Note that the crypts of Lieberkühn open into the intervillar spaces; a solitary lymphoid nodule is shown in the lamina propria.

absorbed nutrients into the lamina propria for distribution to the rest of the body. The process of absorption is discussed later in the chapter.

Electron micrographs of surface absorptive cells demonstrate that they each sport as many as 3000 **microvilli**, approximately 1 μm long, whose tips are covered with a thick **glycocalyx** layer. The glycocalyx coat not only protects the microvilli from autodigestion, but its enzymatic components also function in terminal digestion of dipeptides and disaccharides into their monomers. The actin core of the microvilli is anchored into the actin and intermediate filaments of the cell web. The cytoplasm of surface absorptive cells is rich in organelles, especially endosomes, smooth endoplasmic reticulum, RER, and Golgi apparatus.

The lateral cell membranes of these cells form zonulae occludentes, zonulae adherentes, desmosomes, and gap junctions with adjacent cells. The tight junctions prevent the passage of material via the paracellular route to or from the lumen of the gut.

Goblet Cells

Goblet cells are unicellular glands (see Figs. 17–13 and 17–15) (see Chapter 5). The duodenum has the smallest number of goblet cells, and their number increases toward the ileum. These cells manufacture **mucinogen**, whose hydrated form is **mucin**, a component of **mucus**, a protective layer lining the lumen.

DNES Cells

The small intestine has various types of DNES cells, both open and closed types, that produce paracrine and endocrine hormones (see the section on the stomach and Table 17–2). Approximately 1% of the cells covering the villi and intervillar surface of the small intestine are composed of DNES cells. A small percent of the

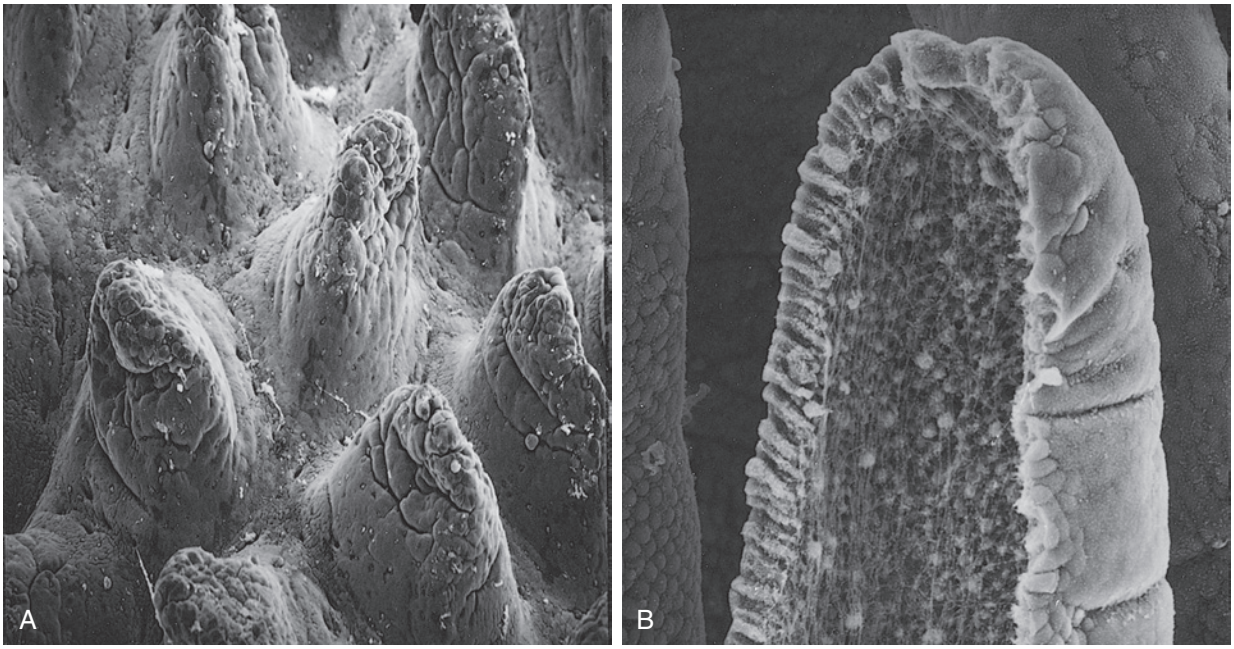


Figure 17–14 Scanning electron micrographs of villi from the mouse ileum. **A**, Observe the villi and the openings of the crypts of Lieberkühn in the intervillar spaces (×160). **B**, Note that the villus is fractured, revealing its core of connective tissue and migrating cells (×500). (From Magney JE, Erlandsen SL, Bjerknes ML, Cheng H. Scanning electron microscopy of isolated epithelium of the murine gastrointestinal tract: morphology of the basal surface and evidence for paracrine-like cells. *Am J Anat*. 1986;177:43-53. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

open form of the DNES cells express the taste receptor protein products of the genes *T2R38*, *T1R1*, *T1R2*, and *T1R3*, thereby acting as "taste cells" in the small intestine distinguishing three G protein receptor linked sensations, namely bitter, sweet, and umami. When sweet components of the intestinal contents are detected, these DNES cells signal the β cells of the islets of Langerhans to release the hormone insulin into the blood stream.

M Cells (Microfold Cells)

Microfold cells phagocytose and transport antigens from the lumen to the lamina propria.

The simple columnar epithelial lining of the small intestine is replaced by squamous-like **M cells** in regions where lymphoid nodules abut the epithelium. These M cells, which are believed to belong to the mononuclear phagocyte system of cells, sample, phagocytose, and transport antigens present in the intestinal lumen.

Lamina Propria

The loose connective tissue of the **lamina propria** forms the core of the villi (described above), which similar to trees of a forest, rise above the surface of the small intestine (Fig. 17–17; see Figs. 17–14 and 17–15). The remainder of the lamina propria, extending down to the muscularis mucosae, is compressed into thin sheets of highly vascularized connective tissue by the numerous tubular intestinal glands, the **crypts of Lieberkühn**. The lamina propria also is rich in lymphoid cells and contains occasional lymphoid nodules, which, as discussed below help protect the intestinal lining from invasion by microorganisms. These lymphoid cells and lymph nodules belong to the GALT (gut-associated lymphoid tissue).

Crypts of Lieberkühn

Crypts of Lieberkühn increase the surface area of the intestinal lining; they are composed of DNES cells, surface absorptive-like columnar cells, goblet cells, regenerative cells, M cells, intermediate cells, and Paneth cells.

Crypts of Lieberkühn are simple tubular (or branched tubular) glands (see Fig. 17–13) that open into the intervillar spaces as perforations of the epithelial lining. Scanning electron micrographs indicate that the base of each villus is surrounded by the openings of several crypts (see Fig. 17–14). These tubular glands are composed of surface absorptive-like columnar cells, goblet cells, DNES cells, M cells (see the section on

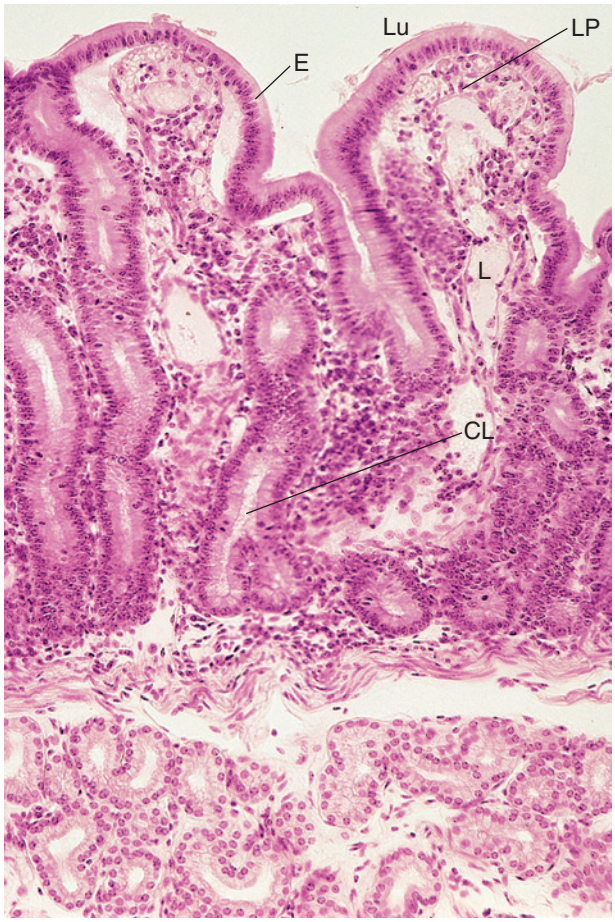


Figure 17–15 Photomicrograph of the duodenal mucosa, displaying the simple columnar epithelium (E), the cellular lamina propria (LP) with its lacteal (L) and the muscularis mucosae. The submucosa houses Brunner glands, a clear indication that this is a section of the duodenum (×132). L, Lacteals of villus.

immunological activity of the lamina propria), regenerative cells, intermediate cells, and Paneth cells.

Surface absorptive-like columnar cells and goblet cells occupy the upper half of the gland. The goblet cells have a short life span; it is believed that after they discharge their mucinogen, they die and are desquamated. The basal half of the gland has no surface absorptive–like columnar cells and only a few goblet cells; instead, most of the cells are intermediate and regenerative cells. Additionally, the crypt epithelium also houses DNES cells, some M cells, and Paneth cells. Only regenerative cells, intermediate cells, and Paneth cells are described here; the others were discussed above.

Regenerative Cells

The **regenerative cells** of the small intestine are stem cells that undergo extensive proliferation to repopulate the epithelium of the crypts, mucosal surface, and villi. These narrow cells appear to be wedged into limited spaces among the newly formed cells (see Fig. 17–13). Their rate of cell division is high, with a relatively short cell cycle of 24 hours. It has been suggested that 5 to 7 days after the appearance of a new cell, that cell has progressed to the tip of the villus and has been exfoliated. Electron micrographs of these undifferentiated cells display few organelles but many free ribosomes. Their single, basally located, oval nuclei are electron-lucent, indicating the presence of a large amount of euchromatin.

Intermediate cells

The intermediate cells constitute the largest cell population of the lining of the crypts of Lieberkuhn; they resemble and are the progeny of regenerative cells. Although their function is to replace defunct cells of the epithelial lining of the intestinal lumen, they have not as yet shown a preference to a particular cell line.

Paneth Cells

Paneth cells produce the antibacterial agent lysozyme.

Paneth cells are clearly distinguishable because of the presence of large, eosinophilic, apical secretory granules (Fig. 17–18; see Fig. 17–13). These pyramid-shaped cells occupy the bottom of the crypts of Lieberkühn and manufacture the antibacterial agent **lysozyme**, defensive proteins **(defensins)**, and **tumor necrosis factor-α**. Unlike the other cells of the intestinal epithelium, Paneth cells have a comparatively long life span of approximately 20 days and secrete lysozyme continuously. Electron micrographs of these cells display a well-developed Golgi apparatus, a large complement of RER, numerous mitochondria, and large apical secretory granules housing a homogeneous secretory product.

Muscularis Mucosae

The **muscularis mucosae** of the small intestine is composed of an inner circular layer and an outer longitudinal layer of smooth muscle cells (see Fig. 17–17). Muscle fibers from the inner circular layer enter the villus and extend through its core to the tip of the connective tissue, as far as the basement membrane. During digestion, these muscle fibers rhythmically contract, shortening the villus several times a minute.

Submucosa

The **submucosa** of the small intestine is composed of dense, irregular fibroelastic connective tissue with a rich lymphatic, neural, and vascular supply. The intrinsic innervation of the submucosa is from the parasympathetic **submucosal (Meissner) plexus**. The submucosa of the **duodenum** is unusual because it houses glands known as ***Brunner glands (duodenal glands)***.

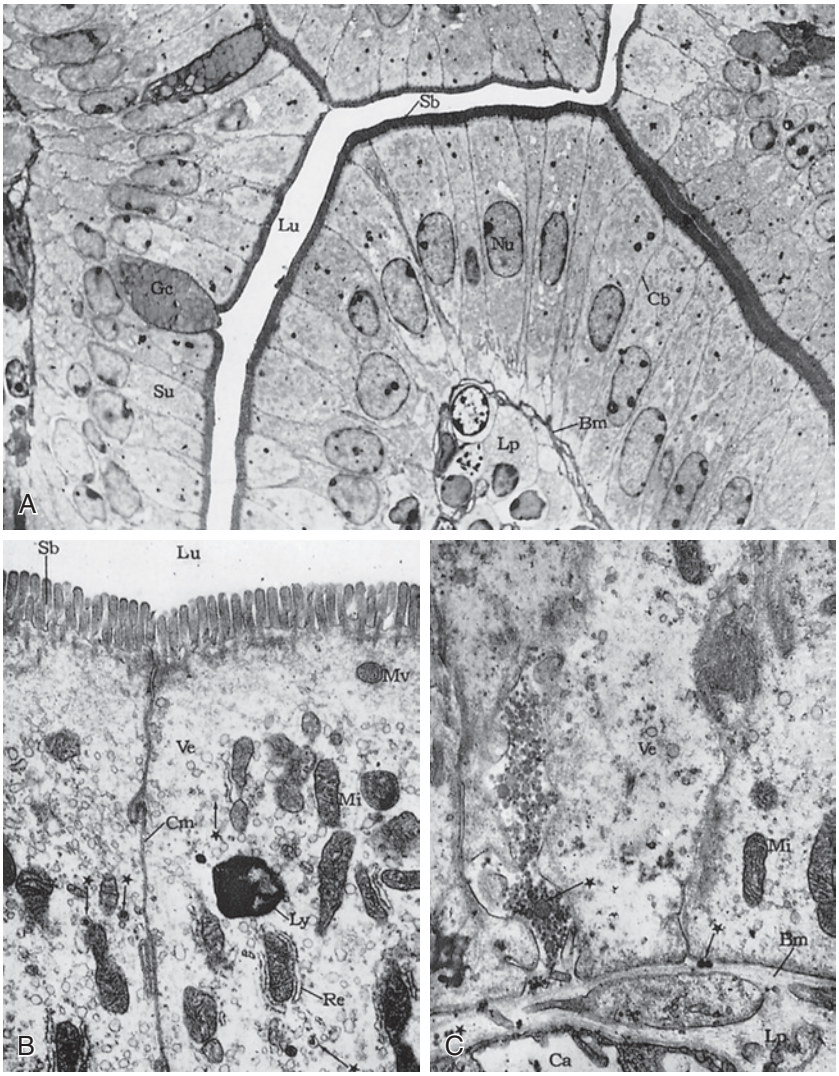


Figure 17–16 Surface absorptive cells from a villus of the mouse jejunum. **A,** Low-magnification electron micrograph displaying two goblet cells (Gc) and numerous surface absorptive cells (Su) (×1744). Note the striated border (Sb) facing the lumen (Lu). Nuclei (Nu) and cell boundaries (Cb) are clearly evident. Observe also that the epithelium is separated from the lamina propria by a well-defined basement membrane (Bm). **B,** A higher magnification electron micrograph of two adjoining surface absorptive cells (×10500). The striated border (Sb) is clearly composed of numerous microvilli that project into the lumen (Lu). The adjoining cell membranes (Cm) are close to each other. Ly, Lysosomes; Mi, mitochondria; Re, rough endoplasmic reticulum; Ve, vesicles; *asterisk* indicates membrane-bound lipid droplets. **C,** Electron micrograph of the basal aspect of the surface absorptive cells (×11200). Bm, Basement membrane; Lp, lamina propria; Mi, mitochondria; Ve, Vesicles; *asterisk* indicates chylomicrons. (From Rhodin JAG. *An Atlas of Ultrastructure*. Philadelphia: WB Saunders; 1963.)

Brunner Glands

Brunner glands produce a mucous, bicarbonate-rich fluid as well as urogastrone (human epidermal growth factor).

Brunner glands are branched, tubuloalveolar glands whose secretory portions resemble mucous acini (see Fig. 17–15). The ducts of these glands penetrate the muscularis mucosae and usually pierce the base of the crypts of Lieberkühn to deliver their secretory product into the lumen of the duodenum. Occasionally, their ducts open into the intervillar spaces. Electron micrographs of the acinar cells display a well-developed RER and Golgi apparatus, numerous mitochondria, and flattened to round nuclei.

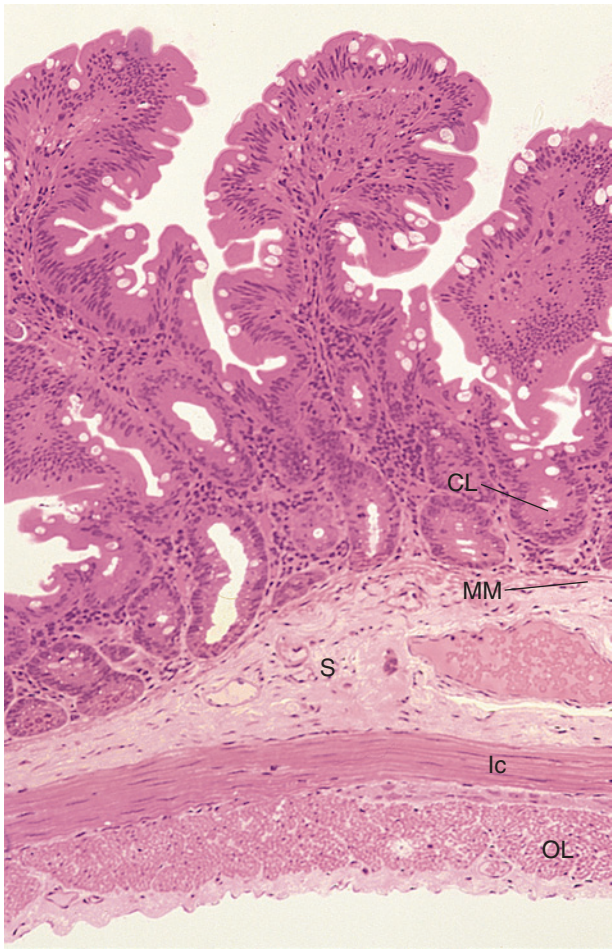


Figure 17–17 Photomicrograph of the mucosa of a monkey jejunum. Observe the well-developed villi, and note that there are no Peyer patches in the lamina propria or any Brunner glands in the submucosa; therefore, this must be a section of the jejunum (×132). CL, Crypts of Lieberkuhn; Ic, inner circular muscle layer of the muscularis externa; MM, muscularis mucosae; OL, outer longitudinal layer of the muscularis externa; S, submucosa.

Brunner glands secrete a mucous, alkaline fluid in response to parasympathetic stimulation. This fluid helps neutralize the acidic chyme that enters the duodenum from the pyloric stomach. The glands also manufacture the polypeptide hormone **urogastrone** (also known as ***human epidermal growth factor***), which is released into the duodenal lumen along with the alkaline buffer. Urogastrone inhibits production of HCl (by directly inhibiting parietal cells) and amplifies the rate of mitotic activity of the regenerative and intermediate cells.

Muscularis Externa and Serosa

The **muscularis externa** of the small intestine is composed of an inner circular layer and an outer longitudinal smooth muscle layer. **Auerbach myenteric plexus**, located between the two muscle layers, is the intrinsic neural supply of the external muscle coat. The muscularis externa is responsible for the peristaltic activity of the small intestine.

Except for the second and third parts of the duodenum which have **adventitia**, the entire small intestine is invested by a **serosa**.

Lymphatic and Vascular Supply of the Small Intestine

Lymph drainage in the small intestine begins as blindly ending lymphatic vessels known as lacteals.

The small intestine has a well-developed lymphatic and vascular supply. Blindly ending lymph capillaries called ***lacteals***, which are located in the cores of villi, deliver their contents into the **submucosal lymphatic plexus**. From here, lymph passes through a series of lymph nodes to be delivered to the thoracic duct, the largest lymph vessel in the body. The thoracic duct empties its content into the circulatory system at the junction of the left internal jugular and subclavian veins.

Capillary loops adjacent to the lacteals are drained by blood vessels that are tributaries of the **submucosal vascular plexus**. Blood from here is delivered to the hepatic portal vein to enter the liver for processing.

Regional Differences

The **duodenum** is the shortest segment of the small intestine, only 25 cm in length. It receives bile from the liver and digestive juices from the pancreas via the common bile duct and pancreatic duct, respectively. These ducts open into the lumen of the duodenum at the **duodenal papilla (of Vater)**. The duodenum differs from the jejunum and ileum in that its villi are broader, taller, and more numerous per unit area. It has fewer goblet cells per unit area than the other segments, and there are **Brunner glands** in its submucosa.

The villi of the **jejunum** are narrower, shorter, and sparser than those of the duodenum. The number of goblet cells per unit area is greater in the jejunum than in the duodenum.

The villi of the **ileum** are the sparsest, shortest, and narrowest of the three regions of the small intestine. The lamina propria of the ileum houses permanent clusters of lymphoid nodules, known as ***Peyer patches***. These structures are located in the wall of the ileum that is opposite the attachment of the mesentery. In the region of Peyer patches, the villi are reduced in height and may even be absent.

Histophysiology of the Small Intestine

In addition to its roles in digestion and absorption, the small intestine exhibits immunological and secretory activities. These activities are considered first, after

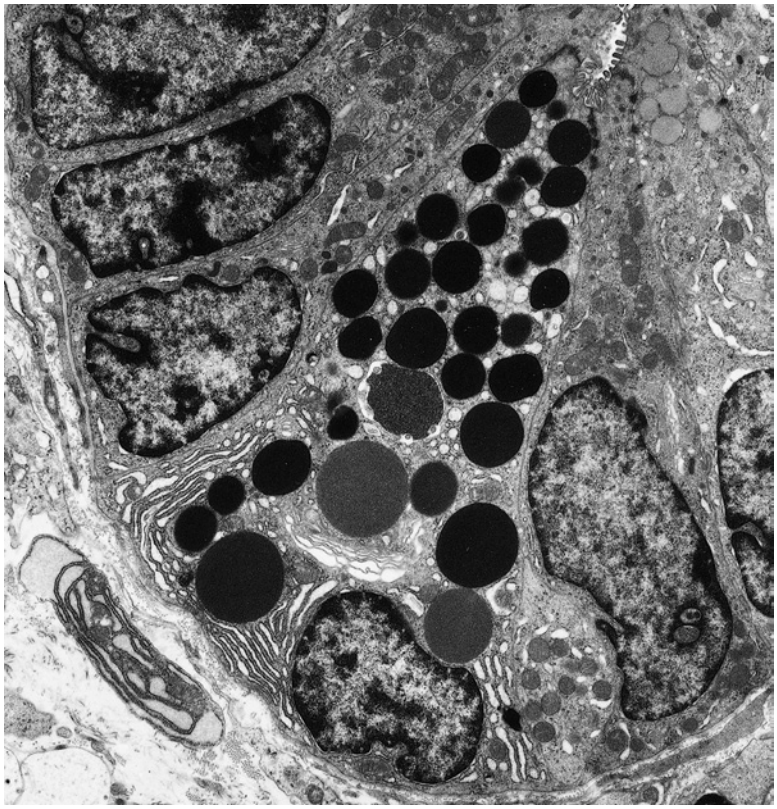


Figure 17–18 Electron micrograph of a Paneth cell from the rabbit ileum (×5900). Note the large, round granules in the cytoplasm of the Paneth cell. (From Satoh Y, Yamano M, Matsuda M, Ono K. Ultrastructure of Paneth cell in the intestine of various mammals. *J Electron Microsc Tech*. 1990;16:69-80. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

CLINICAL CORRELATIONS

Meckel diverticulum is a very common congenital anomaly occurring in about 2% of the Caucasian population. The diverticulum, a remnant of the vitelline duct—an embryonic connection between the midgut and the yolk sac—is a short, wide-mouthed- extension of the distal aspect of the ileum about 100 cm from the cecum. Most Meckel diverticula are asymptomatic, but some can have bleeding and intestinal obstruction. The obstruction is usually caused by intussusception, which is prolapse of the ileum into the diverticulum.

which the primary function, digestion and absorption, of the small intestine is described.

Immunological Activity of the Lamina Propria

Immunoglobulin A produced by plasma cells in the lamina propria is recirculated through the liver and gallbladder.

The lamina propria is rich in plasma cells, lymphocytes, mast cells, extravasated leukocytes, and fibroblasts. Additionally, solitary lymphoid nodules are frequently present in the lamina propria adjacent to the epithelial lining of the mucosa. Moreover, as described previously, the ileum has permanent clusters of lymphoid nodules collectively known as ***Peyer patches***.

Where these lymphoid nodules come into contact with the epithelium, the columnar cells are replaced by **M cells (microfold cells)**, which phagocytose luminal antigens, parasites, viruses, and bacteria (Figs. 17–19 and 17–20). Endocytosed antigens enter the endosomal system of M cells, but instead of being processed, they are packaged in clathrin-coated vesicles, transferred to the basal aspect of the cell, and released into the lamina propria. Antigen-presenting cells and dendritic cells of the lymphoid nodule lying in the immediate vicinity of M cells endocytose the transferred antigens, process them, and present the epitopes to lymphocytes for the initiation of an immune response.

Activated lymphocytes migrate to mesenteric lymph nodes, where they form germinal centers, regions where

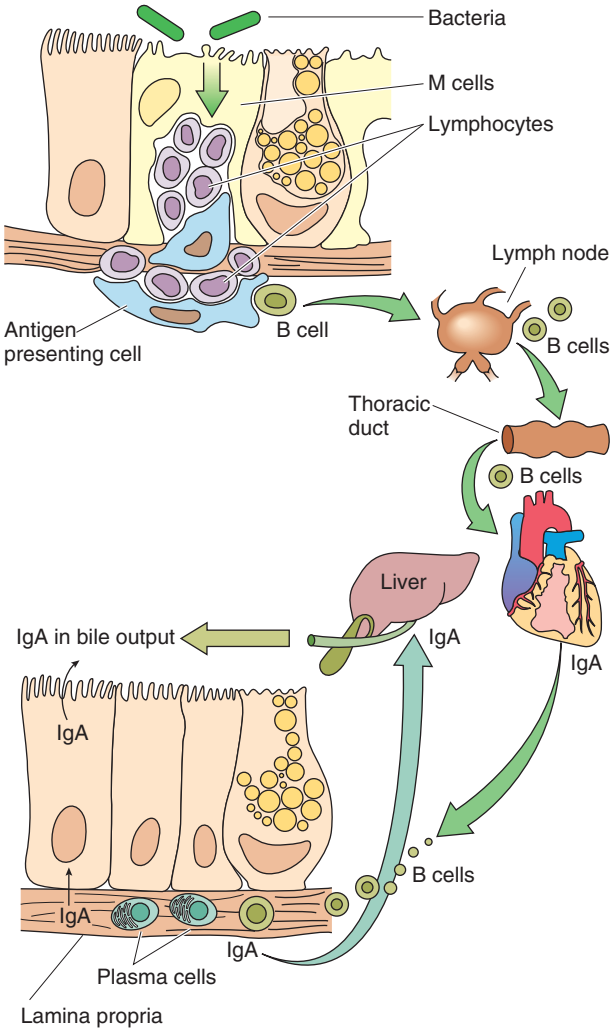


Figure 17–19 Schematic diagram of an M cell and its immunological relationship to the alimentary canal. Observe that immunoglobulin A (IgA) is produced by plasma cells in the lamina propria and, from there, some enters the lumen of the duodenum directly via the surface absorptive cells, whereas most of the IgA enter the hepatic portal system, and hepatocytes of the liver complex it with secretory protein and deliver it into the gallbladder where it is stored with bile. As bile is released into the duodenum, it will be rich in IgA. Therefore, most of the IgA enter the lumen of the duodenum via the bile.

B cells proliferate. The newly formed B cells return to the lamina propria, where they differentiate into **plasma cells** that produce monomeric **immunoglobulin A (IgA)**. Still within the cytoplasm of the plasma cell, two monomeric IgAs are complexed together by **J protein**, also synthesized by the plasma cell, to form **IgA dimers** which are then released by the plasma cells into the lamina propria. The IgA dimers attach to **receptor molecules** of surface absorptive cell that endocytose the IgA–receptor molecule complex and transport it to early endosomes. Within the early endosome, the receptor molecule is modified to become the secretory component of the IgA dimer, now referred to as ***secretory IgA (sIgA)***, and is released via **transcytosis** into the intestinal lumen, where it combats antigens and pathogenic bacteria. But a large portion of the secretory IgA is reabsorbed by the surface absorptive cells to be released into the lamina propria and enter blood vessels that convey it to the **liver**. Hepatocytes endocytose the sIgA, and it becomes a component of bile that is transported into the gallbladder to be released as bile is needed into the duodenum, a route known as the ***enterohepatic circulation***, to defend the body against antigenic onslaught. Thus, much of the luminal IgA enters the intestine through the common bile duct, as a constituent of bile.

Secretory Activity of the Small Intestine

Glands of the small intestine secrete mucus and a watery fluid in response to neural and hormonal stimulation. Neural stimulation, originating in the submucosal plexus, is the principal trigger, but the hormones **secretin** and **cholecystokinin** also play a part in regulating the secretory activities of Brunner glands in the duodenum and of the crypts of Lieberkühn, which collectively produce almost 2 L of slightly alkaline fluid per day.

The DNES cells of the small intestine produce numerous hormones that affect movement of the small intestine and help regulate gastric HCl secretion and the release of pancreatic secretions (see Table 17–2).

CLINICAL CORRELATIONS

The rate of fluid secretion into the small intestine is greatly increased in response to **cholera toxin**. The amount of fluid lost as diarrhea may amount to as much as 10 L/day and, if not replaced, may lead to circulatory shock and death within a few hours. The fluid loss is accompanied by electrolyte imbalance, a contributory factor in the lethal effect of cholera.

Contractions of the Small Intestine

The small intestine participates in two types of contraction: mixing and propulsive.

Contractions of the small intestine may be subdivided into two interrelated phases:

- **Mixing contractions** are more localized and sequentially redistribute the chyme to expose it to the digestive juices.
- **Propulsive contractions** occur as **peristaltic waves** that facilitate the movement of the chyme along the small intestine. Because the chyme moves

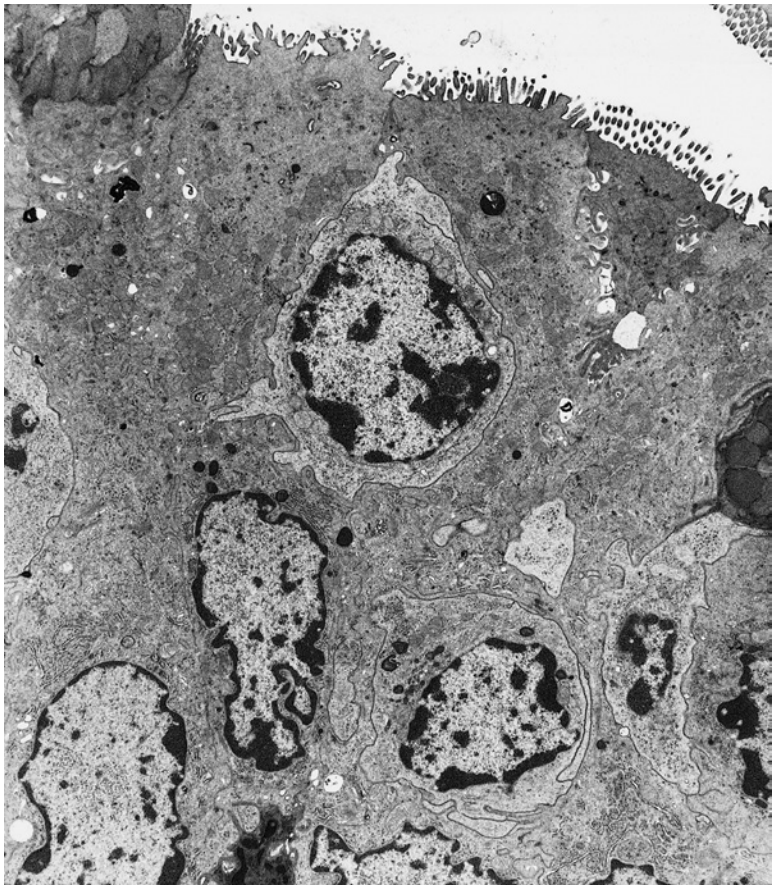


Figure 17–20 Electron micrograph of M cells of the mouse colon (×6665). Observe the electron-dense M cells surrounding the electron-lucent lymphocytes. (From Owen RL, Piazza AJ, Ermak TH. Ultrastructural and cytoarchitectural features of lymphoreticular organs in the colon and rectum of adult BALB/c mice. *Am J Anat*. 1991;190:10-18. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

at an average of 1 to 2 cm/min, it spends 6 to 12 hours in the small intestine. The rate of peristalsis is controlled by neural impulses and hormonal factors. In response to gastric distention, a **gastroenteric reflex** mediated by the **myenteric plexus** provides the neural impetus for peristalsis in the small intestine. The hormones **cholecystokinin**, **gastrin**, **motilin**, **substance P**, and **serotonin** *increase* intestinal motility, whereas **secretin** and **glucagon** *decrease* motility.

CLINICAL CORRELATIONS

If the intestinal mucosa is exposed to profound irritation by toxic substances, the muscularis externa can undergo intense, swift contractions of long duration known as ***peristaltic rush***. These strong contractions propel the chyme into the colon within minutes for elimination as diarrhea.

Digestion

The chyme that enters the duodenum is in the process of being digested by enzymes produced by glands of the oral cavity and stomach. Digestion is intensified in the duodenum by enzymes derived from the exocrine pancreas. The final breakdown of proteins and carbohydrates occurs at the microvilli, where **dipeptidases** and **disaccharidases**, adherent to the glycocalyx, liberate individual amino acids and monosaccharides (mostly **glucose**, **fructose**, and **galactose**). These monomers are transported into the surface absorptive cells by specific carrier proteins; however, dipeptides and tripeptides are also endocytosed by the surface absorptive cells. Lipids are **emulsified** by bile salts into small fat globules that are split into monoglycerides and fatty acids. Bile salts segregate monoglycerides and free fatty acids into **micelles**, 2 nm in diameter, which diffuse into the surface absorptive cells through their plasmalemma.

Absorption

Approximately 6 to 7 L of water, 30 to 35 g of sodium, 0.5 kg of carbohydrates and proteins, and 1 kg of fat are absorbed by the surface absorptive cells of the small intestine each day. Water, amino acids, dipeptides and tripeptides, ions, and monosaccharides enter the **surface absorptive cells** and are released into the intercellular space at the basolateral membrane, processes that rely on specific carrier molecules, ion channels, and aquaporins both at the luminally located entry points and the basolaterally located exit points. These nutrients then enter the capillary beds of the villi and are transported to the liver for processing.

As diagrammed in Figure 17–21, **long-chain fatty acids** and **monoglycerides** enter the smooth endoplasmic reticulum of the surface absorptive cells, where they are **reesterified** to triglycerides. The **triglycerides** are transferred to the Golgi apparatus, where they are combined with a β-lipoprotein coat, manufactured on the RER, to form **chylomicrons**. These large lipoprotein droplets, packaged and released from the Golgi apparatus, are transported to the basolateral cell membrane to be released into the lamina propria. The chylomicrons enter the lacteals, filling these blindly ending lymphatic vessels with a lipid-rich substance known as ***chyle***. Rhythmic contractions of the smooth muscle cells located in the cores of the villi cause shortening of each villus, which acts as a syringe, injecting the chyle from the lacteal into the **submucosal plexus of lymph vessels**. These lymph vessels drain into ever larger lymph vessels and eventually into the largest lymph vessel of the body, known as the *thoracic duct*, which delivers its chyle-rich lymph into the systemic circulation. Therefore, unlike the products of carbohydrate and protein digestion, long-chain fatty acids do not go directly to the liver but enter the systemic circulation.

Unlike long-chained fatty acids just described **short-chain fatty acids** (<12 carbons in length) do not enter

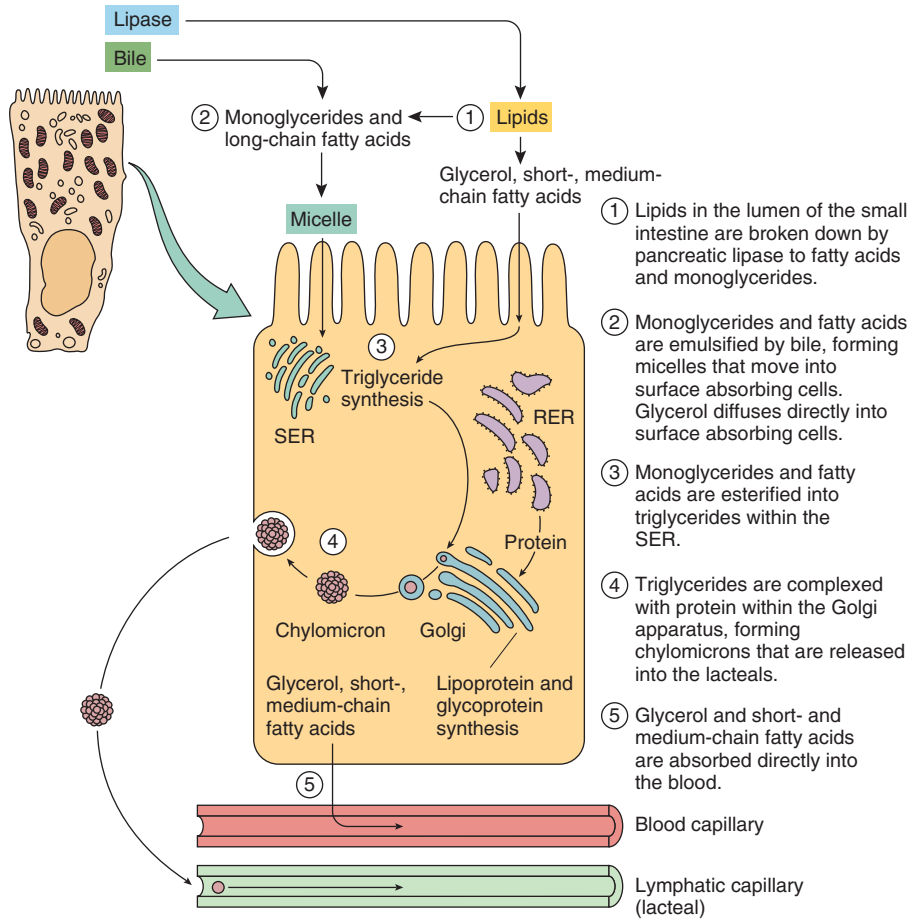


Figure 17–21 Schematic diagram of fat absorption, fat processing, and chylomicron release by surface absorptive cells. RER, Rough endoplasmic reticulum; SER, smooth endoplasmic reticulum.

the smooth endoplasmic reticulum for reesterification. These free fatty acids, which are short enough to be somewhat water soluble, progress to the basolateral membrane of the surface absorptive cell, diffuse into the lamina propria, and enter the capillary loops to be delivered directly to the **liver** for processing.

CLINICAL CORRELATIONS

Malabsorption in the small intestine may occur even though the pancreas delivers its normal complement of enzymes. The various diseases that result in malabsorption are called ***sprue***. An interesting form of sprue, **gluten enteropathy (nontropical sprue)**, is caused by **gluten**, a substance present in rye and wheat, which destroys the microvilli and even villi of susceptible persons. These effects may result from an allergic response to gluten. In patients with this disorder, the surface area available for absorption of nutrients is reduced. Treatment involves elimination of gluten-containing grains from the diet.

LARGE INTESTINE

The large intestine is subdivided into the cecum, colon, rectum, and anus; the appendix is a small, blind outpouching of the cecum.

The **large intestine**, composed of the cecum, colon (ascending, transverse, descending, and sigmoid), rectum, and anus, is approximately 1.5 m long (see Table 17–3). It absorbs most of the water and ions from the chyme it receives from the small intestine as well as much of the gases present in its luminal content and compacts the chyme into feces for elimination. The cecum and the colon are indistinguishable histologically and are discussed as a single entity called the ***colon***. The **appendix**, a blind outpocketing of the cecum, is described separately.

Colon

The **colon** accounts for almost the entire length of the large intestine. It receives chyme from the ileum at the **ileocecal valve**, an anatomical as well as a physiological sphincter that prevents reflux of the cecal content into the ileum.

Histology of the Colon

The colon has no villi but is richly endowed with **crypts of Lieberkühn** that are similar in composition to those of the small intestine, except for the absence of Paneth cells (Figs. 17–22 to 17–25). The number of goblet cells of its simple columnar epithelium increases from the cecum to the sigmoid colon, but throughout most of the colon, the surface absorptive cells are the most numerous cell type. DNES cells, including L cells that secrete the appetite reducing hormone **peptide YY (PYY)**, are also present, although they are few in number. Rapid mitotic activity of the regenerative cells replaces the epithelial lining of the crypts and of the mucosal surface every 6 to 7 days.

The **fibroblast** population is subdivided into two components, one located throughout the **lamina propria** which are just like fibroblasts anywhere else, and the other at the bases of the crypts of Lieberkuhn that migrate along the length of the crypts of Lieberkuhn until they arrive near the opening of the crypts where these fibroblasts differentiate into macrophage-like cells and function as if they were monocyte-derived macrophages. The lamina propria is also richly endowed with components of **GALT**, namely, lymphoid cells and lymphoid nodules. However, there is only a scant amount of lymph drainage in the colon, which accounts for the limited incidence of metastasis of colon cancers.

The muscularis mucosae and submucosa of the colon resemble those of the small intestine. The **muscularis externa** is unusual in that the outer longitudinal layer is not of continuous thickness along the surface, but most of it is gathered into three narrow ribbons of muscle fascicles, known as ***taeniae coli***. The constant tonus maintained by the taeniae coli puckers the large intestine into sacculations, called ***haustra coli***. The **serosa** displays numerous fat-filled pouches, called ***appendices epiploicae***.

Histophysiology of the Colon

The colon functions in absorption of water, electrolytes, and gases as well as in the compaction and elimination of feces.

The colon absorbs water and electrolytes (~1400 mL/day) and compacts and eliminates feces (approximately 100 mL/day).

Feces are composed of water (75%), dead bacteria (7%), roughage (7%), fat (5%), inorganic substances (5%), and undigested protein, dead cells, and bile pigment (1%). The odor of feces varies with the individual and is a function of the diet and bacterial flora, which produce varied amounts of **indole**, **hydrogen sulfide**, and **mercaptans**. The color of feces is due to **urobilin** and **stercobilin**, both the byproducts of bilirubin. Bacterial by-products include riboflavin, thiamin, vitamin B_{12}, and vitamin K.

Bacterial action in the colon produces gases, released as **flatus**, composed of CO_2, methane, and H_2, which then is mixed with the nitrogen and oxygen from swallowed air. The gas is combustible and, very infrequently, it may explode when electrical cauterization is used during sigmoidoscopy or colonoscopy. The large

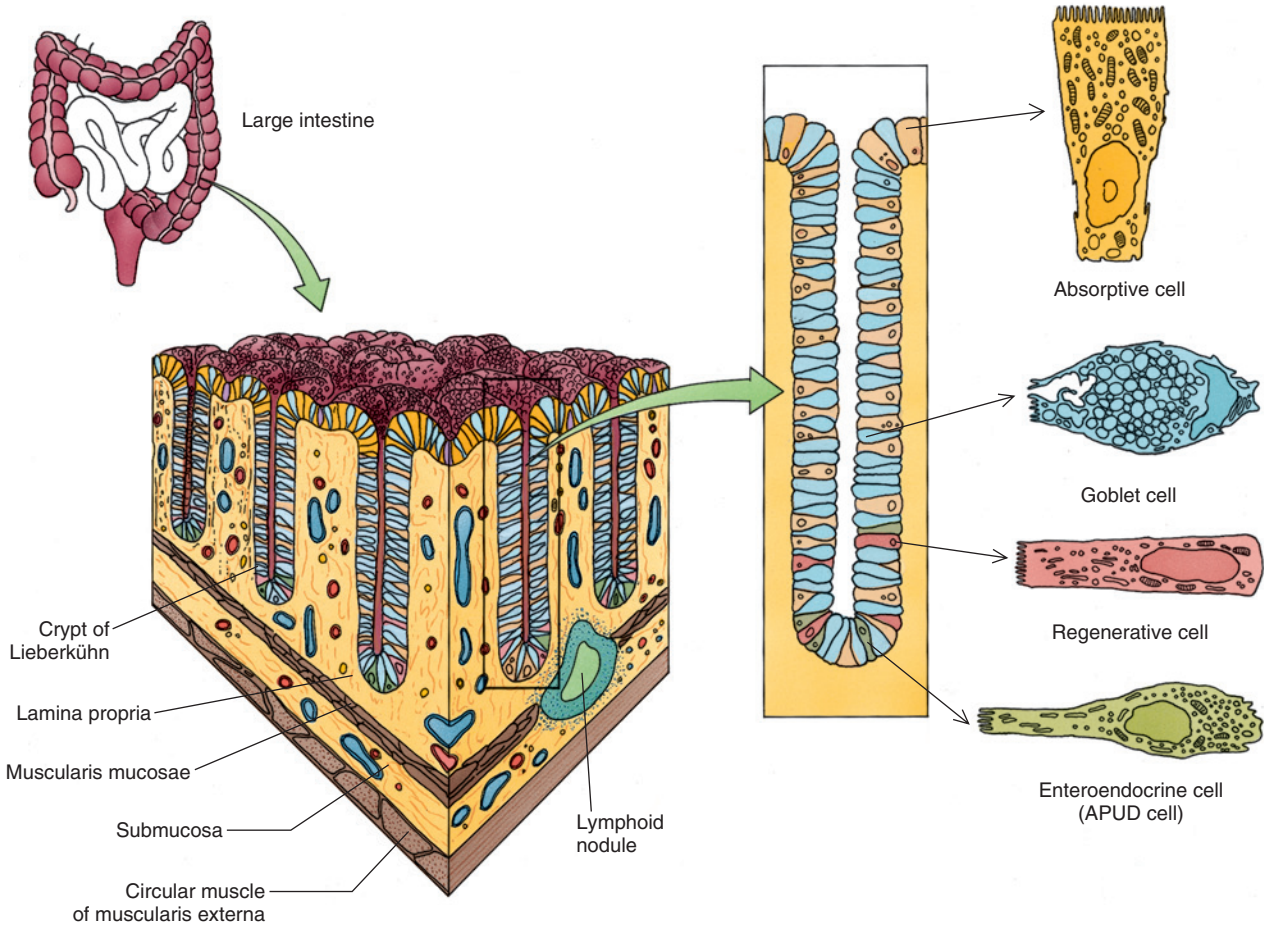


Figure 17–22 Schematic diagram of the colon, crypts of Lieberkühn, and associated cells. APUD, Amine precursor uptake and decarboxylation.

intestine holds 7 to 10 L of gases each day, of which only 0.5 to 1 L is expelled as flatus; the remainder is absorbed through the lining of the colon.

The colon also produces mucus and HCO_3^-. Mucus not only protects the mucosa of the colon but also facilitates the compaction of feces because it is the mucus that permits adherence of the solid wastes into a compact mass. HCO_3^- adheres to the mucus and acts as a buffer, protecting the mucosa from the acid byproducts of bacterial metabolism within the feces.

CLINICAL CORRELATIONS

1. Intense irritation of the colonic mucosa, as in **enteritis**, results in the secretion of large quantities of mucus, water, and electrolytes. Voiding of copious quantities of liquid stool, known as ***diarrhea***, protects the body by diluting and eliminating the irritant. Long-term diarrhea and loss of a large amount of fluid and electrolytes, without a regimen of replacement therapy, may result in circulatory shock and even death.
2. **Pseudomembranous colitis**, an inflammatory disease of the bowels, may result from mercury poisoning, intestinal ischemia, and bronchopneumonia, but most frequently it is due to prolonged antibiotic therapy. Patients most at risk are those who are weak, immunocompromised, and/or elderly. As the intestinal flora is disrupted due to the antibiotic therapy, *Clostridium difficile* assumes a major role in the genesis of this disease whose clinical manifestations include fluid accumulation in the small intestine as well as epithelial shedding and the formation of a thick, viscous membrane composed of fibrin, mucus, neutrophils, and mononuclear cells. The symptoms include a low-grade fever (38° C to 40° C [100.4° F to 104° F]), copious watery diarrhea, severe abdominal cramps, and abdominal tenderness. Mortality is relatively high

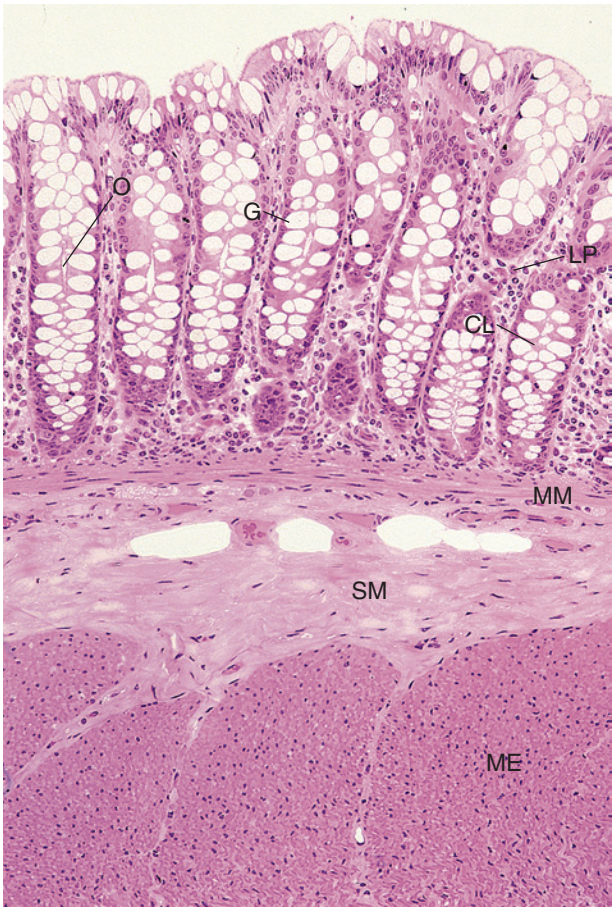


Figure 17–23 Photomicrograph of the monkey colon. Observe that it appears as if most of the cells of the epithelial lining are goblet cells, but, in fact, the surface absorptive cells constitute the largest population of this epithelium (×132). G, Goblet cells, CL, crypts of Lieberkühn; LP, lamina propria; ME, muscularis externa; MM, muscularis mucosae; O, open lumen of crypts of Lieberkuhn; SM, submucosa.

Figure 17–24 Photomicrograph of the crypts of Lieberkühn of the monkey colon. Observe that the base of the crypt displays DNES cells whose granules are basally oriented (×270). E, Diffuse neuroendocrine system (DNES) cell; L, lumen of crypt; P, plasma cell.

CLINICAL CORRELATIONS—cont'd

(10% to 15% of affected individuals) if the condition is not treated in a timely manner by restoring electrolyte balance and maintaining adequate fluid volume by fluid replacement therapy, as much as 10 to 15 L per 24 to 36 hours.

3. Individuals with recurrent abdominal pain, usually beginning between 15 and 30 years of age and who have diarrhea or very loose stool occurring several times a day, may have **Crohn disease**. Accompanying the pain and diarrhea, especially in 15- to 20-year-old children, growth retardation is common (30% of cases). In fact, this is one of the contributing indications to the diagnosis of childhood Crohn disease. The underlying causes of this condition are not understood, but they involve inflammation of the gastrointestinal tract and an immune response, not to the individual's own tissues but to antigens of the patient's bacterial flora. The disease has a genetic component, at least in approximately 50% of the patients with this condition. The prevalent locations that are affected by Crohn disease are the ileum and the colon. Unfortunately, there is no cure for the disease, but the patient may be kept in remission by smoking cessation, decreased meat and dairy intake, an increase in vegetable-based proteins, and the consumption of smaller meals several times a day. Moderate daily exercise and an adequate amount of sleep and rest have also proved beneficial to patients with this condition.

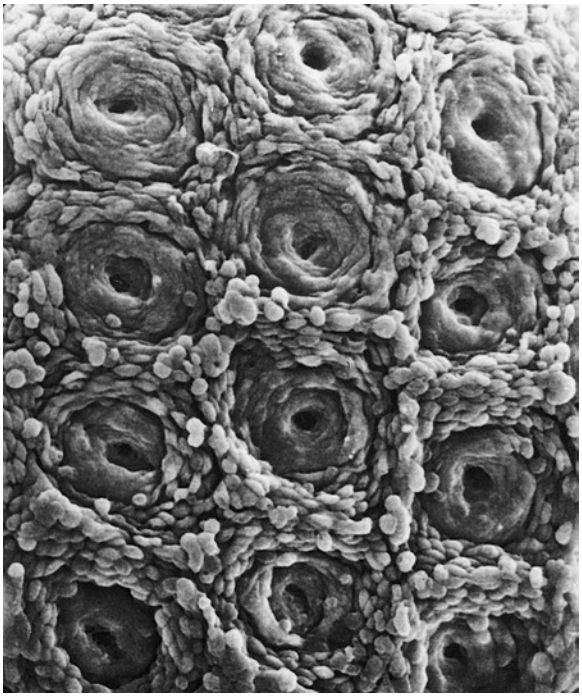


Figure 17–25 Scanning electron micrograph of a monkey colon (×516). Observe the opening of the crypts. (From Specian RD, Neutra MR. The surface topography of the colonic crypt in rabbit and monkey. *Am J Anat*. 1981;160:461-472. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Microbiota of the Colon

The human body is composed of approximately 1 trillion human cells, a number dwarfed by the estimated 10 trillion microbes that inhabit the surface of the skin as well as the lining of the respiratory, genitourinary, and GI tracts. This population of microorganisms is known as the *microbiota* and the collective genome of the microbiota is known as the *microbiome*. The bulk of the microbiota that inhabit the large intestine are commensal organisms. It has been shown that the physiological condition of this intestinal flora directly affects the health of the individual in whom they reside. Because fetuses develop under sterile conditions, it is only during its passage through the birth canal that the baby acquires a microbial flora from the maternal vagina, and thus the baby's flora mirrors that of the mother. It is believed that some of the fecal microbes are swallowed, and later on, during breastfeeding, the baby's intestinal flora becomes expanded. It is only after the maternal milk is supplemented and, later, supplanted by the baby's diet that the intestinal flora resembles that of the adult members of the nuclear family. It was believed that the adult microbial flora may be classified into three enterotypes, according to the predominant organisms that reside in the intestine. However, recent reports suggest that instead of enterotypes, there are gradients of organisms that predominate, and these may be categorized as groups that coexist in the gut lumen. There are two phyla that may be described as the predominant residents in the intestinal lumina of some populations of humans but not in others; therefore, in some individuals, *Prevotella*, whereas, in others, *Bacteriodes* are in the majority. Both of these populations of people of any age groups are considered to be healthy. However, in older, frail, unhealthy individuals, the predominant flora consisted of various species of the genera *Oscillobacter* and *Alistipes*, suggesting such a flora to be indicative of an unhealthy condition. It has also been demonstrated that diet plays a major role in the composition of the intestinal flora and that this flora can be altered within a few days of dietary changes. Indeed, a predominantly Western-type diet has been shown to alter the intestinal flora, which have been suggested to have serious health consequences, leading to conditions such as type 2 diabetes and obesity.

CLINICAL CORRELATIONS

1. It is interesting to note that human breast milk contains nutrients, such as some oligosaccharides, that neonates are unable to digest. Instead it is now believed that these oligosaccharides are actually prebiotics that are designed to sustain the intestinal flora of the newborn.
2. Recent reports have demonstrated that artificial sweeteners have a deleterious effect on the intestinal microbial flora, altering it to present an unhealthy state. These changes to the flora disposed the individuals to obesity as well as to glucose intolerance, a condition that is considered to be a precursor of type 2 diabetes.

Regulation of Appetite

It may appear strange to discuss the regulation of appetite following the description of the colon, but the entire GI tract appears to have roles in this sensation; therefore, it is advisable to understand the structure and function of almost every region of the intestinal tract before treating appetite control.

The control of appetite is a multifactorial process and it has psychological, physical, microbial, and physiological components.

- The psychological component involves the smell, sight, and thoughts of food, but these may be superseded by the physical and physiological components.
- The physical components involve an empty stomach or duodenum that induces a feeling of hunger, whereas a full stomach or duodenum induces a feeling of satiety and a lack of desire for food intake.
- The physical components are really controlled by physiological factors, such as blood nutrient levels;

therefore, the following section concentrates on the physiological aspect of appetite regulation, which involves neuronal and hormonal factors.

- Although the microbial component is not understood, it has been demonstrated that the microbiota residing in the lumen of an individual's colon has a direct influence on an individual's amount of body fat accompanied by a decrease in the blood concentration of hormones, specifically PYY and GLP-1, that are known to increase food intake. Manipulation of the microbiota, specifically by the administration of probiotics and prebiotics, shifts the microbiota reducing the individual's body fat by altering the release of the appropriate DNES-manufactured hormones.

Neuronal Factors of Appetite Control

It has been demonstrated in mice that neurons located in the bed nucleus of the stria terminalis (BNST) project to nuclei of the lateral hypothalamus (the feeding center) whose signals initiate feeding behavior. Conversely, if the BNST neurons stop signaling the nuclei of the lateral hypothalamus, the feeding behavior stops, and the animals stop eating. Unfortunately, at this point it, is not known how the BNST neurons are activated or silenced.

The control of appetite has both positive signals and negative feedback signals. The latter is under the control of the vagus nerve (cranial nerve X), which senses the amount of food in the small intestine and the length of time that the ingested food spends in the various regions of the alimentary canal. The sensory fibers of the vagus provide information to the dorsal vagal complex to provide a sensation of satiety and inhibit further intake of food.

Hormonal Factors of Appetite Control

Most of the hormones that control the appetite are manufactured by the DNES cells of the GI tract. These hormones control not only food intake, but also many regulate the expenditure of energy as well as additional functions of the digestive tract. See Table 17–2 for the list of hormones produced the DNES cells of the GI tract and their functions, including their affects on appetite.

Insulin, produced by the beta cells of the Islets of Langerhans in the pancreas, function in the control of blood glucose level (see Chapter 18) but also reduce appetite. The hormone leptin, produced by adipocytes (and to a limited extent by P/D1 cells [Gr cells] of the stomach and small intestine), inhibits hunger sensation, thereby reducing food intake.

Rectum and Anal Canal

Histologically, the **rectum** resembles the colon, but the crypts of Lieberkühn are deeper and number fewer per unit area (see Table 17–3).

The **anal canal**, the constricted continuation of the rectum, is about 3 to 4 cm long. Its crypts of Lieberkühn are short and few and are no longer present in the distal half of the canal. The mucosa also displays longitudinal folds, the **anal columns (rectal columns of Morgagni)**. These meet one another to form pouch-like outpocketings, the **anal valves** with intervening **anal sinuses**. The anal valves assist the anus in supporting the column of feces.

Anal Mucosa

The **epithelium** of the anal mucosa is *simple cuboidal* from the rectum to the **pectinate line** (at the level of the anal valves), *stratified squamous nonkeratinized* from the pectinate line to the external anal orifice, and *stratified squamous keratinized* (epidermis) at the anus. The **lamina propria**, a fibroelastic connective tissue, houses **anal glands** at the rectoanal junction and **circumanal glands** at the distal end of the anal canal. Additionally, hair follicles and sebaceous glands are present at the anus. The **muscularis mucosae** is composed of an inner circular layer and an outer longitudinal layer of smooth muscle. These muscular layers do not extend beyond the pectinate line.

Anal Submucosa and Muscularis Externa

The **submucosa** of the anal canal is composed of fibroelastic connective tissue. It houses two venous plexuses, the **internal hemorrhoidal plexus**, situated above the pectinate line, and the **external hemorrhoidal plexus**, located at the junction of the anal canal with its external orifice, the **anus**.

The **muscularis externa** consists of an inner circular and an outer longitudinal smooth muscle layer. The inner circular layer becomes thickened as it encircles the region of the pectinate line to form the **internal anal sphincter muscle**. The smooth muscle cells of the outer longitudinal layer continue as a fibroelastic sheet surrounding the internal anal sphincter.

Skeletal muscles of the floor of the pelvis form an **external anal sphincter muscle** that surrounds the fibroelastic sheet and the internal anal sphincter. The external sphincter is under voluntary control and exhibits a constant tonus.

Appendix

The histological appearance of the appendix resembles that of the colon, except it is much smaller in diameter, has a richer supply of lymphoid elements, and contains many more DNES cells in its crypts of Lieberkühn.

The **vermiform appendix** is a 5- to 6-cm-long diverticulum of the cecum with a stellate lumen that usually is occupied by debris. The mucosa of the appendix is composed of a simple columnar epithelium, consisting of surface absorptive cells, goblet cells, and M cells

CLINICAL CORRELATIONS

An increase in the size of the vessels of the submucosal venous plexuses of the anal canal results in the formation of **hemorrhoids**, a condition common in pregnancy and in people older than 50 years. This condition may manifest as painful defecation, appearance of fresh blood with defecation, and anal itching.

As a **rectal examination** is performed by insertion of the index finger through the external anal orifice, the external anal sphincter tightens around the finger. Continued penetration results in activation of the internal anal sphincter, which also tightens around the finger. In males, structures that may be palpated through the anal canal include the bulb of the penis, the prostate, enlarged seminal vesicles, the inferior aspect of the distended bladder, and enlarged iliac lymph nodes; in females, palpable structures include the cervix of the uterus and, in pathological conditions, the ovaries and broad ligament.

where lymphoid nodules adjoin the epithelium (see Table 17–3). The lamina propria is a loose connective tissue with numerous lymphoid nodules and shallow crypts of Lieberkühn. The cells composing these crypts are surface absorptive cells, goblet cells, regenerative cells, numerous DNES cells, and infrequent Paneth cells. The muscularis mucosae, submucosa, and muscularis externa do not deviate from the general plan of the alimentary canal, although lymphoid nodules and occasional fatty infiltration are present in the submucosa. The appendix is invested by a serosa.

Function of the Appendix

Until recently, it has been suggested that the appendix is a vestigial organ with no known functions. Recent studies have suggested otherwise. It is now believed that the lumen of this slender structure houses bacterial biofilm of colonies that represent the normal intestinal flora, and, in the case of pathogenic invasion that annihilates much of the individual's intestinal bacteria, colonies sequestered in the appendix may have the capability of restoring the person's normal flora, thus repairing the biological damage.

CLINICAL CORRELATIONS

The incidence of inflammation of the appendix, **appendicitis**, is greater in teenagers and young adults than in older people; it also occurs more frequently in males than in females. Appendicitis usually is caused by obstruction of the lumen, which results in inflammation accompanied by swelling and an unremitting, severe pain in the lower right quadrant of the abdomen. Additional clinical signs are nausea and vomiting, fever (usually below 102° F), tense abdomen, and an elevated leukocyte count. If the condition is not treated within 1 to 2 days, the appendix may rupture, leading to the onset of peritonitis, which may result in death if untreated.

PATHOLOGICAL CONSIDERATIONS

See Figures 17–26 through 17–29.

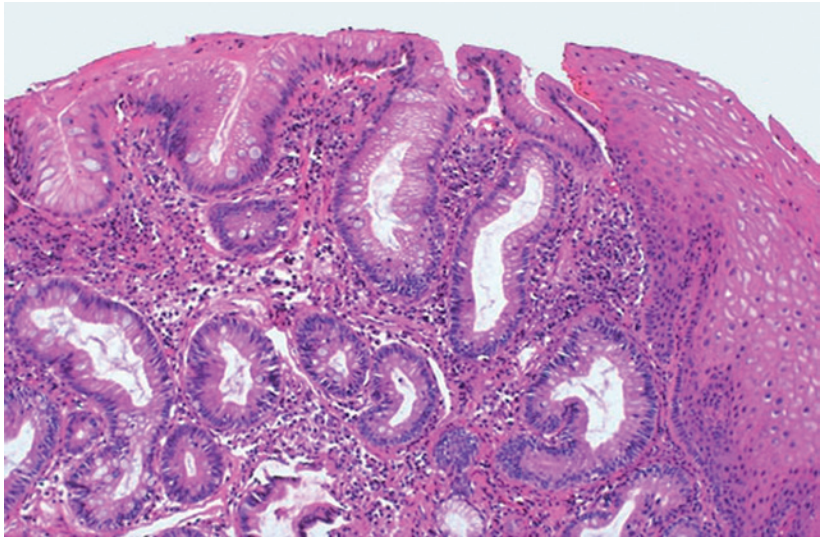


Figure 17–26 Photomicrograph of Barrett esophagus, a condition caused by long-term gastroesophageal reflux in which the esophageal epithelium changes from the normal stratified squamous epithelium (on the right side of the image) to an abnormal columnar epithelium (left side of the image). The epithelium, in this particular patient, displays the presence of goblet cells, reminding one of intestinal epithelium. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:173.)

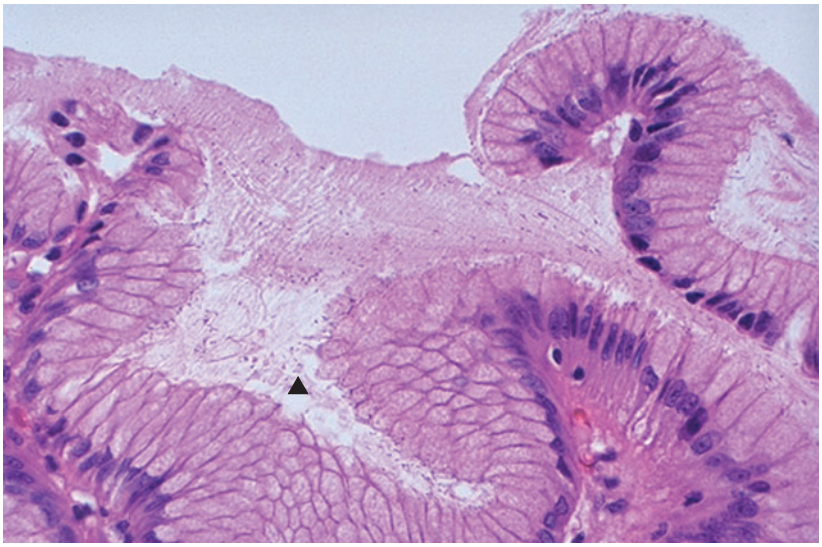


Figure 17–27 Photomicrograph of a stomach whose visible mucous houses small, spiral Gram-negative rods, *Helicobacter pylori*. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:179.)

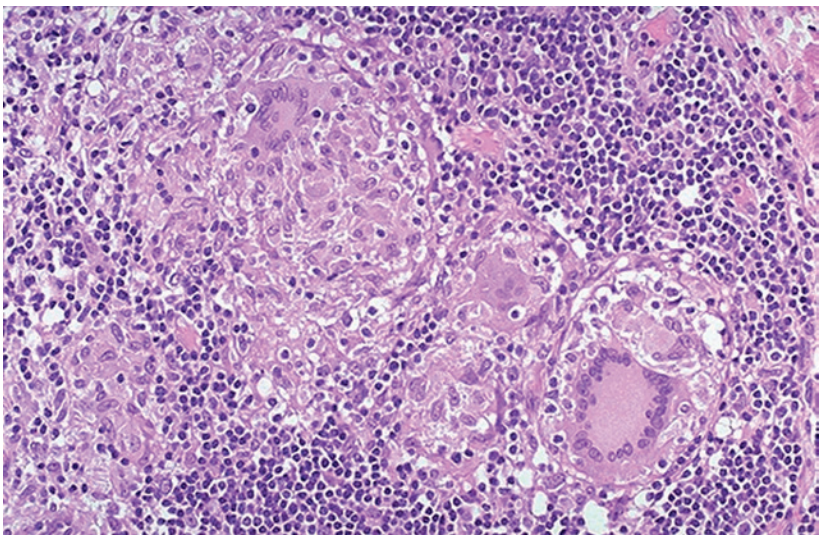


Figure 17–28 Photomicrograph of a patient afflicted with Crohn disease. Note that the inflammation has granulomatous characteristics with the presence of many lymphocytes as well as giant and epithelioid cells. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:192.)

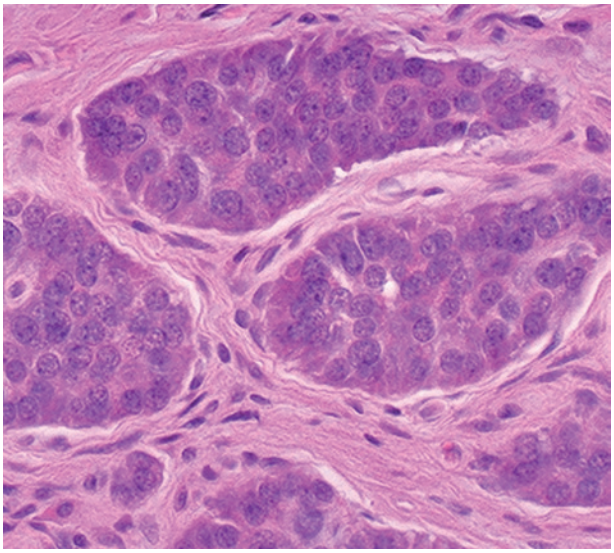
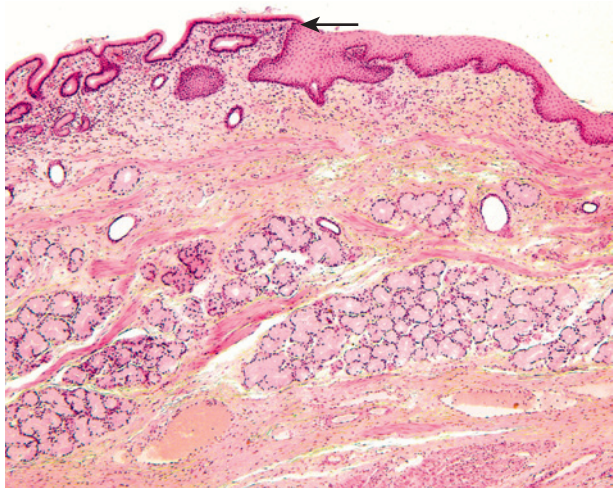


Figure 17–29 Photomicrograph of a patient afflicted with carcinoid tumor of the small intestine. Note that the tumor is composed of clusters of endocrine-resembling small, round cells with round nuclei. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Pathological Basis of Disease*. 2nd ed. Philadelphia: Elsevier; 2010:208.)

REVIEW QUESTIONS

1. A patient presents with Barrett syndrome. The physician should be examining his
   a. esophagus.
   b. stomach.
   c. small intestine.
   d. colon.
   e. rectum.
2. A patient with pernicious anemia has vitamin B_{12} deficiency. The reason for the deficiency is lack of gastric intrinsic factor (GIF). The cell that secretes GIF is
   a. surface-lining cell.
   b. goblet cell.
   c. chief cell.
   d. parietal cell.
   e. mucous neck cell.
3. Ulcers are caused primarily by
   a. *Helicobacter* spp.
   b. *Streptobacillus* spp.
   c. *Staphylococcus* spp.
   d. *Escherichia* spp.
   e. *Campylobacter* spp.
4. An old patient has a low-grade fever, copious severe abdominal cramps, and a copious watery diarrhea. The physician believes his condition to be pseudomembranous colitis, a bacterial infection caused by
   a. *Clostridium* spp.
   b. *Streptobacillus* spp.
   c. *Staphylococcus* spp.
   d. *Escherichia* spp.
   e. *Campylobacter* spp.
5. A patient who has appendicitis will not have which one of the following symptoms?
   a. Pain in the lower right quadrant of the abdomen
   b. Nausea
   c. Vomiting
   d. Fever
   e. Lymphocytopenia
6. The parietal cells of the fundic stomach
   a. manufacture visible mucus.
   b. absorb vitamin B_{12}.
   c. require cholecystokinin to manufacture HCl.
   d. require histamine to manufacture HCl.
   e. require secretin to manufacture HCl.
7. Paneth cells are
   a. present in the crypts of Lieberkuhn of the colon.
   b. are antigen presenting cells.
   c. manufacture and release chondroitin sulfate.
   d. are DNES (diffuse neuroendocrine system) cells.
   e. manufacture and release lysozymes.
8. Long-chain fatty acids are
   a. reesterified in the cisterna of the RER.
   b. reabsorbed by active transport into the surface absorptive cells.
   c. transported into the capillaries of the villus.
   d. emulsified in the lumen of the duodenum by bile.
   e. normally eliminated as a part of feces.
9. Which of the following cells is present in the mucosa of the stomach and manufactures visible mucus?
   a. Surface-lining cells
   b. Mucous neck cells
   c. Parietal cells (oxyntic cells)
   d. M cells
   e. Chief cells
10. 

    The tip of the arrow indicates the junction of the
    a. rectum and the anus.
    b. rectum and the colon.
    c. stomach and the duodenum.
    d. stomach and the esophagus.
    e. esophagus and the pharynx.

ANSWERS

1. A. A patient with Barrett syndrome has a premalignant condition that involves the lower portion of his esophagus, whose stratified squamous epithelium has been replaced by a simple cuboidal epithelium. The stomach, small intestine, colon, and rectum are not involved.
2. D. Gastric intrinsic factor, manufactured by parietal cells of the stomach, facilitates the absorption of vitamin B_{12} from the ileum. Surface-lining cells, goblet cells, and mucous neck cells manufacture mucus. Chief cells manufacture enzymes pepsinogen.
3. A. Ulcers are usually caused by *Helicobacter pylori*, an organism that lives in the mucus layer of the stomach lining. *Streptobacillus* spp. causes rat bite fever, *Staphylococcus* spp. is responsible for varied problems such as skin lesions, toxic shock syndrome, food poisoning, pericarditis, infections of prosthetic devices. *Escherichia* spp. is responsible for diarrhea, nosocomial infections, and urinary tract infections. *Campylobacter* spp. is the cause of gastroenteritis.
4. A. Pseudomembranous colitis, an inflammatory disease of the bowel, may be caused by mercury poisoning, intestinal ischemia, or bronchopneumonia, but, most frequently, it follows prolonged antibiotic therapy that disrupts the intestinal flora, permitting *Clostridium difficile* to flourish, causing the disease. *Streptobacillus* spp. causes rat bite fever. *Staphylococcus* spp. is responsible for varied problems such as skin lesions, toxic shock syndrome, food poisoning, pericarditis, and infections of prosthetic devices. *Escherichia* spp. is responsible for diarrhea, nosocomial infections, and urinary tract infections. *Campylobacter* spp. is the cause of gastroenteritis.
5. E. Patients with appendicitis usually have an increased white blood cell count, not lymphocytopenia. Pain is in the lower right abdominal quadrant, nausea, vomiting, and low fever—below 102° F—are the usual signs of appendicitis.
6. D. Parietal cell (oxyntic cells) of the fundic stomach require histamine, gastrin, and acetylcholine to manufacture hydrochloric acid. Visible mucus is manufactured by surface-lining cells. Vitamin B_{12} is absorbed by surface absorptive cells in the ileum. Cholecystokinin is required for the release of pancreatic enzymes as well as for the contraction of the gallbladder. Secretin is required by the centroacinar cells of the exocrine pancreas to release their bicarbonate-rich medium.
7. E. Paneth cells are located at the base of the crypts of Lieberkuhn of the small intestine and possess large, eosinophilic granules in their cytoplasm. These granules contain an antibacterial agent known as *lysozyme*. Paneth cells do not present antigens and do not manufacture chondroitin sulfate, and they are not members of the diffuse neuroendocrine system of cells (DNES cells).
8. D. Long-chain fatty acids are emulsified in the lumen of the duodenum by bile. Fatty acids and glycerol are reesterified in the smooth endoplasmic reticulum, not in the rough endoplasmic reticulum. Because they are lipids, they can pass through the cell membrane without the need for a carrier protein or an expenditure of energy. They are packaged in the surface absorptive cells into lipoproteins that form chylomicrons and are released into the lamina propria of the villus to enter the lacteals. They are not eliminated as part of feces, although feces do contain about 5% fat.
9. A. Visible mucus is manufactured by the surface-lining cells of the stomach, whereas soluble mucus is manufactured by the mucous neck cells. Parietal cells manufacture HCl as well as gastric intrinsic factor. M cells are antigen-transporting cells that take antigens from the lumen of the gut and deliver them into the lamina propria to the interface of the epithelium with lymphoid nodules. Chief cells synthesize and release pepsinogen.
10. D. The tip of the arrow indicated the junction of the esophagus and the stomach (the gastroesophageal junction). The rectum and the anus have a similar epithelial junction, but the rectum displays crypts of Lieberkuhn. The rectum and the colon both display a simple columnar epithelium. The stomach and the duodenum display villi in the duodenum. The esophagus and larynx both display stratified squamous epithelia.

Digestive System: Glands

18

Extramural glands of the digestive system include the major salivary glands associated with the oral cavity (parotid, submandibular, and sublingual glands), the pancreas, and the liver and gallbladder. Each of these glands has numerous functions aiding the digestive process, and their secretory products are delivered to the lumen of the alimentary tract by a system of ducts.

Saliva produced by salivary glands facilitates the process of tasting food, initiate its digestion, and permit its deglutition (swallowing). These glands also protect the body by secreting the antibacterial agents lysozyme and lactoferrin as well as the secretory immunoglobulin IgA.

The pancreas manufactures a bicarbonate-rich fluid that buffers the acid chyme that the stomach releases into the duodenum and produces enzymes necessary for the digestion of fats, proteins, and carbohydrates. The exocrine secretions of the pancreas are released into the lumen of the duodenum as necessary. In addition, the islets of Langerhans of the pancreas synthesize and release endocrine hormones, including insulin, glucagon, somatostatin, gastrin, and pancreatic polypeptide.

Bile, the exocrine secretion of the liver, is required for proper absorption of lipids, whereas many of the liver's endocrine functions are essential for life. These functions include metabolism of proteins, lipids, and carbohydrates; synthesis of blood proteins and coagulation factors; manufacture of vitamins; and detoxification of blood-borne toxins. The gallbladder concentrates bile and stores it until its release into the lumen of the duodenum.

MAJOR SALIVARY GLANDS

There are three pairs of major salivary glands: parotid, sublingual, and submandibular.

The major salivary glands are the paired parotid, sublingual, and submandibular glands. They are branched **(compound) tubuloacinar glands** whose connective tissue capsule provides septa that subdivide the glands into lobes and lobules. Individual acini are also invested by thin connective tissue elements. The vascular and neural components of the glands reach the secretory units via the connective tissue framework.

Anatomy of Salivary Glands

Each of the major salivary glands has a secretory and a duct portion (Fig. 18–1).

Secretory Portions

The secretory portions of salivary glands are composed of serous and/or mucous secretory cells arranged in acini or tubules that are couched by myoepithelial cells.

The **secretory portions**, arranged in tubules and acini, are composed of three types of cells:

- **Serous cells** are seromucous cells because they secrete both proteins and a considerable amount of polysaccharides. These cells resemble truncated pyramids and have single, round, basally located nuclei, a well-developed rough endoplasmic reticulum (RER) and Golgi complex, numerous basal mitochondria, and abundant apically situated secretory granules rich in **salivary amylase (ptyalin)**, which they secrete along with, **secretory component** (of the secretory IgA molecule), **lactoferrin**, **lysozyme**, and **thiocyanate**. The basal aspects of the lateral cell membranes form tight junctions with each other. Apical to the tight junctions, intercellular canaliculi communicate with the lumen. The plasmalemma basal to the tight junctions forms many processes that interdigitate with those of neighboring cells.
  - **Salivary amylase** is an enzyme that initiates the digestion of complex carbohydrates into sugars.
  - The basal aspect of the serous cells possesses **IgA dimer receptor molecules** that bind IgA dimers manufactured by plasma cells located in the connective tissue. The bound **IgA dimer–IgA dimer receptor molecule complex** becomes internalized into the serous cell and is transferred into early endosomes, where a portion of the receptor molecule is cleaved, and the remainder of the receptor molecule, known as the ***secretory component***, remains attached to the IgA dimer, which becomes known as ***secretory IgA (sIgA)***. The sIgA is released into the saliva, where the secretory component protects it from enzymatic digestion while the immunoglobulin is still capable of combating antigens.
  - **Lactoferrin**, **lysozyme**, and **thiocyanate ions** control the microbial levels of the oral cavity (see later text).
- **Mucous cells** are similar in shape to the serous cells. Their nuclei are also basally located but are flattened instead of being round (Fig. 18–2). The organelle population of these cells differs from that of the serous cells in that mucous secretory cells have fewer

SUMMARY

The **extramural glands** of the digestive system produce materials that aid the digestive process and deliver them to the oral cavity and the duodenum. The extramural glands are the **major salivary glands (parotid**, **submandibular**, and **sublingual glands)** whose ducts lead into the oral cavity and the **pancreas**, **liver** (and its associated **gallbladder**) whose **common bile duct** enters the duodenum. The salivary glands manufacture **saliva** that not only moistens the masticated food but also produces enzymes that begin the digestive process. The pancreas is both an exocrine gland that produces **enzymes** that digest fats, proteins, and carbohydrates and a **bicarbonate-rich fluid** that neutralizes the acidic chyme, as well as endocrine gland that manufactures a series of **hormones**, including **insulin**, **gastrin**, and **glucagon**. The exocrine secretion of the liver, known as ***bile***, is stored and concentrated in the gallbladder until it is needed by the duodenum, where bile emulsifies lipids so that they can be digested. The endocrine secretions of the liver enter the vascular system and are necessary to maintain the life of the individual. The liver also functions in **detoxifying** blood-borne toxins, manufacturing factors required for **coagulation**, producing protein components of blood as well as **vitamins** necessary for life.

KEY WORDS

Bicarbonate-rich fluid; bile; coagulation factors; detoxification; enzymes; extramural glands; gallbladder; gastrin; glucagon; hormones; insulin; liver; major salivary glands; pancreas; parotid gland; sublingual gland; submandibular gland; vitamins

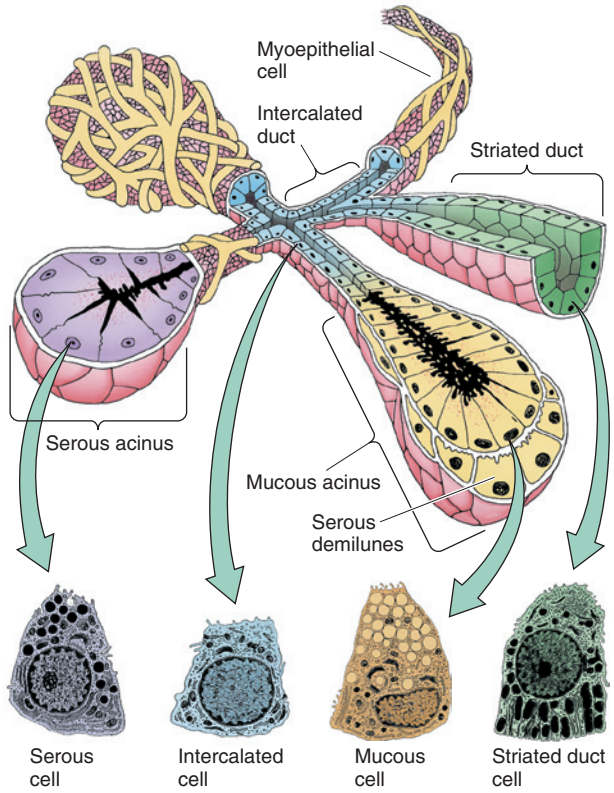


Figure 18–1 Schematic diagram of salivary gland acini, ducts, and cell types.

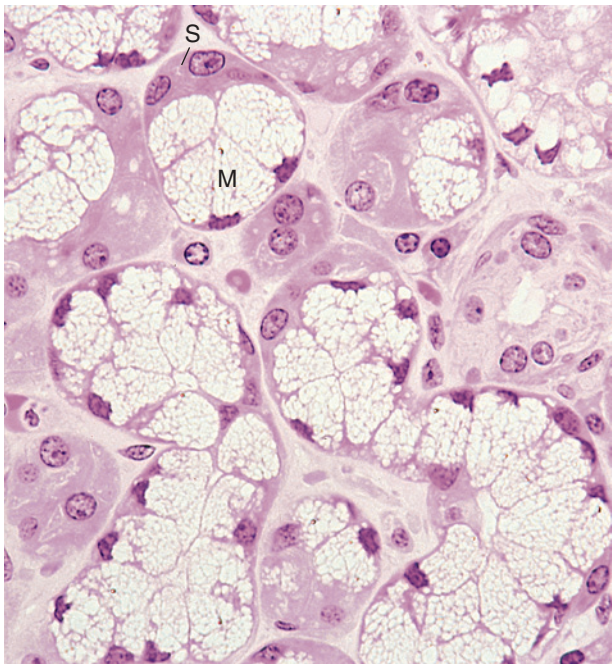


Figure 18–2 Photomicrograph of the monkey sublingual gland displaying mucous acini (M) with serous demilunes (S). Note that serous demilunes may be fixation artifacts (×540).

mitochondria, a less extensive RER, and a considerably greater Golgi apparatus, indicative of the greater carbohydrate component of their secretory product (Fig. 18–3). The apical region of the cytoplasm is occupied by abundant secretory granules housing **mucinogen**, which, when released into the ducts of the gland, becomes hydrated and is known as ***mucin***, a slippery, viscous substance. The intercellular canaliculi and processes of the basal cell membranes are much less extensive than those of serous cells. When mucin contacts and is intermixed with substances present in the oral cavity, it becomes known as ***mucus***.

- **Myoepithelial cells (basket cells)** share the basal laminae of the acinar cells. They have a cell body and several long processes that envelop the secretory acinus and intercalated ducts (see Fig. 18–1). The cell body houses a small complement of organelles in addition to the nucleus and makes hemidesmosomal attachments with the basal lamina. The cytoplasmic processes, which form desmosomal contacts with the acinar and duct cells, are rich in actin and myosin; in electron micrographs, these processes resemble smooth muscle cells. As the processes of myoepithelial cells contract, they press on the acinus, facilitating release of the secretory product into the duct of the gland.

Duct Portions

The ducts of major salivary glands are highly branched and range from very small intercalated ducts to very large principal (terminal) ducts.

The duct portions of the major salivary glands are highly branched structures. The smallest branches of the system of ducts are the **intercalated ducts**, to which the secretory acini (and tubules) are attached. These small ducts are composed of a single layer of low cuboidal cells and possess some myoepithelial cells. Several intercalated ducts merge with each other to form **striated ducts**, composed of a single layer of cuboidal to low columnar cells (see Fig. 18–1). The basolateral membranes of these cells are highly folded, subdividing the cytoplasm into longitudinal compartments that are occupied by elongated mitochondria. The basolateral cell membranes of these cells have **sodium adenosine triphosphatase (Na^+-ATPase)** that pump sodium out of the cell into the connective tissue, thus conserving these ions and reducing the tonicity of saliva. Striated ducts join with each other, forming **intralobular ducts** of increasing caliber, which are surrounded by more abundant connective tissue elements. Ducts arising

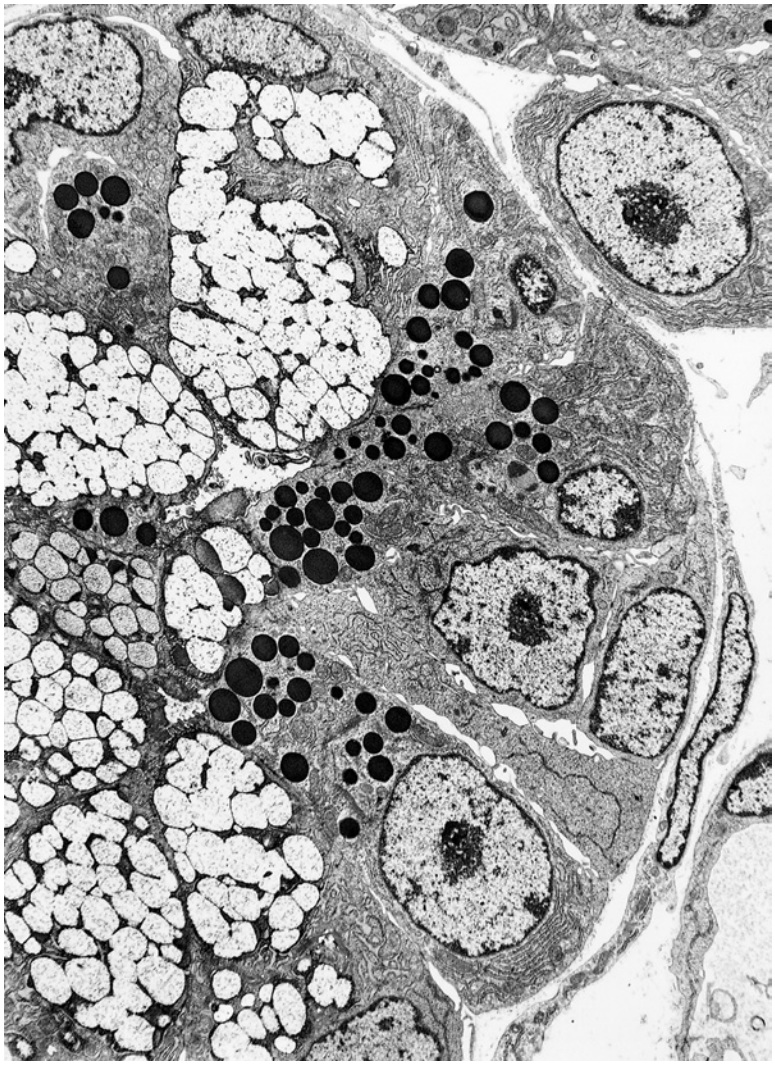


Figure 18–3 Electron micrograph of the rat sublingual gland displaying serous and mucous granules in the cytoplasm of their acinar cells. Note that the nuclei of serous cells are round, whereas the nuclei of mucous cells are flattened. Observe also that the serous secretory products are present as round, dense, dark structures, and the mucous secretory products are mostly dissolved and appear light in color and spongy (×5400). (From Redman RS, Ball WD. Cytodifferentiation of secretory cells in the sublingual glands of the prenatal rat: a histological, histochemical, and ultrastructural study. *Am J Anat*. 1978;153:367-390. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

from lobules unite to form **interlobular ducts**, which, in turn, form **intralobar** and **interlobar ducts**. The **terminal (principal) duct** of the gland delivers saliva into the oral cavity.

Salivon

The acinus, intercalated duct, and striated duct together, according to some authors, constitute the **salivon**, the functional unit of a salivary gland.

Histophysiology of the Salivary Glands

Secretory cells of acini produce primary saliva that is modified by striated ducts to form secondary saliva.

The major salivary glands produce approximately 700 to 1100 mL of saliva per day. Minor salivary glands are located in the mucosa and submucosa of the oral cavity, but they contribute only 3 to 5 mL to the total daily salivary output. To provide such a large salivary output,

the major salivary glands have an extraordinarily rich vascular supply. In fact, it has been estimated that the basal rate of blood flow to salivary glands is 20 times greater than the flow of blood to skeletal muscle. During maximal secretion, the blood flow is correspondingly increased.

Saliva has numerous functions, including lubricating and cleansing of the oral cavity, antibacterial activity, participating in the taste sensation by dissolving food material, initial digestion via the action of salivary amylase (ptyalin) and salivary lipase, aiding swallowing by moistening the food and permitting the formation of bolus, and participating in the clotting process and wound healing because of the clotting factors and epidermal growth factor present in saliva.

The saliva manufactured by the acinar cells, called ***primary saliva***, is isotonic with plasma. The primary saliva, is modified by the cells of the striated ducts by removing sodium and chloride ions from it and secreting potassium and bicarbonate ions into it. Thereafter, the altered secretion, called ***secondary saliva***, is hypotonic.

Acinar cells and duct cells also synthesize the secretory component required to transfer IgA from the connective tissue into the lumen of the secretory acinus or duct (see section on ducts). **Secretory IgA** complexes with antigens in the saliva, mitigating their deleterious effects. Saliva also contains lactoferrin, lysozyme, and thiocyanate ions. **Lactoferrin** binds iron, an element essential for bacterial metabolism; **lysozyme** breaks down bacterial capsules, permitting the entry of **thiocyanate ions**, a bactericidal agent, into the bacteria.

The cells of the salivary gland striated ducts secrete the enzyme **kallikrein** into the connective tissue. Kallikrein enters the bloodstream, where it converts kininogens, a family of plasma proteins, into **bradykinin**, a vasodilator that dilates blood vessels and enhances blood flow to the region.

Role of Autonomic Nerve Supply in Salivary Secretion

The major salivary glands do not secrete continuously. Secretory activity is stimulated via **parasympathetic** and **sympathetic innervation**. Innervation may be intraepithelial (i.e., formation of a synaptic contact between the end-foot and acinar cell) or subepithelial. In subepithelial innervation, the end-feet of axons do not make synaptic contact with the acinar cells; instead, they release their acetylcholine in the vicinity of the secretory cell, at a distance of approximately 100 to 200 nm from its basal plasmalemma. The cell, thus activated, stimulates neighboring cells via **gap junctions** to release their serous secretory product into the lumen of the acinus.

Parasympathetic innervation is the major initiator of salivation and is responsible for the formation of a serous saliva. Acetylcholine, released by the postganglionic parasympathetic nerve fibers, binds to muscarinic cholinergic receptors with consequent release of inositol triphosphate. This effects the liberation of calcium ions, a second messenger, into the cytosol, which facilitates the secretion of serous saliva from the acinar cells.

Initially, **sympathetic innervation** reduces blood flow to the salivons, but that reduction is reversed in short order. Norepinephrine, released by the postganglionic sympathetic fibers, binds to β-adrenergic receptors, resulting in the formation of **cyclic adenosine monophosphate (cAMP, cyclic AMP)**. This secondary messenger activates a cascade of kinases that result in the secretion of the mucous and enzymatic components of saliva by the acinar cells. The mucous element is responsible for the adhesion of food particles to one another in the bolus as well as for the creation of a slippery surface, facilitating swallowing.

Salivary output is enhanced by the taste and smell of food as well as by the process of chewing. Inhibitors of salivation include fatigue, fear, and dehydration; moreover, salivary flow is greatly reduced while the individual is asleep.

Properties of Individual Salivary Glands

Parotid Gland

Although physically the largest of the salivary glands, the two parotid glands produce only about 30% of the total salivary output; the saliva that they produce is serous.

The two **parotid glands**, the largest of the salivary glands, weigh about 20 to 30 g each but produce only approximately 30% of the total output of saliva. Although the parotid gland is said to produce a purely **serous secretion**, the secretory product does have a mucous component. Electron micrographs of the apical regions of serous cells display numerous secretory granules filled with an electron-dense product that has an even more electron-dense core of unknown composition.

The saliva produced by the parotid gland has high levels of the enzyme **salivary amylase (ptyalin)** and secretory IgA. Salivary amylase is responsible for digestion of most of the starch in food, and this digestion continues in the stomach until the acidic chyme inactivates the enzyme. Secretory IgA inactivates antigens located in the oral cavity.

The connective tissue capsule of the parotid gland is well developed and forms numerous septa, which subdivide the gland into lobes and lobules. The duct system follows the distribution detailed earlier. By the 40th year of age, the gland becomes invaded by adipose tissue, which spreads from the connective tissue into the glandular parenchyma.

Sublingual Gland

The paired sublingual glands are very small, are composed mostly of mucous acini with serous demilunes, and produce a mixed saliva.

Each of the paired **sublingual glands**, the smallest of the three major salivary glands, is almond shaped and weighs only 2 to 3 g, and the pair produces only about 5% of the total salivary output. The gland is composed of mucous tubular secretory units, many of which are capped by a small cluster of serous cells, known as ***serous demilunes*** (see Fig. 18–2). Although routine light microscopy demonstrates the presence of serous demilunes, if the tissue is flash frozen, serous demilunes are absent, indicating that they may be fixation artifacts and are merely small clusters of serous cells that deliver their secretion into a lumen in common with the mucous tubular secretory units. It appears that, under normal fixation at room temperature, the mucinogen of the mucous cells become swollen, and they enlarge the cells containing them to such an extent that they squeeze most of the serous cells away from the lumen of the acinus; thus, the serous cells form a cap between the swollen mucous cells and the basement membrane. If the tissue is flash frozen, the mucinogen does not swell and the serous cells remain in their normal position, adjacent to the mucous cells. These serous cells have been shown to secrete lysozyme. The sublingual gland produces a mixed, but mostly mucous, saliva. The intercellular canaliculi are well developed between the mucous cells of the secretory units. Electron micrographs of the cells of the serous demilunes display apical accumulations of secretory vesicles; however, unlike the cells of the parotid and submandibular glands, these vesicles do not have an electron-dense core (see Fig. 18–3).

The sublingual gland has a slender connective tissue capsule, and its duct system does not form a terminal duct. Instead, several ducts open into the floor of the mouth and into the duct of the submandibular gland. Because of the organization of the ducts, some authors consider the sublingual gland to be composed of several smaller glandular subunits.

Submandibular Gland

The paired submandibular glands produce 60% of the total salivary output; although these glands manufacture a mixed saliva, the saliva they produce is mostly serous.

The pair of **submandibular glands** (Fig. 18–4), although only 12 to 15 g in weight each, produces approximately 60% of the total salivary output. About 90% of the acini produce serous saliva, whereas the remainder of the acini manufacture a mucous saliva. Electron micrographs of the apical aspects of the serous cells display electron-dense secretory products, with a

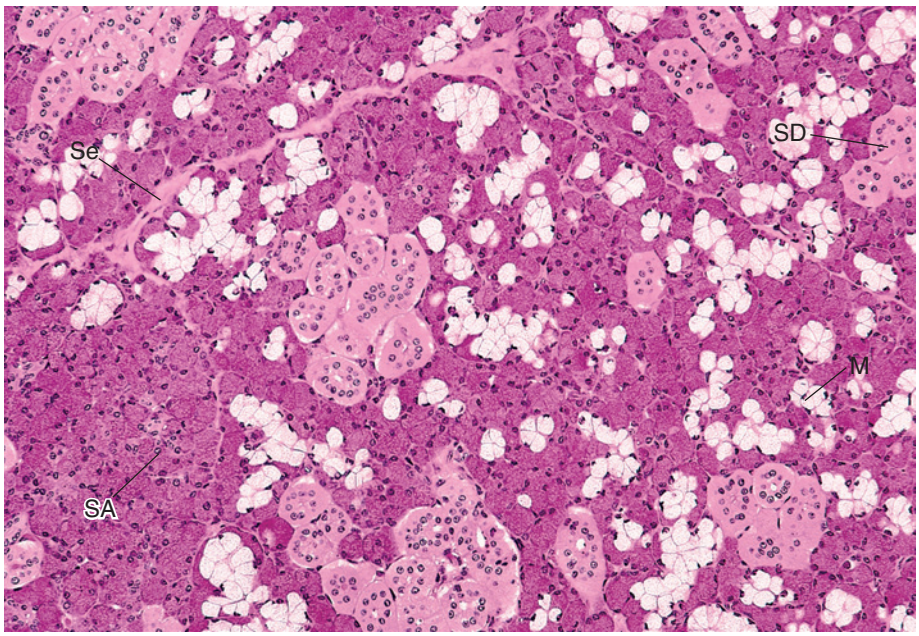


Figure 18–4 Submandibular gland is characterized by the numerous cross-sectional profiles of striated ducts. Note that the ducts appear pale pink in color, and many display a very small but clear lumen. The mucous secretory product has a frothy-looking appearance. Se, Septum; SA, serous acinus; SD, serous demilune; M, mucous cell of the acinus (×132).

denser core, within membrane-bound secretory granules. The number of serous demilunes (see section on the sublingual gland) is limited. The striated ducts of the submandibular gland are much longer than those of the parotid or sublingual glands; therefore, histological sections of this gland display many cross-sectional profiles of these ducts, a characteristic feature of the submandibular gland.

The connective tissue capsule of the submandibular gland is extensive and forms abundant septa, which subdivide the gland into lobes and lobules. Fatty infiltration of the connective tissue elements into the parenchyma is evident by midlife.

CLINICAL CORRELATIONS

1. **Benign pleomorphic adenoma**, a noncancerous salivary gland tumor, usually affects the parotid and the submandibular glands. Surgical removal of the parotid gland must be performed with care because of the presence of the facial nerve plexus within the substance of the gland. Because the facial nerve supplies motor innervation to the muscles of facial expression, damage to its branches during parotidectomy may cause temporary or even permanent paralysis of the muscles served by the damaged fibers.
2. The parotid gland (and occasionally other major salivary glands) is also affected by viral infections, causing **mumps**, a painful disease in children that may result in sterility when it affects adults.
3. About 25% of the cancers involving the parotid gland are considered to be **acinic cell carcinoma**, a slow-growing malignancy that can recur after surgical removal. Occasionally, the carcinoma metastasizes to other organs.

PANCREAS

The pancreas is both an exocrine gland that produces digestive juices and an endocrine gland that manufactures hormones.

The **pancreas**, situated on the posterior body wall, deep to the peritoneum, has four regions: uncinate process, head, body, and tail. It is about 25 cm long, 5 cm wide, and 1 to 2 cm thick, and it weighs approximately 150 g. Its flimsy connective tissue capsule forms septa, which subdivide the gland into lobules. The vascular and nerve supply of the pancreas, as well as its system of ducts, travel in these connective tissue compartments. The pancreas produces exocrine and endocrine secretions. The endocrine components of the pancreas, **islets of Langerhans**, are scattered among the exocrine secretory acini.

Exocrine Pancreas

The exocrine pancreas is a compound tubuloacinar gland that produces daily about 1200 mL of a bicarbonate-rich fluid containing digestive proenzymes. Forty to 50 acinar cells form a round to oval **acinus** whose lumen is occupied by three or four **centroacinar cells**, the beginning of the duct system of the pancreas (Fig. 18–5). The presence of centroacinar cells in the center of the acinus is a distinguishing characteristic of this gland.

Secretory and Duct Portions

*The acinar cells of the pancreas have receptors for the hormone **cholecystokinin (CCK)** and acetylcholine, whereas the centroacinar cells and intercalated ducts have receptors for secretin and for acetylcholine.*

Each **acinar cell** is shaped like a truncated pyramid with its base positioned on the basal lamina separating the acinar cells from the connective tissue compartment. The round nucleus of the cell is basally located and is surrounded by basophilic cytoplasm (Fig. 18–6). The apex of the cell, facing the lumen of the acinus, is filled with proenzyme-containing **secretory granules (zymogen granules)**, whose number diminishes after a meal. The Golgi region, located between the nucleus and the zymogen granules, varies in size in inverse relation to the zymogen granule concentration.

The basal cell membranes of acinar cells have receptors for the hormone **CCK**, released by diffuse neuroendocrine system (DNES) cells of the small intestine, and for the neurotransmitter **acetylcholine**, released by postganglionic parasympathetic nerve fibers. Electron micrographs of acinar cells display an abundance of basally located RER, a rich supply of polysomes, and numerous mitochondria exhibiting matrix granules. The Golgi apparatus is well developed but fluctuates in size, being smaller when the zymogen granules are numerous and larger after the granules release their contents.

The zymogen granules may release their contents individually, or several secretory vesicles may fuse with each other, forming a channel to the lumen of the acinus from the apical cytoplasm.

The **duct system** of the pancreas begins within the center of the acinus with the terminus of the **intercalated ducts**, composed of pale, low cuboidal **centroacinar cells** (see Figs. 18–5 and 18–6). Centroacinar cells and intercalated ducts both have receptors on their basal plasmalemma for the hormone **secretin**, released by DNES cells of the small intestine, and **acetylcholine**, released by postganglionic parasympathetic fibers. Intercalated ducts join each other to form larger **intralobular ducts**, several of which converge to form **interlobular ducts**. These ducts are surrounded by a

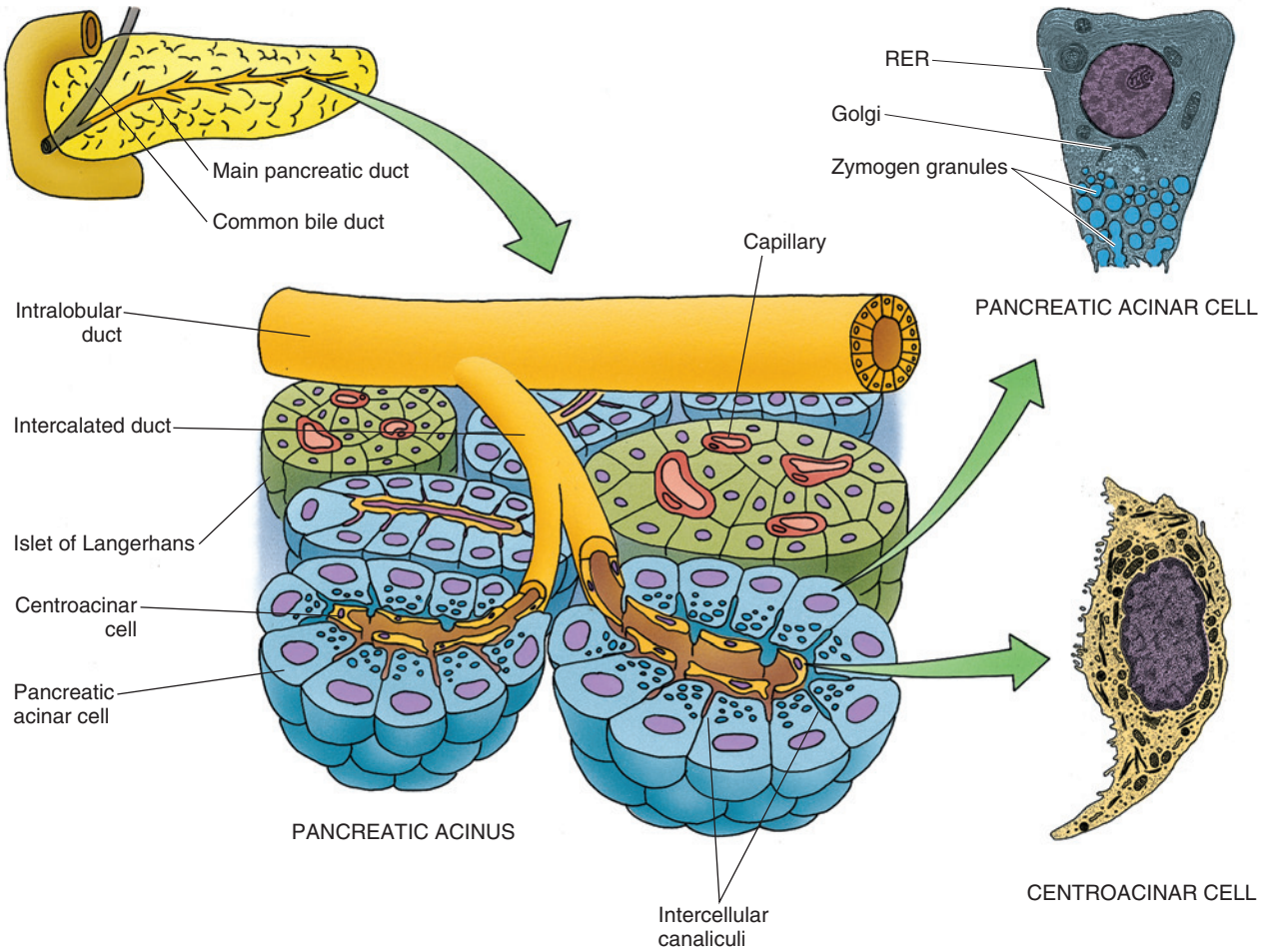


Figure 18–5 Schematic diagram of the pancreas displaying secretory acini, their cell types, and the endocrine islets of Langerhans. RER, Rough endoplasmic reticulum.

considerable amount of connective tissue and deliver their contents into the **main pancreatic duct**, which joins the **common bile duct** before opening in the duodenum at the **papilla of Vater**.

Histophysiology of the Exocrine Pancreas

The acinar cells manufacture and release digestive enzymes, whereas the centroacinar cells and intercalated duct cells release a bicarbonate-rich buffer solution.

The acinar cells of the exocrine pancreas manufacture, store, and release a large number of enzymes: pancreatic amylase, pancreatic lipase, pancreatic cholesterol esterase, ribonuclease (RNase), deoxyribonuclease (DNase), elastase, and the proenzymes trypsinogen, chymotrypsinogen, procarboxypolypeptidase. The cells also manufacture **trypsin inhibitor**, a protein that protects the cell from accidental intracellular activation of trypsin as well as its activation in the pancreatic duct.

- **Pancreatic amylase** breaks down starches, carbohydrates (although it cannot hydrolyze cellulose or chitin), and glycogen into disaccharides.
- **Pancreatic lipase** breaks down fats into fatty acids and monoglycerides.
- **Pancreatic cholesterol esterase** breaks down cholesterol esters into cholesterol and fatty acids.
- **DNase and RNase** break down DNA and RNA, respectively.
- **Elastase** breaks down the major component of elastic fibers, namely elastin.
- The proenzymes trypsinogen, chymotrypsinogen, and procarboxypolypeptidase become converted in the duodenum into trypsin, chymotrypsin, and carboxypolypeptidase.
  - **Trypsin** and **chymotrypsin** break down proteins into short peptides.

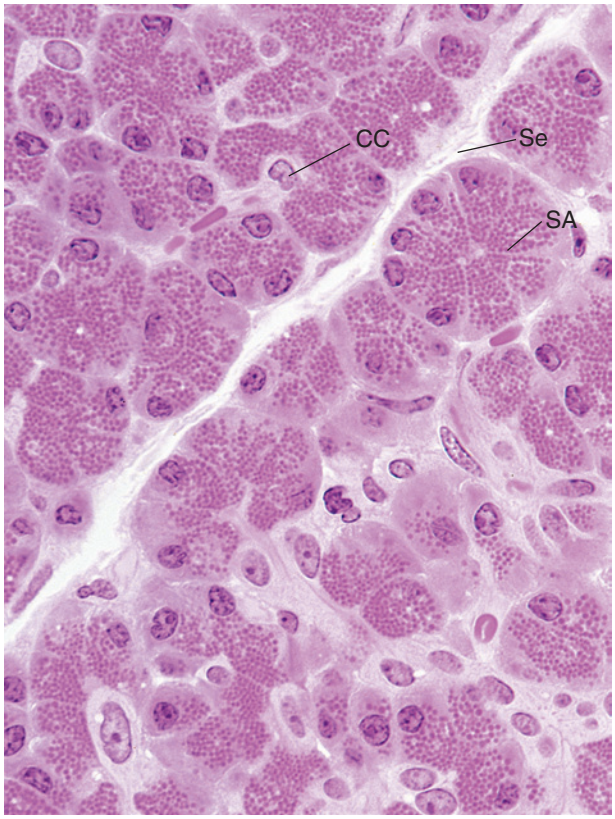


Figure 18–6 Photomicrograph of the monkey exocrine pancreas. Observe that the acini in section appear to be round structures, and much of the acinar cells have many secretory granules, known as *zymogen granules*. CC, Centroacinar cell; Se, septum; SA, serous acinus (×540).

- **Carboxypolypeptidase** breaks down small peptides to form dipeptides and amino acids.

Release of the pancreatic enzymes is effected by the hormone **CCK** manufactured by DNES cells of the small intestine (especially of the duodenum) as well as by **acetylcholine** released by the postganglionic parasympathetic nerve fibers. Both CCK and acetylcholine have to bind to their respective receptors located in the basal plasmalemmae of the pancreatic acinar cells before the enzymes and proenzymes can be released from the acinar cells.

The centroacinar cells and intercalated ducts manufacture a serous, bicarbonate-rich alkaline fluid, which neutralizes and buffers the acid chyme that enters the duodenum from the pyloric stomach. This fluid is enzyme poor, and its release is effected by the hormone **secretin**, produced by enteroendocrine cells of the small intestine in conjunction with **acetylcholine**, released by the postganglionic parasympathetic nerve fibers. Both secretin and acetylcholine have to bind to their respective receptors located in the basal plasmalemmae of the duct cells before the bicarbonate-rich fluid can be released from the duct cells.

Thus, the enzyme-rich and enzyme-poor secretions are regulated separately, and the two secretions may be released at different times or concomitantly.

The assumed mechanism of bicarbonate ion secretion is facilitated by the enzyme **carbonic anhydrase**, which catalyzes the formation of carbonic acid (H_2CO_3) from water (H_2O) and carbon dioxide (CO_2). In the aquatic medium of the cytosol, H_2CO_3 dissociates to form H^+ and HCO_3^-, the HCO_3^- is actively transported into the lumen of the duct, and the hydrogen (H^+) ion is transported into the connective tissue elements.

CLINICAL CORRELATIONS

Occasionally, the pancreatic digestive enzymes become active within the cytoplasm of the acinar cells, resulting in **acute pancreatitis**, which is often fatal. The histological changes involve inflammatory reaction, necrosis of the blood vessels, proteolysis of the pancreatic parenchyma, and enzymatic destruction of adipose cells not only within the pancreas but also in the surrounding region of the abdominal cavity.

Pancreatic cancer is the fifth leading cause of mortality from all cancers, killing about 25,000 people in the United States per year. Fewer than 50% of patients survive more than 1 year, and fewer than 5% survive 5 years. Men are more susceptible to this disease than women. Cigarette smokers have a 70% greater risk for development of pancreatic cancers than nonsmokers.

Endocrine Pancreas

The endocrine pancreas is composed of spherical aggregates of cells, known as islets of Langerhans*, that are scattered among the acini.*

Each **islet of Langerhans** is a richly vascularized spherical conglomeration of approximately 3000 cells. The approximately 1 to 2 million islets distributed throughout the human pancreas constitute the endocrine pancreas. A somewhat greater number of islets are present in the tail than in the remaining regions. Each islet is about 300 μm in diameter and is surrounded by reticular fibers, which also enter the substance of the islet to encircle the network of capillaries that pervade it (Fig. 18–7; see Fig. 18–5).

Cells Composing the Islets of Langerhans

Five types of cells compose the parenchyma of each islet of Langerhans: beta (β) cells, alpha (α) cells, delta (δ) cells (D and D_1 cells), PP cells, and G cells. These cells cannot be differentiated from one another by routine histological examination, but immunocytochemical procedures allow them to be recognized.

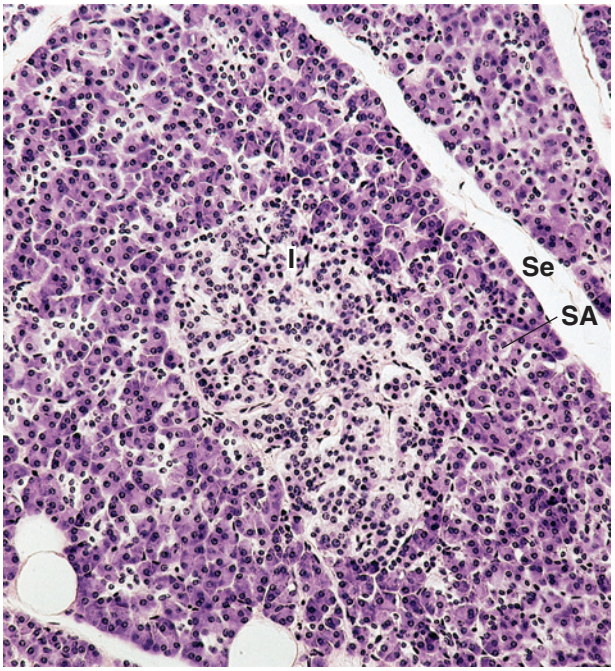


Figure 18–7 Photomicrograph of the human pancreas displaying secretory acini and an islet of Langerhans (I). The histological difference between the exocrine and endocrine pancreas is very evident in this photomicrograph because the Islet is much larger than individual acini and is much lighter in color. Se, Septum; SA, serous acinus (×132).

Electron micrographs also display the features that distinguish the various cells, especially the size and electron density of their granules (Fig. 18–8). Otherwise, the cells do not exhibit any unusual morphological features but resemble cells that specialize in protein synthesis. The characteristic features, locations, and hormones synthesized by these cells are presented in Table 18–1.

Histophysiology of the Endocrine Pancreas

The cells of the islets of Langerhans produce insulin, ghrelin, amylin, glucagon, somatostatin, gastrin, and pancreatic polypeptide.

The two hormones produced in the greatest amounts by the endocrine pancreas—insulin and glucagon—act to decrease and increase blood glucose levels, respectively.

Insulin production begins with synthesis of a single polypeptide chain, **preproinsulin**, on the RER of **β cells**. Within the RER cisternae, this initial product is converted to **proinsulin** by enzymatic cleavage of a polypeptide fragment. Within the *trans* Golgi network, proinsulin is packaged into clathrin-coated vesicles, which lose their clathrin coat as they travel toward the plasmalemma. A segment of the proinsulin molecule near its center is removed by self-excision, thus forming insulin, which is composed of two short polypeptide

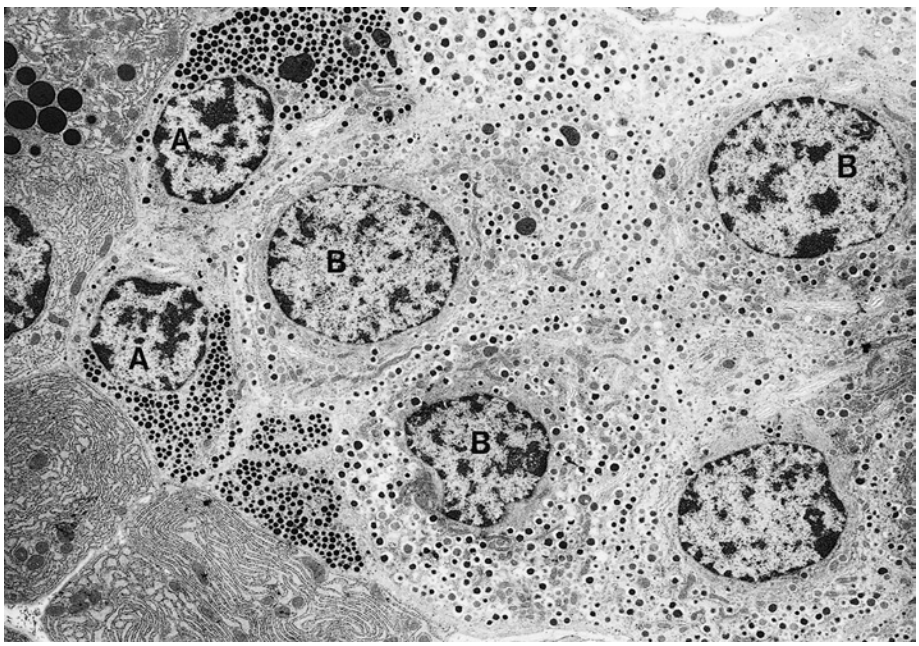


Figure 18–8 Electron micrograph of α cells (A) and β cells (B) of the rabbit islet of Langerhans. Note that the granules of α cells are much more numerous, more tightly packed, smaller, and denser than those of β cells (×5040). (From Jorns A, Grube D. The endocrine pancreas of glucagon-immunized and somatostatin-immunized rabbits. *Cell Tissue Res*. 1991;265:261-273.)

TABLE 18–1 Cells and Hormones of the Islets of Langerhans

Cell	% of Total	Location	Fine Structure of Granules	Hormone and Molecular Weight	Function
β Cell	70	Scattered throughout islet (but concentrated in center)	300 nm in diameter; dense core granule surrounded by a wide electron-lucent halo	Insulin, 6000 Da Amylin, ~3200 Da	Decreases blood glucose levels Inhibits gastric emptying and glucagon release from α cells
α Cell	20	Islet periphery	250 nm in diameter; dense core granule with a narrow electron-lucent halo	Glucagon, 3500 Da	Increases blood glucose levels
δ Cell	5	Scattered throughout islet	350 nm in diameter; electron-lucent homogeneous granule	Somatostatin, 1640 Da	*Paracrine:* inhibits hormone release from endocrine pancreas and enzymes from exocrine pancreas *Endocrine:* reduces contractions of alimentary tract and gallbladder smooth muscles
D D_1				Vasoactive intestinal peptide, 3800 Da	Induces glycogenolysis; regulates smooth muscle tonus and motility of gut; controls ion and water secretion by intestinal epithelial cells
G cell	1	Scattered throughout islet	300 nm in diameter	Gastrin, 2000 Da	Stimulates production of hydrochloric acid by parietal cells of stomach
PP cell (F cell)	1	Scattered throughout islet	180 nm in diameter	Pancreatic polypeptide, 4200 Da	Inhibits exocrine secretions of pancreas
ε Cell (Epsilon cell)	1	Scattered throughout islet	?	Ghrelin	Induces the sensation of hunger and modulates receptive relaxation of the smooth muscle fibers of the muscularis externa of the gastrointestinal tract

chains linked by disulfide bonds. Insulin is released into the intercellular space in response to increased blood glucose levels, as occurs after consumption of a carbohydrate-rich meal.

The released insulin binds to cell-surface insulin receptors on many cells, especially skeletal muscle, liver, and adipose cells. The plasma membranes of these cells also have glucose transport proteins, **glucose transporter protein-4 (GLUT-4)**, which are activated to take up glucose, thus decreasing blood glucose levels. It is interesting that subplasmalemmal vesicles, rich in GLUT-4, are added to the cell membrane during insulin stimulation and returned to their intracellular position when insulin levels are reduced.

Insulin release is induced by

- Elevated blood glucose levels
- Elevated levels of free fatty acids in the blood
- Elevated levels of amino acids in the blood
- Cortisol and growth hormone
- Hormones released by DNES cells of the gastrointestinal (GI) tract: gastric inhibitory peptide, secretin, CCK, and gastrin

- Obesity
- Insulin resistance

Insulin release is inhibited by

- Decreased blood glucose levels
- Leptin
- Somatostatin
- Fasting

Glucagon, a peptide hormone produced by **α cells**, is released in response to low blood glucose levels as well as by the consumption of a meal low in carbohydrate and high in protein. As in insulin production, a prohormone is produced first, and it undergoes proteolytic cleavage to yield the active hormone. Glucagon acts mainly on hepatocytes, causing these cells to activate a cascade of enzymes that eventually leads to the activation of glycogenolytic enzymes. It does that by activating the hepatocyte cell membrane-bound enzyme **adenylyl cyclase** that in turn responds by the formation of the second messenger, **cAMP**. It is the cAMP that activates a **protein kinase regulator protein** that results in the formation of the enzyme **phosphorylase**, which cleaves glycogen into **glucose-1-phosphate (G-1-P)**. The newly formed G-1-P is dephosphorylated, forming glucose, and is released into the bloodstream, increasing blood glucose levels. Glucagon also activates enzymes in fat cells, namely **hormone-sensitive lipase** and **adipose triglyceride lipase**, that break down the stored fats into fatty acids that enter the liver where hepatic enzymes responsible for **gluconeogenesis** (the synthesis of glucose from noncarbohydrate sources) are also activated by glucagon subsequent to the depletion of the intracellular glycogen depot.

Somatostatin, manufactured by one of the two types of **δ cells**, **D cells**, has both paracrine and endocrine effects. The hormone's paracrine effects are to inhibit the release of endocrine hormones by nearby α cells and β cells. Its endocrine effects are on smooth muscle cells of the alimentary tract and gallbladder, reducing the motility of these organs. Somatostatin is released in response to the increased levels of blood glucose, amino acids, or chylomicrons that occur after a meal. Somatostatin also hinders the release of enzymes synthesized by the acinar cells of the pancreas and reduces hydrochloric acid (HCl) production by the parietal cells of the stomach.

Vasoactive intestinal peptide (**VIP**) is produced by the second type of δ cells known as ***D_1 cells***. This hormone induces glycogenolysis and hyperglycemia and regulates intestinal motility and the tone of smooth muscle cells of the wall of the gut. Additionally, VIP also controls the secretion of ions and water by intestinal epithelial cells.

Gastrin, released by **G cells**, stimulates gastric release of HCl, gastric motility and emptying, and the rate of cell division in gastric regenerative cells.

Pancreatic polypeptide, a hormone produced by **PP cells**, inhibits the exocrine secretions of the pancreas and the release of bile from the gallbladder. It also stimulates the release of enzymes by the gastric chief cells while depressing the release of HCl by the parietal cells of the stomach.

Ghrelin, produced by **ε cells (Epsilon cells)**, induces the sensation of hunger and modulates receptive relaxation of the smooth muscle fibers of the muscularis externa of the GI tract

Amylin, a hormone produced by **β cells** and released along with insulin, inhibits emptying of the stomach. It has been suggested that amylin also inhibits the release of glucagon.

Blood Supply of the Pancreas

The arterial blood supply to the pancreas is unusual in that the supply to the exocrine portion and to the endocrine portion of the gland are completely separate. However, the venous drainage is designed so that venous blood from the islets of Langerhans drain directly into the exocrine pancreas, so that the acinar cells have direct access to blood into which the endocrine cells of the islets of Langerhans have delivered their hormones. Therefore, hormones, such as **somatostatin** released by **δ cells** of the islets of Langerhans, can control the secretory function of the acinar cells.

LIVER

The **liver**, weighing approximately 1500 g, is the largest gland in the body. It is located in the upper right-hand quadrant of the abdominal cavity, just inferior to the diaphragm. The liver is subdivided into four lobes—right, left, quadrate, and caudate—of which the first two constitute its bulk (Fig. 18–9A).

Similar to the pancreas, the liver has both endocrine and exocrine functions; unlike the pancreas, however, the same cell (the **hepatocyte**) in the liver is responsible for the formation of the liver's exocrine secretion, **bile**, and its numerous endocrine products. In addition, hepatocytes convert noxious substances into nontoxic materials that are excreted in bile.

General Hepatic Structure and Vascular Supply

The inferior, concave aspect of the liver houses the porta hepatis, through which the portal vein and hepatic artery bring blood into the liver and through which the bile ducts drain bile from the liver.

With the exception of the bare area, the liver is completely enveloped by peritoneum, which forms a simple squamous epithelium covering over the dense, irregular collagenous connective tissue **capsule (Glisson capsule)** of the gland. Glisson capsule is loosely attached

CLINICAL CORRELATIONS

1. Diabetes mellitus is a hyperglycemic metabolic disorder that results from (1) lack of insulin production by β cells of the islets of Langerhans or (2) defective insulin receptors on the target cells. There are two major forms of diabetes mellitus, **type 1** and **type 2** (Table 18–2). The incidence of type 2 is about five to six times that of type 1. If uncontrolled, both types of diabetes may have debilitating sequelae, including circulatory disorders, renal failure, blindness, gangrene, stroke, and myocardial infarcts. The most significant laboratory result indicative of diabetes is elevated blood glucose levels after an overnight fast.

Type 1 diabetes (insulin-dependent diabetes; juvenile-onset diabetes) usually affects persons younger than 20 years of age. It is characterized by the three cardinal signs of **polydipsia** (constant thirst), **polyphagia** (undiminished hunger), and **polyuria** (excessive urination). It has been demonstrated in genetically susceptible mice that too much **zonulin**, a haptoglobin-2 precursor produced by epithelial cells of the small intestine that temporarily opens tight junctions of the intestinal surface absorptive cells, is formed thereby permitting macromolecules of the intestinal lumen to use the paracellular route to enter the lamina propria. Once there, the immune reaction forms antibodies against them, and some of these can attack **β cells** of the **islets of Langerhans**, resulting in type 1 diabetes and other autoimmune diseases such as celiac disease.

Type 2 diabetes (non–insulin-dependent diabetes) is the *most common* and usually affects persons older than 40 years of age.

2. Verner-Morrison syndrome (pancreatic cholera) is characterized by explosive, watery diarrhea that results in hypokalemia (reduced potassium levels) and hypochlorhydria (reduced chloride levels). It is caused by the excessive manufacture and release of vasoactive intestinal peptide due to the adenoma of the D_1 cells that produce this hormone. Frequently, tumors of D_1 cells are malignant.

TABLE 18–2 Comparison of Type 1 and Type 2 Diabetes Mellitus

Type	Common Synonyms	Clinical Characteristics	Patient Weight	Hereditary Component	Islets of Langerhans
Type 1 (insulin-dependent)	Juvenile-onset diabetes; juvenile diabetes; idiopathic diabetes	Abrupt onset of symptoms; age younger than 20 years; decreased blood insulin level; ketoacidosis is common; antibodies present against β cells; possible autoimmune disease; reacts to insulin; polyphagia, polydipsia, polyuria	Normal (or weight loss despite increase in food intake)	About 50% concordance in identical twins; environmental factors important in the development of the disease	Decrease in the size and number of β cells; islets are atrophied and fibrotic
Type 2 (non–insulin-dependent)	Adult-onset diabetes; ketosis-resistant diabetes	Onset after 40 years of age; mild decrease in blood insulin levels; ketoacidosis is rare; no antibodies against β cells; impaired insulin release; insulin-resistant; decrease in number of insulin receptors; impaired postreceptor signaling	80% of the affected individuals are obese	About 90%–100% concordance in identical twins	Some decrease in number of β cells; amylin present in the tissue surrounding β cells

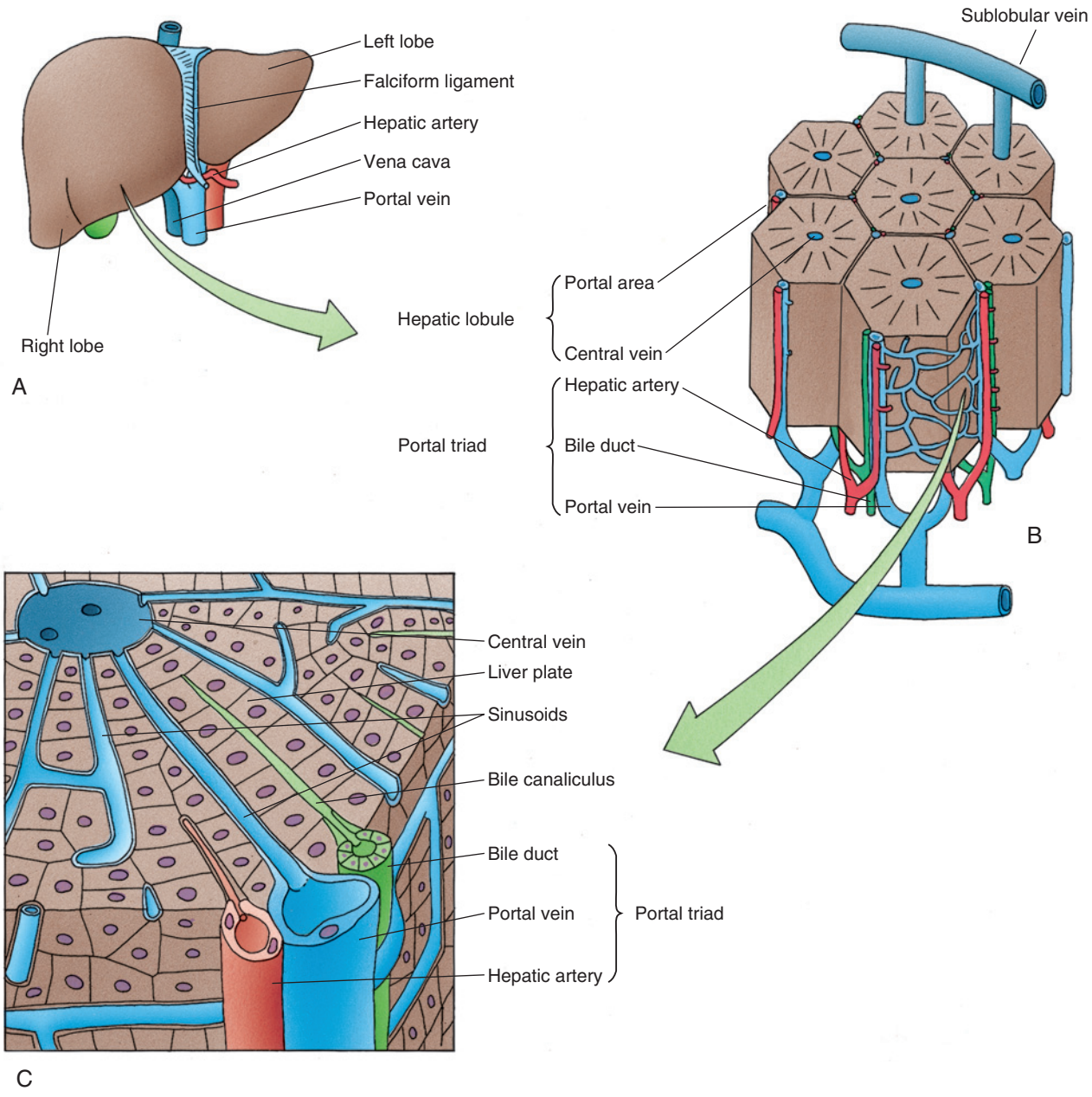


Figure 18–9 Schematic diagram of the liver. **A,** Gross anatomy of the liver. **B,** Liver lobules displaying the portal areas and the central vein. **C,** Portion of the liver lobule displaying the portal area, liver plates, sinusoids, and bile canaliculi.

over the entire circumference of the liver except at the porta hepatis, where it enters the liver, forming a conduit for the blood and lymph vessels and bile ducts. The liver is unusual in that its connective tissue elements are sparse; thus, the bulk of the liver is composed of uniform parenchymal cells, the **hepatocytes**.

The superior aspect of the liver is convex, whereas its inferior region presents a hilum-like indentation, the **porta hepatis**. The liver has a dual blood supply, receiving oxygenated blood from the **left** and **right hepatic arteries** (25%) and hemoglobin-rich blood from the spleen as well as nutrient-rich blood from the digestive tract via the **portal vein** (75%). Both vessels enter the liver at the porta hepatis. Blood leaves the liver at the posterior aspect of the organ through the **hepatic veins**, which deliver their contents into

the inferior vena cava. Bile also leaves the liver at the porta hepatis by way of the right and left hepatic ducts, to be delivered to the **gallbladder** for concentration and storage.

Because the liver occupies a central position in metabolism, all nutrients (except for chylomicrons) absorbed in the alimentary canal are transported directly to the liver via the portal vein. In addition, iron-rich blood from the spleen is routed, by way of the portal vein, directly to the liver for processing. Much of the nutritive material delivered to the liver is converted by the **hepatocytes** into storage products, such as glycogen, to be released as glucose when required by the body.

Hepatocytes are arranged in hexagon-shaped lobules **(classical lobules)** about 2 mm in length and 700 μm in diameter. These lobules are clearly demarcated by slender connective tissue elements (known as *portal tracts*) in animals such as the pig and the camel. However, because of the scarcity of connective tissue and the closely packed arrangement of the lobules in humans, the boundaries of the classical lobules can only be approximated.

Where three classical lobules are in contact with each other, the connective tissue elements are increased, and these regions are known as ***portal areas (portal triads)***. In addition to lymph vessels, portal areas house the following three structures, each of which follows the longitudinal axis of each lobule (see Fig. 18–9B):

- Slender branches of the hepatic artery
- Relatively large limbs of the portal vein
- Interlobular bile ducts (recognized by their simple cuboidal epithelium)

The portal areas are isolated from the liver parenchyma by the **limiting plate**, a sleeve of modified hepatocytes. A narrow space, the **space of Moll**, separates the limiting plate from the connective tissue of the portal area.

Although one would expect six portal areas around each classical lobule, usually only *three* more or less equally distributed portal areas are present at a random section. Along the length of each slender branch of the hepatic artery within the portal area, fine branches, known as ***distributing arterioles***, arise; similar to outstretched arms, they reach toward their counterparts in the neighboring portal areas. Smaller vessels, known as ***inlet arterioles***, branch from the distributing arterioles (or from the parent vessel). In addition, the interlobular bile ducts are vascularized by a **peribiliary capillary plexus**. Venules belonging to the portal vein are also of two sizes: the larger **distributing veins** and the smaller **inlet venules**.

The longitudinal axis of each classical lobule is occupied by the **central vein**, the initial branch of the **hepatic vein**. **Hepatocytes** radiate, similar to spokes of a wheel, from the central vein, forming anastomosing, fenestrated plates of liver cells, separated from each other by large vascular spaces known as ***hepatic sinusoids*** (Fig. 18–10; see Fig. 18–9C). **Inlet arterioles**, **inlet venules**, and branches from the **peribiliary capillary plexus** pierce the limiting plate (of modified hepatocytes) to join the hepatic sinusoids (see Fig. 18–10). As blood enters the sinusoids, its flow slows considerably, and it slowly percolates into the central vein.

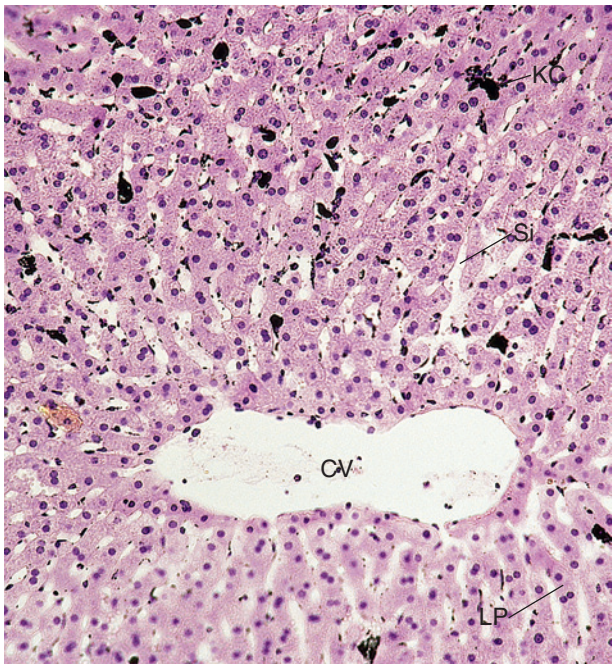


Figure 18–10 Photomicrograph of a dog liver displaying the central vein (CV), liver plates (LP), and sinusoids (Si). This animal was injected with India ink that was phagocytosed by Kupffer cells (KC), which, consequently, appear as black spots (×270).

Because there is only one central vein in each lobule, it receives blood from every sinusoid of that lobule, and its diameter increases as it progresses through the lobule. As the central vein leaves the lobule, it terminates in the **sublobular vein**. Numerous central veins deliver their blood into a single sublobular vein; sublobular veins join each other to form **collecting veins**, which, in turn, form the right and left hepatic veins.

Lymph Flow in the Liver

Lymph formed in the liver flows to the **space of Moll** and enters slender tributaries of the lymph vessel located in the portal area. These lymph vessels merge to form larger and larger structures to form a small number of large vessels that deliver their lymph into the thoracic duct to be transported into the vascular system at the junction of the left internal jugular and left subclavian veins. Lymph from the liver constitutes almost

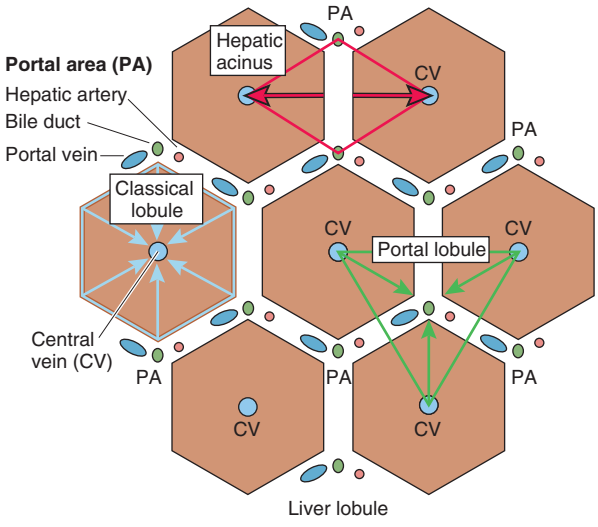


Figure 18–11 Schematic diagram of the three types of lobules in the liver: classic, portal, and liver acinus.

50% of all the lymph formed in the entire body. Because the walls of the liver sinusoids are quite leaky, lymph formed in the liver has a much higher concentration of proteins than lymph formed almost anywhere else in the body.

Three Concepts of Liver Lobules

The three types of liver lobules are the classical lobules, portal lobules, and hepatic acinus (acinus of Rappaport).

There are three basic conceptualizations of the liver lobule (Fig. 18–11). The **classical liver lobule** was the first to be defined histologically because the connective tissue arrangement in the pig liver afforded an obvious rationale. In this concept, blood flows from the periphery to the *center of the lobule* into the central vein. Bile, manufactured by liver cells, enters into small intercellular spaces, **bile canaliculi**, located between hepatocytes and flows to the *periphery of the lobule* to the interlobular bile ducts of the portal areas.

The concept of an exocrine secretion flowing to the periphery of a lobule was not consistent with the situation in the acini of most glands, where the secretion enters a central lumen of the acinus. Therefore, histologists suggested that all hepatocytes that deliver their bile to a particular interlobular bile duct constitute a lobule, called the ***portal lobule***. In histological sections, the portal lobule is defined as the triangular region whose center is the portal area and whose periphery is bounded by imaginary straight lines connecting the three surrounding central veins that form the three apices of the triangle.

A third conceptualization of hepatic lobules is based on blood flow from the distributing arteriole and, consequently, on the order in which hepatocytes degenerate subsequent to toxic or hypoxic insults. This ovoid to diamond-shaped lobule is known as the ***hepatic acinus (acinus of Rappaport)***. It is viewed as three poorly defined, concentric regions of hepatic parenchyma surrounding a distributing artery in the center. The outermost layer, **zone 3**, extends as far as the central vein and is the most oxygen-poor of the three zones. The remaining region is equally divided into two zones (1 and 2); **zone 1** is the richest in oxygen, and **zone 2** has characteristics in between the other two zones.

Hepatic Sinusoids and Hepatocyte Plates

Plates of liver cells delineate vascular spaces between them that are lined by sinusoidal lining cells; the vascular spaces are known as hepatic sinusoids.

Anastomosing **plates of hepatocytes**, no more than two cells thick prior to the age of 7 years and one cell thick after that age, radiate from the central vein toward the periphery of the classical lobule (see Fig. 18–9C). The spaces between the plates of hepatocytes are occupied by hepatic sinusoids, and the blood flowing in these wide vessels is prevented from coming in contact with the hepatocytes by the presence of an endothelial lining composed of **sinusoidal lining cells**. Often, the cells of this endothelial lining do not make contact with each other, leaving gaps of up to 0.5 µm between them. The sinusoidal lining cells also have fenestrae that are present in clusters, known as ***sieve plates***. Thus, particulate matter less than 0.5 µm in diameter may leave the lumen of the sinusoid with relative ease.

Resident macrophages, known as ***Kupffer cells***, are intermingled with the sinusoidal lining cells of the sinusoids (Figs. 18–12 and 18–13). Frequently, phagosomes of Kupffer cells contain endocytosed particulate matter and cellular debris, especially defunct erythrocytes that are being destroyed by these cells. Electron micrographs of Kupffer cells display numerous filopodia-like projections, mitochondria, some RER, a small Golgi apparatus, and an abundance of lysosomes and late endosomes. Because these cells do not make intercellular junctions with the neighboring cells, it has been suggested that they may be migratory scavengers.

Perisinusoidal Space of Disse

The narrow space between a plate of hepatocytes and sinusoidal lining cells is known as the perisinusoidal space of Disse.

The sinusoidal lining cells are separated from the hepatocytes by a narrow **space of Disse (perisinusoidal space)**, and plasma escaping from the sinusoids has free access to this space (Fig. 18–14; see Fig. 18–13).

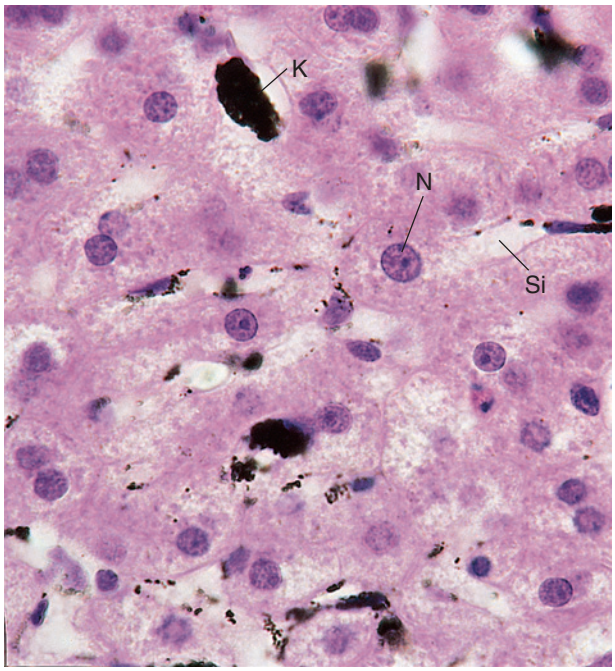


Figure 18–12 Photomicrograph of a canine liver demonstrating plates of hepatocytes, sinusoids (Si), and India ink–containing Kupffer cells (K). N, Nucleus (×540).

Microvilli of the hepatocytes occupy much of the space of Disse; the extensive surface area of the microvilli facilitates exchange of materials between the plasma escaping from the bloodstream and the hepatocytes. Hepatocytes do *not* come into contact with the bloodstream; instead, the space of Disse acts as an intermediate compartment between them.

Although the perisinusoidal space contains type III collagen fibers (reticular fibers) that support the sinusoids, as well as a limited amount of type I and type IV collagen fibers, a basal lamina is absent. Occasionally, nonmyelinated nerve fibers and stellate **perisinusoidal stellate cells** (also known as ***Ito cells*** and ***fat storing cells***) have been noted in this space (see Fig. 18–13). It is believed that perisinusoidal stellate cells store vitamin A; manufacture and release type III collagen into the space of Disse; secrete growth factors required by the liver for generating new hepatocytes; and by differentiating into myofibroblasts, form fibrous connective tissue to replace toxically damaged hepatocytes. They do that in response to the presence of **tumor growth factor β** released by hepatocytes of a compromised liver. In addition **pit cells**, which display short pseudopodia and cytoplasmic granules, have been noted in the perisinusoidal space of mice and rats. These cells, believed to be natural killer cells, are also assumed to be in the human liver.

CLINICAL CORRELATIONS

Chronic inflammation of the liver, caused by factors such as long-term alcoholism, portal hypertension, hepatitis B, hepatitis C, causes hepatocytes to begin to die at a higher than normal rate, and perisinusoidal cells (Ito cells) are induced to differentiate into myofibroblasts and form collagen, eventually resulting in fibrosis and, later, cirrhosis of the liver. Fortunately, these conditions can be reversed if treated in time, but, frequently, the patient will experience liver failure and, in the absence of a liver transplant, death ensues.

Hepatic Ducts

The system of hepatic ducts is composed of cholangioles, canals of Hering, and bile ducts leading to larger and larger bile ducts that finally culminate in the right and left hepatic ducts.

Bile canaliculi anastomose with one another, forming labyrinthine tunnels among the hepatocytes. As these bile canaliculi reach the periphery of the classic lobules, they merge with **canals of Hering**, slender conduits that are formed by hepatocytes in combination with low cuboidal (or ovoid) cells known as ***cholangiocytes***. Bile from the canals of Hering flow into **bile ducts** (composed of cholangiocytes) located in the portal areas of the classical lobules. These bile ducts merge to form increasingly larger conduits, which eventually unite to form the **right hepatic duct** and the **left hepatic duct**. The extrahepatic system of bile ducts is described later. The **ovoid cells** of the canals of Hering are capable of proliferation. The progeny of these oval cells may give rise to cuboidal cells of the bile duct system as well as to hepatocytes.

The cuboidal epithelial cells of the canals of Hering and of the bile ducts secrete a bicarbonate-rich fluid similar to that produced by the duct system of the pancreas. The formation and release of this alkaline buffer are controlled by the hormone secretin, produced by DNES cells of the duodenum. This fluid acts, with fluid from the pancreas, to neutralize the acidic chyme that enters the duodenum.

Hepatocytes

Hepatocytes are 5- to 12-sided polygonal cells, approximately 20 to 30 μm in diameter, that are closely packed together to form anastomosing plates of liver cells, one cell in thickness. These cells exhibit variations in their structural, histochemical, and biochemical properties, depending on their location within liver lobules. Hepatocytes have an approximate life span of 150 days.

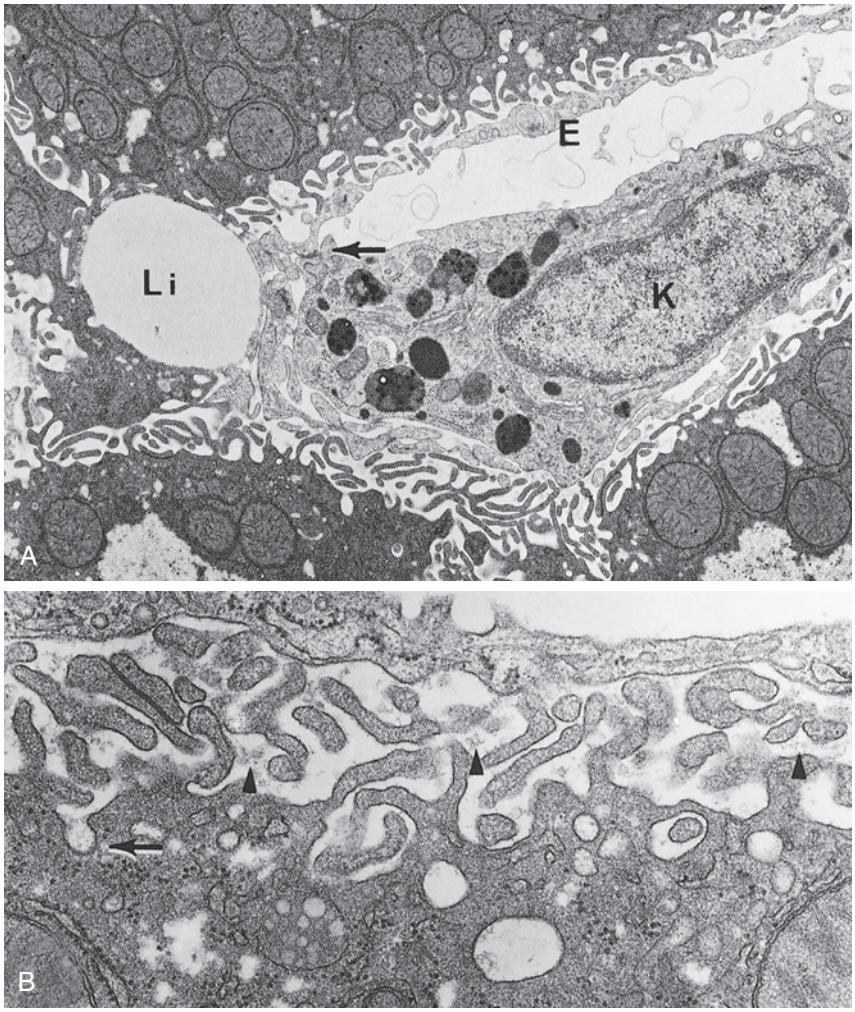


Figure 18–13 Electron micrograph of the shrew liver. **A,** Observe the sinusoid, with its sinusoidal lining cell (E), Kupffer cell (K), and a small region of a lipid droplet (Li)–containing Ito cell (×8885). **B,** A higher magnification of the hepatocyte displays its numerous microvilli *(arrowheads)* protruding into the space of Disse (×29670). The *arrow* indicates the process of pinocytosis. (From Matsumoto E, Hirosawa K. Some observations on the structure of *Suncus* liver with special reference to the vitamin A–storing cell. *Am J Anat*. 1983;167:193-204. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Domains of Hepatocyte Plasmalemma

The plasma membranes of hepatocytes are said to have two domains, lateral and sinusoidal.

Hepatocytes are arranged in such a manner that each cell not only comes in contact with other hepatocytes, but also borders a space of Disse. Thus, the plasmalemma of hepatocytes is said to have **lateral domains** that contact other hepatocytes and **sinusoidal domains** that face the space of Disse.

Lateral Domains

The lateral domains are responsible for the formation of bile canaliculi.

The **lateral domain** of the hepatocyte cell membrane contact the adjacent hepatocyte and form elaborate, labyrinthine intercellular spaces, 1 to 2 μm in diameter, known as *bile canaliculi*, channels that conduct bile between hepatocytes to the periphery of the classical lobules (see Fig. 18–9C). Leakage of bile from bile

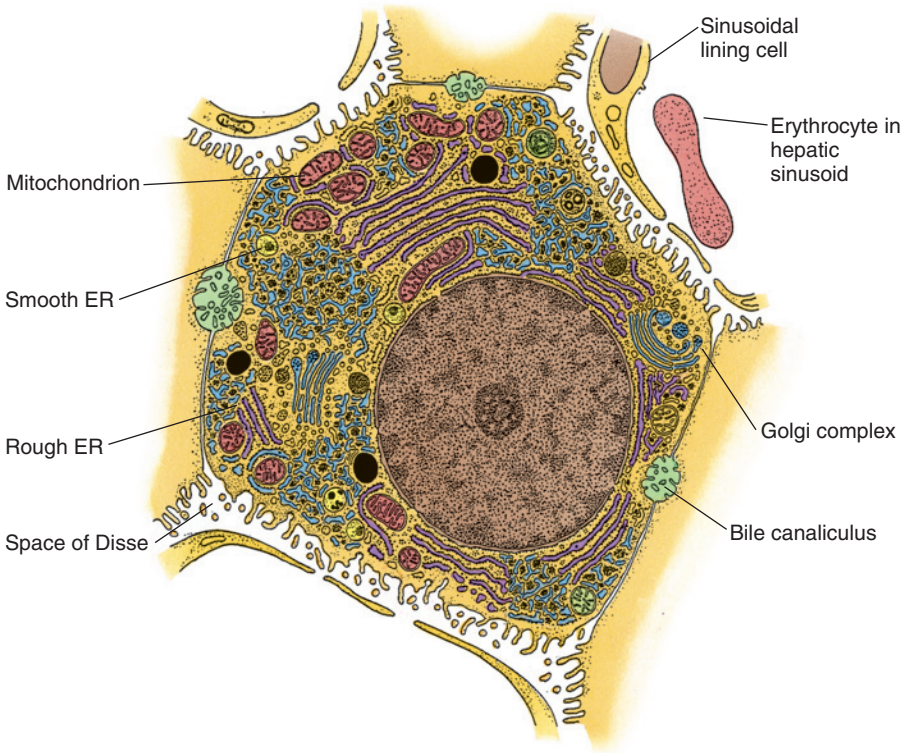


Figure 18–14 Schematic diagram of a hepatocyte indicating its sinusoidal and lateral domains. ER, Endoplasmic reticulum. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

canaliculi is prevented by the formation of tight junctions (fasciae occludentes) between adjoining liver cells, isolating these conduits from the remaining extracellular space.

Short, blunt microvilli project from the hepatocyte into the bile canaliculi, thus increasing the surface areas through which bile can be secreted (see Fig. 18–14). The actin cores of these microvilli mingle with the thickened network of actin and intermediate filaments that reinforce the region of the hepatocyte plasmalemma, which participates in the formation of bile canaliculi.

The cell membranes that form the walls of bile canaliculi display high levels of **Na^+-K^+ ATPase** activity and the enzyme **adenylate cyclase**. The lateral domains also have isolated gap junctions, whereby hepatocytes are able to communicate with each other.

Sinusoidal Domains

The sinusoidal domains form microvilli that protrude into the perisinusoidal space of Disse.

The **sinusoidal domains** of hepatocyte plasma membranes also have microvilli, which project into the space of Disse (see Figs. 18–13 and 18–14). It has been calculated that these microvilli increase the surface area of the sinusoidal domain by a factor of 6, facilitating the exchange of material between the hepatocyte and the plasma in the perisinusoidal space (space of Disse). This cell membrane is rich in mannose-6-phosphate receptors, Na^+-K^+ ATPase, and adenylate cyclase because it is here that the endocrine secretions of the hepatocyte are released and enter the sinusoidal blood, and material carried by the bloodstream is transported into the hepatocyte cytoplasm.

Hepatocyte Organelles and Inclusions

Hepatocytes are large, organelle-rich cells that manufacture the exocrine secretion bile as well as a large number of endocrine secretions; in addition, these cells can perform a large array of metabolic functions.

Hepatocytes constitute only 60% of the total cell number, but they account for about 75% of the weight of the liver. These cells manufacture **primary bile**, which is modified by the epithelial cells lining the bile ducts and gallbladder and become known as ***bile***.

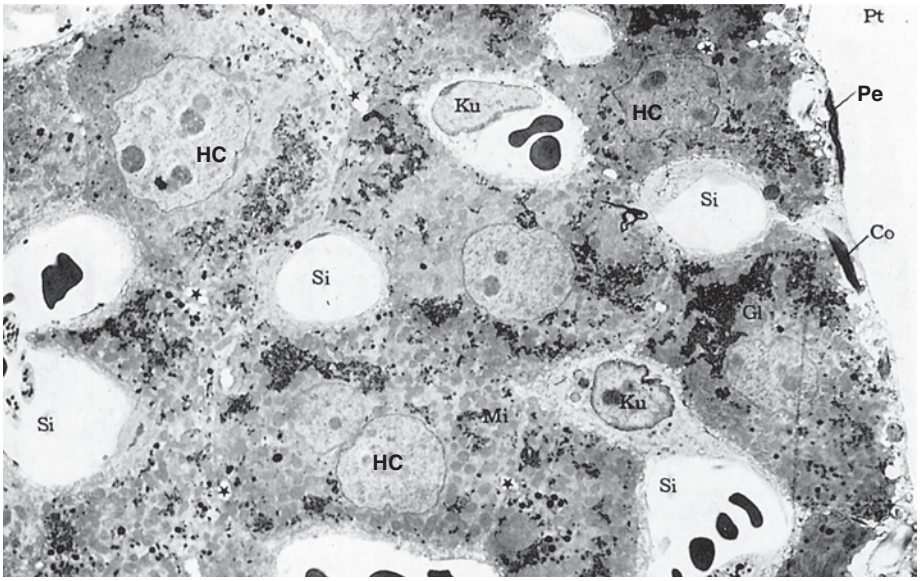


Figure 18–15 Low-magnification electron micrograph of a mouse liver (×2535). The liver is covered over most of its surface by peritoneum (Pe), which overlies the collagenous capsule (Co) of the liver. Observe the sinusoids (Si), Kupffer cells (Ku), and glycogen deposits (Gl) in the hepatocyte (HC) cytoplasm. Bile canaliculi are denoted by *asterisks*. Mi, Mitochondria; PT, peritoneal cavity. (From Rhodin JAG. *An Atlas of Ultrastructure*. Philadelphia: WB Saunders; 1963.)

Approximately 75% of the hepatocytes have a single nucleus, and the remainder have two nuclei. The nuclei vary in size, the smallest ones (50% of the nuclei) being diploid and the larger ones being polyploid, with the largest nuclei reaching 64 N.

Hepatocytes actively synthesize proteins for their own use as well as for export. Thus, they have an abundance of free ribosomes, RER, and Golgi apparatus (Figs. 18–15 and 18–16). Each cell houses several sets of Golgi apparatuses, located preferentially in the vicinity of bile canaliculi.

Because of the high energy requirements of hepatocytes, each cell contains as many as 2000 mitochondria. Cells near the central vein (zone 3 of the liver acinus) have nearly twice as many, but considerably smaller, mitochondria as hepatocytes in the periportal area (zone 1 of the liver acinus). Liver cells also have a rich complement of endosomes, lysosomes, and peroxisomes.

The complement of smooth endoplasmic reticulum (SER) of hepatocytes varies not only by region but also with function. Cells in zone 3 of the liver acinus have a much richer endowment of SER than those in the periportal area. Moreover, the presence of certain drugs and toxins in the blood induces an increase in the SER content of liver cells because detoxification occurs within the cisternae of this organelle.

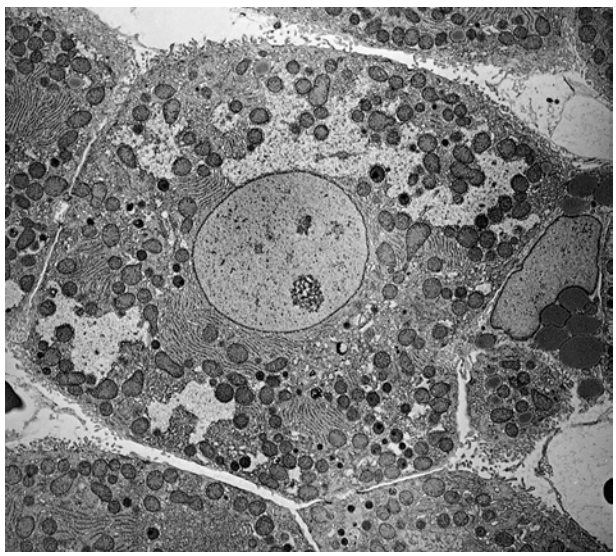


Figure 18–16 Electron micrograph of a rat hepatocyte (×2500). (From Tandler B, Krahenbuhl S, Brass EP. Unusual mitochondria in the hepatocytes of rats treated with a vitamin B_{12} analogue. *Anat Rec*. 1991;231:1-6. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

CLINICAL CORRELATIONS

1. Persons who have consumed hepatotoxic substances, such as alcohol, display an increased number of lipid deposits in their zone 3 hepatocytes. In addition, persons who are taking barbiturates display an increase in the SER content of zone 3 liver cells. Because this zone has the lowest oxygen levels of the three zones, this is the region of the liver acinus that is most susceptible to necrosis in case of severe liver injury.
2. **Wilson disease** is a hereditary condition in which the liver does not eliminate copper by transferring it into bile. Instead, copper accumulates in the eyes, where it appears as green to gold rings in the cornea; in the brain, where it interferes with normal brain function, causing tremors, aphasia, and, occasionally, psychosis; and in the liver, where it causes cirrhosis. If left untreated, the disease is fatal, but the use of a chelating agent, usually penicillamine, binds with copper and facilitates its elimination from the body.

Hepatocytes contain varying amounts of inclusions in the form of lipid droplets and glycogen (Fig. 18–17). The lipid droplets are mostly **very-low-density lipoprotein** and are especially prominent after the consumption of a fatty meal.

Glycogen deposits are present as accumulations of electron-dense granules 20 to 30 nm in size, known as ***β particles***, in the vicinity of SER. The distribution of glycogen varies with hepatocyte location. Liver cells in the vicinity of the portal area (zone 1 of liver acinus) display large clumps of β particles surrounded by SER, whereas pericentral hepatocytes (zone 3 of liver acinus) exhibit diffuse glycogen deposits (see Fig. 18–17). The number of these particles varies with the dietary state of the individual. They are abundant subsequent to feeding and fewer after fasting.

Histophysiology of the Liver

The liver has both exocrine and endocrine roles as well as the protective function of detoxification of toxins and elimination of defunct erythrocytes.

The liver may have as many as 100 different functions, most of which are performed by the hepatocytes. Each of these liver cells produces not only the exocrine secretion bile but also various endocrine secretions. Hepatocytes metabolize the end products of absorption from the alimentary canal, store them as inclusion products, and release them in response to hormonal and nervous signals. Liver cells also detoxify drugs and toxins (protecting the body from their deleterious effects) and transfer secretory IgA from the space of Disse into bile. In addition, Kupffer cells phagocytose blood-borne foreign particulate matter and defunct erythrocytes.

Bile Manufacture

Bile, a fluid manufactured by the liver, is composed of water, bile salts, phospholipids, cholesterol, bile pigments, and IgA.

The liver produces approximately 600 to 1200 mL of bile per day. This fluid, which is mostly water, contains **bile salts (bile acids)**, **bilirubin glucuronide (bile pigment)**, phospholipids, lecithin, cholesterol, plasma electrolytes (especially sodium, bicarbonate, and excess calcium), and IgA. It emulsifies fats in the lumen of the small intestine and assists the surface absorptive cells of the small intestine in absorbing fat, eliminates approximately 80% of the cholesterol synthesized by the liver, and excretes waste products such as bilirubin, the end result of hemoglobin destruction.

Bile salts constitute almost half of the organic components of bile. Most of the bile salts are resorbed from the lumen of the small intestine, enter the liver via the portal vein, are endocytosed by hepatocytes, and are transported into the bile canaliculi for subsequent re-release back into the duodenum **(enterohepatic recirculation of bile salts)**. The remaining 10% of bile salts are manufactured de novo in the SER of hepatocytes by the conjugation of cholic acid, a metabolic byproduct of cholesterol, to either taurine (taurocholic acid) or glycine (glycocholic acid).

CLINICAL CORRELATIONS

Because bile salts are amphipathic molecules, their *hydrophilic* regions are dissolved in aqueous media, and their *hydrophobic (lipophilic)* regions surround lipid droplets. In the lumen of the duodenum, therefore, bile salts emulsify fats and facilitate their digestion. Absence of bile salts prevents fat digestion and absorption, resulting in **fatty stool**.

Bilirubin, a water-insoluble, yellowish green pigment, is the *toxic degradation product* of hemoglobin. As defunct erythrocytes are destroyed by macrophages in the spleen and by Kupffer cells in the liver, bilirubin is released into the bloodstream and is bound to plasma albumin. In this form, known as ***free bilirubin***, it is endocytosed by hepatocytes. The enzyme **glucuronyltransferase**, located in the SER of the hepatocyte, catalyzes the conjugation of bilirubin with glucuronide to form the water-soluble, nontoxic **bilirubin glucuronide (conjugated bilirubin)**. Some of the bilirubin glucuronide is released into the bloodstream, but most of it is excreted into the bile canaliculi for delivery into the alimentary canal for subsequent elimination with the feces (Fig. 18–18).

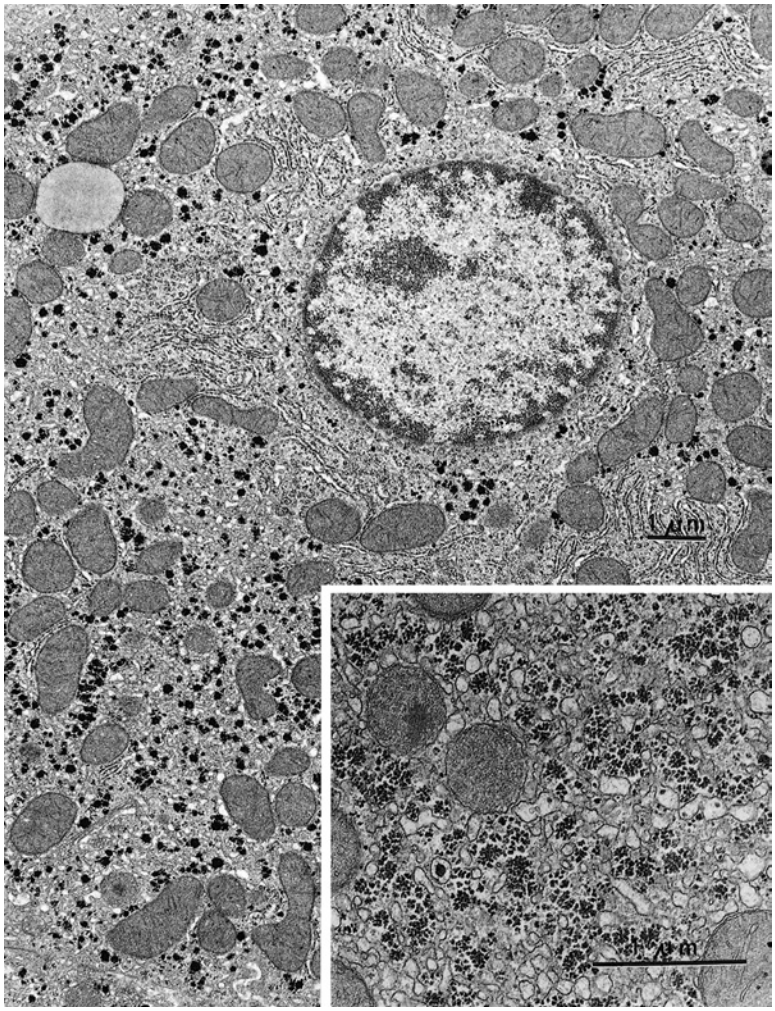


Figure 18–17 Electron micrograph of glycogen and lipid deposits in the pericentral hepatocyte of a rat. Inset shows the presence of glycogen particles at a higher magnification. (From Cardell RR, Cardell EL. Heterogeneity of glycogen distribution in hepatocytes. *J Electron Microsc Tech*. 1987;14:126-139. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Lipid Metabolism

Hepatocytes remove chylomicrons from the space of Disse and degrade them into fatty acids and glycerol.

Chylomicrons released by surface absorbing cells of the small intestine enter the lymphatic system and reach the liver through branches of the hepatic artery. Within hepatocytes, they are degraded into **fatty acids** and **glycerol**. The fatty acids are subsequently desaturated and are used to synthesize phospholipids and cholesterol or are degraded into **acetyl coenzyme A**. Two molecules of acetyl coenzyme A are combined to form **acetoacetic acid**. Much of the acetoacetic acid is converted into β-hydroxybutyric acid and some into acetone. These three compounds are known as ***ketone bodies***. Phospholipids, cholesterol, and ketone bodies are stored in hepatocytes until their release into the space of Disse. In addition, the liver manufactures **very-low-density lipoproteins**, which are also released into the space of Disse as droplets 30 to 100 nm in diameter. If the need arises, hepatocytes can synthesize fats from carbohydrates and proteins, and the newly manufactured fats are released as lipoproteins to be ferried by the bloodstream to adipose cells, where they are stored as triglycerides.

Carbohydrate and Protein Metabolism

Additional responsibilities of the liver include maintenance of normal blood glucose levels, deamination of amino acids, and synthesis of many blood proteins.

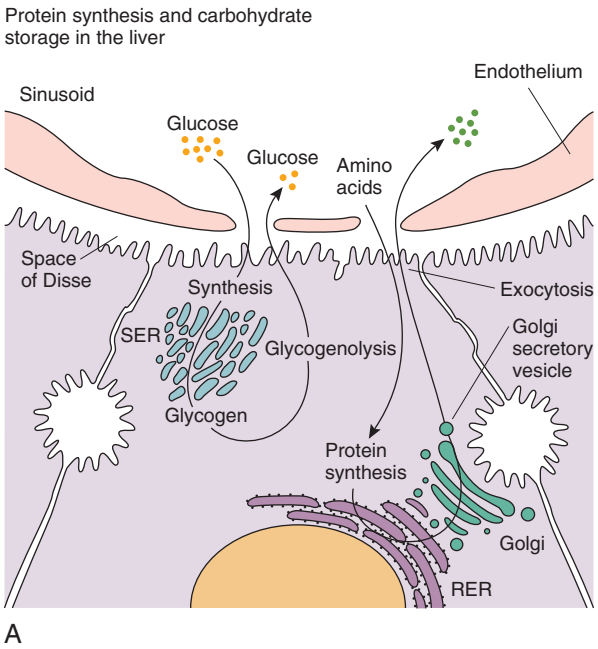


Figure 18–18 Schematic diagram of a hepatocyte function. **A,** Protein synthesis and carbohydrate storage. **B,** Secretion of bile acids and bilirubin. SER, Smooth endoplasmic reticulum.

The liver maintains normal levels of glucose in the blood by transporting glucose from the blood into the hepatocytes and storing it in the form of glycogen. If blood glucose levels drop below normal, hepatocytes hydrolyze glycogen **(glycogenolysis)** into glucose and transport it out of the cells into the space of Disse (see Fig. 18–18). Hepatocytes can also synthesize glucose from other sugars (such as fructose and galactose) or from noncarbohydrate sources (such as amino acids), a process known as ***gluconeogenesis***.

One of the most essential roles of the liver is the elimination of blood-borne ammonia by converting it into **urea**. There are two major sources of ammonia in the body, the deamination of amino acids by hepatocytes and the synthesis of ammonia by bacterial action in the alimentary canal.

CLINICAL CORRELATIONS

1. The yellowish discoloration of the skin that is the hallmark of **jaundice** results from excessively high levels of free or conjugated bilirubin (which are yellowish green) in the bloodstream. The two primary types of jaundice have different causes. A decrease in bilirubin conjugation, because of either hepatocyte malfunction (as in hepatitis) or, more commonly, obstruction of the bile ducts, causes **obstructive jaundice**. Increased hemolysis of erythrocytes, producing so much free bilirubin that hepatocytes, even though unimpaired, cannot eliminate bilirubin rapidly enough, causes **hemolytic jaundice**.
2. **Ketosis** occurs when the concentration of ketone bodies in the blood becomes too high (as in persons with diabetes or starvation). It is recognizable by the typical acetone breath of affected persons. If untreated, ketosis results in decreased blood pH **(acidosis)**, possibly leading to death. The smell of "nail polish remover" (acetone) on the breath of a patient is an indicator that the physician should suspect that patient has diabetes and the proper diagnostic tests should be performed.
3. Excessive blood ammonia levels, indicative of impaired liver function or drastic reduction in blood flow to the liver, may lead to **hepatic coma**, a condition that is incompatible with life.

CLINICAL CORRELATION

If the liver does not convert the ammonia into urea, the individual's blood ammonia level rises to such an extent that the patient goes into hepatic coma, resulting in death.

Approximately 90% of the blood proteins are manufactured by the liver (see Fig. 18–18). These products include

- Factors necessary for coagulation (such as fibrinogen, factor III, accelerator globulin, and prothrombin)

- Proteins required for the complement reactions
- Proteins that function in transport of metabolites
- Albumins
- All of the globulins, except gamma (γ) globulins (antibodies), are also synthesized by the liver

Hepatocytes can also synthesize all of the nonessential amino acids that the body requires.

Vitamin Storage

Vitamin A is stored in the greatest amount in the liver, but vitamins D and B_{12} are also present in substantial quantities. The liver contains enough vitamin stores to prevent deficiency of vitamin A for about 10 months, vitamin D for about 4 months, and vitamin B_{12} for more than 12 months.

Degradation of Hormones and Detoxification of Drugs and Toxins

The liver endocytoses and degrades hormones of the endocrine glands. The endocytosed hormones are either transported into the bile canaliculi in their native form to be digested in the lumen of the alimentary canal or delivered into late endosomes for degradation by lysosomal enzymes.

Drugs, such as barbiturates and antibiotics, and toxins are inactivated by **microsomal mixed-function oxidases** in hepatocytes. These drugs and toxins are usually inactivated in the cisterna of the SER by methylation, conjugation, or oxidation. Occasionally, detoxification occurs in peroxisomes rather than in the SER.

CLINICAL CORRELATIONS

Continued long-term use of certain drugs, such as barbiturates, decreases their effectiveness, requiring the prescription of increased doses. This drug **tolerance** is due to hypertrophy of the SER complement of hepatocytes and a concomitant increase in their mixed-function oxidases. The increase in the organelle size and the enzyme concentration is **induced** by the barbiturate, which is detoxified via oxidative demethylation. In addition, these hepatocytes concurrently become more efficient in detoxifying other drugs and toxins.

Immune Function

Hepatocytes complex IgA with secretory component and release the secretory IgA into the bile canaliculi.

Most of the IgA antibodies formed by plasma cells in the mucosa of the alimentary canal enter the circulatory system and are transported to the liver. Hepatocytes complex the IgA with secretory component and release the complex into bile, which then enters the lumen of the duodenum. Thus, much of the luminal IgA enters the intestine through the common bile duct, accompanying bile. The remainder of the luminal IgA is transported from the intestinal mucosa into the lumen by surface absorptive cells.

Kupffer cells, which are derived from monocyte precursors, are long-lived cells that are located within the lining of the hepatic sinusoids; some may also adhere to the luminal surface of endothelial cells. Kupffer cells have Fc receptors as well as receptors for complement and thus can phagocytose foreign particulate matter. The importance of these cells is appreciable because blood from the portal vein contains a considerable number of microorganisms that enter the bloodstream from the lumen of the alimentary canal. These bacteria become opsonized in the lumen or mucosa of the gut or in the bloodstream. Kupffer cells recognize and endocytose at least 99% of these microorganisms. Kupffer cells also remove cellular debris and defunct erythrocytes from the blood.

Liver Regeneration

The liver has a great ability to regenerate after a hepatotoxic insult or even after three quarters of the liver is excised.

Hepatocytes have a life span of approximately 150 days; thus, mitotic figures are present only infrequently. If hepatotoxic drugs are administered or a portion of the liver is excised, however, hepatocytes proliferate, and the liver regenerates its normal architecture and previous size.

The regenerative ability of the liver of rodents is so enormous that if 75% of the gland is excised, it regenerates to its normal size within 4 weeks. The human liver's regenerative capacity is much less than that of mice and rats. The mechanism of regeneration is controlled by hepatocyte growth factor, transforming growth factor-α, epidermal growth factor, and interleukin-6. Many of these factors are released by the hepatic stellate cells (Ito cells) located in the space of Disse, although hepatocyte growth factor is probably formed by mesenchymal cells of the liver and is bound to heparin in the scant extracellular matrix of the liver. In most cases, the regeneration is due to the replicative capability of the remaining hepatocytes; however, if the hepatotoxic insult is too great, regeneration of the liver is due the mitotic activity of the oval cells of the canals of Hering. Once the liver is regenerated to its normal size, the hepatocytes manufacture and release the cytokine **transforming growth factor-β**, which inhibits mitotic activity in the liver; thus, regeneration ceases.

Gallbladder

The **gallbladder** is a small, pear-shaped organ situated on the inferior aspect of the liver. It is about 10 cm in length and 4 cm in cross section and can store about 70 mL of bile. This organ resembles a sack with a single opening. The bulk of the organ forms the **body**, and the

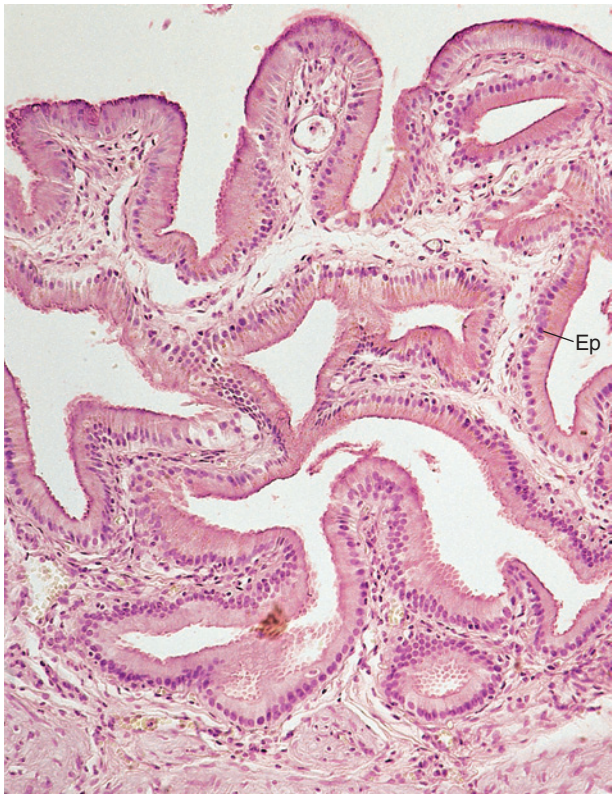


Figure 18–19 Photomicrograph of an empty gallbladder. Observe that the mucosa of the gallbladder is highly folded, indicating that it is empty. Note that the lumen of the gallbladder is lined by a simple columnar epithelium (Ep) (×132).

opening, which is continuous with the **cystic duct**, is called the ***neck***. The neck has an out pocketing, known as *Hartmann pouch*, and it is a region where gallstones frequently lodge. The gallbladder stores and concentrates bile and releases it into the duodenum as required.

Structure of the Gallbladder

The gallbladder is composed of four layers: epithelium, lamina propria, smooth muscle, and serosa/adventitia.

The mucosa of the empty gallbladder is highly folded into tall, parallel ridges (Fig. 18–19). As the gallbladder becomes distended with bile, the plication is reduced to a few short folds, and the mucosa becomes relatively smooth.

The lumen of the gallbladder is lined by a **simple columnar epithelium**, composed of two types of cells: the more common **clear cells** and the infrequent **brush cells** (Fig. 18–20). The oval nuclei of these cells are basally positioned, and the supranuclear cytoplasm displays occasional secretory granules containing mucinogen. In electron micrographs, their luminal surface displays short microvilli coated by a thin layer of glycocalyx. The basal region of the cytoplasm is particularly rich in mitochondria, providing abundant energy for the Na^+-K^+ ATPase pump present in the basolateral cell membrane.

The lamina propria is composed of a vascularized loose connective tissue that is well endowed with elastic and collagen fibers. In the neck of the gallbladder, the lamina propria houses simple tubuloacinar glands, which produce a small amount of mucus to lubricate the lumen of this constricted region. The thin smooth muscle layer of the gallbladder is composed mostly of **obliquely** oriented fibers, whereas others are oriented longitudinally. Although the connective tissue adventitia is attached to the Glisson capsule of the liver, it may be separated from it with relative ease. The nonattached surface of the gallbladder is invested by peritoneum, providing it with a smooth, simple squamous epithelial serosa.

Extrahepatic Ducts

The right and left **hepatic ducts** unite to form the **common hepatic duct**, which is joined by the **cystic duct**, arising from the gallbladder. The merger of these two ducts forms the **common bile duct**, 7 to 8 cm long, which fuses with the pancreatic duct to form the **ampulla of Vater**. The ampulla opens at the duodenal papilla into the lumen of the duodenum.

The opening of the common bile duct and of the pancreatic duct is controlled by a complex of four sphincter muscles, sphincter choledochus, sphincter pancreaticus, sphincter ampullae, and fasciculus longitudinalis, collectively called the ***sphincter of Oddi***. The locations and functions of these muscles are summarized in Table 18–3.

Histophysiology of the Gallbladder

The gallbladder stores, concentrates, and releases bile; bile release is triggered by CCK and vagal stimulation.

The primary functions of the gallbladder are to store, concentrate, and release bile. Bile is constantly manufactured by the liver and must make its way to the gallbladder. This activity requires that the sphincters choledochus, pancreaticus, and ampullae be maintained in a closed position so that the bile backs up the common bile duct and the cystic duct to enter the gallbladder.

Na^+ is actively transported from the basolateral region of the simple columnar epithelium of the gallbladder into the extracellular space and is passively followed by chloride (Cl^-) and water. To compensate for the loss of intracellular ions, apical ion channels permit Na^+ and Cl^- to enter the simple columnar cells,

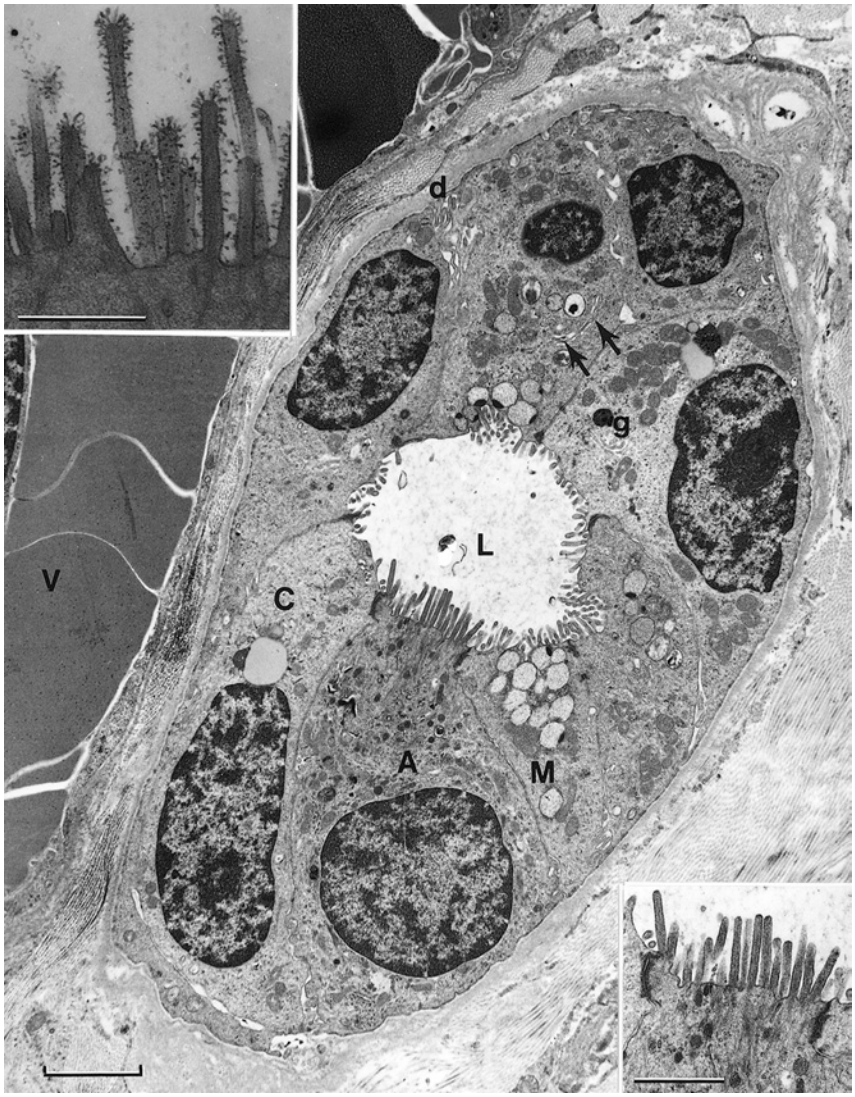


Figure 18–20 Electron micrograph of the human gallbladder diverticulum displaying brush cells (A) and clear cells (C) of the epithelium. d, Interdigitations; g, granules; L, lumen; M, clear cells with mucoid granules. Bar = 2 µm. *Upper inset:* Clear cell microvilli. Bar = 0.5 µm. *Lower inset:* Brush cell microvilli. Bar = 1.0 µm. (From Gilloteaux J, Pomerants B, Kelly T. Human gallbladder mucosa ultrastructure: evidence of intraepithelial nerve structures. *Am J Anat*. 1989;184:321-333. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

reducing the salt (NaCl) concentration of bile. The requirement for osmotic equilibrium drives water from the bile into the simple columnar cell, thus concentrating bile.

The signaling molecule **CCK (cholecystokinin)** is released by I cells (DNES cells) of the duodenum in response to a fatty meal. This molecule comes in contact with CCK receptors on the smooth muscle cells of the gallbladder and causes them to contract intermittently. Concurrently, contact of CCK receptors with the smooth muscle cells of the sphincter of Oddi causes the sphincter muscles to relax. As a result, the rhythmic contractile forces of the gallbladder inject the bile into the lumen of the duodenum. In addition, **acetylcholine**, released by the vagal parasympathetic fibers, stimulates contraction of the gallbladder.

TABLE 18–3 The Sphincter of Oddi and Its Component Parts

Sphincter Muscle	Location and Function
Sphincter choledochus	Surrounds and controls terminal region of common bile duct to stop bile flow into duodenum
Sphincter pancreaticus	Surrounds and controls terminal portion of pancreatic duct to stop pancreatic juices from entering duodenum and prevents entry of bile into pancreatic duct
Sphincter ampullae	Surrounds and controls ampulla of Vater and prevents entry of bile and pancreatic juices into duodenum
Fasciculus longitudinalis	Located in triangular interval delineated by ampulla of Vater, pancreatic duct, and common bile duct; facilitates entry of bile into lumen of duodenum

CLINICAL CORRELATIONS

Gallstones (cholelithiasis) are more common in women than in men and occur most frequently in the fourth decade of life. Approximately 20% of all women and 8% of all men have gallstones. Usually, people are unaware of their presence because gallstones are either small enough to be eliminated with normal bile flow or too large to leave the gallbladder. When they enter and become entrapped in the cystic or common hepatic ducts, gallstones obstruct bile flow and cause excruciating pain. Approximately 80% of gallstones are composed of cholesterol **(cholesterol stones)**; most of the remainder are formed from the calcium salt of bile, calcium bilirubinate **(pigment stones)**, or a combination of cholesterol and calcified bilirubinate. Cholesterol stones are large (1 to 3 cm) and pale yellow, have numerous facets, and are few in number. Pigment stones are smaller (1 cm), black, and ovoid, and they occur in large numbers. Usually, both types of stones are radiolucent.

PATHOLOGICAL CONSIDERATIONS

See Figures 18–21 through 18–23.

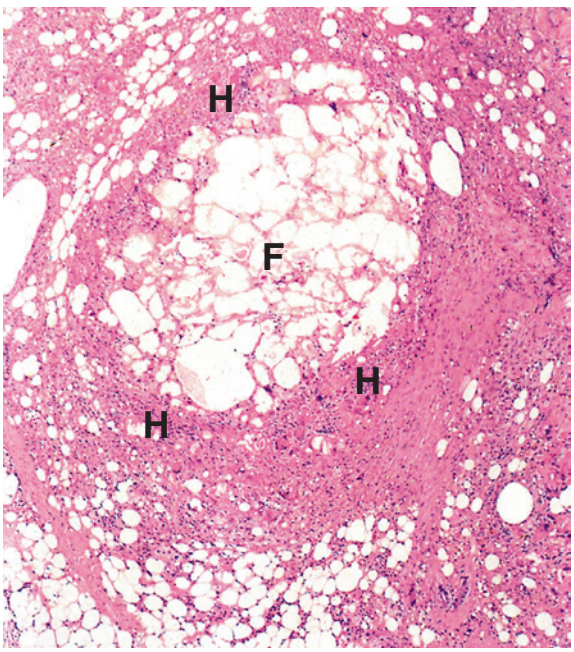


Figure 18–21 Photomicrograph of the pancreas of a patient with acute pancreatitis. Observe the necrotic adipose tissue (F) that is enveloped by macrophages (H) whose cytoplasm is laden with phagocytosed lipid droplets. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:174.)

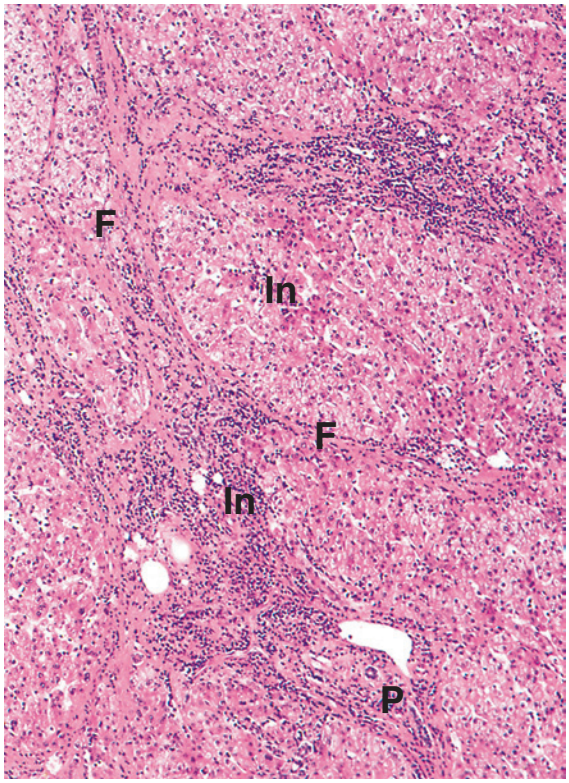


Figure 18–22 Photomicrograph of a patient with progressive chronic hepatitis and consequent cirrhosis. Note that the portal areas (P) are invaded by inflammatory cells, resulting in areas of focal inflammation (In) and fibrosis (F) where portal areas are connected to each other by fibrous connective tissue. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:170.)

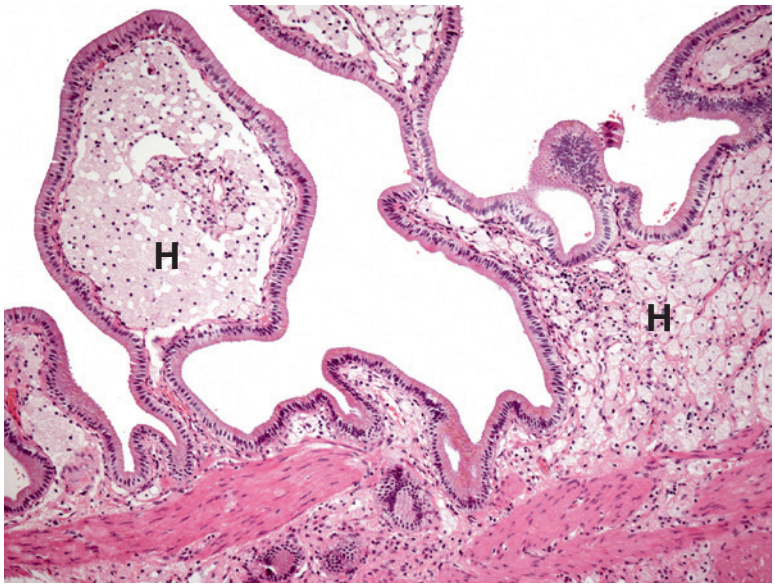
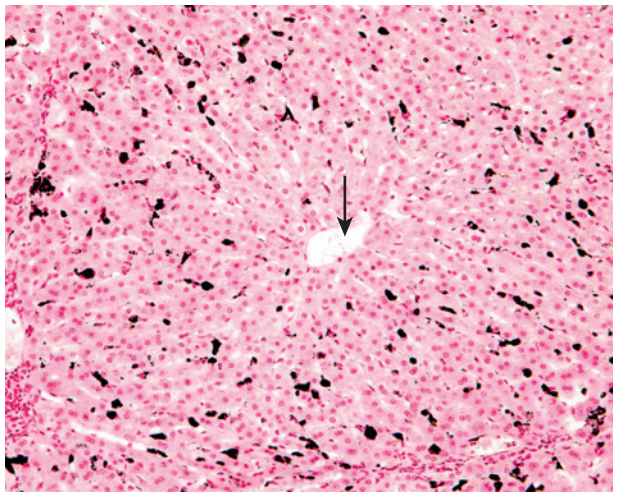


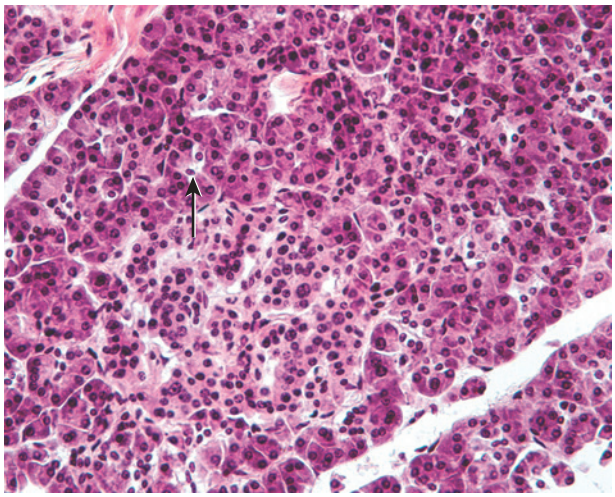
Figure 18–23 Photomicrograph of the gallbladder of a patient with cholesterolosis, a condition that is frequently accompanied by cholesterol-based gallstone. Histological features include the deposition of lipids, including cholesterol, in the lamina propria of the gallbladder. Observe that the lamina propria is inundated by fat-laden macrophages (H). (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:174.)

REVIEW QUESTIONS

1. A patient is seen by her pediatrician because she has mumps. Which of the following salivary glands is most likely to be involved?
   a. Sublingual gland
   b. Glands of von Ebner
   c. Glands of Blandin-Nuhn
   d. Submandibular gland
   e. Parotid gland
2. The acinus of Rappaport is
   a. in the shape of a hexagonal solid.
   b. is related to blood flow.
   c. is related to bile flow.
   d. has a central vein at its center.
   e. receives blood from the sublobular vein.
3. A patient with Verner-Morrison syndrome has
   a. polydipsia.
   b. polyphagia.
   c. adenoma.
   d. polyuria.
   e. polymyalgia.
4. A patient who has a malignancy that causes an overproduction of HCl has a tumor of which of the following cells?
   a. Alpha cells
   b. Beta cells
   c. Delta cells
   d. G cells
   e. PP cells
5. A cell of the pancreas possesses specific proteins that bind secretin. Which of the following is the correct response to that statement?
   a. They are peripheral proteins and result in the release of glucagon.
   b. They are peripheral proteins and result in the release of insulin.
   c. They are integral proteins and result in the release of an enzyme-rich fluid.
   d. They are integral proteins and result in the release of an enzyme-poor fluid.
   e. They are integral proteins and result in the secretion of pancreatic polypeptide.
6. A patient with Wilson disease presents with gold rings in her cornea. The problem is that the patient's liver cannot
   a. eliminate urea.
   b. eliminate copper.
   c. synthesize bilirubin.
   d. conjugate cholic acid.
   e. detoxify toxins.
7. Primary saliva
   a. is manufactured by acinar cells of the secretory acinus.
   b. is formed in the lumina of the intercalated ducts.
   c. is modified by serous demilunes.
   d. is formed in the lumina of striated ducts.
   e. enters the oral cavity.
8. A patient has been a long-term user of prescription barbiturates and has developed an increased tolerance for these substances because her
   a. liver enlarged.
   b. hepatocytes increased in number.
   c. mixed function oxidases decreased.
   d. hepatocytes developed more rough ER.
   e. hepatocytes developed more smooth ER.
9. 

   The tip of the arrow is located in the
   a. space of Disse.
   b. bile duct.
   c. portal area.
   d. central vein.
   e. hepatic sinus.

10.



The tip of the arrow is pointing to which of the following?

a. Acinar cell
b. Beta cell
c. Alpha cell
d. Centroacinar cell
e. Delta cell

ANSWERS

1. E. Mumps, a painful viral infection that occurs usually in children, affects the parotid gland, although the other major salivary glands may also be involved. Glands of von Ebner and glands of Blandin-Nuhn are minor salivary glands that are not affected by the paramyxovirus that causes mumps.
2. B. The acinus of Rappaport is a hypothetical lobule of the liver that is divided into three regions and is based on blood flow into the liver. The classical lobule that contains the central vein in its center is considered to be a hexagonal solid. It is the portal lobule that is based on the flow of bile from the liver. The central vein of the classical lobule delivers its blood to the sublobular vein.
3. C. A patient with Verner-Morrison syndrome (pancreatic cholera) has explosive, watery diarrhea that results in hypokalemia and hypochlorhydria. It is caused by excessive manufacture of the hormone vasoactive intestinal peptide due to adenoma of D_1 cells of the islets of Langerhans that produce this hormone. Polydipsia, polyphagia, and polyuria are signs of type I diabetes mellitus. Polymyalgia is a disease of older individuals and is characterized by very painful stiff hip, shoulders, and neck muscles.
4. D. A patient who has a C-cell tumor produces too much gastrin, which results in excess production of hydrochloric acid. Alpha cells produce glucagon; beta cells produce insulin; delta cells are of two types, D cells that produce somatostatin and D_1 cells that produce vasoactive intestinal peptide; and PP cells produce pancreatic polypeptide.
5. D. Secretin binds to integral proteins of the centroacinar cells of the exocrine pancreas with the resultant release of an enzyme-poor bicarbonate-rich fluid. Secretin does not affect the release of glucagon, insulin, pancreatic polypeptide, or digestive enzymes manufactured by the acinar cell of the pancreas.
6. B. Wilson disease is a hereditary condition in which the liver cannot eliminate copper by transferring it into the bile. Accumulation of copper in the liver causes cirrhosis; in the brain, it disrupts normal brain function, causing tremors, aphasia, and even psychosis. If left untreated, the condition is fatal, but chelating agents, such as penicillamine, bind with the copper and facilitate its removal from the body. The liver of this patient can continue to eliminate urea; form bilirubin, a degradation byproduct of hemoglobin; and conjugate cholic acid to form bile salts and can detoxify toxins.
7. A. Primary saliva is manufactured by cells of the secretory acinus and serous demilunes. It is not modified either by serous demilunes or by the intercalated duct. It is modified but not formed by the striated duct. Primary saliva, modified by the striated duct, is known as *secondary saliva*, and that, not primary saliva, enters the oral cavity.
8. E. A patient develops drug tolerance because his or her hepatocytes develop more smooth endoplasmic reticulum in which detoxification of drugs occurs by the increased amount of mixed function oxidases. The livers of these patients do not increase in size, and their hepatocyte number remains constant. Their rough ER does not increase in size and is not involved in detoxification of drugs.
9. D. The clear area at the tip of the arrow is the central vein, as is evident by the radiating plates of hepatocytes bordering the hepatic sinuses. The space of Disse, a very narrow area between the sinusoidal lining cells and the hepatocytes, is barely visible with the light microscope. Bile ducts are located in the portal area, and they possess a simple cuboidal epithelium. The portal area, a region of connective tissue located where three classical lobules contact each other, houses a branch of the hepatic artery, portal vein, bile duct, and lymph vessel.
10. D. The arrow tip is pointing at a centroacinar cell, the beginning of the duct system of the exocrine pancreas. Acinar cells are much more basophilic than centroacinar cells and surround the centroacinar cells. Beta, alpha, and delta cells belong to the islet of Langerhans, a cluster of lighter-staining cells just below the arrow.

This page intentionally left blank

Urinary System

19

The urinary system removes toxic byproducts of metabolism from the bloodstream by forming **urine**. These actions are performed by the two kidneys, which not only remove the toxins from the bloodstream, but also **conserve** salts, glucose, proteins, and water as well as additional materials essential for proper health. Because of these eliminating and conserving functions, the kidneys also help regulating **blood pressure**, **hemodynamics**, and the **acid–base balance** of the body. Urine is delivered from the kidneys into the two **ureters**, from which it passes to a storage organ, the **urinary bladder**. During voiding, the urinary bladder is emptied via the **urethra**, which delivers the urine to outside the body. In addition, the kidneys have an endocrine function in that they produce **renin**, **erythropoietin**, and **prostaglandins**, among others, and they also convert a circulating not very active form of vitamin D_3 **(25-OH-vitamin D_3)** to the active vitamin, known as ***calcitriol*** **(1,25-$[OH]_2$ vitamin D_3)**, and also has the ability to synthesize glucose from noncarbohydrate sources, the process known as ***gluconeogenesis***. Interestingly, the rate of gluconeogenesis by the kidneys can equal that of the liver.

INTAKE AND LOSS OF WATER FROM THE BODY

On a daily basis, an individual's intake of water is approximately 2.1 L, which is gained in the form of liquid plus the water content of food. An additional 200 mL of water is formed by the body due to carbohydrate oxidation.

Water loss is due to a number of sources: insensible loss, loss due to sweat, loss in the feces, loss due to the kidneys:

- **Insensible water loss** occurs in two ways:
  - Via the process of breathing, an individual loses approximately 350 mL of water per day.
  - Via diffusion through the skin, an individual loses approximately 350 mL per day.
- **Water loss due to sweating** is only about 100 mL per day, but that changes due to very hot weather conditions, and during strenuous exercise, the loss may be as much as 10 L per day.
- **Water loss in feces** is approximately 100 mL per day.
- **Water loss through the kidneys** is approximately 1400 mL per day.

Therefore, the loss of water (2.3 L) equals the amount of water an individual takes in (2.3 L), and it is the responsibility of the kidneys to adjust the loss of water in urine to ensure this balanced water conservation. Because the kidneys play such an important role in the body's water balance, much of this chapter is dedicated to the discussion of the structure and functions of the kidneys.

KIDNEY

The kidneys have a concave region, known as the hilum, *where the ureter, renal vein, renal artery, and lymph vessels pierce the kidney.*

The kidneys are large, reddish, bean-shaped organs situated retroperitoneally on the posterior abdominal wall. Because of the position of the liver, the right kidney is approximately 1 to 2 cm lower than the left. Each kidney is about 11 cm long, 4 to 5 cm wide, and 2 to 3 cm thick and is embedded in perirenal fat and lies with its convex border situated laterally and its concave **hilum** facing medially. Branches of the renal artery and vein, lymph vessels, and ureter pierce the kidney at its hilum. The ureter is expanded at this region, forming the **renal pelvis**. A fat-filled extension of the hilum deeper into the kidney is called the ***renal sinus***.

The kidney is invested by a thin, loosely adhering **capsule**, consisting mainly of dense, irregular collagenous connective tissue with occasional elastic fibers and smooth muscle cells.

Overview of Kidney Structure

The kidney is subdivided into an outer cortex and an inner medulla.

A hemisected view of the kidney shows that it is separated into a cortex and a medulla (Fig. 19–1). The cortical region appears dark brown and granular, whereas the medulla contains 6 to 12 discrete, pyramid-shaped, pale, striated regions, the **renal pyramids**. The base of each pyramid is oriented toward the cortex, constituting the corticomedullary border, whereas its apex, known as the ***renal papilla***, points toward the hilum. The apex is perforated by 20 or so openings of the **ducts of Bellini**; this sieve-like region is known as the ***area cribrosa***. The apex is surrounded by a cup-like space, the **minor calyx**, which, joining two or three

SUMMARY

The **urinary system** is composed of the paired **kidneys**, paired **ureters**, a single **urinary bladder**, and the single **urethra**. The two kidneys filter the entire blood supply of the individual every 5 minutes but form only 1 mL of urine per minute by removing urea and other toxins from the blood while conserving water, salts, glucose, and proteins. They also maintain the acid–base balance of the body, regulate blood pressure, manufacture hormones, assist in maintaining the body's homeostasis, and convert the inactive form of vitamin D to the active form. Each kidney has a **cortex** and a **medulla**. The basic functional unit of the kidney is the **uniniferous tubule**, composed of **nephrons** and **collecting tubules**. Nephrons resemble a hairpin and have several distinct regions: **renal corpuscle**, **proximal convoluted tubule**, **pars recta of the proximal tubule** (also known as the ***descending thick limb of Henle loop***), desc**ending thin limb of Henle loop**, **Henle loop**, **ascending thin limb of Henle loop**, ascen**ding thick limb of Henle loop** (also known as the ***pars recta of the distal tubule***), **macula densa**, **distal convoluted tubul**e, and the **connecting tubule**. Connecting tubules of the nephrons drain into **cortical collecting tubules**. Several nephrons drain into each **cortical collecting tubule**. Several cortical collecting tubules join to form **medullary collecting tubules**, several of which form the large **papillary collecting ducts** that deliver their urine into the **minor calyces**. Each kidney has several minor calyces that drain into a few **major calyces** that deliver their urine into the **ureters**. The two ureters drain into the **urinary bladder** that stores urine until it is voided via the **urethra**.

KEY WORDS

Calyces; collecting ducts; collecting tubules; connecting tubules; cortex; distal tubule; Henle loop; kidneys; macula densa; medulla; nephrons; proximal tubule; renal corpuscle; ureters; urethra; urinary bladder; urinary system; uriniferous tubule

neighboring minor calyces, forms a **major calyx**. The three or four major calyces are larger spaces that empty into the **renal pelvis**, the expanded continuation of the proximal portion of the ureter. Neighboring pyramids are separated from each other by material resembling the cortex, the **cortical columns** (of Bertin).

The portion of the cortex overlying the base of each pyramid is known as a ***cortical arch***. Macroscopically, three types of substances may be observed in the cortex: (1) red, dot-like granules, **renal corpuscles**; (2) convoluted tubules, the **cortical labyrinth**; and (3) longitudinal striations, **medullary rays**, which are cortical continuations of material located in the renal pyramids.

A renal pyramid, with its associated cortical arch and cortical columns, represents a **lobe** of the kidney. Hence, the human kidney is a multilobar organ. Each medullary ray with part of the cortical labyrinth surrounding it is considered a kidney **lobule** (bounded by **interlobular arteries**), which continues into the medulla as a cone-shaped structure.

CLINICAL CORRELATIONS

1. During fetal development, the lobes of the kidney are accentuated by deep clefts, but this characteristic is normally obliterated in adults. When the lobation is retained after infancy, the condition is known as ***lobated kidney***.
2. Another anomalous kidney development is known as ***polycystic kidney disease***, which presents varied morphological features according to the severity of the affliction; it involves the appearance of thin-walled cysts on and in the kidneys.

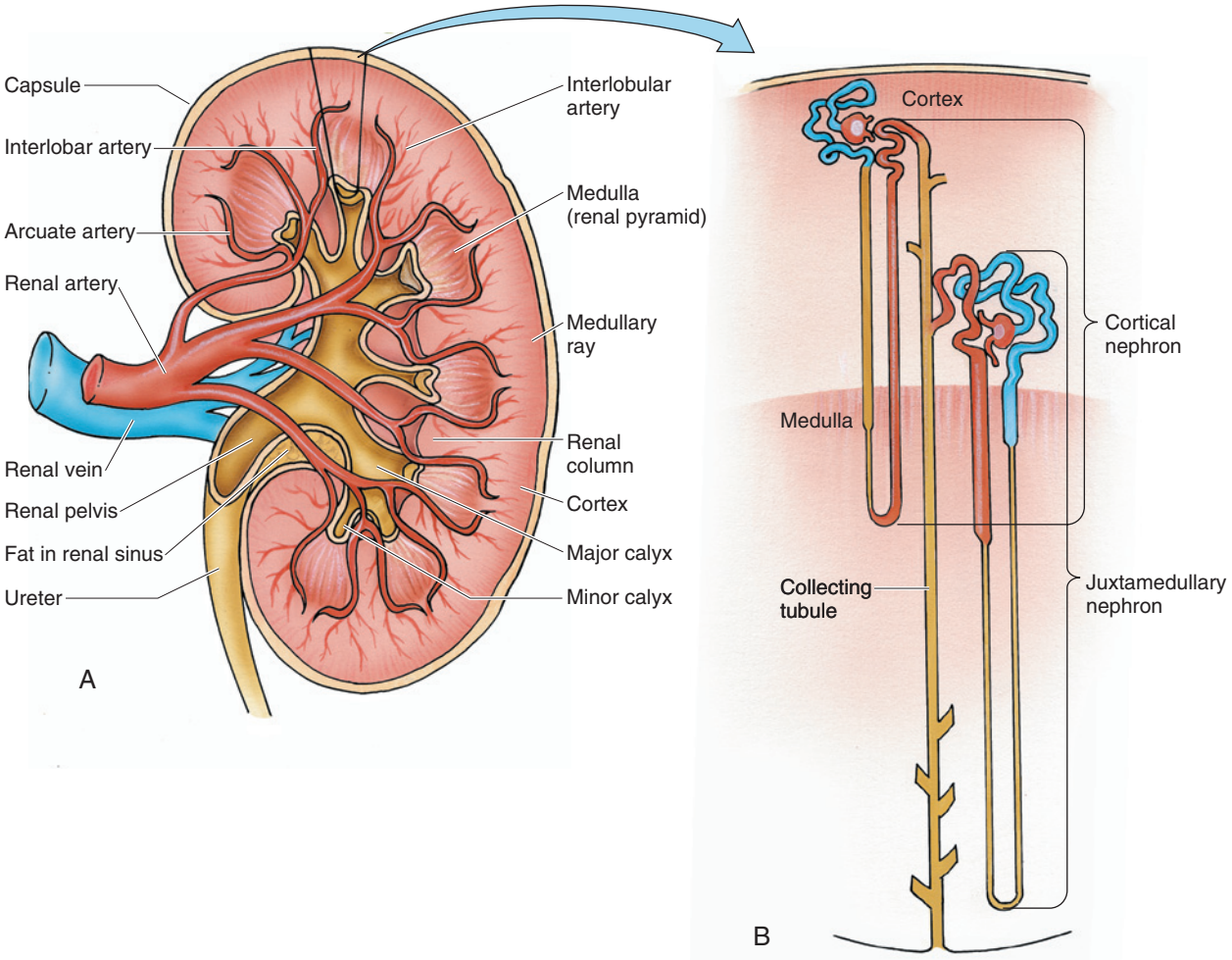


Figure 19–1 The kidney. **A,** Schematic diagram of a hemisected kidney illustrating its morphology and circulation. **B,** Arrangement of cortical and juxtamedullary nephrons.

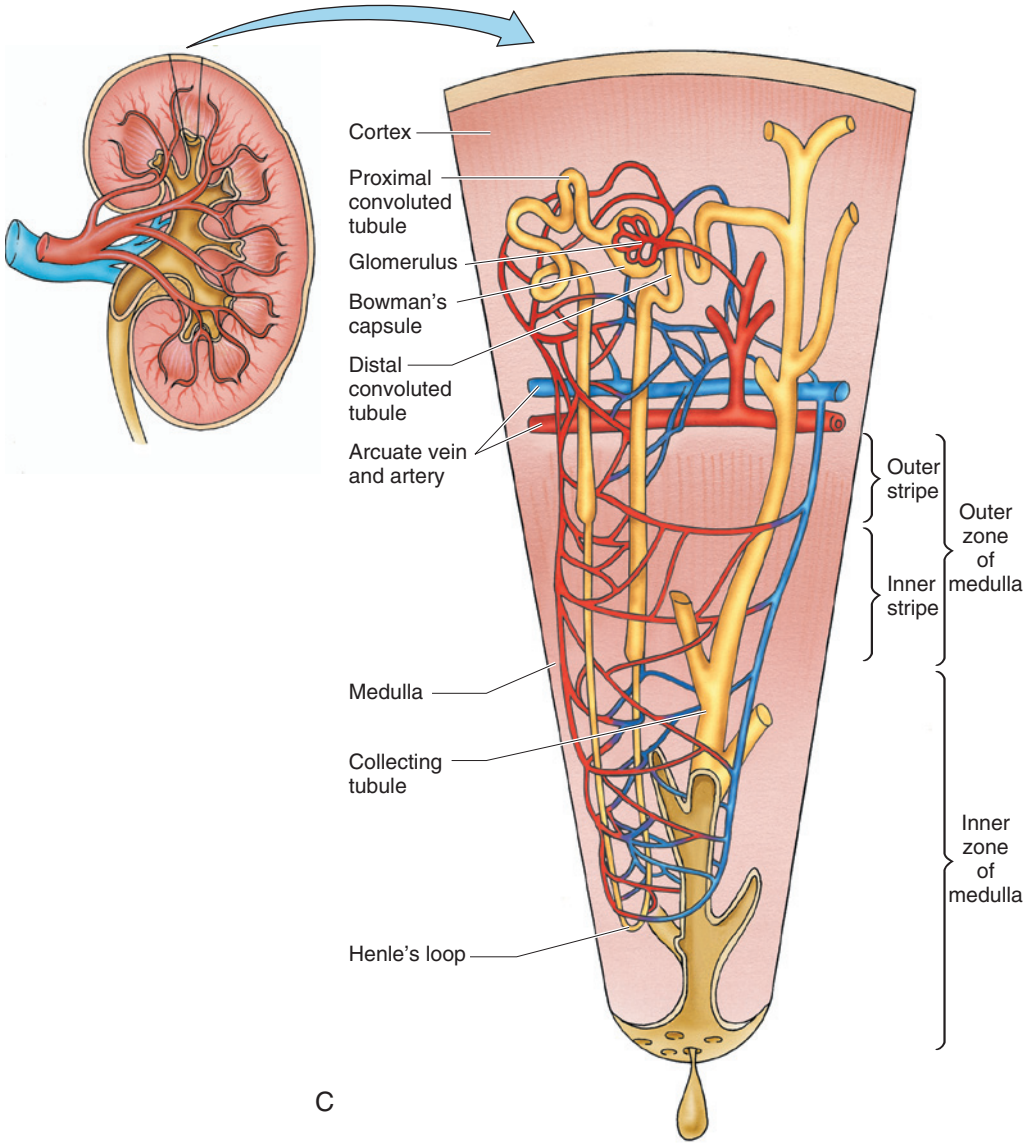


Figure 19–1, cont'd **C,** The uriniferous tubule and its vascular supply and drainage. The juxtamedullary nephron extends much deeper into the medulla than does the cortical nephron.

Uriniferous Tubules

The uriniferous tubule, the functional unit of the kidney, is composed of a nephron and a collecting tubule.

The functional unit of the kidney is the **uriniferous tubule**, a highly convoluted structure that modifies the fluid passing through it to form **urine** as its final output. This tubule consists of two parts, each with a different embryological origin, the **nephron** and the **collecting tubule** (see Fig. 19–1). There are approximately 1.3 million nephrons in each kidney. Several nephrons are drained by a single collecting tubule, and multiple collecting tubules join in the deeper aspect of the medulla to form larger and larger ducts. The largest of these ducts, the **ducts of Bellini**, perforate the renal papilla at the area cribrosa.

Uriniferous tubules are densely packed so that the connective tissue **stroma** of the kidney is scant. The entire uriniferous tubule is epithelial in nature and is, therefore, separated from the connective tissue stroma by an intervening **basal lamina**. Much of the connective tissue is occupied by the rich vascular supply of the kidney. The functional relationship between the vascular supply and the uriniferous tubules is discussed later in the chapter.

Nephron

There are two types of nephrons, depending on the location of their renal corpuscles and the length of their Henle loop.

Two types of nephrons are found in the human kidney: shorter **cortical nephrons**, subdivided into two groups, superficial and midcortical nephrons, neither of which extend deep into the medulla and the longer **juxtamedullary nephrons**, whose renal corpuscle is located in the cortex—at the corticomedullary junction—and whose tubular parts extend deep into the medulla (see Fig. 19–1). The specific locations of the two types of nephrons, the cellular composition of their various regions, and the specific alignments of these regions in register with one another permit the subdivision of the medulla into an **outer zone** and an **inner zone**. The outer zone of the medulla is further subdivided into an **outer stripe** and an **inner stripe**. Unless otherwise noted, all of the descriptions in this textbook refer to juxtamedullary nephrons, even though they constitute only 15% of all nephrons.

Each juxtamedullary nephron is about 40 mm long. The constituent parts of the nephron are modified to perform specific physiological functions. **Bowman capsule**, with its attendant glomerulus, filters the fluid expressed from the bloodstream. The subsequent tubular portions of the nephron (i.e., the **proximal tubule**, the **thin limbs of Henle loop**, and the **distal tubule**) modify the filtrate to form urine.

CLINICAL CORRELATIONS

Because nephrons cannot be regenerated as the individual ages, there is a reduction in the number of nephrons. It has been reported that, after middle age (around 40 years of age), there is an annual 1% loss in the number of nephrons, so that a person who is 75 years old has 35% fewer nephrons than she or he did at age 40. Fortunately, the remaining nephrons compensate for the decrease by adapting to the deteriorated condition, and, provided that all other conditions remain normal, they are able to maintain normal physiological status.

Renal Corpuscle

The renal corpuscle is composed of a tuft of capillaries, the glomerulus, surrounded by Bowman capsule.

The **renal corpuscle**, an oval to round structure about 200 to 250 µm in diameter, is composed of a tuft of capillaries, the **glomerulus**, which is invaginated into **Bowman capsule**, the dilated, pouch-like, proximal end of the nephron (Figs. 19–2 to 19–4; see Fig. 19–1). During development, the capillaries become invested

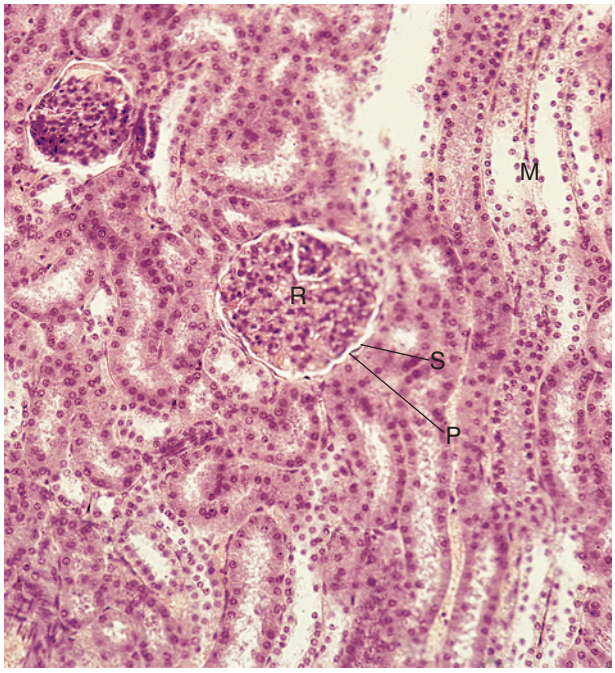


Figure 19–2 Photomicrograph of the kidney cortex in a monkey illustrating renal corpuscles (R), medullary ray (M), and cross-sectional profiles of the uriniferous tubules. A portion of the urinary space (S) is clearly evident at the periphery of the renal corpuscle and is bounded by the simple squamous epithelium composing the parietal layer (P) of Bowman capsule (×132).

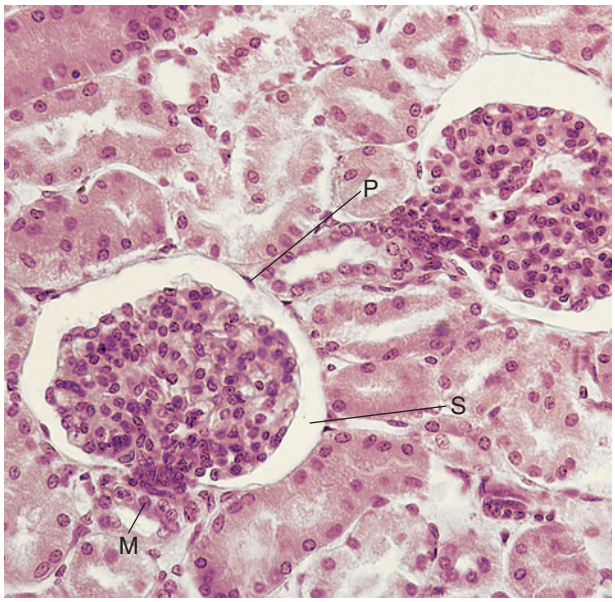


Figure 19–3 Photomicrograph of the monkey renal corpuscle surrounded by cross-sectional profiles of proximal and distal tubules. The macula densa (M) and the parietal layer of Bowman capsule (P) are clearly evident as they enclose the clear space, a part of the urinary space (S) (×270).

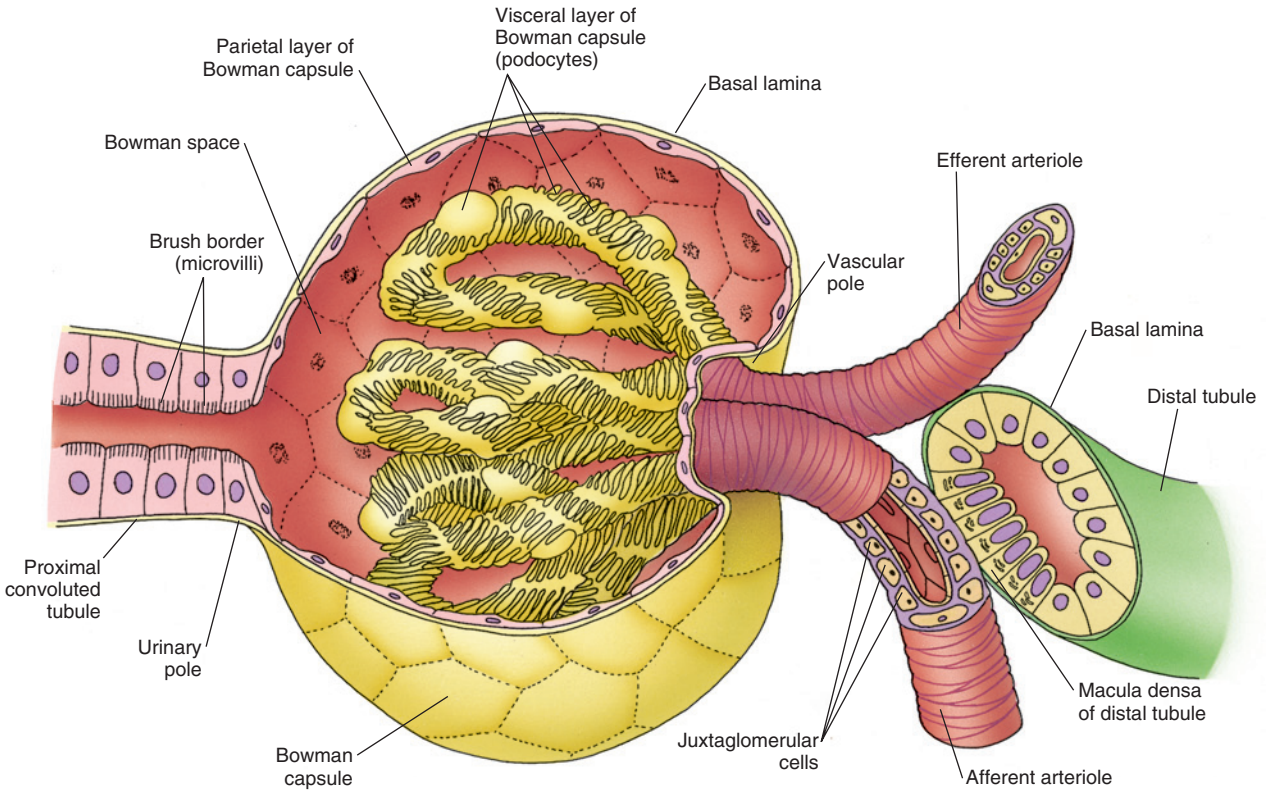


Figure 19–4 Diagram of a renal corpuscle and its juxtaglomerular apparatus.

by the blind end of the tubular nephron, almost as if a hand were to push into the end of an expanded balloon. Hence, the space inside Bowman capsule, known as ***Bowman space (urinary space)***, is decreased in volume. The glomerulus is in intimate contact with the **visceral layer of Bowman capsule**, composed of modified epithelial cells called ***podocytes***. The outer wall surrounding Bowman space, composed of simple squamous epithelial cells (sitting on a thin basal lamina), is the **parietal layer** (see Fig. 19–4).

The region where the vessels supplying and draining the glomerulus enter and exit Bowman capsule is known as the ***vascular pole***, whereas the region of continuation between the renal corpuscle and the proximal tubule, which drains Bowman space, is called the ***urinary pole***. The glomerulus is supplied by the short, straight **afferent glomerular arteriole**, a branch of the **interlobular artery**, and drained by the **efferent glomerular arteriole**; hence, the glomerulus is a completely arterial capillary bed. Although the outer diameter of the afferent arteriole is greater than that of the efferent arteriole, their luminal diameters are approximately equal.

The efferent glomerular arteriole presents greater resistance to blood flow, resulting in higher capillary pressures in the glomerulus than in other capillary beds. Filtrate leaking out of the glomerulus enters Bowman space through a complex **filtration barrier** composed of the endothelial wall of the glomerular capillary, the basal lamina, and the visceral layer of Bowman capsule.

Glomerulus

The glomerulus is composed of tufts of fenestrated capillaries supplied by the afferent glomerular arteriole and drained by the efferent glomerular arteriole.

The **glomerulus** is formed as several tufts of anastomosing capillaries that arise from branches of the afferent glomerular arteriole. The connective tissue component of the afferent arteriole does not enter Bowman capsule, and the normal connective tissue cells are replaced by a specialized cell type known as ***mesangial cells***. There are two groups of mesangial cells. **Extraglomerular mesangial cells** are located at the vascular pole, and pericyte-like **intraglomerular mesangial cells** are situated within the renal corpuscle (Figs. 19–5 and 19–6).

Intraglomerular mesangial cells are probably phagocytic and function in resorption of the basal lamina. Mesangial cells may also be contractile because they

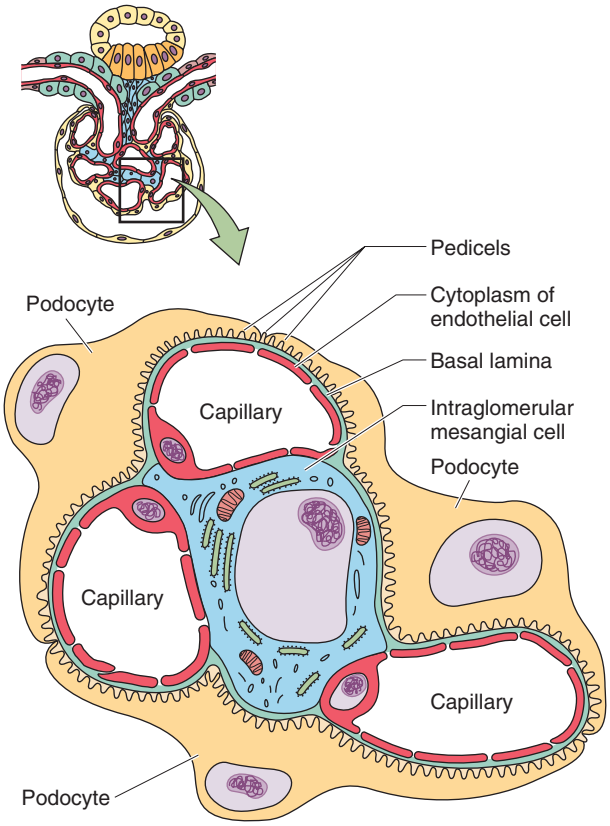


Figure 19–5 Relationship between the intraglomerular mesangial cell, podocytes, and glomerulus.

have receptors for vasoconstrictors such as **angiotensin II** and **atrial natriuretic peptide** and thus reduce blood flow through the glomerulus. Mesangial cells also synthesize various cytokines, such as interleukin-1, endothelins, platelet-derived growth factor, prostaglandin E_2, as well as mesangial matrix. Moreover, they, along with podocytes and the glomerular basement membrane, provide physical support to the capillaries of the glomerulus. The glomerulus is composed of fenestrated capillaries (Fig. 19–7; see Figs. 19–5 and 19–6) whose endothelial cells are highly attenuated, except for the region containing the nucleus; their fenestrae are usually not covered by a diaphragm. The pores are large, ranging between 70 and 90 nm in diameter; hence, these capillaries act as a barrier only to formed elements of the blood and to macromolecules whose effective diameter exceeds the size of the fenestrae (e.g., albumin, 69,000 daltons [Da]).

Basal Lamina. Investing the glomerulus is a glomerular basal lamina (~300 nm thick), consisting of three layers (see Figs. 19–6 and 19–7). The middle dense layer, the **lamina densa**, is about 100 nm in thickness and consists of type IV collagen, however, not the usual one composed of α_1 and α_2 chains that are present in lamina densa of basal laminae in other regions of the body but the α_3, α_4, and α_5 chains. Less electron-dense layers, the **laminae rarae**, which contain **laminin**, **fibronectin**, and the highly hydrated polyanionic proteoglycans, perlecan and agrin, rich in **heparan sulfate**, are located on either side of the lamina densa. Some refer to a **lamina rara interna**, between the endothelial cells of the capillary and the lamina densa, and the **lamina rara externa**, between the lamina densa and the visceral layer of Bowman capsule. Fibronectin and laminin assist the pedicels and endothelial cells to maintain their attachment to the lamina.

CLINICAL CORRELATIONS

Mutations in the α_3 and α_4 chains of type IV collagen result in **Alport syndrome**, an autosomal recessive form that is distinguished by loss of hearing, vision problems, and nephritis accompanied by microscopic hematuria. Patients with Alport syndrome frequently suffer from kidney failure and may, eventually, require a kidney transplant.

Visceral Layer of Bowman Capsule

The visceral layer of Bowman capsule is composed of epithelial cells that become modified and are known as podocytes.

The visceral layer of Bowman capsule is composed of epithelial cells that are highly modified to perform a filtering function. These large cells, called ***podocytes***, bear numerous long, tentacle-like cytoplasmic extensions, **primary (major) processes**, which follow but usually do not come in close contact with the longitudinal axes of the glomerular capillaries (see Fig. 19–7). Each primary process bears many **secondary processes**, also known as ***pedicels***, arranged in an orderly fashion. Pedicels completely envelop most of the glomerular capillaries by interdigitating with pedicels from neighboring major processes of different podocytes (Figs. 19–8 and 19–9). Podocytes also manufacture **glomerular endothelial growth factor** that functions not only in maintaining the integrity of the endothelial cells of the glomerulus but also prompts them, when the need arises, to enter the cell cycle.

Pedicels have a well-developed glycocalyx composed of the negatively charged sialoprotein **podocalyxin**, **podoplanin**, and **podoendin**, which form an electrostatic repulsion that contributes to the formation of the filtration barrier. Pedicels rest on the lamina rara externa of the basal lamina and possess **$\alpha_3\beta_1$ integrins** in their plasmalemma that assists them to adhere to the basal lamina. Their cytoplasm is devoid of organelles but does house microtubules and microfilaments. Interdigitation occurs in such a fashion that narrow clefts, 20 to 40 nm

Figure 19–6 Electron micrograph of a region of the human kidney glomerulus containing red blood cells (×4594). Note the association between the intraglomerular mesangial cell and the podocytes around the glomerular capillaries. BS, Bowman space; CL, capillary lumen; E, endothelial cell; M, mesangial cells; V, podocyte. (From Brenner BM, Rector FC. *The Kidney*. 4th ed. Vol 1. Philadelphia: WB Saunders; 1991.)

in width, known as ***filtration slits***, remain between adjacent pedicels. Filtration slits are not completely open; instead, they are covered by a thin **filtration slit diaphragm**, which extends between neighboring pedicels and acts as a part of the filtration barrier (Fig. 19–10; see Fig. 19–7). The slit diaphragm has a central bar, on either side of which are rows of pores 14 nm^2 in area. The slit diaphragm of the two neighboring pedicels bind to each other via the extracytoplasmic moieties of the transmembrane proteins **nephrin**. The intracytoplasmic component of nephrin is connected to actin filaments by two interlinking proteins, **CD2 associated protein** and **podocin**.

The cell body of the podocyte is not at all unusual in organelle content. It houses the irregularly shaped nucleus as well as rough endoplasmic reticulum, Golgi apparatus, and numerous free ribosomes.

Filtration Process. Fluid leaving the glomerular capillaries is driven by the **blood pressure** in the glomerular capillary bed (60 mm Hg), but that is resisted by the **colloid osmotic pressure** of the blood in the glomerulus (32 mm Hg) and the hydrostatic pressure of the fluid, known as the ***oncotic pressure***, in the urinary space (18 mm Hg). Therefore, there is a **net filtration pressure** of 10 mm Hg, which drives fluid into the urinary space. In order for the fluid to leave the glomerular capillaries it has to pass through the fenestrae of the capillary endothelial cells and this fluid is filtered by the basal lamina. The lamina densa traps larger molecules (>69,000 Da), whereas the polyanions of the laminae rarae impede the passage of negatively charged molecules and molecules that are incapable of deformation. The fluid containing small molecules, ions, and macromolecules that penetrates the lamina densa, must

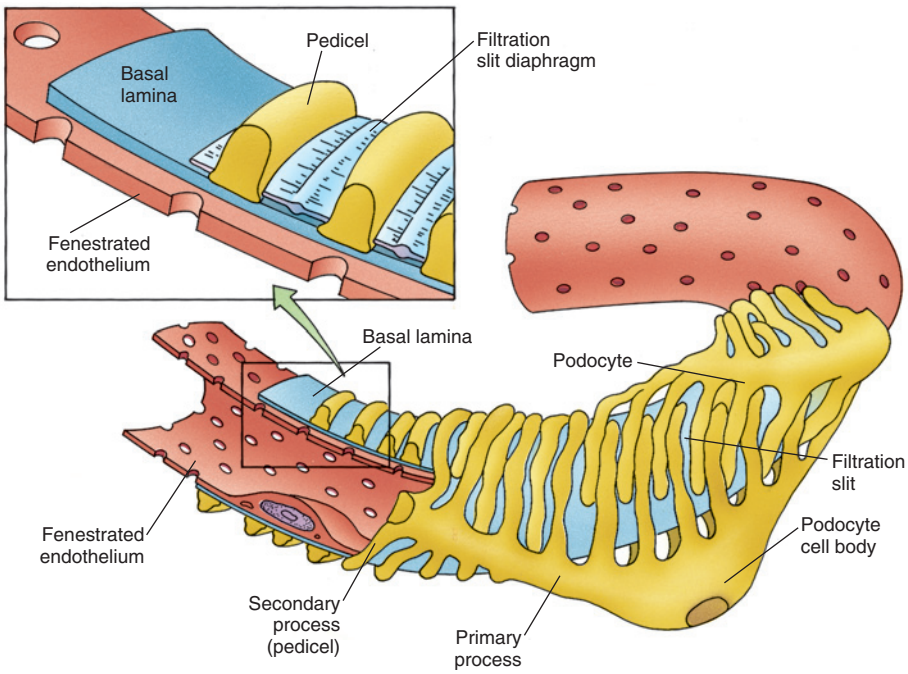


Figure 19–7 Schematic diagram of the interrelationship of the glomerulus, the podocytes, the pedicels, and the basal laminae.

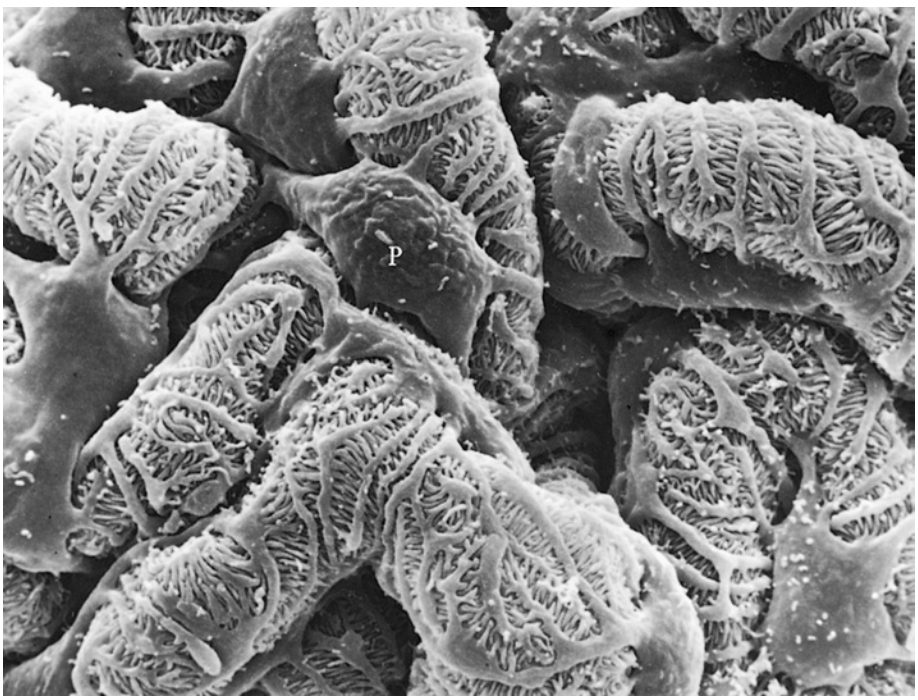


Figure 19–8 Scanning electron micrograph of podocytes and their processes from the kidney of a rat (×4700). P, Podocytes. (From Brenner BM, Rector FC. *The Kidney*. 4th ed. Vol 1. Philadelphia: WB Saunders; 1991.)

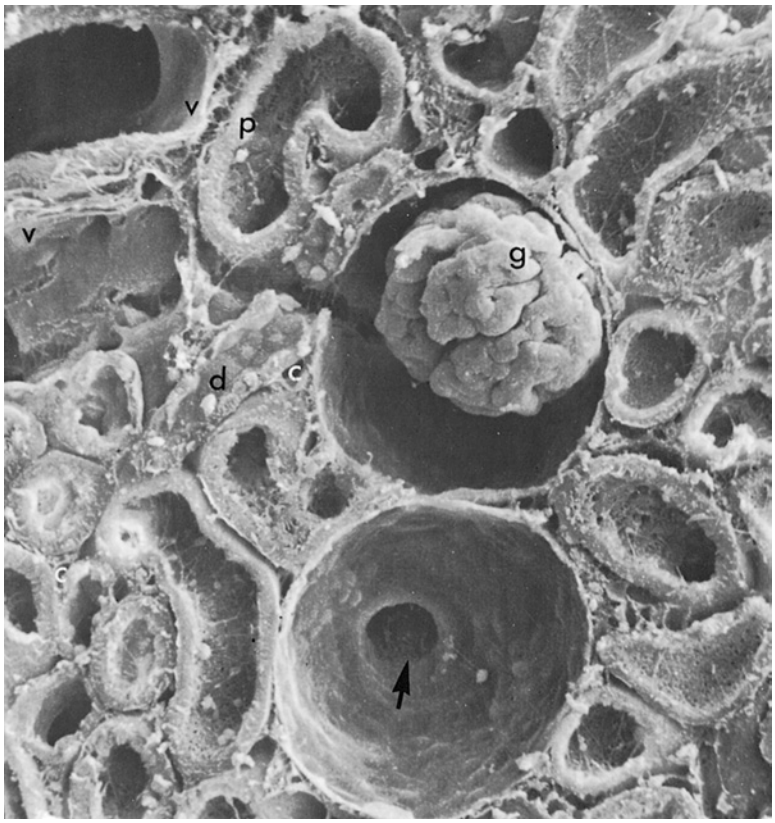


Figure 19–9 Scanning electron micrograph of the rat renal cortex displaying a renal corpuscle with its glomerulus (g) (×543). The renal corpuscle below it does not have its glomerulus, so the urinary pole *(arrow)* is evident. C, Capillaries; d, distal convoluted tubule; P, proximal convoluted tubule; v, blood vessels. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

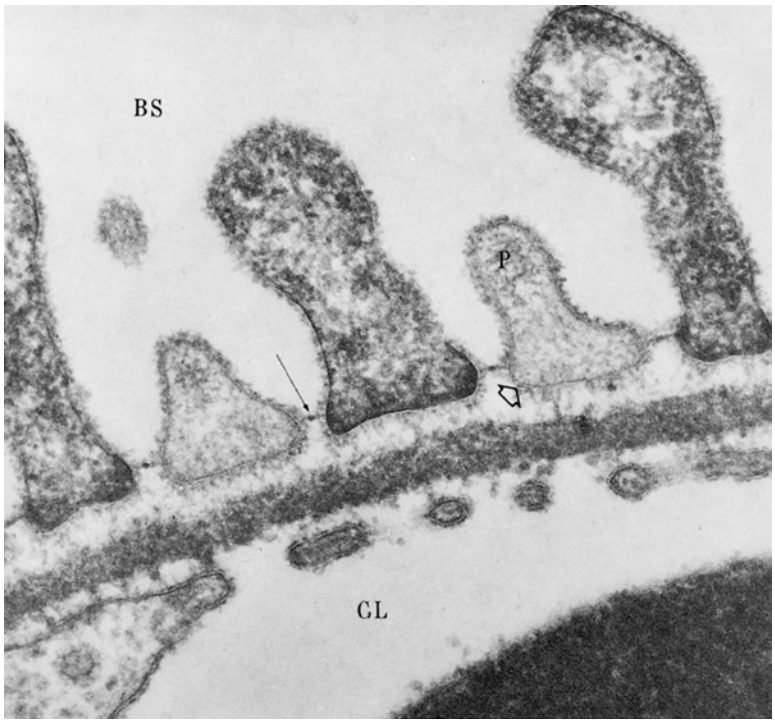


Figure 19–10 Electron micrograph of pedicels and diaphragms bridging the filtration slits of a glomerulus in a rat (×86700). BS, Bowman space; CL, capillary lumen. *Hollow arrow* indicates the laminae rara externa; the *arrow* indicates the filtration slit diaphragm. (From Brenner BM, Rector FC. *The Kidney*. 4th ed. Vol 1. Philadelphia: WB Saunders; 1991.)

pass through the pores in the slit diaphragm of the filtration slits; if the macromolecules are uncharged and if they are 1.8 nm or less in diameter, they can pass without any hindrance through the slit diaphragm. If the uncharged macromolecules are greater than 4 nm in diameter they cannot pass through the slit diaphragm. The fluid entering Bowman space, is called the ***glomerular ultrafiltrate***. The rate at which the ultrafiltrate enters the urinary space per unit time (usually in mL/min) is referred to as the ***glomerular filtration rate (GFR)***.

Because the basal lamina traps larger macromolecules, it would become clogged were it not continuously phagocytosed by **intraglomerular mesangial cells** and replenished by both the visceral layer of Bowman capsule (podocytes) and glomerular endothelial cells.

CLINICAL CORRELATIONS

1. The presence of albumin in the urine **(albuminuria)** is the result of increased permeability of the glomerular endothelium. Among the causes of this condition are vascular injury, hypertension, mercury poisoning, and exposure to bacterial toxins.
2. The basal lamina may also become impaired because of the deposition of antigen–antibody complexes that are filtered from the glomeruli or from the reaction of antibasement membrane antibody with the basal lamina itself. Both of these instances produce types of **glomerulonephritis**.
3. In cases of **lipoid nephrosis**, the basal laminae are not congested with antibodies, but adjacent pedicels appear to fuse with one another. This disease is one of the most prevalent kidney disorders in children.
4. Individuals with **diabetic kidney disease** present with **proteinuria**, which has been suggested to be a function of damaged or altered podocytes. Some of these problems may be due to apoptosis of podocytes resulting in areas denuded of these cells where proteins can leak into the urinary space; other problems may be due to alterations in the morphology of the **slit diaphragms** again permitting the escape of proteins into Bowman space; or some conditions result in podocyte hypertrophy with a resultant detachment of the enlarged cells, again forming denuded regions where proteins can escape into the urinary space.

Proximal Tubule

The proximal tubule has two regions: the proximal convoluted tubule and the pars recta of the proximal tubule.

Bowman space drains into the **proximal tubule** at the **urinary pole**. In this junctional region, sometimes called the *neck* of the proximal tubule (negligible in humans), the simple squamous epithelium of the parietal layer of Bowman capsule joins the simple cuboidal epithelium of the proximal tubule (see Fig. 19–4). The proximal tubule, constituting much of the renal cortex, is approximately 60 µm in diameter and about 14 mm long. The tubule consists of a highly tortuous region, the **pars convoluta (proximal convoluted tubule)**, located near renal corpuscles, and a straighter portion, the **pars recta (descending thick limb of Henle loop)**, which descends in medullary rays within the cortex and then within the medulla to become continuous with the **loop of Henle** at the junction of the outer and inner stripes.

Viewed with the light microscope, the convoluted portion of the proximal tubule is composed of a simple cuboidal type of epithelium with an eosinophilic, granular appearing cytoplasm (Fig. 19–11; see Fig. 19–3). These cells have an elaborate striated border and an intricate system of interlocking and interwoven lateral cell processes. Thus, the lateral cell membranes are usually indistinguishable with the light microscope. The height of the cells varies with their functional state—from a low cuboidal to an almost high cuboidal epithelium.

The method and rapidity of fixation modify the microscopic morphology of the proximal convoluted tubule because its lumen is kept open by fluid pressure. Ideal fixation demonstrates wide open, empty lumina and no clumping of the striated border. However, paraffin sections usually display mostly occluded lumina; fluted and ragged-appearing striated borders; few, basally placed nuclei per cross-section of the tubule; and a lack of distinct lateral cell membranes. The cuboidal cells sit on a well-defined basement membrane, easily demonstrated by the periodic acid–Schiff reaction. Each cross-section is composed of approximately 10 to 20 cells, but because these cells are large, usually only six to eight nuclei are included in the plane of section (see Fig. 19–3).

On the basis of the ultrastructural features of its component cells, the proximal tubule can be subdivided into three regions:

- The first two thirds of the pars convoluta is designated $\mathbf{S_1}$.
- The remainder of the pars convoluta and much of the pars recta is designated $\mathbf{S_2}$.
- The remainder of the pars recta is designated $\mathbf{S_3}$.

Cells of the $\mathbf{S_1}$ **region** have long (1.3 to 1.6 µm), closely packed microvilli and a system of intermicrovillar caveolae, known as ***apical canaliculi***, that extend into the apical cytoplasm (Fig. 19–12). This system is more extensive during active diuresis, suggesting that it functions in resorption of proteins during tubular clearing of the **glomerular ultrafiltrate**. Mitochondria, Golgi apparatus, and other normal cellular components are present in these cells. Elaborate lateral and basal

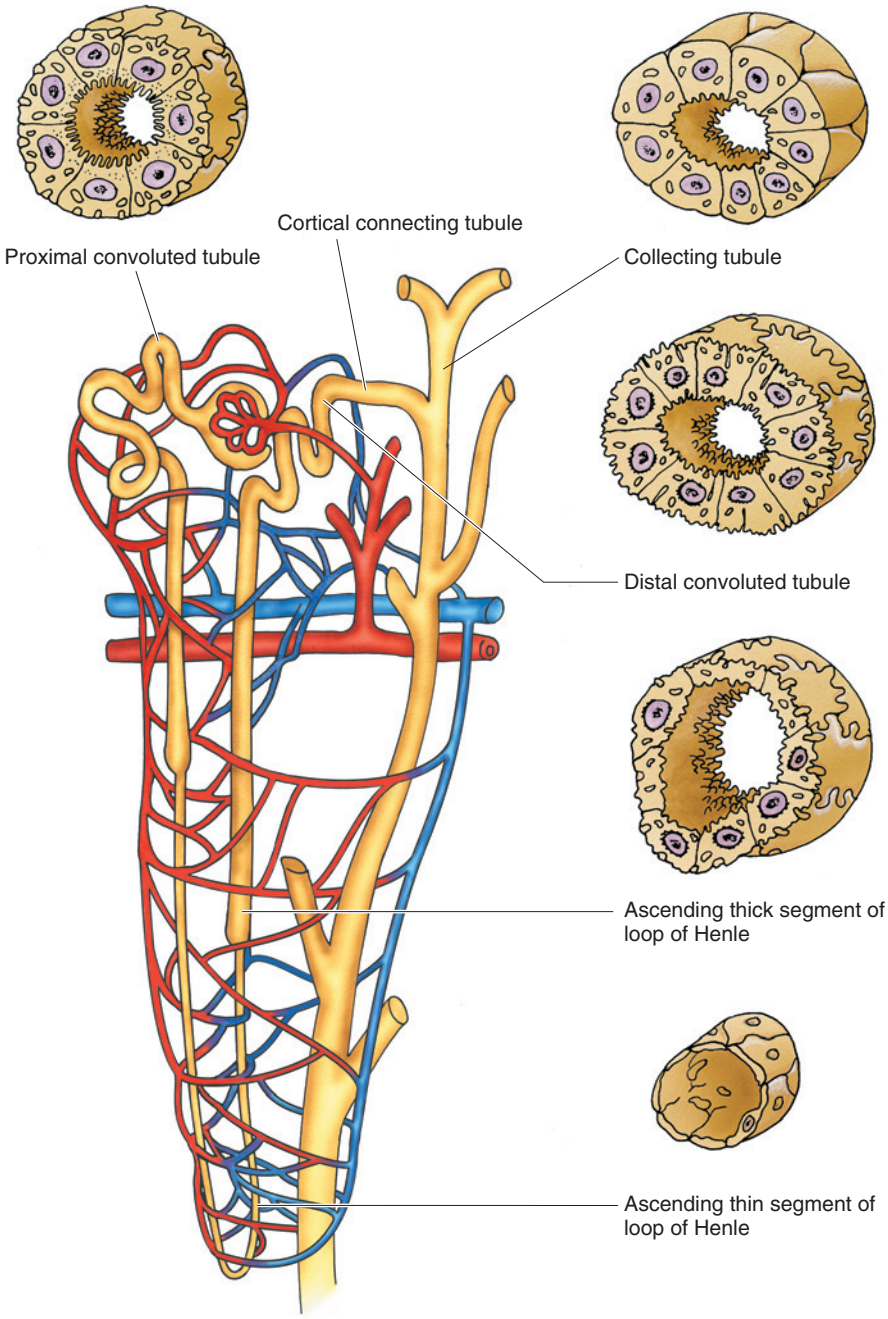


Figure 19–11 Schematic diagram of the uriniferous tubule and its cross-sectional morphology as viewed with the light microscope.

processes may extend almost the entire height of the cell. These processes are long and narrow and usually accommodate elongated, tubular mitochondria.

Cells composing the **S_2 region** are similar to those of the S_1 region, but they have fewer mitochondria and apical canaliculi, have less elaborate intercellular processes, and are lower in height.

Cells of the **S_3 region** are low cuboidal with few mitochondria. These cells have only infrequent intercellular processes and no apical canaliculi.

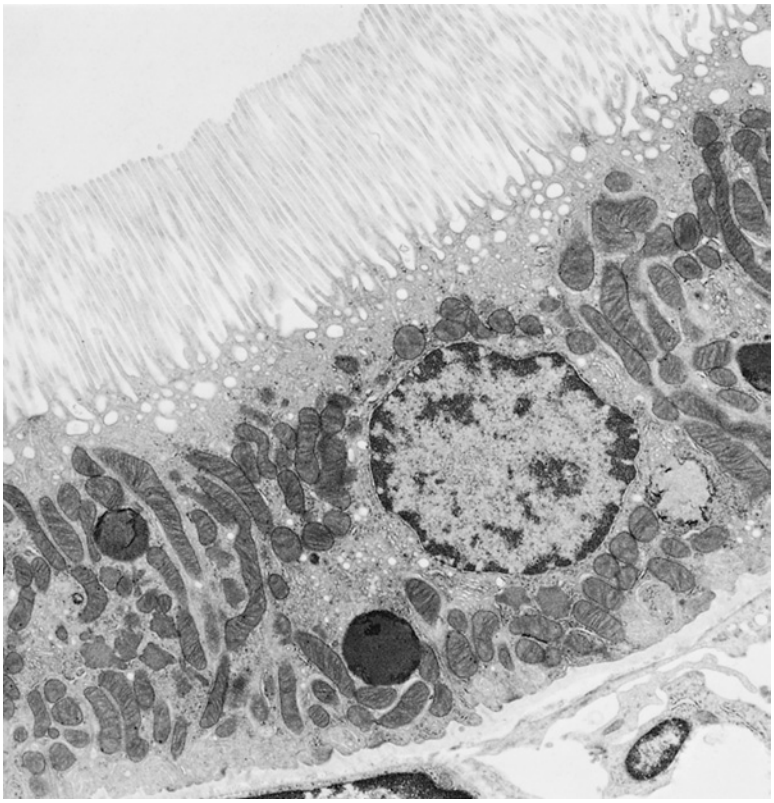


Figure 19–12 Electron micrograph of the S_1 segment of the rat proximal tubule (×7128). (From Brenner BM, Rector FC. *The Kidney*. 4th ed. Vol 1. Philadelphia: WB Saunders; 1991.)

About 67% to perhaps as much as 80% of sodium, chloride (Cl^-), and water is resorbed from the glomerular ultrafiltrate and transported into the connective tissue stroma by cells of the proximal tubule. Sodium is actively pumped out of the cell at the basolateral cell membranes by a sodium pump associated with sodium-potassium adenosine triphosphatase (Na^+-K^+ ATPase). The sodium (Na^+) is followed by chloride, mostly through the tight junctions between cuboidal cells (that is via the **paracellular route**) but also via co-transport with sodium ions along the apical (luminal) cell membranes to maintain electrical neutrality and is also followed by water to maintain osmotic equilibrium. The water also passes mostly through tight junctions between the cuboidal cells as well as through aquaporin 1 channels located in the basolateral cell membrane. In addition, all of the glucose, amino acids, and protein in the glomerular ultrafiltrate are resorbed by the vacuolar endocytic apparatus of the cells of the proximal tubule. Moreover, the proximal tubule excretes a limited amount of H^+ ions into the urine and also eliminates the organic solutes (e.g., catecholamines, bile salts, and oxalate), drugs (e.g., penicillin), and toxins that must be rapidly excreted from the body. Proximal tubules are able to monitor the ultrafiltrate by the presence of a single **primary cilium** on the luminal aspect of each cuboidal cell.

Thin Limbs of Henle Loop

The thin limbs of the loop of Henle have three regions: the descending thin limb, Henle loop, and the ascending thin limb.

The pars recta of the proximal tubule continues as the **thin limb of Henle loop** (see Fig. 19–11). This thin tubule, whose overall diameter is about 15 to 20 µm, is composed of squamous epithelial cells with an average height of 1.5 to 2 µm. The length of the thin segments varies with the location of the nephron (see Fig. 19–1). In cortical nephrons, the thin segment is only 1 to 2 mm long or may be completely absent. Juxtamedullary nephrons have much longer thin segments, 9 to 10 mm in length, and they form a hairpin-like loop that extends deep into the medulla as far down as the renal papilla. The region of the loop continuous with the pars recta of the proximal tubule is called the ***descending thin limb* (of Henle loop)**, the hairpin-like bend is **Henle loop**, and the region that connects Henle loop to the pars recta of the **distal tubule** is known as the ***ascending thin limb (of Henle loop)***.

TABLE 19–1 Cell Types Composing the Thin Limbs of Henle Loop

Cell Type	Location	Fine Structural Features
Type I	Cortical nephrons	Squamous cells with no lateral processes and no interdigitations
Type II	Juxtamedullary nephrons; descending thin limb of the outer zone of the medulla	Squamous cells with numerous, long, radiating processes that interdigitate with those of neighboring cells; fascia occludentes between cells; infoldings of the basal plasmalemma
Type III	Juxtamedullary nephrons; descending thin limb of the inner zone of the medulla	Squamous cells with fewer processes and interdigitations than those of type II
Type IV	Juxtamedullary nephrons; ascending thin limb	Squamous cells with numerous, long, radiating processes that interdigitate with those of neighboring cells as in type II cells; no infoldings of the basal plasmalemma

The nuclei of the cells composing the thin limbs bulge into the lumen of the tubule; hence, in paraffin section, these limbs resemble capillaries in cross-section (see Fig. 19–11). They may be distinguished from capillaries, in that their epithelial lining cells are slightly thicker, their nuclei stain less densely, and their lumina contain no blood cells.

The fine structure of the epithelial cells constituting the thin segments is not unusual. They present a few short, stubby microvilli on their luminal surfaces and a few mitochondria in the cytoplasm surrounding the nucleus. Numerous processes project from the basal portion of the cell to interdigitate with those of neighboring cells.

It is possible to differentiate among four types of epithelial cells composing different regions of Henle loop according to their fine structural features. The locations and fine structural features of the four cell types are listed in Table 19–1.

The descending thin limb is highly permeable to water due to the presence of numerous aquaporin 1 water channels; it is moderately permeable to urea, and slightly permeable to sodium, chloride, and other ions. The major difference between the ascending and descending thin limbs is that the ascending thin limb is only slightly permeable or almost impermeable to water. The significance of this difference in water permeability is discussed below.

Distal Tubule

The distal tubule has three regions: the pars recta (the ascending thick limb of Henle loop), the macula densa, and the pars convoluta (the distal convoluted tubule).

The **distal tubule** is subdivided into the **pars recta**, which, as the continuation of the ascending thin limb of Henle loop, is also known as the ***ascending thick limb of Henle loop***, and the **pars convoluta (distal convoluted tubule)**. Interposed between the ascending thick limb and the distal convoluted tubule is a modified region of the distal tubule called the ***macula densa***.

The ascending thick limb of Henle loop is 9 to 10 mm in length and 30 to 40 µm in diameter. It joins the ascending thin limb at the junction of the inner stripe with the inner zone of the medulla and ascends straight up through the medulla to reach the cortex. The low cuboidal epithelial cells composing the ascending thick segment have centrally placed, round to slightly oval nuclei and a few club-shaped, short microvilli. Although the lateral aspects of these cells interdigitate with each other, the interrelationships between neighboring cells are not nearly as elaborate as in the proximal convoluted tubules. Basal interdigitations are much more extensive, however, and the number of mitochondria is greater in these cells than in those of the proximal convoluted tubules. Moreover, these cells form highly efficient zonulae occludentes with their neighboring cells.

The thick ascending limb is ***not*** permeable to water or urea. In addition, its cells have **1-sodium, 1-potassium, 2-chloride co-transporters (pumps)** that function in the active transport of 2 ions of chloride, and 1 ion of sodium and potassium each from the lumen of the tubule into the cell of the thick ascending limb. At the same time, sodium-potassium ATPase pumps of the basal cell membrane (contacting the basal lamina) deliver equimolar quantities of K^+ ions into the cell and Na^+ ion out of the cells as well as Na^+-H^+ co-transporters at the luminal surface that deliver a limited amount of hydrogen ions into the lumen, mildly acidifying the ultrafiltrate and resorb sodium ions from the lumen into the cell. Thus, as the filtrate reaches the cortex of the kidney within the lumen of the distal tubule, its salt concentration is low and its urea concentration remains high. These cells also manufacture **Tamm-Horsfall protein (uromodulin)**, which they release into the lumen of the thick ascending limb to impede the formation of kidney stones as well as decreasing the chances of urinary tract infection.

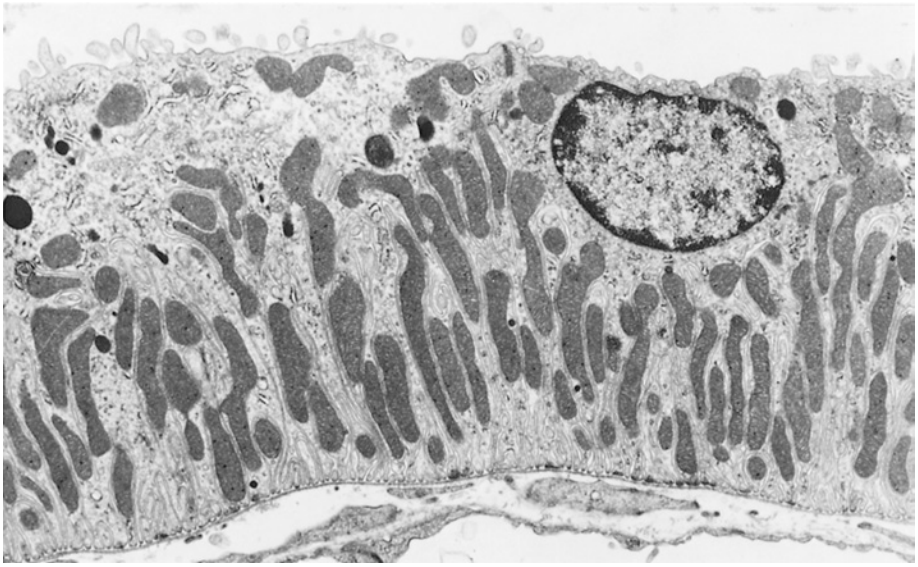


Figure 19–13 Electron micrograph of the distal convoluted tubule (×8100). (From Brenner BM, Rector FC. *The Kidney*. 4th ed. Vol 1. Philadelphia: WB Saunders; 1991.)

As the ascending thick limb of the Henle loop passes near its own renal corpuscle, it lies between the afferent and efferent glomerular arterioles. This region of the distal tubule is called the ***macula densa***, a component of the **juxtaglomerular apparatus** (described in the next section). Because the cells of the macula densa are tall and narrow, the nuclei of these cells are much closer together than those of the remainder of the distal tubule, thus appearing dark and dense, hence the name macula densa.

Distal convoluted tubules are short (4 to 5 mm) with an overall diameter of 25 to 45 μm. In paraffin sections, the lumina of these tubules are wide open, the granular cytoplasm of the low cuboidal lining epithelium is paler than those of proximal convoluted tubules, and because the cells are narrower, more nuclei are apparent in tubular cross-section. The ultrastructure of these cells demonstrates a clear, pale cytoplasm with a few, blunt apical microvilli (Fig. 19–13). Nuclei are more or less round and apically located, having one or two dense nucleoli. Mitochondria are not as numerous, and the basal interdigitations are not as extensive as those of the ascending thick limb of Henle loop.

Because distal convoluted tubules are much shorter than proximal convoluted tubules, any section of the kidney cortex presents many more cross-sections of proximal convoluted tubules than cross-sections of distal convoluted tubules. Indeed, the ratio of cross-sections of proximal to distal convoluted tubules surrounding any renal corpuscle is usually 7:1.

Distal convoluted tubules usually ascend slightly above their own renal corpuscles and drain into the arched portion of the collecting tubules.

Similar to the thick ascending limbs, the distal convoluted tubule is impermeable to water and urea. However, in the basolateral plasmalemma of its cells, high Na^+-K^+ ATPase activity powers sodium-potassium exchange pumps. Thus, in response to the hormone **aldosterone**, these cells can actively resorb almost all of the remaining sodium (and, passively, chloride) from the lumen of the tubule into the renal interstitium. In addition, potassium and hydrogen ions are actively secreted *into* the lumen, thus controlling the body's extracellular fluid potassium level and the acidity of urine, respectively.

Juxtaglomerular Apparatus

The juxtaglomerular apparatus has three components: the macula densa of the distal tubule, juxtaglomerular cells of the afferent glomerular arteriole, and extraglomerular mesangial cells.

The **juxtaglomerular apparatus** consists of the **macula densa** of the distal tubule, **juxtaglomerular cells** of the adjacent afferent (and, occasionally, efferent) glomerular arteriole, and the **extraglomerular mesangial cells** (also known as *polkissen*, *lacis cells*, and *polar cushions*). These structures are illustrated schematically in Figure 19–14.

The cells of the **macula densa** are tall, narrow, pale cells with centrally placed nuclei (Fig. 19–15; see Figs. 19–2 to 19–4 and Fig. 19–14). Because of the narrowness of these cells, the densely staining nuclei are near to each other; collectively, viewed with the light microscope, they appear as a dense spot. With the electron microscope, these cells demonstrate numerous

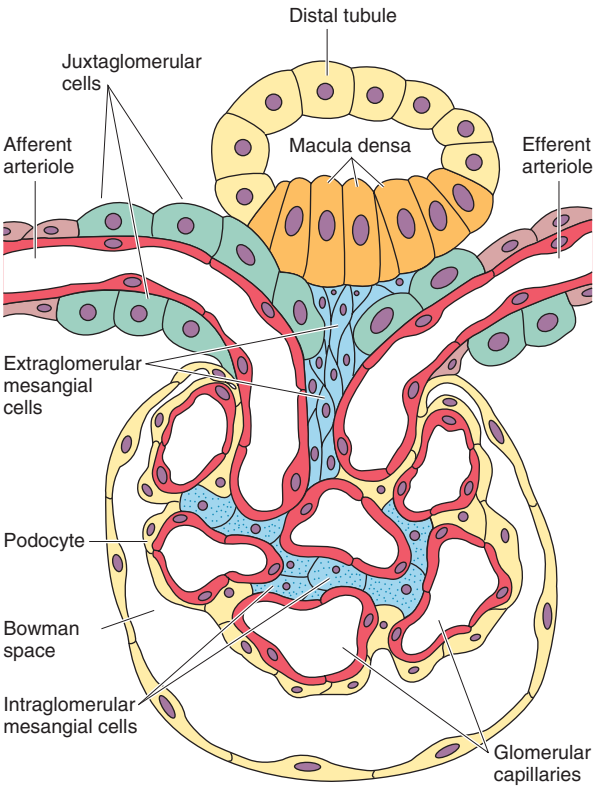


Figure 19–14 Schematic diagram of the juxtaglomerular apparatus.

microvilli, small mitochondria, and an infranuclearly located Golgi apparatus (see Fig. 19–15).

The **juxtaglomerular cells**, modified smooth muscle cells located in the tunica media of afferent (and, occasionally, efferent) glomerular arterioles, are richly innervated by sympathetic nerve fibers. The nuclei of these cells are round instead of elongated. Juxtaglomerular cells contain specific granules demonstrated to be the proteolytic enzyme **renin** (see Fig. 19–15). Small quantities of **angiotensin-converting enzyme (ACE)**, **angiotensin I**, and **angiotensin II** are also present in these cells.

Juxtaglomerular cells and the cells of the macula densa have a special spatial relationship because the basal lamina, normally present between epithelium and other tissues, is absent at this point, permitting intimate contact between cells of the macula densa and the juxtaglomerular cells.

The extraglomerular mesangial cells, the third member of the juxtaglomerular apparatus, occupy the space bounded by the afferent arteriole, macula densa, efferent arteriole, and vascular pole of the renal corpuscle. These cells may contain occasional granules and are probably contiguous with the intraglomerular mesangial cells. The functional significance of the juxtaglomerular apparatus is discussed later in the chapter.

Collecting Tubules

Collecting tubules, composed of a simple cuboidal epithelium, convey and modify the ultrafiltrate from the nephron to the minor calyces of the kidney.

Collecting tubules are not part of the nephron. They have different embryological origins, and it is only later in development that they meet the nephron and join it to form a continuous structure. The distal convoluted tubules of several nephrons join to form a short **connecting tubule** that leads into the collecting tubule (Fig. 19–16; see Fig. 19–11). The glomerular ultrafiltrate that enters the collecting tubule is modified and delivered to the medullary papillae. Collecting tubules are about 20 mm long and have three recognized regions (see Fig. 19–1):

- Cortical
- Medullary
- Papillary

Cortical collecting tubules are located in the medullary rays and are composed of two types of cuboidal cells (see Figs. 19–2 and 19–11):

- **Principal cells (light cells)** have oval, centrally located nuclei; a few small mitochondria; and short, sparse microvilli. The basal membranes of these cells display numerous infoldings. Because the lateral cell membranes are not plicated, they are clearly evident with the light microscope. These cells possess numerous aquaporin-2 channels that are very sensitive to **antidiuretic hormone (ADH)** and become completely permeable to water, thereby permitting these cells to resorb water from the ultrafiltrate, thus ***concentrating*** urine.
- **Intercalated cells (dark cells)** display numerous apical vesicles 50 to 200 nm in diameter, microplicae on their apical plasmalemma, and an abundance of mitochondria. The nuclei of these cells are round and centrally located. There are two types of intercalated cells, **type α cells**, whose luminal membrane possesses H^+-ATPase that functions in transporting H^+, even against high concentration gradients, into the lumen of the tubule, thus acidifying urine and **type β cells** whose basolateral membrane possesses H^+-ATPase and functions in resorbing H^+ and secreting HCO_3^-, thus making the urine less acidic.

Medullary collecting tubules are of larger caliber because they are formed by the union of several cortical collecting tubules (see Fig. 19–11). Those in the **outer zone** of the medulla are similar to the cortical collecting tubules in that they display both principal and intercalated cells, whereas tubules of the **inner zone** of the medulla have principal cells only (Fig. 19–17). The cells

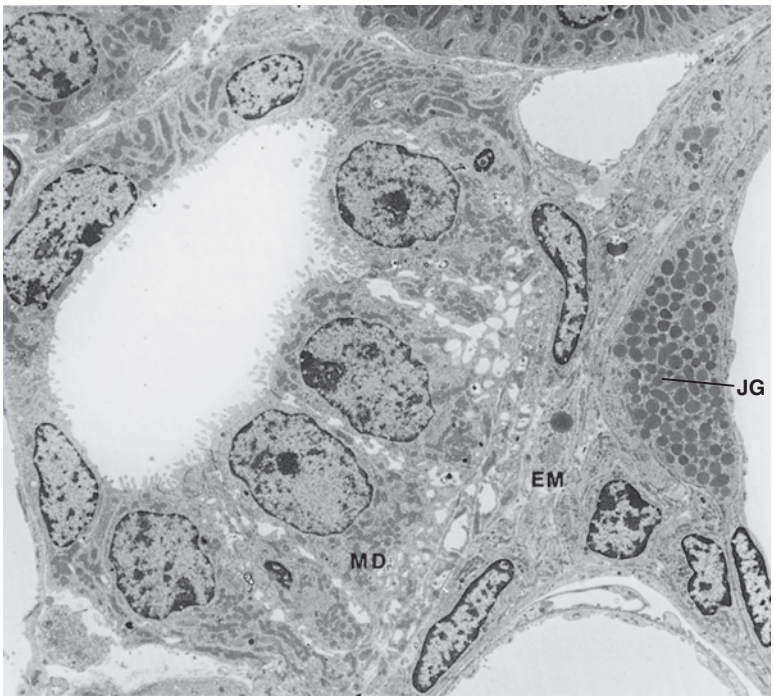


Figure 19–15 Electron micrograph of the juxtaglomerular apparatus from the kidney of a rabbit. The macula densa (MD), juxtaglomerular cell (JG; containing electron-dense granules), and extraglomerular mesangial (EM) cells are displayed (×2552). (From Brenner BM, Rector FC. *The Kidney*. 4th ed. Vol 1. Philadelphia: WB Saunders; 1991.)

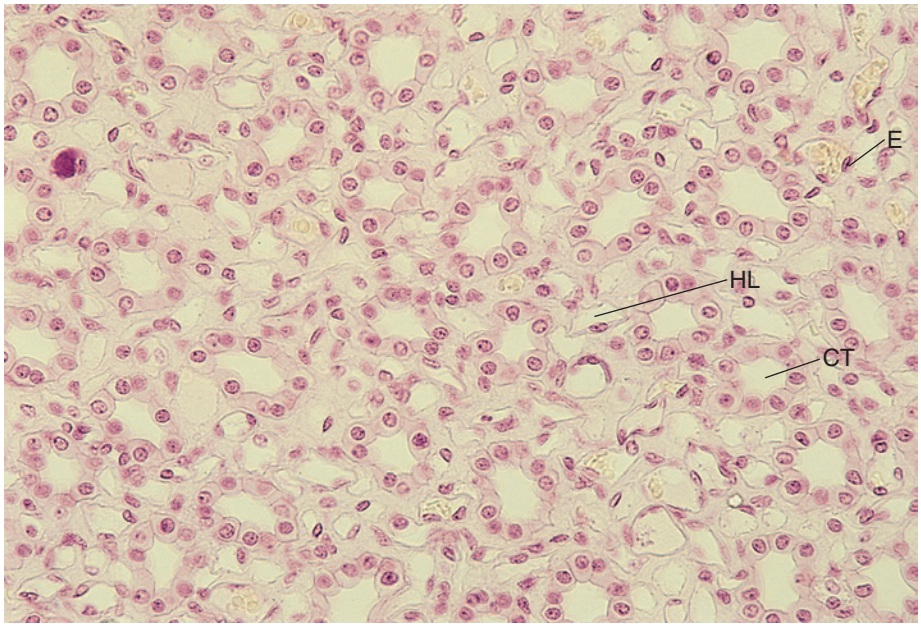


Figure 19–16 The medulla of the kidney displays the simple cuboidal epithelium of the collecting tubules (CT) as well as the simple squamous epithelium of the thin limbs of Henle loop (HL) and the endothelial cells (E) of the vasa recta. Note that the connective tissue components are sparse and consists mostly of vascular elements (×270).

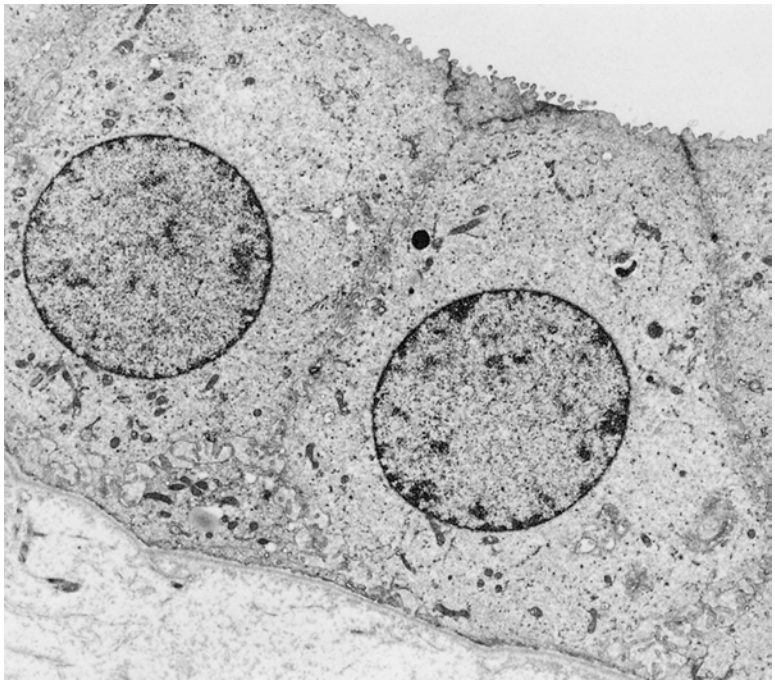


Figure 19–17 Electron micrograph of a collecting duct from a rabbit kidney (×4790). (From Brenner BM, Rector FC. *The Kidney*. 4th ed. Vol 1. Philadelphia: WB Saunders; 1991.)

of these ducts are also able to excrete hydrogen ions into the lumen of the duct thereby acidifying urine.

Papillary collecting tubules (ducts of Bellini) are each formed by the confluence of several medullary collecting tubules. These are large ducts, 200 to 300 µm in diameter, and they open at the area cribrosa of the renal papilla to deliver the urine that they convey into the minor calyx of the kidney. Each duct of Bellini collects urine from as many as 5000 nephrons. These ducts are lined by tall columnar principal cells only where each cell possesses a primary cilium that probably is responsible for monitoring the urine in its lumen.

Collecting tubules are impermeable to water. However, in the presence of **ADH**, they become permeable to water (and in the medullary collecting tubules to urea). Thus, in the absence of ADH, urine is copious and hypotonic, and, in the presence of ADH, the volume of urine is low and concentrated.

Renal Interstitium

The renal interstitium is a very flimsy, scant amount of loose connective tissue housing three types of cells: fibroblasts, macrophages, and interstitial cells.

The kidney is invested by a dense, irregular collagenous type of connective tissue with some elastic fibers interspersed among the bundles of collagen. This capsule is not attached firmly to the underlying cortex. As blood vessels enter the hilum, they travel in a thin connective tissue cover, some of which is derived from the capsule. The cortical region has only delicate connective tissue elements that constitute less than 10% of the cortical volume and is associated mostly with the basement membranes investing the uriniferous tubules and their vascular supply. The two cellular components of the cortical connective tissue are **fibroblasts** and cells that are believed to be **interstitial dendritic cells**, members of the mononuclear phagocytic system.

The medullary interstitial connective tissue component is more extensive than that found in the cortex; in fact, it occupies more than 20% of the volume of the medulla. Embedded in this connective tissue are the various components of the uriniferous tubules as well as the extensive vascular network located in the medulla. The cell population of this connective tissue consists of four cell types:

- Fibroblasts
- Macrophages
- Pericytes
- Interstitial cells

The cortical interstitium possesses only **fibroblasts** and **macrophages**, whereas the medullary interstitium is composed of all four cell types. Interstitial cells are described in the next paragraph.

Interstitial cells appear to be situated like the rungs of a ladder, one on top of the other, and are most

numerous between straight collecting ducts and between the ducts of Bellini. Interstitial cells have elongated nuclei and numerous lipid droplets. It is believed that these cells synthesize **medullipin I**, a substance that is converted in the liver to **medullipin II**, a potent vasodilator that lowers blood pressure.

Renal Circulation: Arterial Supply

The two kidneys receive 20% of the total blood volume (1200 mL) per minute via the large branches of the abdominal aorta known as the renal arteries.

The two kidneys receive an extremely extensive blood supply (1200 mL every minute) via the large **renal arteries**, direct branches of the abdominal aorta (see Fig. 19–1). Before entering the hilum of the kidney, the renal artery bifurcates into an anterior and a posterior division, which, in turn, subdivide to form a total of five **segmental arteries**. The branches of any one segmental artery do not form anastomoses with the branches of other segmental arteries. Hence, if blood flow through one of these arteries is blocked, circulation to the region of the kidney supplied by the affected vessel is interrupted. Therefore, the kidney is said to be subdivided into vascular segments, with each segment supplied by a specific artery.

The first subdivisions of the segmental arteries are called ***lobar arteries***, one for each lobe of the kidney. These in turn branch to form two or three **interlobar arteries**, which travel between the renal pyramids to the corticomedullary junction. At the corticomedullary junction, these arteries form a series of vessels (perpendicular to the parent vessel) that, to a large extent, remain at that junction, occupying the same curved plane. Because these arteries describe a slight arc over the base of the renal pyramid, they are called ***arcuate arteries***.

Although arcuate arteries once were believed to anastomose with each other, more recent studies suggest that terminal branches of these arteries do not join each other. Instead, terminal branches, as all other branches of the arcuate arteries, ascend into the cortex, forming interlobular arteries.

Interlobular arteries ascend within the cortical labyrinth approximately halfway between neighboring medullary rays. Hence, they travel in the interstices between any two lobules. Many branches arise from the interlobular arteries. These branches supply the glomeruli of renal corpuscles and are known as ***afferent glomerular arterioles***. Some of the interlobular arteries ascend through the cortex to perforate the kidney capsule. Here they contribute to the formation of the capsular plexus. Most of the interlobular arteries, however, terminate as afferent glomerular arterioles.

Each glomerulus is drained by another arteriole, the **efferent glomerular arteriole**. There are two types of efferent glomerular arterioles, those draining glomeruli of cortical nephrons and those draining glomeruli of juxtamedullary nephrons.

Efferent glomerular arterioles from *cortical nephrons* are short and branch to form a system of capillaries, the **peritubular capillary network**. This capillary bed supplies the entire cortical labyrinth, with the obvious exception of the glomeruli. Thus, the kidney possesses a dual capillary bed, one responsible for the formation of the ultrafiltrate, the glomerulus with a high hydrostatic pressure of about 60 mm Hg, and the second, the peritubular capillary bed with a low hydrostatic pressure of about 12 mm Hg. This hydrostatic pressure differential permits the exceptionally efficient reabsorption of the fluid conserved by the uriniferous tubules.

It is thought that the endothelial cells of the peritubular capillary network (and perhaps connective tissue cells of the cortex and outer medulla) manufacture and release 90% of the body's hormone **erythropoietin**; the remaining 10% is formed by hepatocytes.

The efferent glomerular arterioles, derived from glomeruli of juxtamedullary nephrons as well as from glomeruli located in the lower quadrant of the cortex, each give rise to 10 to 25 long, hairpin-like capillaries that dip deep into the medulla (Figs. 19–18 and 19–19). Their descending limbs have a narrow bore and are called ***arteriolae rectae***, and their ascending limbs are much greater in diameter and are called ***venae rectae***; frequently, these vessels are simply called ***vasa recta***. The hairpin-like shape of the vasa recta, which closely follows and wraps around the two limbs of Henle loop and the collecting tubule, is essential in the physiology of urine concentration (see later text).

Renal Circulation: Venous Drainage

The arcuate veins receive blood from the cortex via the stellate veins and interlobular veins and from the medulla via the venae rectae; arcuate veins are drained by the interlobar veins that deliver their blood into the renal vein.

Venae rectae deliver their blood to **arcuate veins**, vessels that follow the paths of the same-named arteries. Blood is thus drained from the medulla. Cortical blood is collected into a star-shaped system of subcapsular veins called ***stellate veins***, which are tributaries of the **interlobular veins**. Stellate veins also receive blood from the terminal portions of the efferent glomerular arterioles. The interlobular veins, paralleling the same-named arteries, deliver their blood to the **arcuate veins**. Hence, arcuate veins drain both the medulla and the cortex. Arcuate veins are tributaries of **interlobar**

Figure 19–18 Electron micrograph of the arteria recta of a rat kidney. (From Takahashi-Iwanaga H. The three-dimensional cytoarchitecture of the interstitial tissue in the rat kidney. *Cell Tissue Res*. 1991;264:269-281.)

veins that unite, near the hilum, to form the **renal vein**. This large vein delivers the blood to the inferior vena cava. Note the absence of lobar and segmental veins in contrast to the presence of such named arteries in the arterial system of the kidney.

Lymphatic Supply of the Kidney

Lymph vessels of the kidney probably follow the larger arteries.

The **lymphatic supply** of the kidney is not completely understood. It is believed that most lymphatic vessels follow the larger arteries. According to most authors, the lymphatic supply of the kidney may be subdivided into *superficial* and *deep* aspects located in the subcapsular region and in the medulla, respectively. The two systems may or may not join each other near the hilum, where they form several large lymphatic trunks. Lymph nodes in the vicinity of the vena cava and the abdominal aorta receive lymph from the kidneys. There are lymph vessels in the cortex that do not follow the larger arteries, but they do drain their lymph into a plexus of lymph vessels at the hilum.

Renal Innervation

Most nerve fibers that reach the kidney are unmyelinated, sympathetic fibers that form the renal plexus, traveling along the renal artery. The cell bodies of these fibers are probably located in the aortic and celiac plexuses. Sympathetic fibers are distributed by branches of

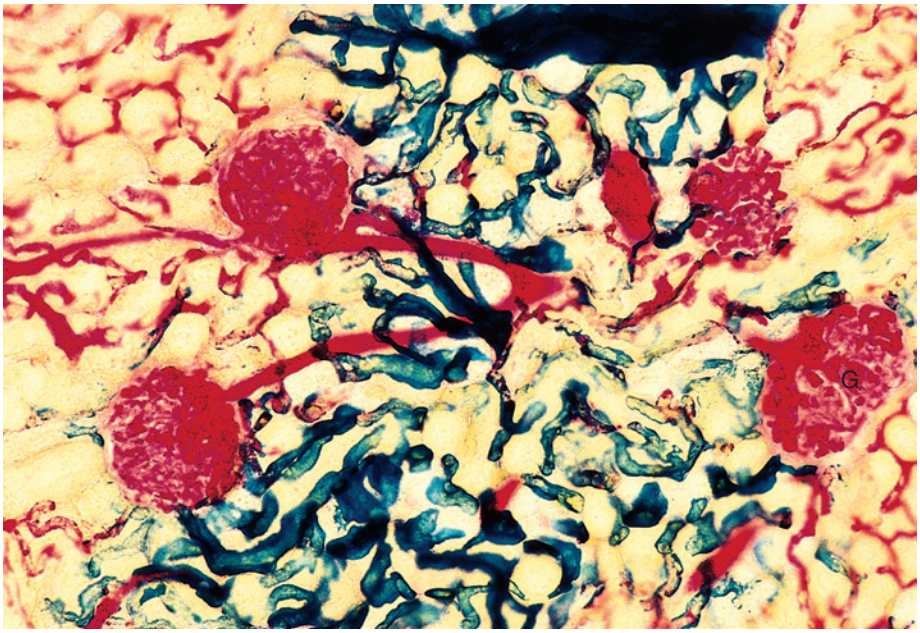


Figure 19–19 Injected kidney displaying the rich vascular supply of the kidney cortex. Note that the glomeruli are clearly evident (×132).

the renal arterial tree, and these vessels are modulated by some of these fibers. Additional sympathetic fibers reach the epithelium of the renal tubules, the juxtaglomerular and interstitial cells, and the capsule of the kidney. Sensory fibers and parasympathetic fibers (probably from the vagus nerve) have also been described in the kidney.

General Functions of the Kidney

The kidneys play a role in excretion as well as in regulation of body fluid composition and volume. Specifically, they regulate solute components (e.g., sodium, potassium, chloride, glucose, amino acids) and acid–base balance. Thus, during the summer, when a great deal of fluid is lost through perspiration, the urinary output is reduced in volume and increased in osmolarity. During the winter months, when fluid loss through perspiration is minimal, the urinary output is increased in volume, and the urine is dilute.

In addition, the kidneys excrete detoxified end products; regulate the osmolality of urine; and secrete substances such as medullipin I, renin, prostaglandins, and erythropoietin. They can also function in gluconeogenesis.

Finally, the kidneys regulate blood pressure and, in the presence of parathyroid hormone, aid in the conversion of a less active form of vitamin D_3 (25-OH-vitamin D_3) to **calcitriol ($1,25\text{-}[OH]_2$-vitamin D_3)**, its most active form, that is responsible for the increased absorption of calcium and phosphate ions by the digestive system and their transport into the extracellular fluid compartment of the body. Although all of these functions are important aspects of kidney histophysiology, only the mechanism of urine formation is discussed in the chapter.

Recapitulation of the Mechanism of Urine Formation

The two kidneys receive about one-fifth the total blood volume (1200 mL) per minute, and they manufacture about 1 to 2 mL of urine per minute.

The two kidneys receive a large volume of circulating blood because the renal arteries are large and are direct branches of the abdominal aorta. Inulin, a fructose polymer, can be used to measure the **GFR (glomerular filtration rate)**. Such studies have shown that the entire blood supply circulates through the two kidneys every 5 minutes. Thus, approximately 1200 mL of blood enters the two kidneys each minute, from which 125 mL/min of glomerular filtrate is formed in the average male, which is the typical GFR for an adult human. Thus, 180 L of glomerular filtrate is formed each day, of which only 1.5 to 2.0 L is excreted as urine. Therefore, every day, at least 178 L of the glomerular filtrate is resorbed by the two kidneys, and only about 1% of the total glomerular filtrate is excreted.

Filtration in the Renal Corpuscle

Fluid component from the blood passes through the filtration barrier to become the ultrafiltrate.

As blood passes from the afferent glomerular arteriole into the glomerulus, it encounters a region of differential pressure, where the intracapillary blood pressure (60 mm Hg) is greater than the two opposing forces, namely the colloid osmotic pressure of the blood (32 mm Hg) and the oncotic pressure, that is the fluid pressure, in Bowman space (18 mm Hg), forcing fluid from the capillary into that space. Therefore, the net effect, the **filtration force**, is 10 mm Hg. The fluid entering Bowman space is called the ***(glomerular) ultrafiltrate***.

Because of the tripartite **filtration barrier** (endothelial cell, basal lamina, filtration slit or diaphragm), cellular material and large macromolecules cannot leave the glomerulus; thus, the ultrafiltrate is similar to plasma (without its constituent macromolecules). Molecules greater than 69,000 Da (e.g., albumin) are trapped by the basal lamina. In addition to molecular weight, the molecular shape and charge of a molecule and the functional state of the filtration barrier all influence the ability of a molecule to traverse the filtration barrier. Because the filtration barrier has negatively charged components, macromolecules that are negatively charged are less able to cross the filtration barrier compared with positively charged or neutral macromolecules.

Resorption in the Proximal Tubule

The proximal tubule is the site of mass movement, where a tremendous amount of electrolytes, glucose, amino acids, protein, and water is conserved.

The **ultrafiltrate** leaves Bowman space via the urinary pole to enter the proximal convoluted tubule, where modification of this fluid begins. Materials resorbed from the lumen of the proximal tubule enter the tubular epithelial cells, from which they are exocytosed into the interstitial connective tissue. Here, the resorbed substances gain entrance to the rich peritubular capillary network and thus are returned to the body via the bloodstream.

Most resorption of materials from the ultrafiltrate occurs in the proximal tubule. Normally, the following amounts are absorbed in the proximal tubule: 100% of proteins, glucose, amino acids; almost 100% of bicarbonate ions; 65% to 80% of sodium and chloride ions; and 65% to 80% of the water.

The Na^+-K^+ ATPase–powered **sodium pumps** in the basolateral plasma membrane of the proximal tubule cell pump sodium into the renal interstitium. This movement of sodium ions out of the cell at the basolateral membrane causes sodium in the lumen of the

tubule to leave the ultrafiltrate and enter the cell through its apical cell membrane. In this fashion, the net sodium movement is from the ultrafiltrate into the renal connective tissue. To maintain electrical neutrality, chloride ions passively follow sodium. Further, to maintain osmotic equilibrium, water passively follows sodium (by osmosis).

Additionally, energy-requiring pumps, located in the apical plasmalemma of proximal tubule cells, cotransport amino acids and glucose with sodium into the cell to be released into the renal interstitium. However, as the glucose and amino acids become depleted in the ultrafiltrate of the proximal tubule the cotransport in the medullary portion of the proximal tubule switches from glucose and amino acids to chloride. Therefore, much of the Cl^- resorption is due to its cotransport with sodium in the medullary portion of the proximal tubule. It should be noted that that some of the chloride resorption occurs via dedicated chloride channels. Proteins, brought into the cell by pinocytotic vesicles, are degraded by hydrolytic enzymes within late endosomes.

Each day, as much as 140 g of glucose, 430 g of sodium, 500 g of chloride, 300 g of bicarbonate, 18 g of potassium ions, 54 g of protein, and approximately 142 L of water are conserved by the proximal tubules of the kidney.

The proximal tubule also releases certain substances into the tubular lumen. These include hydrogen (H^+), ammonia, phenol red, hippuric acid, uric acid, organic bases, and ethylenediaminetetraacetate as well as certain drugs, such as penicillin.

Henle Loop and the Countercurrent Multiplier System

The long Henle loop of the juxtamedullary nephron is responsible for the establishment of the countercurrent multiplier system.

The osmolarity of the glomerular ultrafiltrate is the same as that of circulating blood. This osmolarity is not altered by the proximal tubule because water has left its lumen in response to the movement of ions. However, the osmotic pressure of *formed urine* is different from that of blood. The osmotic pressure differential is established by the remaining regions of the uriniferous tubule. Interestingly, the osmolarity and volume of urine vary, indicating that the kidneys can modulate these factors.

A gradient of osmolarity, increasing from the corticomedullary junction to deep into the medulla, is maintained in the renal medullary interstitium. The long loops of Henle of **juxtamedullary nephrons** aid not only in the creation but also in the maintenance of this osmotic gradient via a **countercurrent multiplier system** (Fig. 19–20). The cells of the thin descending limb of Henle loop are freely permeable to water and moderately permeable to urea but only slightly permeable to salts (including sodium and chloride). Therefore, the movement of water reacts to the osmotic forces in its microenvironment. The thin ascending limb is almost completely impermeable to water, but to a very limited extent, salts can enter the cells of the tubule to be reabsorbed or leave the cells and enter the lumen of the tubule to be excreted depending on the conditions of the interstitium. It is important to understand at this point (to be explained later) that *urea enters* the lumina of both the descending and ascending thin limbs of Henle loop.

The thick ascending limb of Henle loop is completely impermeable to water; however, **1-sodium**, **1-potassium**, **2-chloride co-transporters (pumps)** actively remove 2 chloride ions and one sodium and potassium ions each from the lumen of the tubules and these enter the cells of the ascending thick limb of Henle loop. At the same time, sodium-potassium ATPase pumps of the basal cell membrane (contacting the basal lamina) deliver equimolar quantities of K^+ ions into the cell (from the renal interstitium) and Na^+ ion (along with chloride ions via an energy requiring system) out of the cells (into the renal interstitium). Additionally, Na^+-H^+ co-transporters at the luminal surface deliver a limited amount of hydrogen ions into the lumen, mildly acidifying the ultrafiltrate and resorb sodium ions from the lumen into the cell. However, urea cannot enter or leave the lumen of the ascending thick limb. As the filtrate ascends, it contains fewer and fewer ions but the same amount of water; hence, the amount of salts that may be transferred out into the interstitium decreases. Thus, a gradient of salt concentration is established in the renal interstitium so that the highest interstitial osmolarity is deep in the medulla, and the osmolarity of the interstitium decreases toward the cortex.

Because the medulla is tightly packed with thick and thin (ascending and descending) limbs of Henle loop and collecting tubules, the gradient of osmolarity that is established is pervasive and affects all the tubules equally (see Fig. 19–20).

Therefore, keeping the foregoing in mind, we can recap the movement of ions and water, once again starting as the ultrafiltrate, which, as the reader should recall, is isotonic with blood as it leaves the pars recta of the proximal tubule. As the ultrafiltrate descends in the thin descending limb of Henle loop, it loses water *(reducing volume and increasing osmolarity),* reacting to the osmotic gradient of the interstitium, so that the intraluminal filtrate more or less becomes equilibrated with that of the surrounding connective tissue. This fluid of high osmolarity now ascends in the thin ascending limb of Henle loop, which is mostly impermeable to water but not to salts. Thus, the volume of the ultrafiltrate does not change (i.e., the volume is the same

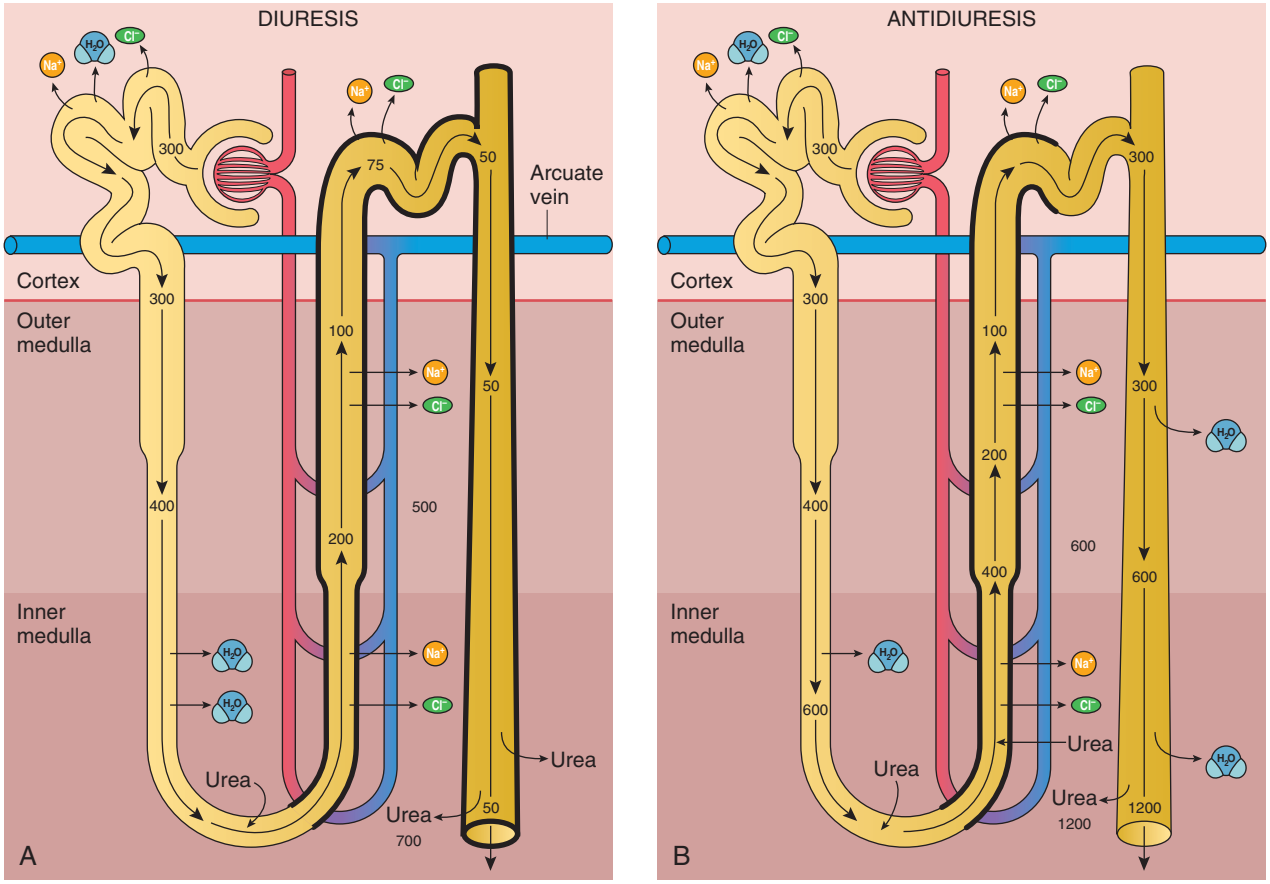


Figure 19–20 Histophysiology of the uriniferous tubule. **A,** In the absence of antidiuretic hormone (ADH) (diuresis). **B,** In the presence of ADH (antidiuresis). Numbers indicate milliosmoles per liter. Areas outlined by a *thick line* indicate that the tubule is impermeable to water. In the presence of ADH, the collecting tubule changes so that it becomes permeable to water, and the concentration in the interstitium of the inner medulla increases. The vasa recta is simplified in this drawing because it encompasses the entire uriniferous tubule (see Fig. 19–1).

when the ultrafiltrate leaves the ascending thin limb as when it entered it), but the osmolarity of the ultrafiltrate inside the tubule adjusts to the osmolarity of the interstitium.

The fluid entering the ascending thick limb of Henle loop passes a region that is impermeable to water but has 1-sodium, 1-potassium, 2-chloride co-transporters (pumps), which removes chloride, sodium, and potassium ions from the lumen, Because water cannot leave the lumen, the ultrafiltrate becomes *hypotonic, but its volume remains constant* as it ascends to the cortex in the ascending thick limb. The chloride and sodium that were transferred from the lumen of the ascending thick limb into the connective tissue are responsible for the establishment of a concentration gradient in the renal interstitium of the outer medulla.

Monitoring the Filtrate in the Juxtaglomerular Apparatus

When cells of the macula densa detect a low sodium concentration in the ultrafiltrate, they cause juxtaglomerular cells to release the enzyme renin, which converts angiotensinogen to angiotensin I.

The cells of the macula densa probably monitor the filtrate volume and sodium concentration. If sodium concentration is below a specific threshold, macula densa cells do two things:

- They cause dilation of the afferent glomerular arterioles, thus increasing blood flow into the glomerulus.
- They instruct juxtaglomerular cells to release the enzyme renin into circulation.

TABLE 19–2 Effects of Angiotensin II

Function	Result
Acts as a potent vasoconstrictor	Increased blood pressure
Facilitates synthesis and release of aldosterone	Resorption of sodium and chloride from lumen of distal convoluted tubule
Facilitates release of ADH	Resorption of water from lumen of collecting tubule
Increases thirst	Increased tissue fluid volume
Inhibits renin release	Feedback inhibition
Facilitates release of prostaglandins	Vasodilation of afferent glomerular arteriole, thus maintaining glomerular filtration rate

ADH, Antidiuretic hormone.

The enzyme **rennin** converts **angiotensinogen**, normally present in the bloodstream, into **angiotensin I**, a mild vasoconstrictor. In the capillaries of the lungs, but also to a lesser extent in those of the kidneys and other organs of the body, **ACE (angiotensin converting enzyme)** converts angiotensin I to **angiotensin II**, a hormone with numerous biological effects (Table 19–2). As a potent vasoconstrictor, angiotensin II reduces the luminal diameter of blood vessels, thus constricting the *efferent glomerular arterioles*, further increasing pressure within the glomerulus. The increased intraglomerular pressure along with the increased volume of blood flow results in the increased GFR of a larger volume of blood. Angiotensin II also influences the adrenal cortex to release **aldosterone**, a hormone that acts primarily on cells of the distal convoluted tubules, increasing their resorption of sodium and chloride ions. Additionally, angiotensin II facilitates the release of **ADH** from the posterior pituitary (neurohypophysis), which causes the collecting tubules to become permeable to water thereby resorbing water from the lumen and releasing it into the renal interstitium (see following section on the loss of water and urea from filtrate in collecting tubules).

Loss of Water and Urea From Filtrate in Collecting Tubules

Antidiuretic hormone (vasopressin) causes the conservation of water and the excretion of a concentrated urine.

The filtrate that leaves the distal convoluted tubule to enter the collecting tubule is hypotonic. As the collecting tubule passes through the medulla to reach the area cribrosa, it is also subject to the same osmotic gradients as the ascending and descending limbs of Henle loop. In the absence of **ADH**, the cells of the collecting tubule and, to a lesser extent, of the distal convoluted tubule are completely impermeable to water (see Fig. 19–20). Therefore, the filtrate, or urine, is not modified in the collecting tubule, and the urine remains dilute (hypotonic).

Under the influence of ADH, however, the cells of the collecting tubule (and, in animals other than humans and monkeys, the distal convoluted tubules) become freely permeable to water and the medullary collecting tubules also to urea. As the filtrate descends through the renal medulla in the collecting tubule, it is subject to the osmotic pressure gradients established by the hairpin-like loops of Henle and the vasa recta (see the next section on vasa recta), and water leaves the lumina of the collecting tubules to enter the interstitium. Hence, the urine, *in the presence of ADH*, becomes **concentrated** and **hypertonic**.

In addition, the concentration of urea becomes extremely high in the lumen of the collecting tubule, and, in the presence of ADH, it passively enters the interstitium of the inner medulla. Thus, much of the concentration gradient of the renal interstitium in the inner medulla is due to the presence of **urea**, whereas, in the outer medulla, **sodium** and **chloride** are responsible for the establishment of the concentration gradient.

The action of ADH is believed to be dependent on V_2 receptors located in the basolateral plasma membranes of **principal cells** of the collecting ducts. Once ADH binds to a V_2 receptor,

- Gs proteins are activated.
- Adenylate cyclase generates cAMP.
- Aquaporin-2 channels (AQP2) as well as AQP3 and AQP4 are inserted into the luminal plasma membrane (Table 19–3).
- Water, from the lumen of the collecting duct, enters the cell.
- Water leaves the cell via aquaporin 3 (AQP 3) and aquaporin 4 (AQP 4) channels (that are always present in the basolateral cell membranes) to enter the renal interstitium.

CLINICAL CORRELATIONS

One of the causes contributing to **chronic essential hypertension** is the presence of elevated levels of angiotensin II. Elevated blood levels of angiotensin II were once believed to be due to the excessive release of renin from the juxtaglomerular cells of the juxtaglomerular apparatus. It is now realized that the increased activity of angiotensin-converting enzyme, rather than the renal release of renin, is directly responsible for elevating the concentration of angiotensin II.

TABLE 19–3 Types of Aquaporins and Their Locations in the Uriniferous Tubule

Aquaporins	Location	Function
Aquaporin-1 (AQP 1)	Proximal tubule and thin descending limb of Henle loop	These segments are always permeable to water.
Aquaporin-2 (AQP 2)	In the presence of ADH, they are in the luminal surface of principal cells of the collecting ducts. In the absence of ADH, they are stored in apically located vesicles of principal cells of collecting ducts.	In the presence of ADH AQP 2 channels are inserted into the luminal membranes of principal cells, and water can traverse the cell to enter the renal interstitium.
Aquaporins-3 and -4 (AQP 3 and AQP 4)	Always present in the basolateral cell membranes of the principal cells of collecting ducts	The basolateral cell membranes of the principal cells of collecting ducts are always permeable to water.

ADH, Antidiuretic hormone.

- The increase in the interstitial fluid volume causes an increase in blood pressure thereby increasing GFR.

CLINICAL CORRELATIONS

Congenital nephrogenic diabetes insipidus is an X-linked disorder evidenced clinically in male infants only, although it may also have a certain degree of clinical penetrance in female infants. This condition, in affected males, manifests itself in the formation of copious quantity of dilute urine due to the malformation of the V_2 receptor. Additional symptoms include fever, vomiting, and hypernatremia, and extreme dehydration. The blood level of antidiuretic hormone (ADH) is normal or somewhat elevated; however, the aberrant ADH receptor cannot activate Gs proteins, and, consequently, aquaporins are not inserted into the luminal plasma membranes of collecting ducts, resulting in the inability to concentrate urine.

Vasa Recta and Countercurrent Exchange System

The lumen of the arterial limb of the vasa recta has a smaller diameter than that of the venous limb; both limbs are freely permeable to electrolytes and water.

The vasa recta helps maintain the osmotic gradient in the medulla because both arterial and venous limbs are freely permeable to water and salts (Fig. 19–21). Moreover, as indicated previously, the luminal diameter of the arterial limb is smaller than that of the venous limb. Therefore, as the blood courses down the arterial limb, it loses water and gains salts, and as it returns via the venous limb, it loses salts and gains water, thus acting as a **countercurrent exchange system**.

This mechanism ensures that the system of osmotic gradients remains undisturbed because the osmolarity of the blood in the vessels is more or less equilibrated with that of the interstitium. However, the volume of salts and fluid being brought in by the arterial limb is less than that being taken away by the venous limb. This exchange system causes salt and water to be resorbed (returned back to the body) because of the concentration gradient in the renal medulla.

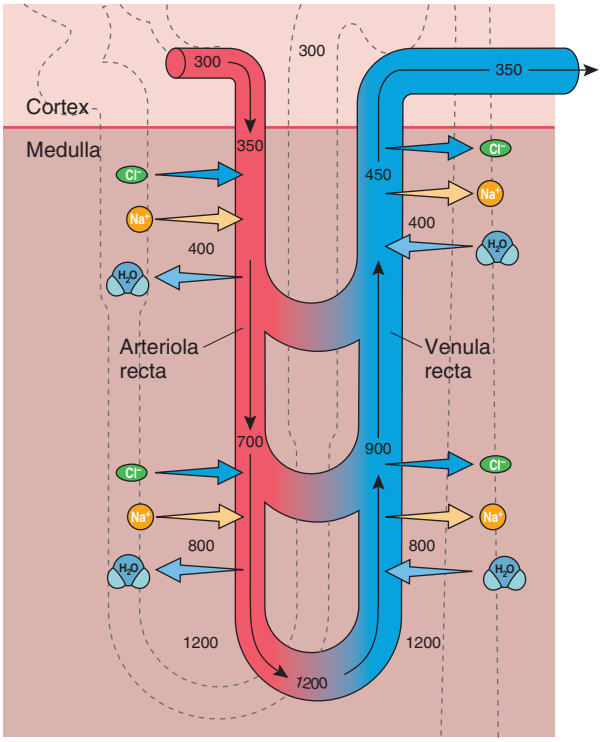


Figure 19–21 Histophysiology of the vasa recta. Numbers represent milliosmoles per liter. The arteriola recta is smaller in diameter than the venula recta.

The structure and function of the various regions of the uriniferous tubule are presented in Table 19–4.

TABLE 19–4 Structure and Function of the Uriniferous Tubule

Region of Uriniferous Tubule	Major Functions	Miscellaneous Comments
Renal corpuscle: Simple squamous epithelium, fused basal laminae, podocytes	Filtration	Filtration barrier: endothelial cell, fused basal laminae, filtration slits
Proximal tubule: Simple cuboidal epithelium	Resorption of 65%–80% of water, sodium, and chloride (reducing volume of ultrafiltrate); resorption of 100% of protein, amino acids, glucose, and bicarbonate	Sodium pump in basolateral membrane; ultrafiltrate is isotonic with blood
Descending thin limb of Henle loop: Simple squamous epithelium	Completely permeable to water and slightly permeable to salts (reducing volume of ultrafiltrate)	Ultrafiltrate is hypertonic with respect to blood; urea enters lumen of tubule
Ascending thin limb of Henle loop: Simple squamous epithelium	Impermeable to water, permeable to salts; sodium and chloride leave tubule to enter renal interstitium	Ultrafiltrate is hypertonic with respect to blood; urea leaves renal interstitium and enters the lumen of tubule
Ascending thick limb of Henle loop: Simple cuboidal epithelium	Impermeable to water; chloride and sodium leave tubule to enter renal interstitium	Ultrafiltrate becomes hypotonic with respect to blood; sodium-chloride pump in basolateral cell membrane is responsible for establishment of osmotic gradient in interstitium of outer medulla
Macula densa: Simple columnar cells	Monitors sodium level and volume of ultrafiltrate in lumen of distal tubule	Contacts and communicates with juxtaglomerular cells
Juxtaglomerular cells: Modified smooth muscle cells	Synthesize and release renin into bloodstream	Renin initiates the reaction for the eventual formation of angiotensin II (see Table 19–2)
Distal convoluted tubule: Simple cuboidal epithelium	Responds to aldosterone by resorbing sodium and chloride for lumen	Ultrafiltrate becomes more hypotonic (in the presence of aldosterone); sodium pump in basolateral membrane; potassium is secreted into the lumen
Collecting tubule: Simple cuboidal epithelium	In the presence of ADH, water and urea leave the lumen to enter the renal interstitium	Urine becomes hypertonic in the presence of ADH; urea in interstitium is responsible for gradient of concentration in interstitium of the inner medulla

ADH, Antidiuretic hormone.

EXCRETORY PASSAGES

The excretory passages of the urinary system consist of the minor and major calyces, the pelvis of the kidney, the ureter, the single urinary bladder, and the single urethra.

Calyces

Each minor calyx accepts urine from the renal papilla of a renal pyramid; as many as four minor calyces may deliver their urine to a major calyx.

The renal papilla of each renal pyramid fits into a **minor calyx**, a funnel-shaped chamber that accepts urine leaving the ducts of Bellini at the area cribrosa (see Fig. 19–1). The portion of the apex of the pyramid that projects into the minor calyx is covered by **transitional epithelium**, which acts as a barrier, separating the urine from the underlying interstitial connective tissue. Deep to the lamina propria is a thin muscular coat composed entirely of smooth muscle. This muscular layer propels the urine into a **major calyx**, one of three or four larger funnel-shaped chambers, each of which collects urine from two to four **minor calyces**. The major calyces are similar in structure to the minor calyces as well as to the expanded proximal region of the ureters, the **renal pelvis**. The walls of the excretory passages thicken from the minor calyces to the urinary bladder.

Ureter

The ureters deliver urine from the kidneys to the urinary bladder.

Each **ureter** is about 3 to 4 mm in diameter, is approximately 25 to 30 cm long, and pierces the base of the

urinary bladder. The ureters are hollow tubes consisting of

- A mucosa, which lines the lumen
- A muscular coat (muscularis)
- A fibrous, connective tissue covering

The **mucosa** of the ureter presents several folds, which project into the lumen when the ureter is empty but are absent when the ureter is distended. The **transitional epithelial lining**, three to five cell layers in thickness, overlies a layer of dense, irregular fibroelastic connective tissue, which constitutes the **lamina propria**. As always, the epithelium is separated from the underlying lamina propria by a basal lamina.

The **muscularis** of the ureter is composed of two predominantly inseparable layers of smooth muscle cells. The arrangement of the layers is opposite that found in the digestive tract because the outer layer is arranged circularly and the inner layer is longitudinally disposed. This arrangement is true for the proximal two thirds of the ureter, but in the lower third, near the urinary bladder, a third muscle layer, whose fibers are oriented longitudinally, is added onto the existing surface of the existing muscle coat. Hence, the muscular fiber orientation in the lower one third of the ureter is **outer longitudinal**, **middle circular**, and **inner longitudinal**. However, it should be noted that, just as in the digestive tract, these muscle layers are arranged in a helical configuration, where the pitch of the helices varies from short to long, thus giving the appearance of circular or longitudinal orientations.

The **fibrous outer coat** of the ureter is unremarkable. At its proximal and distal terminals, it blends with the capsule of the kidney and the connective tissue of the bladder wall, respectively. Contrary to expectation, urine does not pass down the ureter because of gravitational forces; instead, muscular contraction of the ureteric wall establishes peristalsis-like waves that convey urine to the urinary bladder. As the ureters pierce the posterior aspect of the base of the bladder, they pass obliquely for about 2 to 3 cm through the muscular wall of the urinary bladder. Because the muscular wall of the urinary bladder is under constant tonus, the portions of the ureters that pass through this muscular wall become compressed, preventing the reflux of urine back into the ureters. As the ureters open into the bladder lumen, a valve-like flap of mucosa hangs over each ureteric orifice, also contributing to the prevention of regurgitation of urine from the bladder back into the ureters.

Urinary Bladder

The urinary bladder stores urine until it is ready to be voided.

The **urinary bladder** is essentially an organ for storing urine until the pressure becomes sufficient to induce the urge for micturition, or voiding. It has major anatomical regions, the larger **body** and the much smaller **neck**, where the body expands to store urine and the neck connects the bladder to the urethra. Histologically, the bladder has a **mucosa** composed of a **transitional epithelium (urothelium)** and an underlying connective tissue layer, the **lamina propria** surrounded by a thick **smooth muscle coat**, which is enveloped by a **serosa** on its posterior aspect, whereas its anterior aspect has an **adventitia** that causes the bladder to adhere to the anterior abdominal wall. The mucosa of the bladder (Figs. 19–22 and 19–23) is arranged in numerous folds, which disappear when the bladder becomes distended with urine. During distention, the large, round, **dome-shaped cells** of the transitional epithelium become stretched and change their morphology to become flattened.

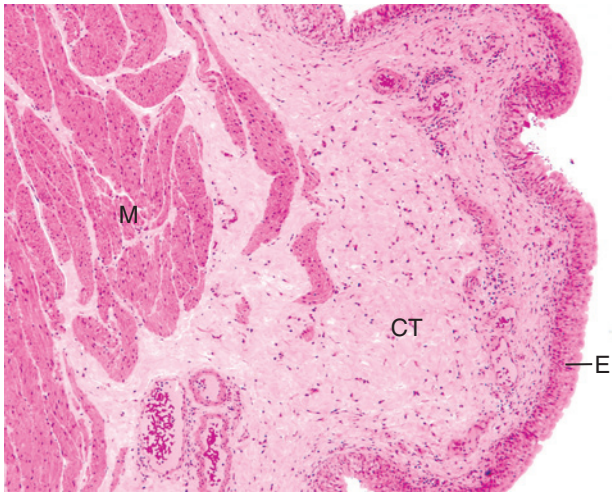


Figure 19–22 Low-power photomicrograph of a monkey urinary bladder. Observe the epithelium (E), subepithelial connective tissue (CT), and muscular coat (M) of the bladder (×58).

The accommodation of cell shape is performed by a unique feature of the **transitional epithelial cell plasmalemma**, which is composed of a mosaic of specialized, rigid, thickened regions, **plaques**, interspersed by normal cell membrane, **interplaque regions**. When the bladder is empty, the plaque regions are folded into irregular, angular contours, which disappear when the cell becomes stretched. These rigid plaque regions, anchored to intracytoplasmic filaments, resemble gap junctions, but this similarity is superficial only.

Plaques appear to be impermeable to water and salts; thus, these cells act as osmotic barriers between the urine and the underlying lamina propria. The superficial cells of the transitional epithelium are held together by desmosomes and, possibly, by tight junctions, which also aid the establishment of the osmotic barrier by preventing the passage of fluid between the cells.

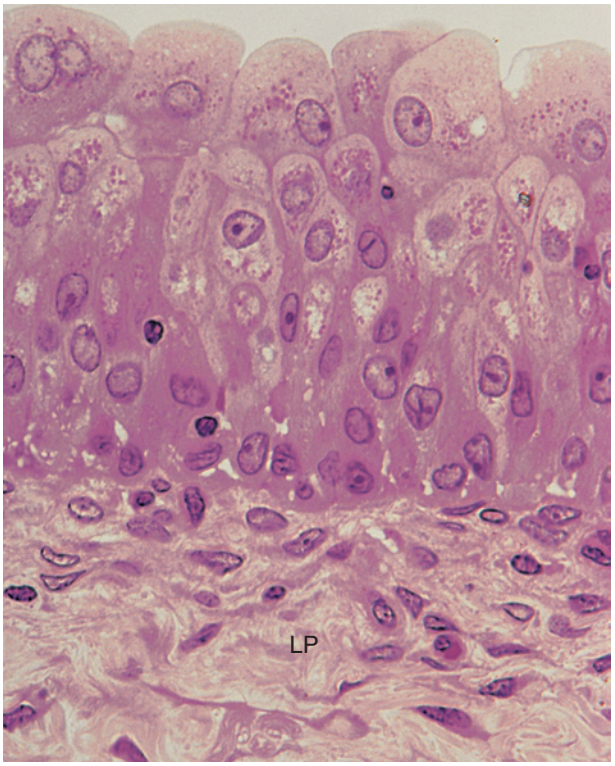


Figure 19–23 Photomicrograph of transitional epithelium from the bladder of a monkey. Observe the very large dome-shaped cells abutting the lumen (×540).

The triangular region of the bladder, whose apices are the orifices of the two ureters and the urethra, is known as the ***trigone***. The mucosa of the trigone is always smooth and is never thrown into folds. The embryonic origin of the trigone differs from that of the remainder of the bladder.

The lamina propria of the bladder may be subdivided into two layers: a more *superficial*, dense, irregular collagenous connective tissue and a *deeper*, looser layer of connective tissue composed of a mixture of collagen and elastic fibers. The lamina propria contains no glands, except at the region surrounding the urethral orifice, where **mucous glands** may be found. Usually, these glands extend only into the superficial layer of the lamina propria. They secrete a clear viscous fluid that apparently lubricates the urethral orifice.

The muscular coat of the urinary bladder, known as the ***detrusor muscle***, is composed of three interlaced layers of smooth muscle, which can be separated only in the region of the neck of the bladder. Here, they are arranged as a thin inner longitudinal layer, a thick middle circular layer, and a thin outer longitudinal layer. The middle circular layer is interwoven with elastic fibers and forms the **internal sphincter muscle** around the internal orifice of the neck of the bladder just before it joins the urethra. The constant tonus of this muscle averts the emptying of the bladder until the fluid pressure becomes strong enough to relax the internal sphincter and initiate voiding. Fortunately, in humans and many domesticated animals, emptying can be controlled by the voluntary nervous system because of the presence of a second sphincter muscle. As the urethra passes through the floor of the pelvis, a region known as the *urogenital diaphragm*, **skeletal muscle fibers** form the **external sphincter of the bladder**, which is, in reality, a sphincter of the **membranous urethra**, the first of the two (in females) or three (in males) regions of the urethra (see following section on the urethra). This sphincter muscle is under voluntary control, and even though the detrusor muscle contracts and the internal sphincter muscle of the bladder relaxes to attempt to empty the bladder, voluntary contraction of the external sphincter prevents urine from entering the urethra (the innervation of the bladder and the control of micturition is described in the next section).

The adventitia of the bladder is composed of a dense, irregular collagenous type of connective tissue containing a generous amount of elastic fibers. Certain regions of the adventitia are covered by a serosa, a peritoneal reflection onto the wall of the bladder, whereas other regions may be surrounded by fat.

CLINICAL CORRELATIONS

Cancer of the bladder is one of the most common cancers, and fully 50% of the cases involve individuals who smoke, although, in 30% of the cases, it is attributed to exposure to carcinogens such as naphthylamine and benzidine in the workplace. This condition may occur in younger individuals, but it is more frequent in older males (66%) than in older females (33%) and almost always begins in the transitional epithelium of the bladder. Symptoms of bladder cancer are blood in the urine, with or without pain and later painful and frequent urination. Bladder cancer is usually diagnosed early enough to be cured, but remission does occur, and patients should be monitored for the remainder of their lives. However, annually, almost 200,000 people die worldwide from bladder cancer.

Micturition

Micturition (urination; emptying of the urinary bladder) is a process that is controlled by both the autonomic nervous system and by the voluntary nervous system. The autonomic control of the urinary bladder has two components, **sensory** and **motor**. The sensory fibers originate in stretch receptors and they monitor how much the bladder wall is stretched by the accumulated urine. The motor component is under the control of parasympathetic nerves whose preganglionic fibers,

originating from preganglionic parasympathetic neurons located in spinal cord segments S2 and S3, synapse on postganglionic parasympathetic neurons located in small parasympathetic ganglia residing in the wall of the urinary bladder. Postganglionic fibers from these ganglia innervate the detrusor muscle and the internal sphincter of the bladder. When the bladder is stretched to a certain extent the signals from the stretch receptors initiate contraction of the detrusor muscle and relaxation of the internal urinary sphincter so that the bladder can expel the urine. However, for actual urination to occur, the somatic motor fibers carried by the pudendal nerve must permit the relaxation of the external sphincter of the bladder, thus opening the lumen of the membranous portion of the urethra. The average individual voids about 1.5 L to 2 L of urine per day.

Urethra

The urethra conveys urine from the urinary bladder to outside the body.

The urinary bladder is drained by a single tubular structure, the **urethra**, which communicates with the outside, permitting elimination of urine from the body. As the urethra pierces the **perineum (urogenital diaphragm; floor of the pelvis)**, skeletal muscle fibers form the **external sphincter muscle** surrounding the urethra. This muscle permits voluntary control of micturition (see previous section). The urethra of the male is longer than that of the female and has a dual function, acting as a route for urine as well as for semen.

CLINICAL CORRELATIONS

Loss of voluntary control over the **external sphincter muscle** of the urethra causes **urinary incontinence**, a condition affecting primarily older women.

Female Urethra

The female urethra is about 4 to 5 cm in length and 5 to 6 mm in diameter. It extends from the urinary bladder to the external urethral orifice just above and anterior to the opening of the vagina. Normally, the lumen is collapsed, except during micturition. It is lined by a **transitional epithelium** near the bladder and by a **stratified squamous nonkeratinized epithelium** along the remainder of its length. Interspersed in the epithelium are patches of pseudostratified columnar epithelium. The mucosa is arranged in elongated folds because of the organization of the fibroelastic **lamina propria**. Along the entire length of the urethra are numerous clear, mucus-secreting **glands of Littre**.

A thin, vascular, erectile coat surrounds the mucosa, resembling the corpus spongiosum of the male. The muscular layer of the urethra is continuous with that of the bladder but is composed of two layers only, an inner longitudinal and an outer circular smooth muscle layer. As the urethra pierces the perineum (urogenital diaphragm), a sphincter of skeletal muscle surrounds it and permits voluntary control of micturition.

Male Urethra

The male urethra is about 20 cm long, and its three regions are named according to the structures through which it passes.

- **Prostatic urethra**. Three to 4 cm long, lies entirely in the prostate gland. It is lined by a transitional epithelium and receives the openings of many tiny ducts of the prostate, the prostatic utricle (a rudimentary homologue of the uterus), and the paired ejaculatory ducts.
- **Membranous urethra**. One to 2 cm long. This segment is so named because it passes through the perineal membrane (urogenital diaphragm). It is lined by stratified columnar epithelium interspersed with patches of pseudostratified columnar epithelium.
- **Spongy urethra (penile urethra)**. Longest portion of the urethra (15 cm long), passes through the length of the penis, terminating at the tip of the glans penis as the external urethral orifice. This segment is so named because it is located in the corpus spongiosum. It is lined by stratified columnar epithelium interspersed with patches of pseudostratified columnar and stratified squamous, nonkeratinized epithelia. The enlarged terminal portion of the urethra in the glans penis (the **navicular fossa**) is lined by stratified squamous, nonkeratinized epithelium.

The **lamina propria** of all three regions is composed of a loose fibroelastic connective tissue with a rich vascular supply. It houses numerous **glands of Littre**, whose mucous secretion lubricates the epithelial lining of the urethra.

CLINICAL CORRELATIONS

Urinary tract infections (UTI) usually affect the urinary bladder **(acute cystitis)**; however, in some cases, the infection may travel up the ureters and involve the kidneys **(pyelonephritis)**. In the case of acute cystitis, the symptoms are frequent urination and painful or burning sensations during voiding. However, if these conditions are accompanied by fever and pain in the lower back and, occasionally, vomiting, all of which developed in a short period of time, the physician should suspect pyelonephritis. In most cases, the infection is caused by the invasion of the urinary tract by ***Escherichia coli (E. coli)***. Because the most common source of *E. coli* is the anus, UTIs are more frequent in females than in males because the proximity of the urethra to the anus. Additionally, in women who have undergone

CLINICAL CORRELATIONS—cont'd

hysterectomy, the connection of the bladder to the anterior abdominal wall is weakened, and the bladder tends to prolapse, thus making emptying the bladder more difficult. The trapped urine has the tendency to permit the proliferation of *E. coli* in the bladder, thereby increasing the chances of acute cystitis. In most cases, antibiotic treatment eliminates acute cystitis. Pyelonephritis is a very serious disease that should be treated with antibiotics and, at times, even by surgical intervention.

PATHOLOGICAL CONSIDERATIONS

See Figures 19–24 through 19–26.

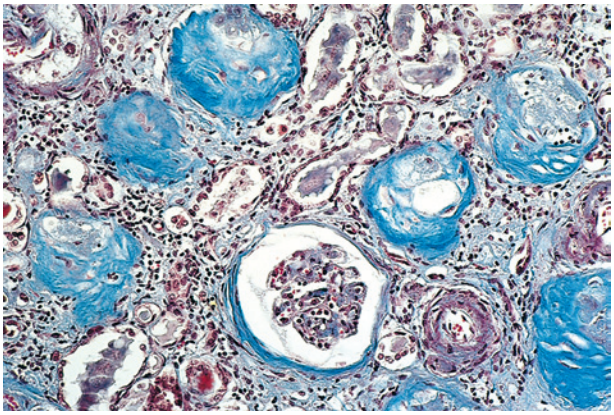


Figure 19–24 Photomicrograph of the kidney of a patient with chronic glomerulonephritis. Observe in this Masson trichrome–stained tissue that most of the glomeruli have been replaced blue-stained collagen fibers. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:925.)

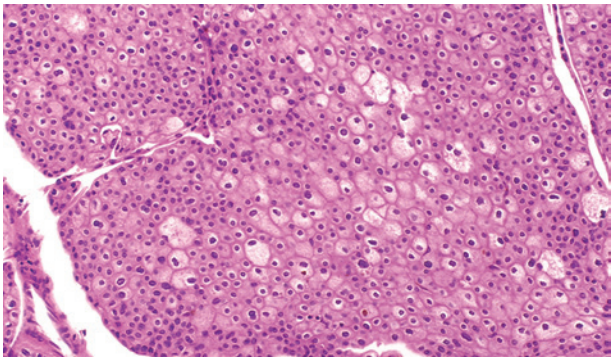


Figure 19–25 Photomicrograph of the kidney of a patient with chromophobe type renal cell carcinoma as evidenced by the presence of pale chromophobe-like cells that display a perinuclear halo. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:955.)

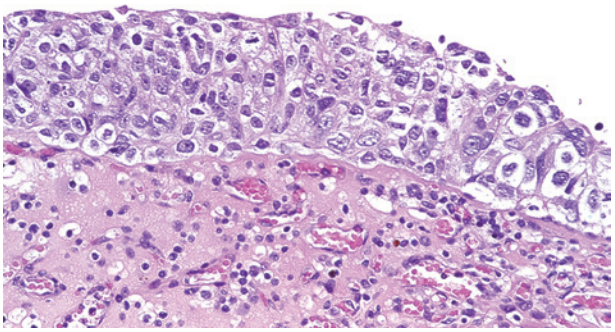
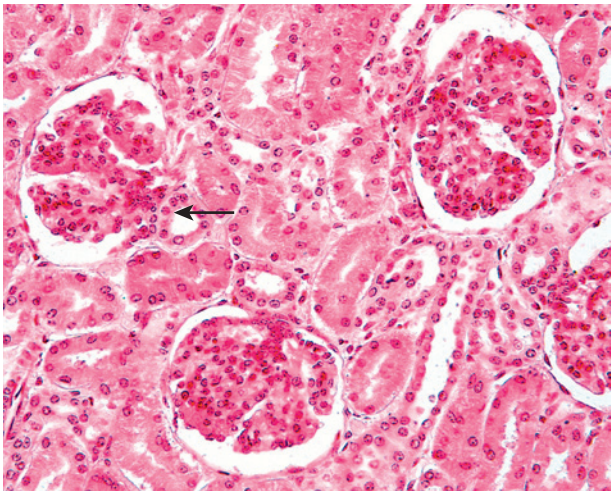
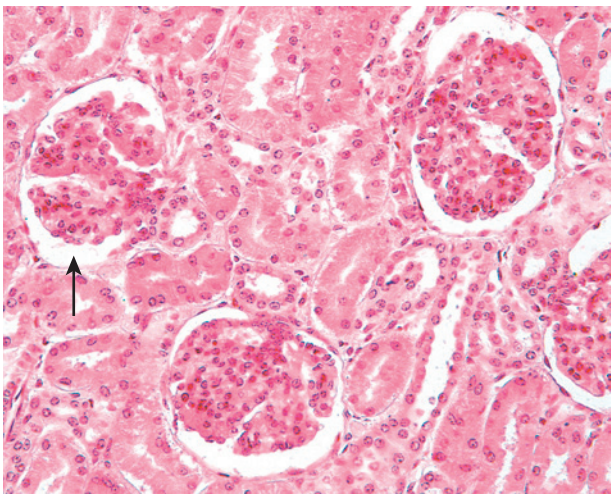


Figure 19–26 Photomicrograph of the urinary bladder of a patient with flat carcinoma in situ. Observe the characteristic enlarged, pleomorphic nuclei of the transitional epithelial cells. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:967.)

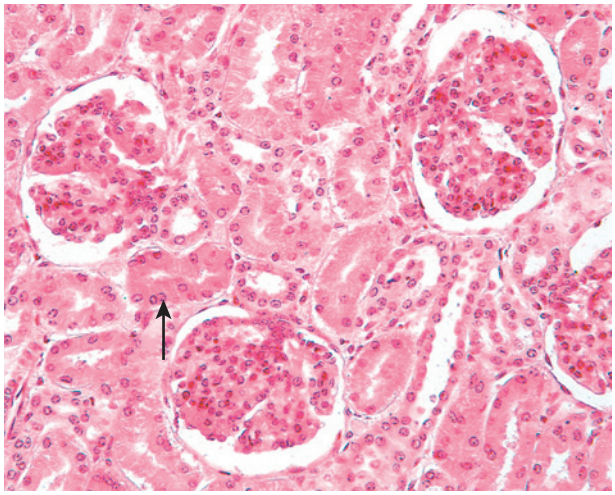
REVIEW QUESTIONS

1. A patient with Alport syndrome has vision problems, hearing loss, and nephritis. This inherited disorder is caused by mutations in
   a. type I collagen.
   b. type II collagen.
   c. type III collagen.
   d. type IV collagen.
   e. type VII collagen.
2. The greatest reduction in the volume of the ultrafiltrate occurs in which of the following?
   a. Proximal tubule
   b. Descending thin limb of Henle loop
   c. Ascending thin limb of Henle loop
   d. Ascending thick limb of Henle loop
   e. Collecting duct
3. Which of the following regions of Henle loop has a hypotonic ultrafiltrate?
   a. Descending thick limb
   b. Descending thin limb
   c. Henle loop
   d. Ascending thin limb
   e. Ascending thick limb
4. Blood from the efferent glomerular arteriole enters the
   a. glomerulus.
   b. peritubular capillary network.
   c. interlobular artery.
   d. arcuate artery.
   e. stellate vein.
5. Renin is released by which of the following structures?
   a. Proximal tubules
   b. Macula densa
   c. Descending thin limb of Henle loop
   d. Juxtaglomerular cells
   e. Collecting duct
6. 

   The structure at the tip of the arrow
   a. releases renin.
   b. releases angiotensin I.
   c. is a proximal convoluted tubule.
   d. adjoins afferent glomerular arteriole.
   e. is located at the urinary pole of the renal corpuscle.
7. 

   The space containing the tip of the arrow is normally occupied by
   a. air.
   b. plasma.
   c. serum.
   d. ultrafiltrate.
   e. urine.

8.



The cell at the tip of the arrow
a. adds amino acids into the lumen.
b. removes sodium ions from the lumen.
c. removes urea from the lumen.
d. adds chloride ions into the lumen.
e. adds water into the lumen.

9. Which of the following structures is located in the cortex of the kidney?
   a. Arteria recta
   b. Interlobar artery
   c. Minor calyx
   d. Efferent glomerular arteriole
   e. Henle loop
10. Which of the following is secreted by juxtaglomerular cells of the kidney?
   a. Angiotensinogen
   b. Vasopressin
   c. Renin
   d. Rennin
   e. Erythropoietin

ANSWERS

1. D. Patients with Alport syndrome, an autosomal recessive disorder, have mutations in their genes that code for α_3 and α_4 chains of their type IV collagen. These patients frequently suffer from kidney failure and may require a kidney transplant. Types I, II, III, and VII collagens are not affected by this mutation.
2. A. It is in the proximal tubule that approximately 65 to even as high as 80% of the fluid volume is resorbed in the proximal tubule. Of the 20% to 35 % of the water volume that is left, a high percent leaves the descending thin limb, but some of it reenters the ascending thin limb of Henle loop. The ascending thick limb is impermeable to water, and the permeability of the collecting duct varies, depending on the presence or absence of antidiuretic hormone (vasopressin).
3. E. The ascending thick limb of Henle loop is completely impermeable to water; however, it has chloride (and sodium) pumps that drive salts out of the ultrafiltrate, making it hypotonic (as little as 100 mOsm/L). The descending thin limb and Henle loop both lose water and gain salts; therefore, they have a very high osmolarity. The ascending thin limb is mostly impermeable to water but is freely permeable to salts, which leave the lumen, but the ultrafiltrate still has a high osmolarity.
4. B. Blood from the efferent glomerular arteriole of cortical nephrons enters the paratubular capillary network. Blood from the glomerulus enters the efferent glomerular arteriole. The interlobular arteriole delivers blood to the afferent glomerular arteriole. Blood from the arcuate artery enters the interlobular arteriole. The stellate veins drain the capsule of the kidney, the terminal portions of the efferent glomerular arterioles, and the periarterial capillary plexus and deliver their blood into the interlobular veins.
5. D. Renin is manufactured and released by juxtaglomerular cells, modified smooth muscle cells of the afferent (and also efferent) glomerular arterioles. The proximal tubules, macula densa, thin limbs of Henle, and the collecting duct do not possess renin.
6. D. The structure at the tip of the arrow is the macula densa, the part of the distal tubule (not of the proximal tubule) that measures the flow rate and the osmolarity of the ultrafiltrate. It lies adjacent to the afferent glomerular arteriole and, if needed, induces the juxtaglomerular cells, the modified smooth muscle cells of that vessel, to release its stored renin into the blood stream. Renin, an enzyme, cleaves angiotensinogen, a small peptide manufactured by hepatocytes, to form angiotensin I. The macula densa is located at the vascular pole, not the urinary pole, of the renal corpuscle.
7. D. The space occupied by the arrow is known as *Bowman space* (urinary space) and is normally filled with ultrafiltrate. It never contains air. Blood is filtered by the glomerular capillaries and the podocytes filter plasma. Serum is the fluid that is expressed from blood after clotting. Urine is formed as the ultrafiltrate is modified by the nephron and the collecting ducts.
8. B. The *arrow* indicates a cell of the proximal convoluted tubule, which removes sodium and chloride ions as well as water and amino acids from the ultrafiltrate in the lumen of the tubule. It does not add or remove ammonia.
9. D. Efferent glomerular arterioles drain the glomerulus, and, therefore, it is in the renal cortex. All other structures listed are in the medulla of the kidney.
10. C. The juxtaglomerular cells manufacture and release the enzyme renin that cleave angiotensinogen, manufactured by the liver, to form angiotensin I. Vasopressin (antidiuretic hormone) is manufactured by cells of the hypothalamus and released by the neurohypophysis. Rennin is an enzyme that curdles milk proteins. Erythropoietin is manufactured by the endothelial cells of the peritubular capillary network (and perhaps connective tissue cells of the cortex and outer medulla).

This page intentionally left blank

Female Reproductive System

20

The **female reproductive system** consists of the internal reproductive organs (the paired ovaries and oviducts, the uterus, and the vagina) (Fig. 20–1) and the external genitalia (the clitoris, the labia majora, and the labia minora).

The reproductive organs are incompletely developed and remain in a state of rest until **gonadotropic hormones** secreted by the pituitary gland signal the initiation of puberty. Thereafter, many changes take place in the entire reproductive system, including further differentiation of the reproductive organs, culminating in **menarche**, the first menstrual flow, ranging from about 9 to 15 years of age with an average age of 12.7 years. After the first menstrual flow, the menstrual cycle, which involves many hormonal, histological, and psychological changes, is repeated approximately each month (28 days) throughout the entire reproductive years unless it is interrupted by pregnancy. As a woman nears the end of her reproductive years, her menstrual cycles become less regular as hormonal and neurological signals begin to change, initiating **menopause**. Eventually, menstrual cycles cease; after menopause, limited involution of the reproductive organs occurs. Thus, the female reproductive system is controlled by complex orchestrations of hormonal, neurological, and psychological factors.

Although the mammary glands are not considered part of the female reproductive system, their physiology and function are so closely associated with the reproductive system that they are discussed in this chapter.

OVARIES

The ovaries, covered by the germinal epithelium, are indistinctly divided into a cortex and a medulla.

The paired **ovaries**, located within the pelvis, are almond-shaped bodies 3 cm long, 1.5 to 2 cm wide, and 1 cm thick and each weighs approximately 14 g. The ovaries are suspended in the **broad ligament of the uterus** by an attachment called the ***mesovarium***, a special fold of the peritoneum that conveys blood vessels to the ovaries (see Fig. 20–1).

Embryonic Development of the Ovaries

In the absence of the Y chromosome, which is responsible for the development of the male gonads, the default formation of **ovaries** will occur, which begins prior to the fourth week of development with the formation of the epithelially covered **gonadal ridges** on the posterior aspects of the abdominal wall. By the fifth week of development cells, derived from the epithelial cover penetrate the substance of the gonadal ridges to form a group of cells, known as the ***primitive sex cords***. Within a few days of the formation of the primitive sex cords, cells from the yolk sac, known as ***primitive germ cells***, invade the gonadal ridges and undergo cell division, and their rapid proliferation enlarges the gonadal ridges, and by the sixth week of development, these gonadal ridges form structures known as ***indifferent gonads***—indifferent because they do not as yet exhibit male or female characteristics. Within the next few days, these primitive sex cords burrow deeper into the medulla of the gonadal ridges only to undergo apoptosis and die. By the seventh week of development, another group of cells from the epithelial cover burrows into the *cortical aspect* of the gonadal ridges to establish **cortical sex cords**. Shortly after their formation these **cortical sex cords** dissociate, forming individual cells that migrate to and form a single layer of cells around each individual **primitive germ cell**, which is now known as an ***oogonium*** and the surrounding cells become known as ***follicular cells***. Because there are many more oogonia present than there are follicular cells to surround them, only 500,000 oogonia become surrounded by follicular cells, and the oogonia without follicular cells surrounding them are forced into apoptosis, undergo **atresia**, and die.

At this point each oogonium, surrounded by a single layer of flattened follicular cells, enters the first meitic division and are renamed to be known as ***primary oocytes***. The primary oocyte and its flat follicular cells are collectively known as a ***primordial follicle***. In order to bring the meiotic activity of the primary oocyte to a halt, the follicular cells release the signaling molecule **oocyte maturation inhibitor**, forcing the primary oocyte to remain in the **diplotene** stage of **meiosis I** until the time of ovulation. Primary oocytes remain in that phase until just before ovulation, when they are triggered, in response to the surge of **luteinizing hormone (LH)** and by **meiosis-inducing substance** to complete their first meiotic division, forming the secondary oocyte and the first polar body. Therefore, at birth, there are approximately 1 to 2 million primordial follicles present in the two ovaries. Of these, about 600,000 become atretic over the next decade or so of life, and at menarche a young woman has only about 300,000 to 400,000 follicles. Generally, ovulation will

SUMMARY

The **female reproductive system** is composed of the paired **ovaries** and **oviducts**; the **uterus** and the **vagina**; as well as the **external genitalia**, namely the **clitoris**, **labia minora**, and the **labia majora**. Although the **mammary glands (breasts)** are modified sweat glands, they are also discussed under the rubric of this system. The ovaries house **germ cells**, and, in the sexually mature female, these cells undergo maturation and begin their meiotic division. A single **secondary oocyte** is **ovulated** every month. The secondary oocyte enters the oviduct, where it may or may not be **fertilized** by a haploid **spermatozoon**. If pregnancy does not occur, the uterus sheds part of its **endometrium** every month via a process known as ***menstruation***. If the secondary oocyte is fertilized, it completes its meiotic division and is known as an ***ovum***, a haploid cell. The **pronucleus** of the ovum merges with the pronucleus of the spermatozoon to form a diploid cell, known as the ***zygote***, which undergoes mitotic divisions to form a **morula**. As the morula continues to develop, it forms a **blastocyst** that travels into the uterus and **implants** itself into the wall of the uterus where, in cooperation with the uterine endometrium, forms a **placenta** and completes its development into an **embryo**. At the time of birth, the embryo passes through the vagina, and the newborn baby is nourished by the milk produced by the mother's mammary glands.

KEY WORDS

Blastocyst; breasts; embryo; endometrium; external genitalia; female reproductive system; fertilization; germ cells; implantation; mammary glands; menstruation; morula; ovaries; oviducts; ovulation; ovum; placenta; pronucleus; secondary oocyte; spermatozoon; uterus; vagina; zygote

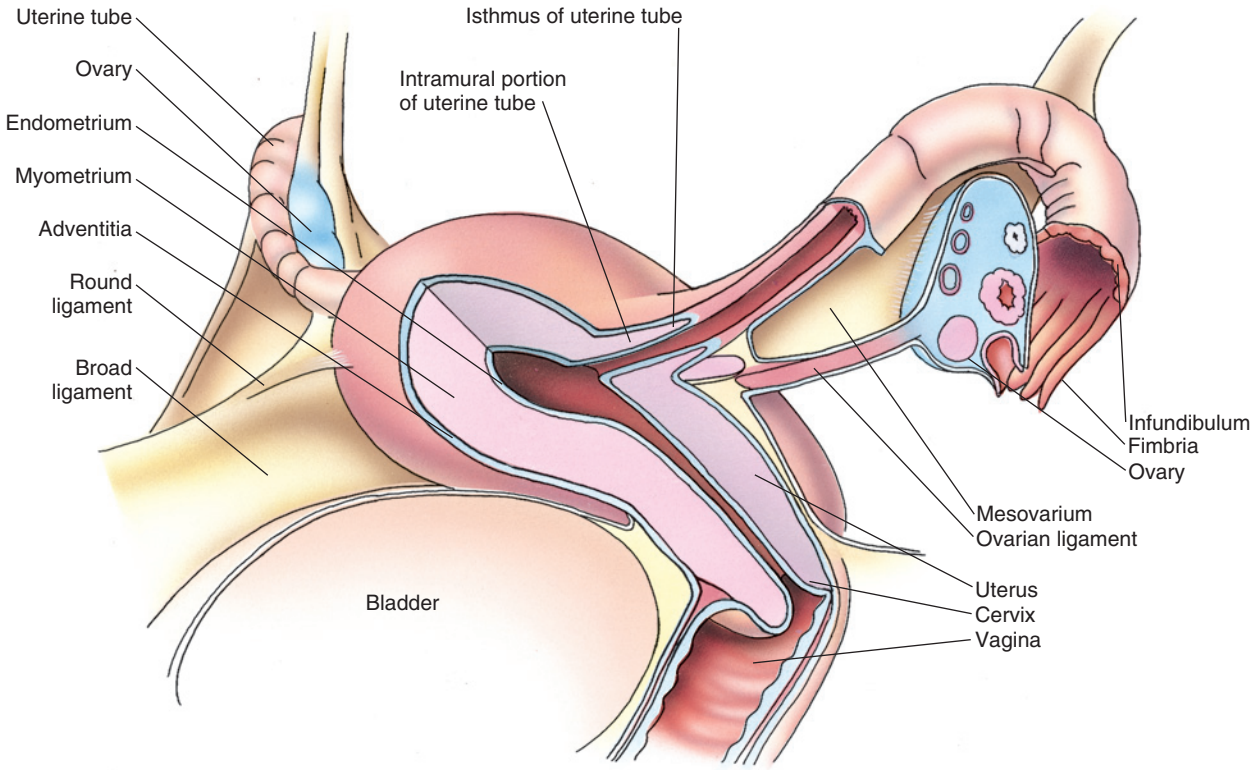


Figure 20–1 Schematic diagram of the female reproductive tract. Note that the ovary is sectioned to show the developing follicles and that the uterus and the fallopian tube are both opened to display their respective lumina.

occur every 28 days for the next 30 to 40 years, with one oocyte released each month, for a total of about 450 oocytes released over the reproductive period. The remaining follicles degenerate and die over the same period of time.

General Description of the Ovaries

The surface epithelium covering the ovaries, called the ***germinal epithelium***, is a modified peritoneum. This low cuboidal epithelium, derived from the **mesothelial epithelium** covering the developing ovaries, was originally believed to give rise to the germ cells; although this is now known to be untrue, the name persists. Directly beneath this epithelium is the **tunica albuginea**, a poorly vascularized, dense, irregular collagenous connective tissue capsule whose collagen fibers are oriented more or less parallel to the ovary surface. Each ovary is subdivided into the highly cellular **cortex** and a **medulla**, which consists mostly of a richly vascularized loose connective tissue. The blood vessels of the medulla are derived from the ovarian arteries. Histologically, however, the division between the cortex and the medulla is indistinct.

CLINICAL CORRELATION

The development of a female is the default condition that requires the absence of a Y chromosome. If a Y chromsome is present, two of its genes, ***SRY* (sex-determining region of the Y chromosome)** and ***SOX9*** (a gene that codes for a transcription factor known by the same name) become activated. The former expresses the protein known as **testis determining factor**, which inhibits the regions of the X chromosome that code for the development of a female. The protein SOX9 has several functions, including the prompting of **Sertoli cells** to form **anti-Mullerian hormone (AMH)** that prevents the formation of the female reproductive tract (see Chapter 21 on the male reproductive system) and also activates **fibroblast growth factor-9** that encourages Sertoli cell proliferation and thereby the continued synthesis and release of the protein SOX9, prompting a continuous supply of AMH and development of an embryo of the male gender.

Ovarian Cortex

The ovarian cortex is composed of the connective tissue stroma that houses ovarian follicles in various stages of development.

The **ovarian cortex** of a sexually mature female is composed of a connective tissue framework, the **stroma** (also known as the ***interstitial compartment***), housing fibroblast-like **stromal cells** (also known as ***interstitial cells***) as well as **ovarian follicles** in various stages of development (Fig. 20–2A).

The Ovarian Cortex at Onset of Puberty

The pulsatile release of gonadotropin-releasing hormone has the major responsibility for the initiation of puberty.

Before the onset of puberty, all of the follicles of the ovarian cortex are in the **primordial follicle** stage. The decapeptide **gonadotropin-releasing hormone (GnRH**, also known as ***luteinizing hormone-releasing hormone***, ***LHRH***), produced by the neurosecretory neurons of the arcuate nucleus and preoptic area of the hypothalamus, plays a major role in initiating puberty.

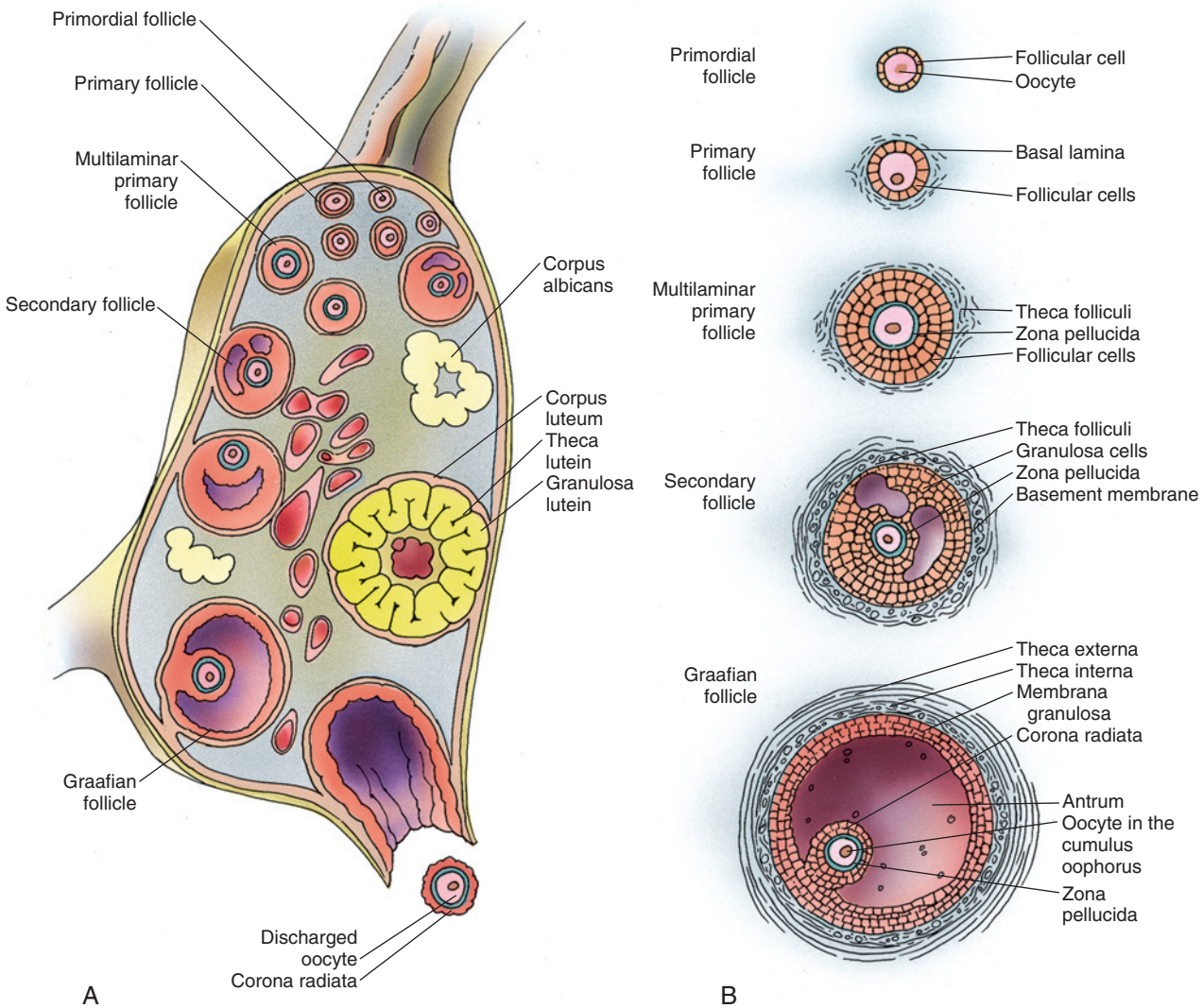


Figure 20–2 Diagram of ovarian structure and follicular development. Observe that a corpus luteun as well as a corpus albicans is also displayed in the ovary. Note that all of the stages of follicular development, from the primordial follicle stage to the graafian follicle stage, are presented.

TABLE 20–1 Pulsatility Rate of LHRH Release

Rate of Release	Direct Results	Effects of Direct Results
Less than 60 minutes	Downregulates LHRH receptor formation	Anovulation due to lack of gonadotropin responsivity
Greater than 90 minutes	Inadequate stimulation of basophils	Anovulation and amenorrhea
Between 60 and 90 minutes	Adequate number of LHRH receptors on basophils	Normal ovulatory cycle

LHRH, Luteinizing hormone-releasing hormone.

It is interesting that release of LHRH is pulsatile, occurring approximately every 90 minutes, and that its half-life in the bloodstream is only about 2 to 4 minutes. The pulsatility of LHRH release is a prerequisite not only for the onset of menarche but also for the maintenance of the normal ovulatory and menstrual cycles throughout the reproductive life of the female.

The pulsatile release of **GnRH** (and by the simple, nonpulsatile release of the hormone **leptin**) results in a similar, pulsatile, release of gonadotropins **(follicle-stimulating hormone [FSH]** and **LH)** from the basophils of the anterior pituitary that culminates in the commencement of follicular development and the onset of the ovulatory cycle (Table 20–1). The ovulatory cycle, follicular development, and hormonal interrelationships are described next.

Ovarian Follicles

Ovarian follicles mature through four developmental stages: primordial, primary, secondary, and graafian.

Ovarian follicles are surrounded by stromal tissue and consist of a **primary oocyte** and its associated **follicular cells (granulosa cells)** arranged in a single spherical layer or several concentric layers around the primary oocyte. Follicular cells, similar to the germinal epithelium, are derived from the **mesothelial epithelium** and possibly also from a second source, the primitive sex cords of the **mesonephros**, a precursor of the metanephros, the structure that develops into the definitive kidney.

There are two stages of follicular development based on the growth of the follicle; additionally, they are also categorized by the development of the oocyte and of the follicular cells (Table 20–2; see Fig. 20–2B):

- Nongrowing follicles
  - *Primordial* follicles (also categorized as **nongrowing follicles**)
- Growing follicles
  - Unilaminar and multilaminar *primary* follicles
  - *Secondary* (antral) follicles
  - *Graafian* (mature) follicles

The development of the primary follicles is *independent* of FSH; instead, the differentiation and proliferation of the follicular cells are triggered by as yet uncharacterized local factors secreted probably by the follicular cells and the primary oocytes of the ovary. Secondary and later follicles, however, are under the influence of FSH. Follicular development usually culminates in the release of a single oocyte (ovulation).

Primordial Follicles

Primordial follicles, composed of a single layer of flattened follicular cells that surround the primary oocyte, are separated from the ovarian stroma by a basement membrane; they are considered to be the basic reproductive units of the ovary.

Primordial follicles, the most primitive follicles, are abundant before birth, after which they become fewer in number; they are considered to be the basic reproductive units of the ovary. The primordial follicle is composed of a **primary oocyte** surrounded by a single layer of flattened **follicular cells** (Figs. 20–3 and 20–4).

The **primary oocyte**, arrested in the **dictyate stage of prophase** of **meiosis I**, due to the release of the factor known as ***oocyte maturation inhibitor*** by the follicular cells surrounding it, is a spherical cell about 25 μm in diameter. The primary oocyte will not resume its meiotic division until ovulation, even if that occurs 30 to 40 years later. It has a large, acentric nucleus containing a single nucleolus. The nucleoplasm appears vesicular because of the uncoiled chromosomes. The organelles include numerous mitochondria, abundant Golgi complexes, rough endoplasmic reticulum (RER) displaying only a few ribosomes, and occasional annulate lamellae. Moreover, primary oocytes house vesicles that occupy the cortical region of the cell, just beneath the plasmalemma. These vesicles are known as ***cortical granules***, and they house proteolytic enzymes that will function during the process of fertilization (see the section on fertilization).

The squamous **follicular cells** completely surround the primary oocyte and are attached to each other by desmosomes. They are separated from the connective tissue stroma by a basal lamina.

Primary Follicles

There are two types of primary follicles, unilaminar and multilaminar, depending on the number of layers of follicular cells that surround the primary oocyte.

Primordial follicles develop into **primary follicles** (see Fig. 20–3) distinguished as a result of changes in the

TABLE 20–2 Stages of Ovarian Follicular Development

Stage	FSH Dependent	Oocyte	Zona Pellucida	Follicular Cells or Granulosa	Liquor Folliculi	Theca Interna	Theca Externa
Primordial follicle	No	Primary oocyte	None	Single layer of flat cells	None	None	None
Unilaminar primary follicle	No	Primary oocyte	Present	Single layer of cuboidal cells	None	None	None
Multilaminar primary follicle	No	Primary oocyte	Present and microvilli of primary oocyte form gap junctions with filopodia of corona radiata cells	Several layers of follicular cells (now called *granulosa cells*)	None	Present	Present
Secondary follicle	Yes	Primary oocyte	Present with gap junctions	Spaces develop between granulosa cells	Accumulate in spaces between granulosa cells	Present	Present
Graafian follicle	Yes, until it becomes the dominant follicle	Primary oocyte surrounded by corona radiata in cumulus oophorus	Present with gap junctions	Form membrana granulosa and cumulus oophorus	Fills the antrum	Present	Present

FSH, Follicle-stimulating hormone.

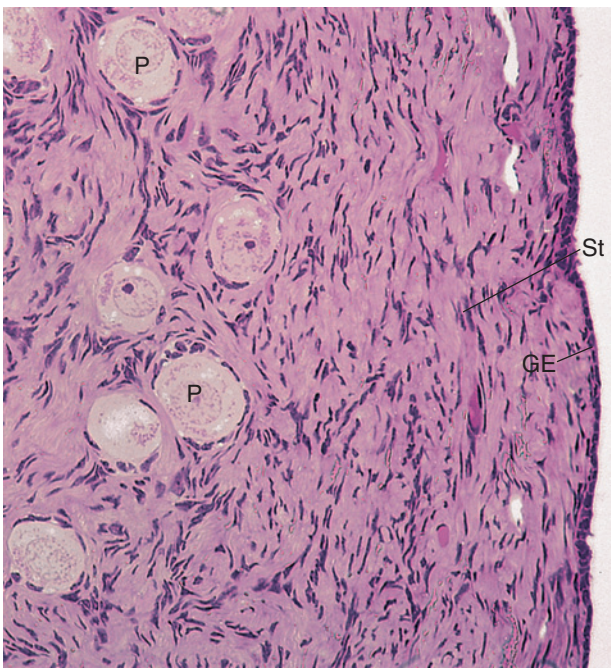


Figure 20–3 Light micrograph of the ovarian cortex demonstrating mostly primordial follicles (P), which are primary oocytes surrounded by follicular cells. The germinal epithelium (GE) as well as the ocarian stroma (St) of the cortex are also evident in this photomicrograph (×270).

primary oocyte, the follicular cells, and the surrounding stromal tissue.

The **primary oocyte** grows to about 100 to 150 µm in diameter with an enlarged nucleus (sometimes these nuclei are called ***germinal vesicles***). Several Golgi complexes are scattered throughout the cell, the RER becomes rich with ribosomes, free ribosomes are abundant, and mitochondria are numerous and dispersed throughout the cell.

Follicular cells become cuboidal in shape. As long as only a single layer of follicular cells encircles the oocyte, the follicle is called a ***unilaminar primary follicle***. Both the follicular cells and the primary oocyte participate in the transformation of the primordial follicle into the primary follicle by expressing transcription factors such as **newborn oogenesis homeobox**, **spermatogenesis and oogenesis helix-loop-helix 1 and 2**, and **forkhead box L2**. All of these factors as well other signaling molecules, such as **epidermal growth factor**, **insulin-like growth factor**, and **calcium ions**, act at the level of the microenvironment of a particular developing primary follicle so that other neighboring primordial follicles are not prompted into becoming primary follicles. In fact, other local factors, such as phosporylated **forkhead box O3**, manufactured by the primary oocyte, inhibits the transformation of the primordial follicle into a primary follicle.

Figure 20–4 Electron micrograph of a primordial ovarian follicle of a rat ovary (×6200). Observe the oocyte surrounded by follicular cells. (From Leardkamolkarn V, Abrahamson DR. Immunoelectron microscopic localization of laminin in rat ovarian follicles. *Anat Rec*. 1992;233:41–52. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Multilaminar Primary Follicle

When the follicular cells proliferate and stratify, forming several layers of cells around the primary oocyte, the follicle is called a ***multilaminar primary follicle***, and the follicular cells are more commonly referred to as ***granulosa cells***. The proliferative activity of the granulosa cells is due to the growth factors **activin**, **bone morphogenic protein-15**, and **growth differentiation factor-9** produced by the primary oocyte.

During this stage, an amorphous substance (the **zona pellucida**) appears, separating the oocyte from the surrounding follicular cells. The zonula pellucida is composed of four different glycoproteins, ZP_1, ZP_2, ZP_3, and ZP_4, secreted by the oocyte (see later section on fertilization for the functions of these glycoproteins). Filopodia of the follicular cells invade the zonula pellucida and come in contact with the oocyte plasmalemma and form gap junctions with each other (composed of **connexin 43**) as well as gap junctions with the microvilli of the primary oocyte (using **connexin 37**). It is through these connexin 37–based gap junctions that follicular cells communicate with the primary oocyte throughout follicular development. The presence of gap junctions are necessary for the oocyte to be able to progress through meiosis.

Stromal cells begin to be organized around the multilaminar primary follicle, forming an inner **theca interna**, composed mostly of a richly vascularized cellular layer, and an outer **theca externa**, composed mostly of fibrous connective tissue. This process of reorganization of the stromal cells as well as the growth of the primary oocyte in diameter are due to the secretion of **kit ligand (stem cell factor)** by the granulosa cells. As kit ligand is being released, both the primary oocyte and the granulosa cells in the immediate vicinity of the primary oocyte express **kit ligand receptors**, and the binding of kit ligand to its receptors effects changes in the primary oocyte and the stromal cells. The cells composing the theca interna also possess **LH receptors** on their plasmalemma, and these cells assume the ultrastructural characteristics of steroid-producing cells. Their cytoplasm accumulates numerous lipid droplets and has abundant smooth endoplasmic reticulum, and the cristae of their mitochondria are tubular. These theca interna cells produce the male sex hormone **androstenedione**, which enters the granulosa cells, where it is converted by the enzyme **aromatase** into the estrogen **estradiol**. The granulosa cells are separated from the theca interna by a thickened basal lamina.

Secondary (Antral) Follicles

Secondary follicles are similar to primary follicles except for the presence of accumulations of liquor folliculi among the granulosa cells.

The multilaminar primary follicle continues to develop and increase in size, reaching up to 200 µm in diameter. A large spherical follicle is formed with numerous layers of granulosa cells around the primary oocyte (whose size from this point on remains constant due to the continued release of **oocyte maturation inhibitor** by the theca interna cells). Several intercellular spaces develop within the mass of granulosa cells and become filled with a fluid known as ***liquor folliculi***. Once the multilaminar primary follicle displays the presence of liquor folliculi, it is known as a ***secondary follicle*** (although some refer to this phase as a **tertiary follicle** or **antral follicle**) (Fig. 20–5; see Fig. 20–2B).

Continued proliferation of the granulosa cells of the secondary follicle depends on **FSH** released by basophil cells of the anterior pituitary. Under the influence of FSH, the number of layers of the granulosa cells increases, as does the number of liquor folliculi–containing intercellular spaces. This fluid, an exudate of plasma, contains glycosaminoglycans, proteoglycans, and steroid-binding proteins produced by the granulosa cells. Moreover, it contains the hormones **progesterone**, **estradiol**, **inhibin**, **folliostatin (folliculostatin)** and **activin**, which regulate the release of LH and FSH. In addition, FSH (along with estrogen) induces the granulosa cells to manufacture receptors for LH, which become incorporated into their plasmalemma.

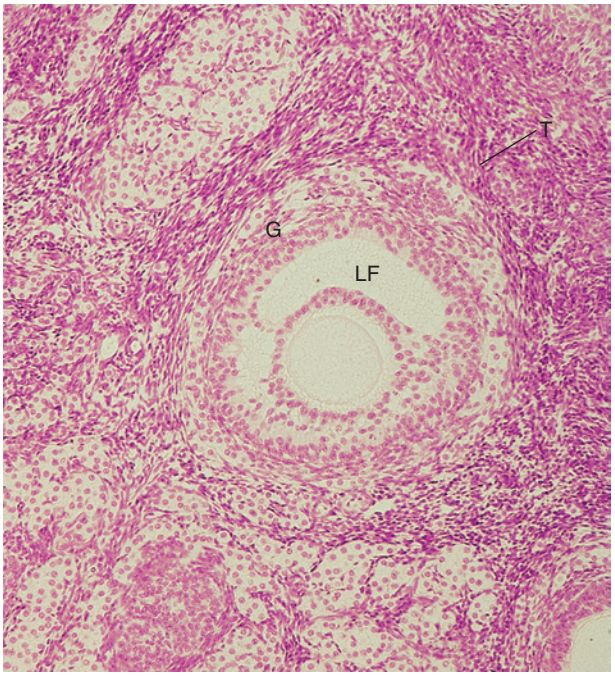


Figure 20–5 Light micrograph of a secondary follicle. Observe the primary oocyte and the follicular fluid surrounded by membrana granulosa. Note also the presence of the basement membrane between the granulosa cells (G) and the theca interna (T). LF, Liquor folliculi (×132).

Graafian (Mature) Follicles

Graafian follicles, also known as mature follicles*, may be as large as the entire ovary; it is these follicles that undergo ovulation.*

Continued proliferation of the granulosa cells and continued formation of liquor folliculi result in the formation of a **graafian (mature) follicle** whose diameter reaches 2.5 cm by the time of ovulation. The graafian follicle may be observed as a transparent bulge on the surface of the ovary, nearly as large as the ovary itself.

As more fluid is produced, individual droplets of liquor folliculi coalesce to form a single, fluid-filled **(liquor folliculi)** chamber, the **antrum**. The granulosa cells become rearranged so that the primary oocyte is now surrounded by a small group of granulosa cells that project out from the wall into the fluid-filled antrum. This structure is called the ***cumulus oophorus***. The loosely arranged low cuboidal granulosa cells immediately adjacent to the zona pellucida move slightly away from the oocyte, but their filopodia remain within the zona pellucida, maintaining contact with the primary oocyte. This single layer of granulosa cells that immediately surrounds the primary oocyte is called the ***corona radiata***. At this time, two different types of granulosa cells may be distinguished, **cumulus granulosa cells** and the **membrana (mural) granulosa cells** (Table 20–3), where the membrana granulosa cells form the wall of the antrum and the cumulus granulosa cells function in directing the development of the primary oocyte.

Toward the end of this stage, stromal cells become enlarged, and the theca interna is invaded by capillaries that nourish them as well as the avascular granulosa cells. Most of the follicles that reach this stage of development undergo atresia, but some of the granulosa cells associated with the atretic follicles do not degenerate; instead, they form **interstitial glands**, which secrete small amounts of androgens until menopause is concluded. A few secondary follicles continue to develop into mature follicles.

The theca interna cells, separated from the membrana granulosa by a basement membrane, continue to display **LH receptors** and under the influence of LH synthesize **androstenedione (androgen)**, the male sex hormone. The androgens are conveyed across the basement membrane and enter the cells of the membrana granulosa, where the androgens are transformed by the enzyme **aromatase** into **estradiol (estrogen)**.

TABLE 20–3 Types of Granulosa Cells

Cells	Characteristics of the Cells
Membrana granulosa • Granulosa mural cells	• Abut the basement membrane • Have LH and FSH receptors • Function in steroidogenesis due to the presence of the enzyme aromatase (estradiol, progesterone) • Produces the regulatory hormones activin, inhibin, folliculostatin, and insulin-like growth factors I. • Form the bulk of the corpus luteum
• Antral granulosa cells	• Line the antrum • Are not active in steroidogenesis
Cumulus oophorus granulosa cells	• Surround the oocyte • Contact the oocyte plasmalemma by their filopodia • Do not possess many LH receptors • Divide to form cells of the membrana granulosa • Are ovulated along with the oocyte

FSH, Follicle-stimulating hormone; LH, luteinizing horome.

The theca externa is a dense irregular collagenous connective tissue endowed with smooth muscle cells. It possesses a rich vascular supply that provides oxygen and nutrients for the theca interna, both the membrana and cumulus granulosa cells, as well as for the primary oocyte.

Continued formation of liquor folliculi causes the cumulus oophorus, composed of the primary oocyte, the corona radiata, and associated follicular cells, to become detached from its base to float freely within the liquor folliculi (see Fig. 20–2B).

Ovulation

The process of releasing the secondary oocyte from the graafian follicle is known as ovulation.

By the fourteenth day of the menstrual cycle, estrogen produced mostly by the developing graafian follicle, but also by secondary follicles, causes elevation of blood estrogen to levels high enough to have the following effects:

- Negative feedback inhibition shuts off FSH release by the anterior pituitary.
- A sudden surge of LH is released by basophils of the anterior pituitary.

The surge in LH levels results in increased blood flow to the ovaries, and capillaries within the theca externa begin leaking plasma, resulting in edema. Concomitant with edema formation, histamine, prostaglandins, and collagenase are released in the vicinity of the graafian follicle. Additionally, plasminogen activator levels, the enzyme that catalyzes the conversion of plasminogen to plasmin, increase in the follicle, and the newly formed plasmin facilitates the proteolysis of the membrana granulosa, permitting ovulation to occur (Fig. 20–6 and Table 20–4).

In addition, the LH surge is responsible for the following events:

1. A local factor, **maturation promoting factor**, is released by the primary oocyte.
2. Under the influence of maturation promoting factor, composed of **cyclin B** and **cyclin-dependent kinase**, the primary oocyte of the graafian follicle resumes and completes its first meiotic division, resulting in the formation of two daughter cells, the **secondary oocyte** and the **first polar body**. Because of the uneven distribution of the cytoplasm, the first polar body is composed of a nucleus surrounded by only a narrow rim of cytoplasm.
3. The newly formed secondary oocyte enters the **second meiotic division** and is arrested in **metaphase** and remains at that stage until fertilization.
4. The presence and continued formation of proteoglycans and hyaluronic acid by the granulosa cells attract water, thus causing an even greater increase not only in the size of the graafian follicle but also in the loosening of the membrana granulosa.
5. Just before ovulation, the surface of the ovary, where the graafian follicle is pressing against the tunica albuginea, loses its blood supply.
6. This thinned, avascular region becomes blanched and is known as the ***stigma***. The connective tissue at the stigma degenerates, as does the wall of the graafian follicle in contact with the stigma, forming an opening between the peritoneal cavity and the antrum of the graafian follicle.
7. Through this opening, the secondary oocyte and its attendant follicular cells and some of the liquor folliculi are gently released from the ovary, resulting in **ovulation**. Although the average menstrual cycle is 28 days long, some cycles are longer, and others are shorter; however, ovulation is always on the fourteenth day before the beginning of menstruation.
8. The remnants of the graafian follicle are converted into the corpus hemorrhagicum and then the corpus luteum.
9. It would appear from the previous description that the entire process of the recruitment of the primordial follicle into a graafian follicle and ovulation of the secondary oocyte occurs in approximately 14 days; however, that is not so. Instead, the time period requires is much greater, for it takes almost 10 months for the primordial follicle to become a secondary follicle and a subsequent 2 months for ovulation to occur. All of the oocyte and the granulosa cells of follicles that have reached the secondary follicle stage (but are not going to ovulate)

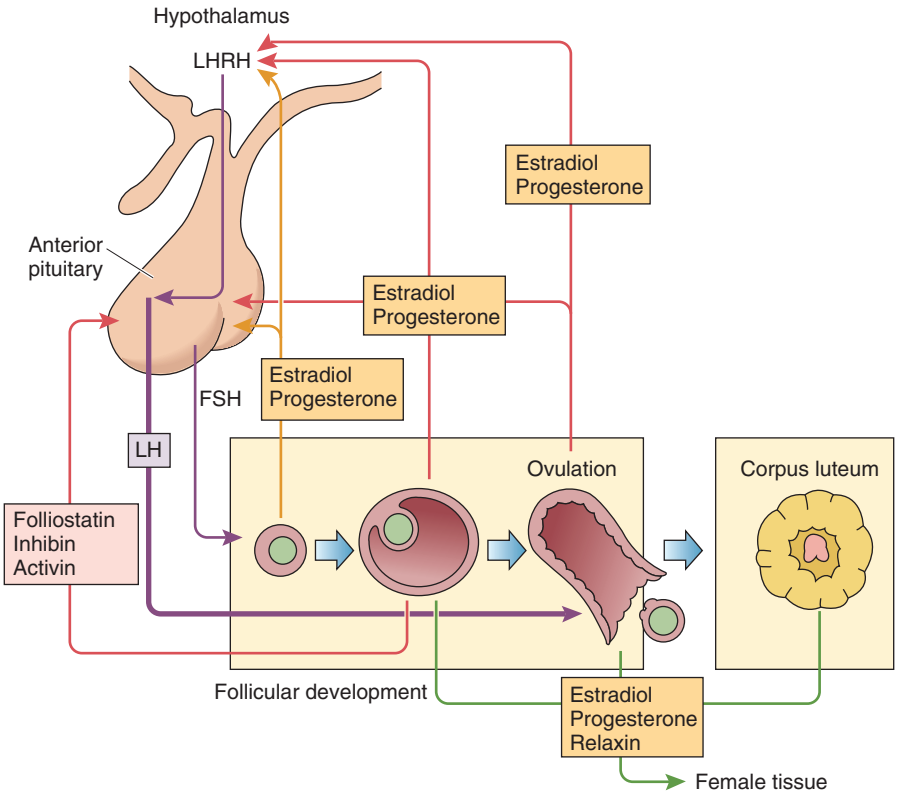


Figure 20–6 Schematic diagram illustrating the hormonal interactions between the hypothalamo-pituitary axis and the female reproductive system. Note that folliostatin and inhibin both suppress follicle-stimulating hormone (FSH) release, whereas activin facilitates the release of FSH. LHRH, Gonadotropin-releasing hormone; LH, luteinizing hormone.

undergo atresia and degenerate, but their theca cells do not undergo apoptosis; instead, they dedifferentiate into stromal cells.

10. Usually, only one of the follicles that reach the graafian follicle stage will ovulate, and that is known as the ***dominant follicle***.
11. All of the follicles that have reached the graafian follicle stage are FSH dependent, except for the dominant follicle that begins to produce large quantities of the hormone **inhibin** that shuts off FSH release by the pituitary gland (but does not act on GnRH release by the hypothalamus).
12. Once FSH is no longer produced, those follicles that are FSH dependent undergo atresia, whereas the dominant follicle progresses to ovulation.

The distal, fimbriated end of the oviduct, which presses against the ovary, whisks the secondary oocyte and follicular cells into the **infundibulum** of the **oviduct** to begin the journey into the ampulla, where the oocyte may be fertilized (see Fig. 20–1). If it is not fertilized within approximately 24 hours, the secondary oocyte degenerates and is phagocytosed. The process of fertilization is discussed later in the chapter.

Corpus Luteum

The corpus luteum, formed from the remnants of the graafian follicle, is a temporary endocrine gland that manufactures and releases hormones that support the uterine endometrium.

After the secondary oocyte and its associated follicular cells are ovulated, the remainder of the graafian follicle collapses and becomes folded; some of the ruptured blood vessels leak blood into the follicular cavity, forming a central clot. The resulting structure is known as the ***corpus hemorrhagicum***. As the clot is removed by phagocytes, continued high levels of **LH**, in conjunction with the hormones estradiol, insulin-like growth factors I and II, human chorionic gonadotrophin, and prolactin convert the corpus hemorrhagicum into a temporary structure known as the ***corpus luteum***, which functions as an endocrine gland (Fig. 20–7). This highly vascularized structure is composed of granulosa-lutein cells (modified granulosa cells) and theca-lutein cells (modified theca interna cells). The basement membrane between the former theca interna and the membrana granulosa disintegrates and the

TABLE 20–4 Major Hormones Involved in the Female Reproductive System

Hormone	Source	Function
Gonadotropin-releasing hormone (LHRH)	Hypothalamus	Stimulates release of FSH and LH from anterior pituitary gland
Prolactin-inhibiting factor	Hypothalamus	Inhibits release of prolactin by acidophils of anterior pituitary gland
Follicle-stimulating hormone (FSH)	Basophils of anterior pituitary gland	Stimulates secretion of estrogen and development of ovarian follicles (from secondary follicle onward)
Luteinizing hormone (LH)	Basophils of anterior pituitary gland	Stimulates formation of estrogen and progesterone; promotes ovulation and formation of corpus luteum
Estradiol	Granulosa cells of ovary; granulosa-lutein cells of corpus luteum; and the placenta	Inhibits release of FSH and LHRH; triggers surge of LH; causes proliferation and hypertrophy of myometrium of uterus; causes development of female sexual characteristics, including breasts and body fat; stimulates milk production before and at parturition
Progesterone	Granulosa cells of the ovary; theca-lutein and granulosa-lutein cells of the corpus luteum; placenta	Inhibits the release of LHRH from the hypothalamus and LH from the basophils of the anterior pituitary; causes the development of the uterine endometrium and regulates the viscosity of the mucus produced by the glands of the uterine cervix; causes the development of female sexual characteristics, including breasts; suppresses T cell–mediated rejection of the fetus
Inhibin	Granulosa cells of the ovary; granulosa-lutein cells of the corpus luteum	Inhibits FSH secretion by basophils of the anterior pituitary
Activin	Oocyte	Promotes granulosa cell proliferation
Human chorionic gonadotropin (hCG)	Placenta	Assists in the maintenance of the corpus luteum; promotes the release of progesterone
Human placental lactogen (human chorionic somatomammotropin)	Placenta	Promotes mammary gland development during pregnancy; promotes lactogenesis
Chorionic thyrotropin	Placenta	Stimulates thyroid hormone release
Insulin-like growth factors I and II	Placenta	Stimulates cytotrophoblast growth and development
Endothelial growth factor	Placenta	Supports trophoblast development and function
Fibroblast growth factor	Placenta	Induces cytotrophoblast proliferation
Colony stimulating factor	Placenta	Induces cytotrophoblast proliferation
Tumor necrosis factor	Placenta	Inhibits cytotrophoblast proliferation
Leptin	Placenta	Assists in transplacental transport of nutrients; maintains maternal nutrient status
Relaxin	Placenta	Facilitates parturition by softening the fibrocartilage of the pubic symphysis; softens the cervix and facilitates its dilation in preparation for parturition
Oxytocin	Hypothalamus via the posterior pituitary	Stimulates smooth muscle contraction of the uterus during orgasm and during parturition; stimulates contraction of myoepithelial cells of the mammary gland, assisting in milk ejection

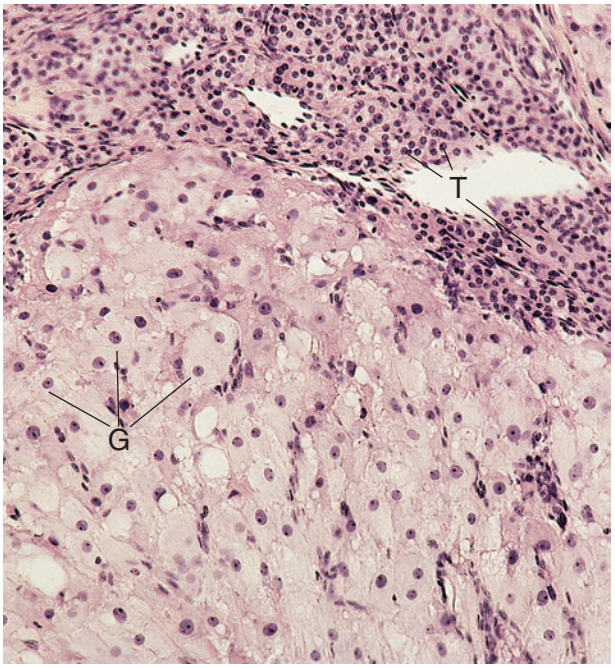


Figure 20–7 Light micrograph of the corpus luteum. Note the difference between the large granulosa-lutein (G) and small theca-lutein (T) cells (×132).

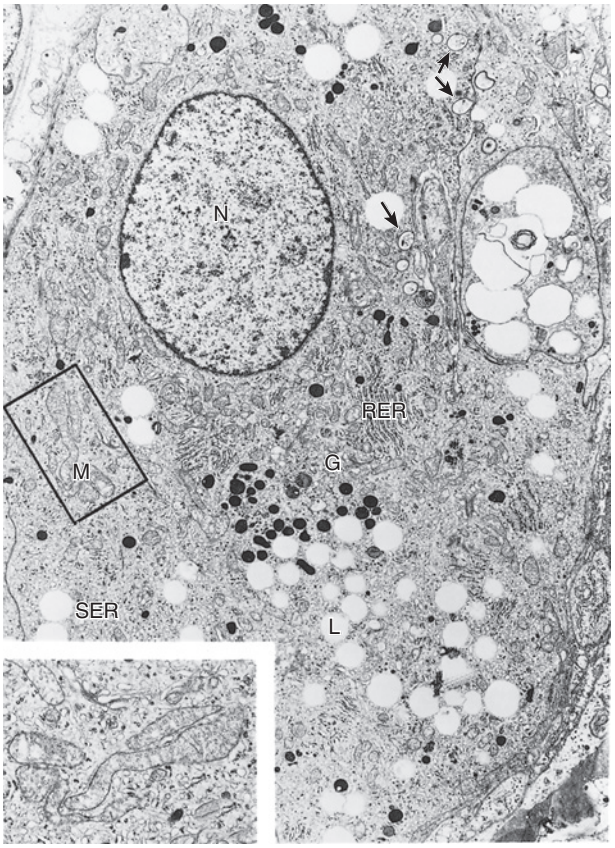


Figure 20–8 Electron micrograph of a rhesus monkey granulosa-lutein cell with its large acentric nucleus and numerous organelles. G, Golgi apparatus; L, lipid droplet; M, mitochondria (displayed at a higher magnification in *inset, lower left*); N, nucleus; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum (×6800). (From Booher C, Enders AC, Hendrick X, Hess DL. Structural characteristics of the corpus luteum during implantation in the rhesus monkey *[Macaca mulatta]*. *Am J Anat*. 1981;160:1736. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

blood vessels of the theca interna, in response to the **angiogenic factors**, **fibroblast growth factor**, and **vascular endothelial growth factor**, invade the membrane granulosa. The maintenance of the corpus luteum is **LH dependent**.

Granulosa-Lutein Cells

Granulosa cells of the graafian follicle differentiate into hormone-producing granulosa-lutein cells.

The granulosa cells remaining in the central region of the follicle account for about 80% of the cell population of the corpus luteum. They become modified into large, pale-staining cells (30 to 50 μm in diameter) called ***granulosa-lutein cells***. These cells have many long microvilli and develop all of the organelles necessary for steroid production, including abundant smooth endoplasmic reticulum and RER, abundant mitochondria, several well-developed Golgi complexes, and some lipid droplets scattered throughout the cytoplasm (Fig. 20–8). The granulosa-lutein cells produce **progesterone** and convert androgens produced by the theca-lutein cells into **estrogens**.

- The production of progesterone is dependent on the presence of l**ow-density lipoprotein (LDL) cholesterol receptors** on the basal plasmalemmae of these cells as well as on the presence of **steroidogenic acute regulatory (StAR) proteins**. LDL cholesterol receptors transfer LDL cholesterol into the granulosa lutein cells and StAR proteins transfer the LDL cholesterol into the mitochondria of these cells. Within the mitochondria the LDL cholesterol is transformed into **pregnelonone**, which, in the cytoplasm, is converted into **progesterone**.
- The conversion of androgens, synthesised in the theca lutein cells and transferred into the granulosa lutein cells, into estrogens is dependent on the enzyme **aromatase**.

Theca-Lutein Cells

Theca-lutein cells, derived from the cells of the theca interna, secrete progesterone, androgens, and some estrogens.

The **theca interna cells** at the periphery of the corpus luteum account for about 20% of the luteal cell population. These dark-staining cells remain small (15 μm in diameter) but become modified into hormone-secreting cells known as ***theca-lutein cells***. They specialize in the production of some **progesterone** and estrogens, and **androgens**.

Progesterone and estrogens secreted by granulosa-lutein and theca-lutein cells inhibit the secretion of LH and FSH, respectively. The absence of FSH prevents the development of new follicles, thus preventing a second ovulation. If pregnancy does not occur, the absence of LH leads to degeneration of the corpus luteum, forming the **corpus luteum of menstruation**. If pregnancy occurs, **human chorionic gonadotropin (hCG)**, secreted by the placenta, maintains the corpus luteum for 3 months. Now called the ***corpus luteum of pregnancy***, it grows to a diameter of 5 cm and continues to secrete hormones necessary for the maintenance of pregnancy. Although the placenta becomes the main site of production of the various hormones involved in maintaining pregnancy 2 to 3 months after its formation, the corpus luteum continues to form these hormones for several months.

Corpus Albicans

As the corpus luteum degenerates and is being phagocytosed by macrophages, fibroblasts enter, manufacture type I collagen, and form a fibrous structure known as the corpus albicans.

The corpus luteum of menstruation (or of pregnancy) is invaded by fibroblasts, becomes fibrotic, recruits **T cells**, and ceases to function. Its remnants undergo autolysis, a process known as ***luteolysis***, because the T cells release the cytokines **interferon-γ** that recruits macrophages. The newly recruited macrophages release **tumor necrosis factor α (TNF-α)**, which induces granulosa lutein and theca lutein cells to undergo apoptosis and the macrophages phagocytose the remnants of the apoptotic cells. The fibrous connective tissue that forms in the place of the corpus luteum is known as the ***corpus albicans*** and remains for some time before being reabsorbed. The remnants of the corpus albicans persist as a scar on the surface of the ovary.

Atretic Follicles

Follicles that undergo degeneration are known as atretic follicles.

The ovaries contain many follicles in various stages of development. Most follicles degenerate before reaching the mature stage, but multiple graafian follicles develop during each menstrual cycle. Nevertheless, once a dominant follicle forms, the remaining FSH-dependent follicles undergo atresia; the resulting **atretic follicles** are eventually phagocytosed by macrophages. Thus, normally, only the single dominant follicle ovulates during each menstrual cycle. Occasionally, two separate follicles develop to maturity and ovulate, leading to fraternal twins if both oocytes are fertilized. Although only about 2% of all follicles reach the mature stage and are primed to undergo ovulation, fewer than 1 in 20 of them actually do ovulate. Of all the follicles present in the ovaries at menarche, just 0.1% to 0.2% develop to maturity and undergo ovulation.

Ovarian Medulla

The ovarian medulla is a richly vascularized fibroelastic connective tissue housing connective tissue cells, interstitial cells, and hilar cells.

The central region of the ovary, the **medulla**, is composed of fibroblasts loosely embedded in a collagen-rich meshwork containing elastic fibers (see Fig. 20–2A). The medulla also contains large blood vessels, lymph vessels, and nerve fibers. The medulla of the premenstrual human ovary has a few clusters of epithelioid **interstitial cells** that secrete estrogens. In mammals having large litters, the ovaries contain many clusters of these interstitial cells, which collectively are called the ***interstitial gland***. In humans, most of these interstitial cells involute during the first menstrual cycle and have little, if any, function.

Hilar cells constitute another group of epithelioid cells in the ovarian medulla. These cells have a similar configuration of organelles and contain the same substances in their cytoplasm as Leydig cells of the testes. These cells secrete androgens.

Oviducts (Fallopian Tubes)

The oviducts act as a conduit for spermatozoa to reach the primary oocyte and to convey the fertilized egg to the uterus.

The **oviducts**, or **fallopian tubes**, are paired, muscular-walled tubular structures approximately 12 cm long, each with an open, free end and an open attached end (see Fig. 20–1). The oviducts become continuous with the wall of the uterus at their attached ends, where they traverse the uterine wall to open into its lumen. The free ends open into the peritoneal cavity close to the ovaries.

The oviducts are divided into four anatomical regions:

- Beginning at the free end is the **infundibulum**, whose open end is fringed with projections called

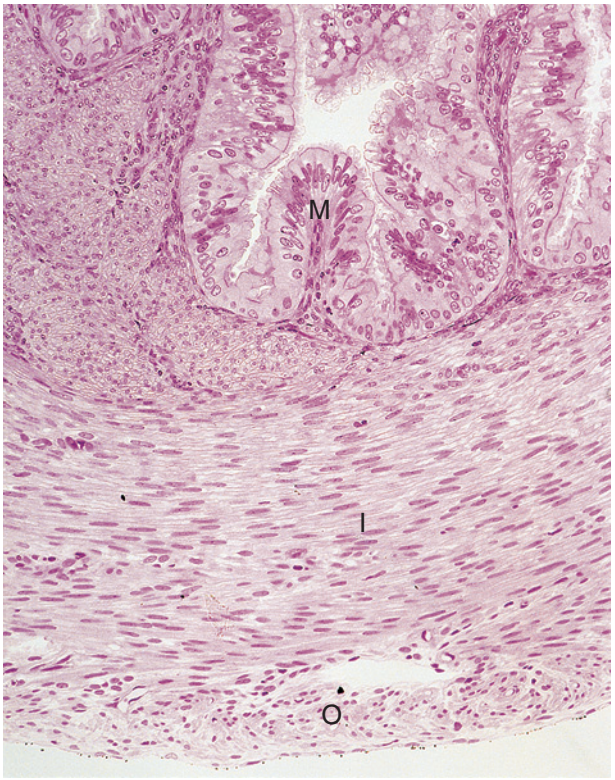


Figure 20–9 Light micrograph of the oviduct in cross section. Observe the outer longitudinal (O) and inner circular (I) muscle layers and the mucosa (M). Note that the mucosa is thrown into folds that reduce the size of the lumen (×132).

fimbriae. These fimbriae help capture the ovulated secondary oocyte.

- The longest region is the expanded **ampulla**, where fertilization usually takes place.
- The **isthmus** is the narrowed portion between the ampulla and the uterus.
- The attached end is known as the ***intramural region*** that passes through the uterine wall to open into the lumen of the uterus.

The oviducts are covered by visceral peritoneum. Their walls are composed of three layers (Fig. 20–9):

- Mucosa
- Muscularis
- Serosa

The **mucosa** is characterized by many longitudinal folds. These folds are present in all four regions of the oviduct but are most pronounced in the ampulla, where they branch; in the other regions, the mucosal folds are reduced to low elevations. The **simple columnar epithelium** that lines the lumen is tallest in the infundibulum and shortens as the oviduct approaches the uterus. Two different cell types constitute this epithelium:

- Nonciliated peg cells
- Ciliated cells

Peg cells have no cilia. They have a secretory function, providing a nutritive and protective environment for maintaining spermatozoa on their migration route to reach the secondary oocyte. Products within the secretions of the peg cells facilitate **capacitation** of spermatozoa, a process whereby spermatozoa become fully mature and capable of fertilizing the ovum. It is not known whether human spermatozoa require capacitation because they are capable of in vitro fertilization of the ovum without being exposed to the female reproductive tract. If there is such a requirement, the sojourn in the female reproductive tract necessitates only a minimal amount of time. Secretory products also provide nutrition and protection to the ovum; if the ovum is fertilized, the same secretions provide nutrients to the embryo during the initial phases of its development. The secretions of peg cells coupled with the movement of the fluid toward the uterus inhibit microorganisms from the uterus moving to the oviduct and into the peritoneal cavity. The number of peg cells is progesterone dependent in that they increase in number if progesterone is present.

The **cilia** of **ciliated cells** beat in unison toward the uterus. As a result, the fertilized ovum, the spermatozoa, and the viscous liquid produced by the peg cells are all propelled toward the uterus (Fig. 20–10). The number of ciliated cells is also estrogen dependent in that they, too, increase in number if estrogen is present.

The **lamina propria** of the oviduct mucosa is unremarkable because it is composed of loose connective tissue containing fibroblasts, mast cells, lymphoid cells, collagen, and reticular fibers. The **muscularis** consists of poorly defined inner circular and outer longitudinal layers of smooth muscle. Loose connective tissue also fills spaces between the bundles of muscle. A simple squamous epithelium provides the **serosal covering** of the oviduct. The loose connective tissue between the serosa and the muscularis contains many blood vessels and autonomic nerve fibers.

Because the oviducts are so richly vascularized with mostly large veins, contractions of the muscularis during ovulation constrict the engorged veins. This constriction causes distention of the entire oviduct and brings the fimbriae into contact with the ovary, thereby aiding the capture of the released secondary oocyte. Continued rhythmic contractions of the layers of the muscularis, coupled with the beating of the cilia within, help propel the captured oocyte to the uterus.

Uterus

The uterus is a muscular organ consisting of a fundus, body, and cervix.

The uterus, a single, thick, pear-shaped structure located in the midline of the pelvis, receives at its broad, closed end the terminals of the paired oviducts. The

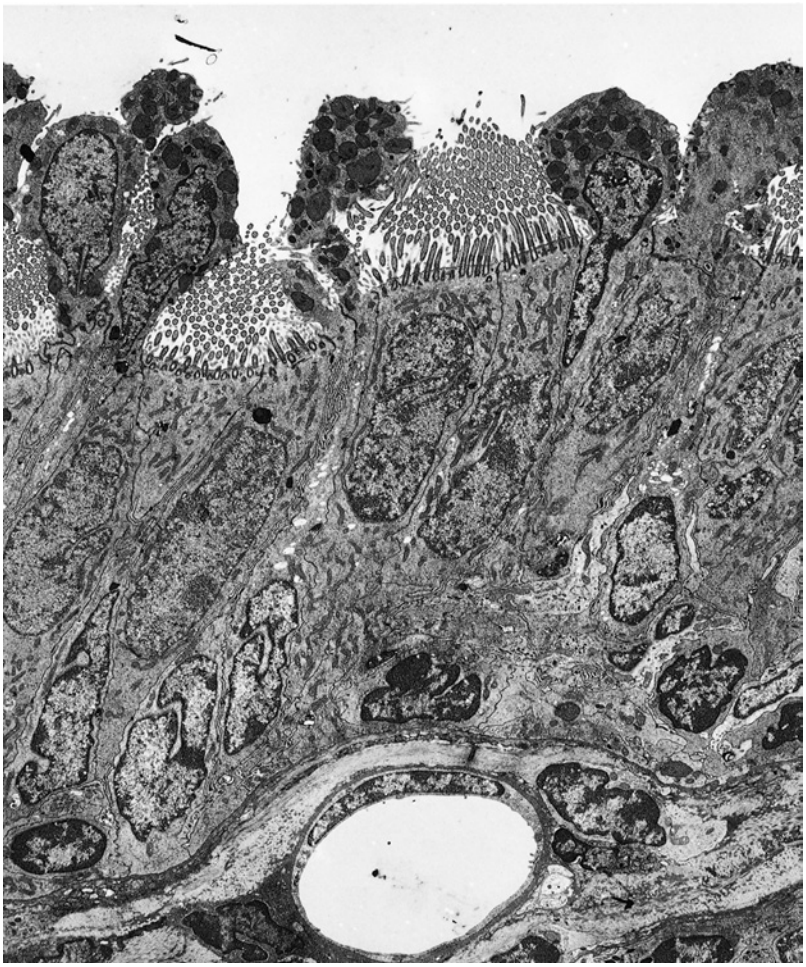


Figure 20–10 Electron micrograph of the oviduct epithelium. Note the bulbous apices of the peg cells as well as the cilia of the ciliated cells (×40000). (From Hollis DE, Frith PA, Vaughan JD, et al. Ultrastructural changes in the oviductal epithelium of merino ewes during the estrous cycle. *Am J Anat*. 1984;171:441-456. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

uterus is a robust muscular organ about 7 cm long, 4 cm wide, and 2.5 cm thick. The lumen of the non-gravid uterus is only about 10 mL in volume, and, by the time of parturition, it has increased to more than 5 L. The uterus is divided into three regions (see Fig. 20–1):

- **Body**. Broad portion into which the oviducts open.
- **Fundus**. Rounded base located superior to the exit ports of the oviducts in the body.
- **Cervix**. Narrow circular portion that protrudes and opens into the vagina.

Body and Fundus

The uterine wall of the body and the fundus is composed of an **endometrium**, **myometrium**, and either an **adventitia** or a **serosa**.

Endometrium

The endometrium is the mucosal lining of the uterus, consists of two layers, the superficial functionalis and the deeper located basalis.

The endometrium, or mucosal lining of the uterus, is composed of a simple columnar epithelium and a lamina propria. The epithelium is composed of **nonciliated secretory columnar cells** and **ciliated cells**, whereas the lamina propria houses simple branched **tubular glands** that extend as far as the myometrium (Fig. 20–11). Although the glandular cells resemble those of the surface epithelium, there are no ciliated cells in the glands. The dense, irregular collagenous connective tissue of the **lamina propria** is highly cellular and

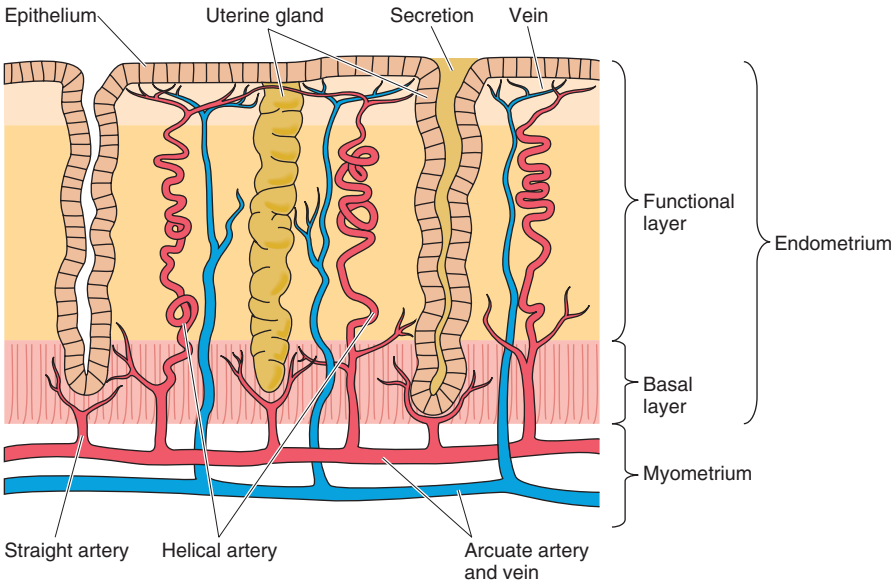


Figure 20–11 Diagram of the uterine endometrium characterized by the basal and functional layers. Observe that the basal layer is supplied by the straight arteries, whereas the functionalis layer is served by the coiled vessels known as the *helical arteries*.

contains star-shaped cells, macrophages, leukocytes, and an abundance of reticular fibers. The morphological and physiological alterations that occur in the endometrium during the phases of the menstrual cycle are controlled by various hormones (see later text).

The endometrium consists of two layers (see Fig. 20–11):

- **Functionalis**. A thick, superficial layer, whose thickness ranges between 1 and 7 mm depending on the stage of the menstrual cycle; it is this layer that is sloughed at menstruation.
- **Basalis**. A deep, narrow layer, approximately 1 mm in thickness. This layer is not sloughed during menstruation. The glands and connective tissue elements of the basalis proliferate and thereby regenerate the functionalis during each menstrual cycle.

The **functionalis** is vascularized by numerous coiled **helical arteries**, which originate from the **arcuate arteries** of the stratum vasculare, located in the middle layer of the myometrium. The coiled arteries give rise to a rich capillary network that supplies the glands and connective tissue of the functionalis. It is the helical arteries that permit the development of the **hemochorioendothelial type of placenta** as well as the process of **menstruation**, the hormone-dependent casting off of the functional layer of the endometrium. Another set of arteries, the **straight arteries**, also originate from the arcuate arteries but are much shorter and supply only the basalis.

Myometrium

The myometrium is composed of inner longitudinal, middle circular, and outer longitudinal layers of smooth muscle.

The thick muscular wall of the uterus, the **myometrium**, is composed of *three layers* of smooth muscle. **Longitudinal muscle** makes up the *inner* and *outer layers*, whereas the richly *vascularized middle layer* contains mostly **circularly** arranged smooth muscle bundles. This richly vascularized region, houses the **arcuate arteries** and is called the ***stratum vasculare***. As the uterus narrows toward the cervix, the smooth muscle tissue diminishes and is replaced by fibrous connective tissue. At the cervix, the myometrium is composed of dense, irregular collagenous connective tissue containing elastic fibers and only a small number of scattered smooth muscle cells.

The size and number of the myometrial muscle cells are related to estrogen levels. The muscle cells are largest and most numerous during pregnancy, when estrogen levels are the highest; they are smallest after the conclusion of menstruation, when estrogen levels are low. When estrogen is absent, the myometrial muscle atrophies, with some cells succumbing to **apoptosis**. Although most of the increase in uterine size during pregnancy is related to **hypertrophy** of the smooth muscle cells, the smooth muscle cell population also increases, suggesting that **hyperplasia** also occurs.

However, it is unclear whether the increase in cell number results only from division of smooth muscle cells or also from differentiation of undifferentiated cells into smooth muscle fibers.

Sexual stimulation causes moderate uterine contractions. During menstruation, the contractions may be painful in some women. Powerful, rhythmic contractions of the pregnant uterus during delivery expel the fetus and later the placenta from the uterus. The process of uterine contractions during parturition is due to hormonal actions:

- The myometrium and the fetal membranes produce **prostaglandins**.
- The posterior pituitary gland releases the hormone **oxytocin**.
- Prostaglandins and oxytocin stimulate uterine contractions.
- After delivery, oxytocin continues to stimulate uterine contractions, which inhibit excessive blood loss from the detachment site of the placenta.

Uterine Serosa or Adventitia

Because the uterus is tipped anteriorly and lies against the bladder, much of its anterior portion is covered by **adventitia** (connective tissue without an epithelial covering); thus, this area is **retroperitoneal**. The fundus and posterior portion of the body are covered by a **serosa**, composed of a layer of squamous mesothelial cells resting on areolar connective tissue; therefore, this area is intraperitoneal.

CLINICAL CORRELATIONS

The presence of endometrial tissue in the pelvis or in the peritoneal cavity is known as ***endometriosis***. This often painful condition may cause dysmenorrhea and even infertility. The origin of endometrial tissue outside the uterus is not known, but three theories have been proposed.

The **regurgitation theory** proposes that menstrual flow escapes from the uterus via the fallopian tubes to enter the peritoneal cavity. The **metaplastic theory** suggests that the epithelial cells of the peritoneum differentiate into endometrial cells. The **vascular (lymphatic) dissemination theory** proposes that endometrial cells enter vascular (or lymphatic) channels during menstruation and are distributed by the blood (or lymph) vascular system.

These extrauterine endometrial tissues also undergo cyclic changes. Hemorrhaging of this tissue may cause adhesions and extreme pain. If endometriosis is not corrected, the pelvic viscera may be embroiled in a fibrotic mass, possibly resulting in sterility.

Cervix

The terminal end of the uterus (the cervix) extends into the vagina.

The **cervix** is the terminal end of the uterus that protrudes into the vagina (see Fig. 20–1). The lumen of the cervix is lined by a **mucus-secreting simple columnar epithelium**; however, its external surface, where the cervix protrudes into the vagina, is covered by a **stratified squamous nonkeratinized epithelium** similar to that of the vagina. The wall of the cervix consists mostly of dense, collagenous connective tissue containing many elastic fibers and only a few smooth muscle fibers. Cervical mucosa contains branched **cervical glands**. Although the cervical mucosa changes during the menstrual cycle, it does not slough during menstruation.

At the midpoint in the menstrual cycle, around the time of ovulation, the cervical glands secrete a serous fluid that facilitates entry of the spermatozoa into the uterus. At other times and during pregnancy, the secretions of the cervical glands become more viscous, forming a plug of thickened mucus in the orifice of the cervix, thus thwarting the entry of sperm and microorganisms into the uterus. The hormone **progesterone** regulates the changes in the viscosity of the cervical gland secretions.

At the time of parturition, another luteal hormone, **relaxin**, induces lysis of collagen in the cervical walls. This results in a softening of the cervix, thus facilitating cervical dilation.

CLINICAL CORRELATIONS

The **Papanicolaou (Pap smear) technique** is a diagnostic tool for detecting cervical cancer. It is performed by aspirating cervicular fluid from the vagina or taking scrapings directly from the cervix. The tissue or fluid is prepared and stained on a microscope slide and then examined for variations in the cell populations to detect anaplasia, dysplasia, and carcinoma.

Cervical carcinoma is one of the most common cancers in women, although it is rare in virgins and in nulliparous women (women who have not given birth). The incidence increases in women with multiple sex partners and herpes infections. It develops from the stratified squamous nonkeratinized epithelium of the cervix, where it is called ***carcinoma in situ***. If detected by Pap smear in this stage, it can usually be successfully treated with surgery. If not detected early, however, it may invade other areas and metastasize, thus changing to **invasive carcinoma**, which carries a poor prognosis.

Menstrual Cycle

The menstrual cycle is divided into the menstrual, proliferative (follicular), and secretory (luteal) phases.

Ideally, the average menstrual cycle is a 28-day cycle. Although the successive events constituting the cycle

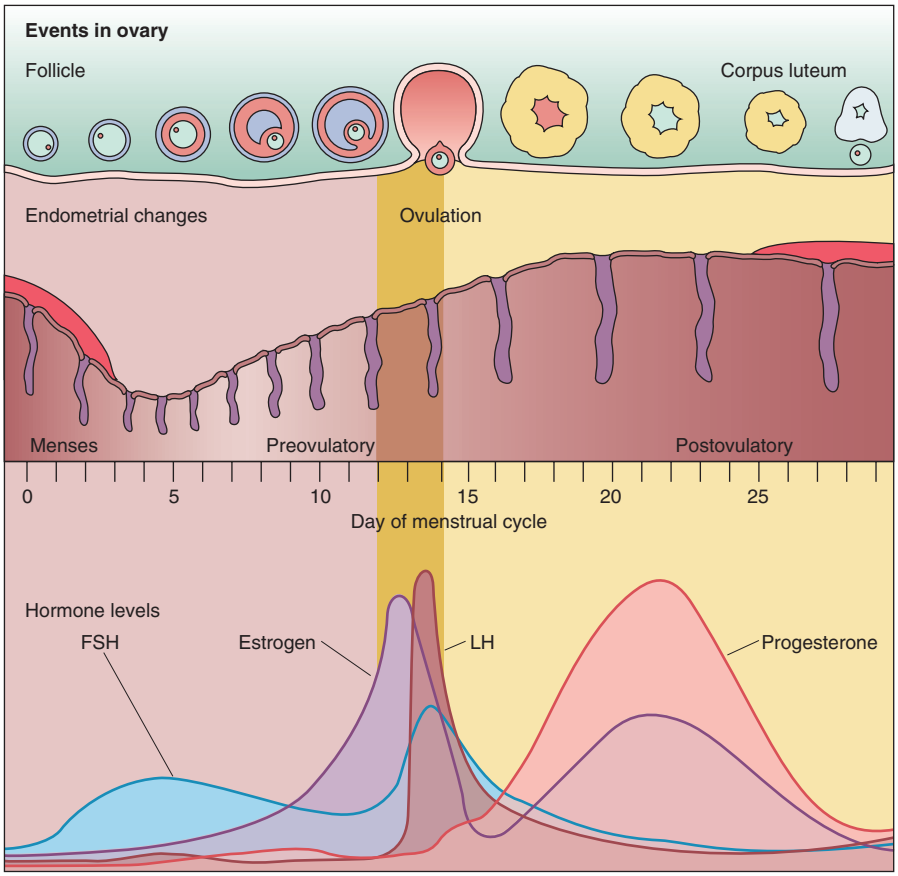


Figure 20–12 Diagram correlating the events in follicular development, ovulation, hormonal interrelationships, and the menstrual cycle. Note that the levels of estrogen and luteinizing hormone (LH) are highest at the time of ovulation. FSH, Follicle-stimulating hormone.

occur continuously, they can be described in three phases: **menstrual phase**, **proliferative (follicular) phase**, and **secretory (luteal) phase** (Fig. 20–12). These histologically recognizable phases depend on the levels of estradiol and progesterone.

Menstrual Phase (Day 1 to Days 3 or 4)

The menstrual phase is characterized by the desquamation of the functionalis layer of the endometrium.

Menstruation, which begins on the day that bleeding from the uterus starts, occurs if fertilization does not take place. In this case, the corpus luteum becomes nonfunctional about 14 days after ovulation, thus reducing the levels of **progesterone** and **estradiol**.

A couple of days before bleeding begins, the functionalis layer of the endometrium becomes deprived of blood as the coiled (helical) arteries are intermittently constricted. After 2 days or so, the coiled arteries become permanently constricted, reducing oxygen to the functionalis layer, leading to a shutdown of the glands, invasion by leukocytes, ischemia, and eventual **necrosis** of the **functionalis**. Shortly thereafter, the coiled arteries dilate again; however, because these coiled arteries have been weakened by the previous events, they rupture. The disgorged blood removes patches of the functionalis to be discharged as a **hemorrhagic discharge (menses)**, initiating menstruation on day 1.

Although the entire functionalis layer of the endometrium is sloughed, it is not completely released from the wall immediately; rather, this process continues for 3 to 4 days. During a normal menstrual period, the approximate blood loss is only 35 mL, although in some women it may be greater. It is important to note that during the menstrual phase there is an inhibition of the blood clotting mechanism.

Before and during the menstrual phase, the basalis layer of the endometrium continues to be vascularized by its own straight arteries and thus remains viable. The basal cells of the glands of the basalis begin to proliferate, and the newly formed cells migrate to the surface

to begin reepithelialization of the connective tissue wound of the uterine lumen. These events commence the proliferative phase.

Proliferative (Follicular) Phase (Days 4 or 5 to Day 14)

The proliferative phase is characterized by a reepithelialization of the lining of the endometrium and renewal of the functionalis.

The proliferative phase (also called the ***follicular phase*** because it occurs at the same time as the development of the ovarian follicles) begins when menstrual flow ceases, on about day 4 or 5, and continues through day 14. The proliferative phase is characterized by reepithelialization of the lining of the endometrium; reconstruction of the glands, connective tissue, and the coiled arteries of the lamina propria; and renewal of the functionalis. The process of proliferation is driven by the increasing levels of the hormone **estradiol** produced by the granulosa cells of the developing follicles but especially by the **dominant follicle** of the ovary. Estradiol binds to **estradiol receptors** in the stromal cells forming **estradiol-estradiol receptor complexes** that act as transcriptional factors that activate dozens of genes. These genes code for paracrine type **growth factors** that in turn act on epithelial and endothelial cells, inducing their proliferation.

During this phase, the functional layer becomes much thicker (up to 2 to 3 mm) because of the proliferation of the cells in the base of the glands, whose blood supply remained intact, were unaffected during the menstrual phase. As stated previously, it is these cells that are responsible for the formation of the epithelial lining of the uterus as well as for the establishment of new glands in the functionalis. These tubular glands are straight, not yet coiled, but their cells begin to accumulate glycogen, as do the cells of the stroma that proliferated to renew the stroma of the functionalis. The coiled arteries that were lost in the menstrual phase are replaced but are not tightly coiled and reach only two thirds of the way into the functionalis.

By the fourteenth day of the menstrual cycle (ovulation), the functionalis layer of the endometrium has been fully restored to its previous status with a full complement of epithelium, glands, stroma, and coiled arteries.

Secretory (Luteal) Phase (Days 15 to 28)

The secretory phase is characterized by thickening of the endometrium as a result of edema and accumulated glycogen secretions of the highly coiled endometrial glands.

The secretory phase (or **luteal phase)** commences after ovulation and is driven by the hormone **progesterone** released by the **granulosa lutein cells** of the **corpus luteum** and to a limited extent by **estradiol** produced by the theca lutein cells of the corpus luteum. Stromal cells possess **progesterone receptors** and the **progesterone–progesterone receptor complexes (P-PR complex)** not only act as transcription factors but also decrease the expression of estradiol receptors. Additionally, P-PR complexes activate genes that code for enzymes that inactivate estradiols. Moreover, P-PR complexes activate genes responsible for differentiation of the endometrium, making it receptive for the arrival of the early embryo.

During this phase, the endometrium continues to thicken as a result of edema and accumulated glycogen secretions of the endometrial glands, which become *highly convoluted* and *branched*. The secretory products first accumulate in the basal region of the cytoplasm of the cells constituting the endometrial glands. As more secretory product is manufactured, the secretory granules move apically and are released into the lumen of the gland. This **glycogen-rich material** will nourish the conceptus before the formation of the placenta.

Most of the changes that result in the thickening of the endometrium are attributed to the functionalis, although the lumina of the glands located in the basalis are also filled with secretory product (Fig. 20–13). The

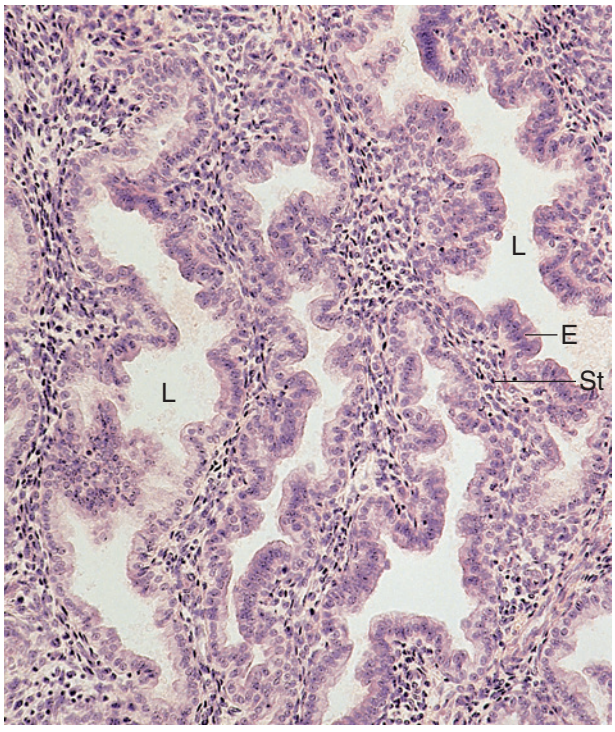


Figure 20–13 Light micrograph of the endometrium (E) of the uterus in the luteal phase. Note the lumina (L) of the glands surrounded by stromal cells (St) (×132).

coiled arteries of the functionalis attain full development, becoming more coiled and extending fully into the functional layer, by the twenty-second day. Thus, at this point in the secretory phase, the endometrium is about 6 to 7 mm thick.

The secretory phase completes the cycle as the 28th day approaches, presaging the menstrual phase of a new menstrual cycle. The menstrual phase is driven by the *reduction* in the hormones that promote the growth and development of the functionalis layer of the endometrium.

Fertilization and Implantation and Placental Development

Fertilization

Fertilization, the fusion of the sperm and the oocyte, occurs in the ampulla of the oviduct.

The oocyte and its attendant follicular cells are transported down the oviduct by the beating of the cilia of the ciliated cells of the epithelial lining and by rhythmic contractions of the smooth muscle of the oviduct (Fig. 20–14). The nutrient-rich fluid produced by peg cells of

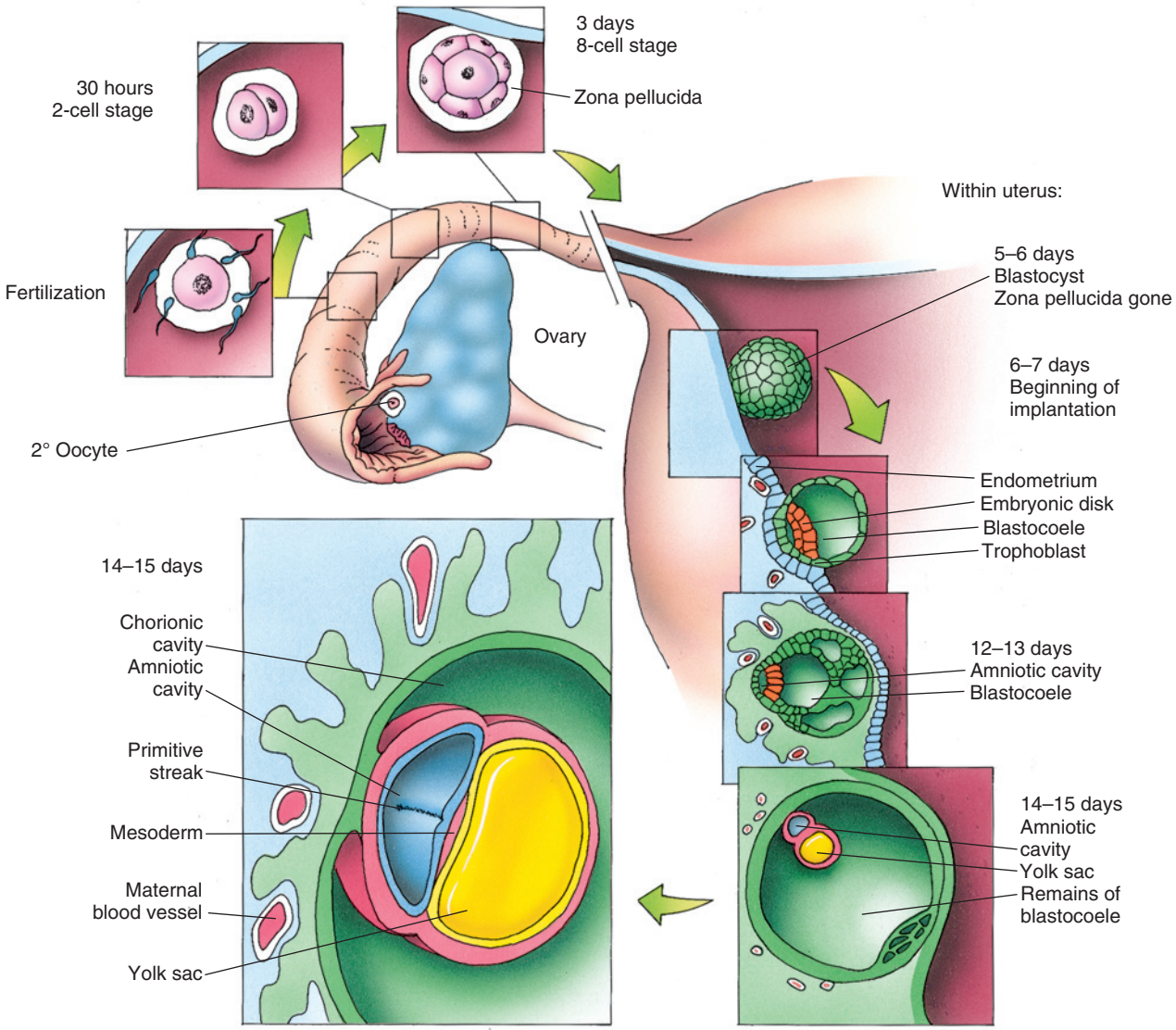


Figure 20–14 Diagram of the process of fertilization, zygote formation, morula and blastocyst development, and implantation.

the mucosal epithelium nourishes the oocyte on its way to the uterus.

Spermatozoa, introduced into the vagina during sexual intercourse, pass through the cervix, the uterine lumen, and up the oviduct to the ampulla to encounter the secondary oocyte. In order for a spermatozoon to have the ability to fertilize a secondary oocyte, it has to progress through three stages: maturation, capacitation, and hyperactivity.

- The process of **maturation** occurs in the male reproductive tract; prior to maturation, the spermatozoon can travel only in a circular direction, and, after maturation, it can travel in a forward direction. While in the male reproductive tract, the spermatozoon is subject to a high concentration of **fertilization promoting peptide (FPP)** that prevents the spermatozoon from undergoing capacitation.
- Once the spermatozoon is deposited in the female reproductive tract, the level of FPP is greatly reduced and the reduced level of FPP prompts the spermatozoon to begin **capacitation**. The process of capacitation entails a modification of the acrosomal membrane, in that cholesterol and certain glycoproteins are removed from the membrane, making it more permeable to calcium ions and facilitating a stonger bond between the membranes of the spermatozoon and of the oocyte.
- The increased entry of calcium ions into the spermatozoon elevates **cAMP** levels within the spermatozoon, inducing it to be more vigorous and a stronger swimmer, a condition known as ***hyperactivity***. Due to its enhanced abilities, the spermatozoon has an increased capability to pass through the zona pellucida to reach the secondary oocyte.

Fertilization usually occurs in the ampulla (Fig. 20–15). At this time, the cells of the corona radiata still surround the **zona pellucida**, a glycoprotein covering of the secondary oocyte. The zona pellucida is a gel-like substance consisting of four related glycoproteins named *ZP1*, *ZP2*, *ZP3*, and *ZP4*. These glycoproteins act to prevent polyspermy; that is, they ensure that only a single spermatozon is able to bind to the secondary oocyte. **ZP3** binds to the first spermatozoon to reach the zona pellucida and triggers the spermatozon to initiate the acrosome reaction (see later text). **ZP2** assists ZP3 in binding the spermatozoon to the zona pellucida. **ZP1** acts to cross link ZP2 with ZP3 so that they are no longer able to bind to spermatozoa, thus taking further steps to prevent polyspermy. The role of ZP4 is not yet understood.

The **acrosome reaction** results in the release of the acrosomal enzymes into the zona pellucida. The liberated enzymes, especially the inner acrosomal membrane-bound enzyme **acrosin**, decrease the viscosity of the zona pellucida, facilitating the flagellar movement of the spermatozoa to propel the sperm toward the oocyte. Once the spermatozoon penetrates the entire width of the zona pellucida, it enters the **perivitelline space**, located between the zona pellucida and the oocyte cell membrane, and can reach the oocyte.

When the spermatozoon contacts the secondary oocyte membrane its integral protein molecules, known as ***fertilin***, bind with the oocyte's **integrin** and **CD9 molecules** ensuring a strong attachment between them. This contact between the sperm and the oocyte is responsible for the **cortical reaction**, which is an additional process that prevents **polyspermy**. The cortical reaction has a fast and a slow component.

- The **fast component** involves a change in the resting membrane potential of the oocyte plasma membrane that prevents contact between the oocyte and another sperm. This alteration of the membrane potential lasts only a few minutes.
- The **slow component** involves the release of the contents of numerous cortical granules located in the oocyte's cytoplasm into the perivitelline space. Enzymes, such as **ovastacin**, within the cortical granules act to hydrolyze ZP2 and ZP3 molecules, the sperm receptors, in the zona pellucida, thus preventing additional spermatozoa from reaching the oocyte. The alteration in the ZP proteins cause the zona pellucida to become a firmer, thicker gel that prevents other spermatozoa from penetrating it.

At this time, the **nucleus (male pronucleus)** and **centrosome** of the spermatozoon enter the cytoplasm of the secondary oocyte, triggering it to resume and complete its second meiotic division. This results in an unequal division of the cytoplasm, forming two haploid cells, the **ovum** and the **second polar body**. The resultant haploid nucleus of the ovum **(female pronucleus)** and the male pronucleus both lose their nuclear envelopes as they travel toward each other and their chromosomes mingle with each other forming a new cell, known as the ***zygote*** with the diploid number of chromosomes and thus completing the event of fertilization. The zygote enters its first mitosis, forming two daughter cells a process that initiates the development of a new embryo. It should be recognized that the mitotic spindle apparatus of the embryo is derived from the spermatozoon, whereas the mitochondria and much of the cytoplasm is derived from the ovum.

The window of time between ovulation and fertilization is about 24 hours. If fertilization does not occur during this period, the oocyte degenerates and is phagocytosed by macrophages.

Implantation

Implantation is the process that occurs as the blastocyst becomes embedded in the uterine endometrium.

As the zygote continues its journey through the oviduct on its way to the uterus, it undergoes numerous mitotic divisions, becoming the spherical cluster of cells known

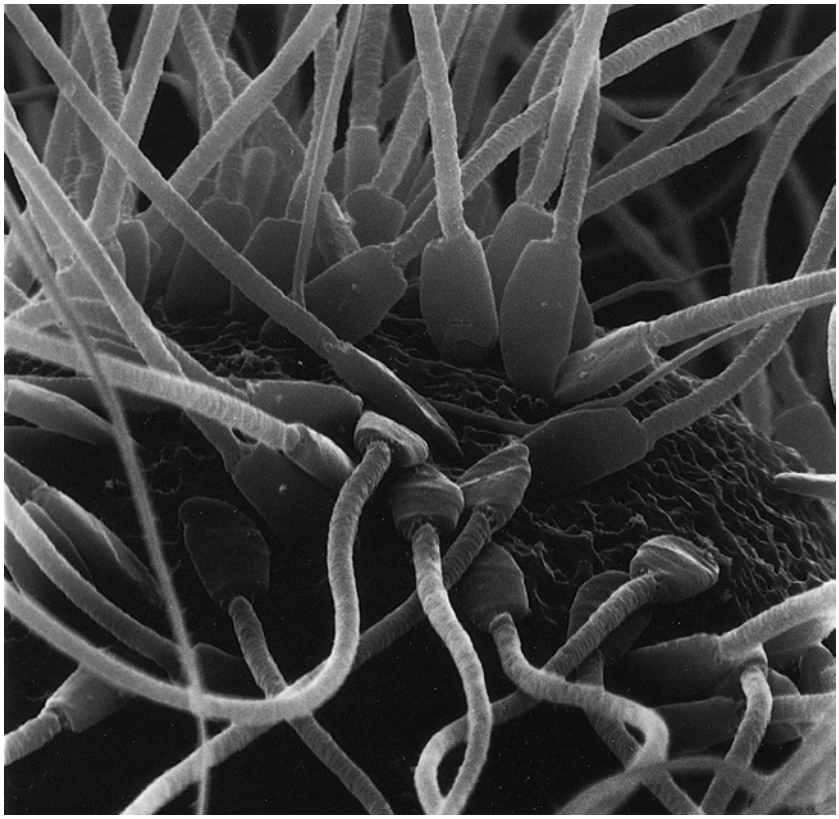


Figure 20–15 Scanning electron micrograph of fertilization. Observe that the large number of spermatozoa are trying to make their way through the cells of the corona radiata, but only a single spermatozoon will be able to fertilize the egg (×5700). (From Phillips DM, Shalgi R, Dekel N. Mammalian fertilization as seen with the scanning electron microscope. *Am J Anat*. 1985;174:357-372. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

as the ***morula*** (see Fig. 20–14). With further divisions and modifications, the morula is transformed into the **blastocyst**, composed of a hollow ball of cells, whose lumen contains a somewhat viscous fluid and a few cells at one pole. The peripheral cells are known as ***trophoblasts***, and the cells trapped inside the blastocyst are the **embryoblasts.** The blastocyst enters the uterine cavity about 4 to 6 days after fertilization, and on the sixth or seventh day, it begins to embed itself into the uterine wall, a process known as ***implantation***. The trophoblasts of the blastocyst stimulate the transformation of the star-shaped **stromal cells** of the uterine endometrium into pale-staining **decidual cells**, whose stored glycogen probably provides nourishment for the developing embryo.

The embryoblasts develop into the embryo, whereas **trophoblast cells** give rise to the embryonic portion of the placenta. Trophoblast cells proliferate rapidly, forming an inner conglomeration of individual cells, which are mitotically active and are known as ***cytotrophoblasts***, and a thicker outer syncytium of cells that do not undergo mitosis, called ***syncytiotrophoblasts***.

The **cytotrophoblasts** proliferate, with the new cells joining the syncytiotrophoblasts. As the syncytiotrophoblasts increase in number, they form vacuoles that coalesce into large, labyrinthine spaces known as ***lacunae***. Continued growth of the syncytium erodes the endometrium. This process permits deep penetration of the blastocyst into the wall of the endometrium, and, by the eleventh day of gestation, the endometrial epithelium seals over the implantation site.

Placenta

The placenta is a vascular tissue derived from the uterine endometrium as well as from the developing embryo.

At parturition, or birth, the placenta is a highly vascularized disk-shaped structure, about 18 cm in diameter and 2.5 cm thick in its middle and weighs about 600 g.

Development of the Placenta

Continued erosion of the highly vascular endometrium by the **syncytiotrophoblasts** also erodes the maternal

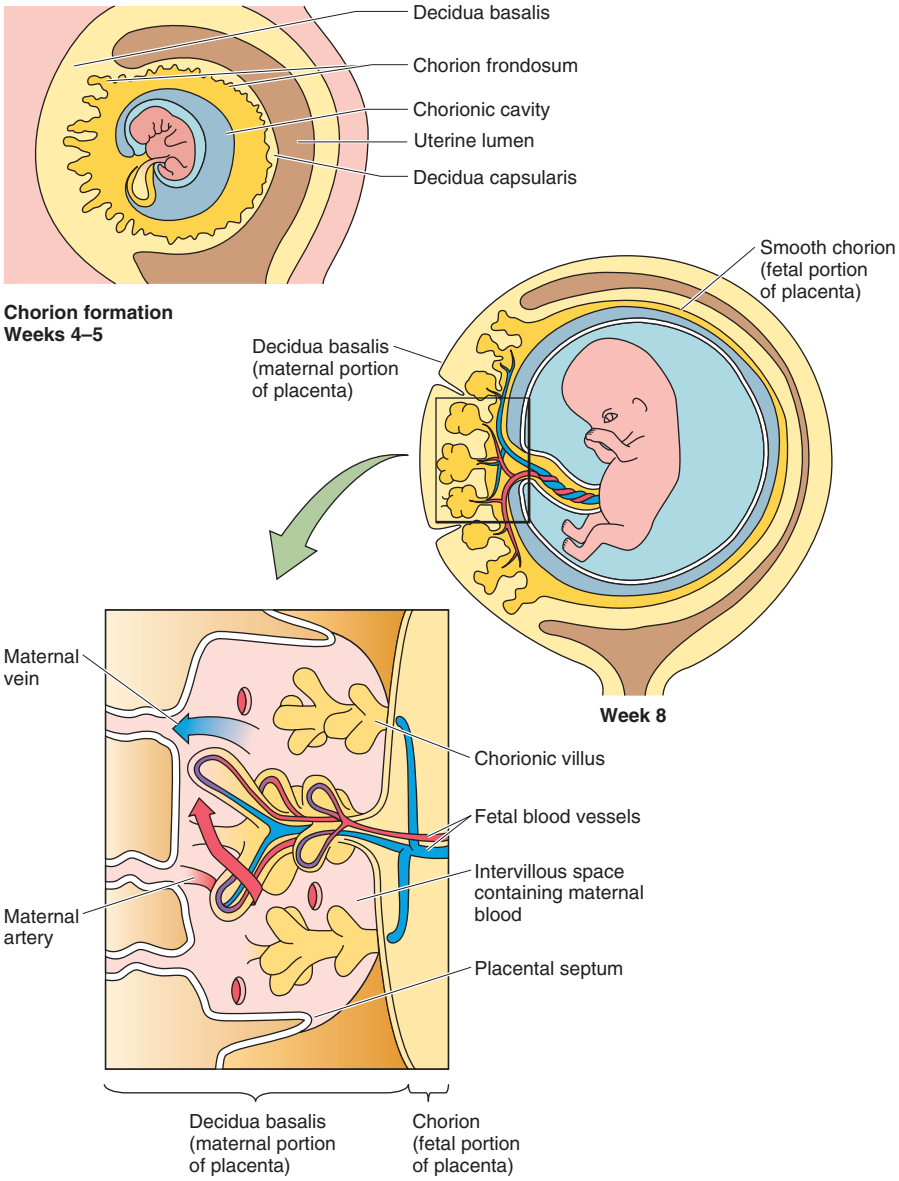


Figure 20–16 Diagram of chorion and decidua formation as well as circulation within the placenta.

blood vessels. The blood from these vessels empties into the lacunae of the syncytiotrophoblasts that surround the embryo. Thus, the maternal blood provides nourishment for the developing embryo. With further growth and development, the **placenta**, more accurately known as the ***hemochorioendothelial placenta***, begins to be formed with the resultant separation between the blood of the developing embryo and that of the mother (maternal blood). The reason that it is referred to as a *hemochorioendothelia placenta* is because only three layers, the fetal endothelial vessels of the placenta, embryonic connective tissue, and a layer of trophoblasts, are interposed between the maternal blood and the fetal blood.

The remaining trophoblast cells give rise to the **chorion**, which develops and evolves into the **chorionic plate**, which gives rise to the **chorionic villi** (Fig. 20–16).

The developing trophoblasts induce changes in the endometrium in their vicinity, altering it to begin the formation of the maternal portion of the placenta. This altered maternal tissue, called the ***decidua***, is subdivided into three regions:

- The **decidua capsularis** is interposed between the uterine lumen and the developing embryo.

- The **decidua basalis** is interposed between the developing embryo and the myometrium.
- The **decidua parietalis** composes the balance of the decidua.

Initially, the entire embryo is surrounded by decidua in order to nourish it. The region of the chorion in contact with the decidua capsularis forms short, insubstantial villi, thus remaining smooth surfaced; that region of the chorion is known as the ***chorion laeve***. The region of the decidua basalis, however, becomes highly vascularized by maternal blood vessels; it is in this region that the placenta develops. The region of the chorionic plate in contact with the decidua basalis forms extensive chorionic villi, known as ***primary villi***; thus, this region of the chorion is known as the ***chorion frondosum***.

The primary villi are composed of both syncytiotrophoblasts and cytotrophoblasts. With further development, extraembryonic mesenchymal cells enter the core of the primary villi, converting them into **secondary villi** (Fig. 20–17). The connective tissue of the secondary villi becomes vascularized by extensive capillary beds, which are linked to the developing vascular supply of the embryo.

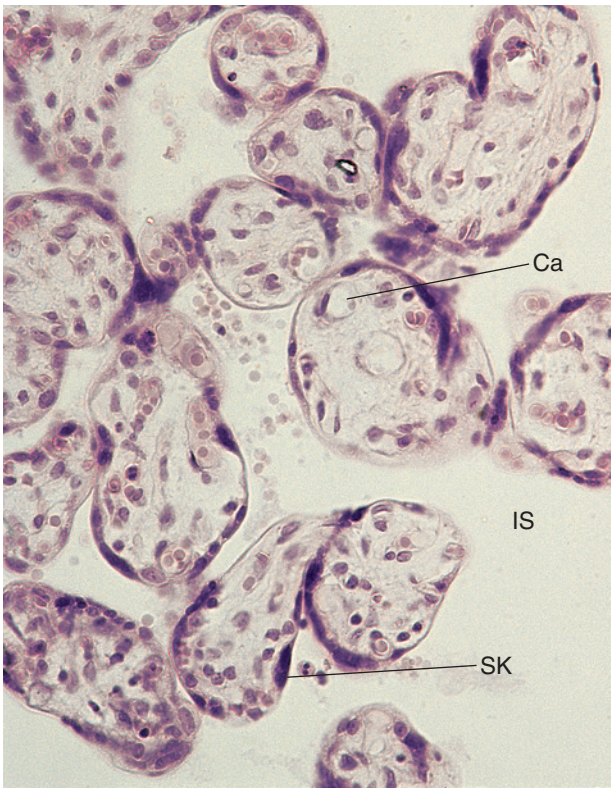


Figure 20–17 Light micrograph of cross sections of the chorionic villi of the placenta. Observe the cytotrophoblasts as well as the syncytiotrophoblasts covering the chorionic villi. Ca, Capillary; IS, intervillous space; SK, syncytial knot (×270).

As development continues, the cytotrophoblast population decreases because these cells join the syncytium and contribute to its growth. The decidua basalis forms large vascular spaces, **lacunae**, that are compartmentalized into smaller regions by **placental septa**, extensions of the decidua. Secondary villi project into these vascular spaces and are surrounded by maternal blood that is delivered to and drained from the lacunae by maternal blood vessels of the decidua basalis.

Most of the villi are not anchored to the decidua basalis but are suspended in maternal blood of the lacunae similar to roots of vegetables grown in hydroponic environments; these are known as ***free villi***. The villi anchored to the decidua basalis are called ***anchoring villi***. Capillaries of free and anchoring villi are near the surface of the villi and are separated from the maternal blood by a slight amount of connective tissue and the syncytiotrophoblasts covering the secondary villus. Thus, as indicated previously in the definition of the hemochorioendothelial placenta, maternal blood and fetal blood do not intermix; instead, nutrients and oxygen from the maternal blood diffuse through the cytotrophoblasts (and their basal lamina) syncytiotrophoblasts, connective tissue, and endothelial cells (and their basal lamina) of the capillaries of the villi to reach the fetal blood. These structures form the **placental barrier**. Certain substances, such as water, oxygen, carbon dioxide, small molecules, some proteins, lipids, hormones, drugs, and some antibodies (especially immunoglobulin G) can penetrate the placental barrier, whereas most macromolecules cannot. Some of these substances, such as IgG, are able to penetrate the placental barrier via specific carrier-mediated transport.

In addition to being the site where nutritious substances, waste, and gases are exchanged between maternal and fetal blood, the placenta, specifically the syncytiotrophoblasts, serves as an endocrine organ, secreting **hCG**, **chorionic thyrotropin**, **progesterone**, **estrogen**, **human chorionic somatomammotropin** hormone (also known as *lactogenic hormone*), endothelial growth factor, platelet-derived growth factor, fibroblast growth factor, TNF-α, transforming growth factor, insulin-like growth factor I, insulin-like growth factor II, colony-stimulating factor, as well as interleukin 1, interleukin 3, leptin, and relaxin. Also, stromal connective tissue cells of the decidua form the **decidual cells**, which enlarge and synthesize **prolactin** and **prostaglandins** (see Table 20–4).

Maternal Changes During Pregnancy

On the average, at full term, the mother gains approximately 12.5 kg (Table 20–5), has gastroesophageal reflux, has bouts of constipation, experiences reduction in bile delivery to the duodenum with a resultant

TABLE 20–5 Sources of Weight Gain in the Gravid Mother at Term

Source of Weight Gain	Weight (kg)
Fetus	3.5
Placenta	0.6
Amniotic fluid	0.8
Uterus	1.0
Breasts	0.4
Blood	1.5
Extravascular fluid	1.5
Maternal fat gained (as fat reserve)	3.2
Total	12.5

CLINICAL CORRELATIONS

1. The blastocyst usually implants into the upper third of the anterior or posterior wall of the uterus, and it is in that location that the placenta will begin to develop. Occasionally, in 1 of 200 pregnancies, implantation occurs lower down in the uterus, near the cervix, where the endometrium is much thinner and the connective tissue stroma is much denser. As the placenta begins to develop and enlarge, it covers partially or completely the opening of the cervix, making normal, vaginal delivery an untenable option. This condition is referred to as ***placenta previa*** and usually necessitates the delivery of the baby via a cesearean section.
2. The **placenta** was believed to be a sterile organ; however, recent reports indicate that it harbors nonpathogenic microbes. Interestingly, the **placental microbiota** does not resemble that of the vagina; instead, it resembles the microbiota of the oral cavity. The phyla represented included Proteobacteria, Fusobacteria, Firmucites, Bacteriodetes, and Tenericutes. It has been suggested that this is the first exposure of the newborn to his or her microbiome because the newborn possesses many of the bacteria present in the placenta. Additionally, the newborn also receives vaginal microbiota during normal delivery as well as microorganisms from individuals who come in physical contact with the neonate.

formation of gallstones, has an increased glomerular filtration rate and urine production, has an increased blood volume, increased heart rate, and reduced diastolic blood pressure. Additionally, because the diaphragm is displaced, the mother's expiratory reserve volume is reduced by almost half.

Vagina

The vagina, a fibromuscular sheath, is composed of three layers: mucosa, muscularis, and adventitia.

The vagina is a fibromuscular tubular structure 8 to 9 cm in length connected to the uterus proximally and the vestibule of the external genitalia distally. The vagina consists of three layers: **mucosa**, **muscularis**, and **adventitia**.

The lumen of the vagina is lined by a thick **stratified squamous nonkeratinized epithelium** (150 to 200 µm thick), although some of the superficial cells may contain some keratohyalin. Langerhans cells in the epithelium function in antigen presentation to T lymphocytes housed in the inguinal lymph nodes. The epithelial cells are stimulated by estrogen to synthesize and store large deposits of **glycogen**, which is released into the lumen as the vaginal epithelial cells are sloughed. Naturally occurring vaginal bacterial flora metabolize the glycogen, forming **lactic acid**, which is responsible for the low pH in the lumen of the vagina, especially at the midpoint of the menstrual cycle. The lowered pH also helps restrict pathogenic invasion.

The **lamina propria** of the vagina is composed of a loose fibroelastic connective tissue containing a rich vascular supply in its deeper regions. It also contains numerous lymphocytes and neutrophils that reach the lumen by passing through intercellular spaces during certain periods in the menstrual cycle, where they participate in immune responses. Although the vagina does not contain glands, there is an increase in vaginal fluid during sexual stimulation, arousal, and intercourse that serves to lubricate its lining. The fluid is derived from the transudate present in the lamina propria combined with secretions from glands of the cervix.

The **muscularis** layer of the vagina is composed of smooth muscle cells arranged so that the mostly longitudinal bundles of the external surface intermingle with the more circularly arranged bundles near the lumen. A sphincter muscle, composed of skeletal muscle fibers, encircles the vagina at its external opening.

Dense, fibroelastic connective tissue constitutes the **adventitia** of the vagina, attaching it to surrounding structures. Contained within the adventitia is a rich vascular supply with a vast venous plexus and nerve bundles derived from the pelvic splanchnic nerves.

External Genitalia

The external genitalia (vulva) are composed of the labia majora, labia minora, vestibule, and clitoris.

The **labia majora** are two folds of skin heavily endowed with adipose tissue and a thin layer of smooth muscle. The homologue of these structures in the male is the scrotum, with the smooth muscle layer corresponding

to the dartos muscle of the scrotum. The labia majora are covered with coarse hair on their external surface but are devoid of hair on their smooth inner surface. Numerous sweat glands and sebaceous glands open on both surfaces.

The **labia minora**, located medial and slightly deep to the labia majora, are the homologues of the urethral surface of the penis in the male. The labia minora are two smaller folds of skin devoid of hair follicles and adipose tissue. Their core is composed of a spongy connective tissue containing elastic fibers arranged in networks. They contain numerous sebaceous glands and are richly supplied with blood vessels and nerve endings.

The cleft situated between the right and left labia minora is the **vestibule**, a space that receives secretions of the **glands of Bartholin**, paired mucus-secreting glands, and many small **minor vestibular glands**. Also located in the vestibule are the orifices of the urethra and the vagina. In virgins, the orifice of the vagina is narrowed by a thin fold of epithelially enclosed fibrovascular tissue called ***hymen***.

The **clitoris**, the female homologue of the penis, is located between the folds of the labia minora superiorly, where the two labia minora unite to form the prepuce over the top of the **glans clitoridis**. The clitoris is covered by stratified squamous epithelium and is composed of two **erectile bodies** containing numerous blood vessels and sensory nerves, including Meissner and pacinian corpuscles, which are sensitive during sexual arousal.

Mammary Glands

The mammary glands are compound tubuloalveolar glands that consist of 15 to 20 lobes radiating out from the nipple and are separated from each other by adipose and collagenous connective tissue.

Mammary glands secrete milk, a fluid that contains proteins, lipids, and lactose as well as lymphocytes and monocytes, antibodies, minerals, and fat-soluble vitamins, to provide the proper nourishment for the newborn.

The mammary glands develop in the same manner and are of the same structure in both sexes until puberty, when changes in the hormonal secretions in females cause further development and structural changes within the glands. Secretions of **estradiol** and **progesterone** from the ovary (as well as from the placenta) and **prolactin** from the acidophils of the anterior pituitary gland and human somatomammotropin from the placenta initiate development of **lobules** and **terminal ductules**. Full development of the ductal portion of the breast requires **glucocorticoids** and further activation by anterior pituitary **somatotropin**.

Concomitant with these events is an increase in connective tissue and adipose tissue within the stroma, causing the gland to enlarge. Full development occurs at about 20 years of age with minor cyclic changes during each menstrual period, whereas major changes occur during pregnancy and in lactation. After age 40 years or so, the secretory portions as well as some of the ducts and connective tissue elements of the breasts begin to atrophy, and they continue this process through menopause.

The glands within the breasts are classified as **compound tubuloacinar glands**, consisting of 15 to 20 lobes radiating out from the nipple and separated from each other by adipose and collagenous connective tissue. Each lobe is drained by its own **lactiferous duct**, leading directly to the **nipple**, where it opens onto its surface. Before reaching the nipple, each of the ducts is dilated to form a **lactiferous sinus** for milk storage and then narrows before passing through the nipple.

Resting Mammary Glands

Acini are not developed in the resting mammary gland.

Resting (nonsecreting or **nonlactating)** mammary glands of nonpregnant women have the same basic architecture as the lactating (active) mammary glands, except that they are smaller and without developed acini, which occur during pregnancy only. Near the opening at the nipple, lactiferous ducts are lined by a stratified squamous (keratinized) epithelium. The lactiferous sinus and the lactiferous duct leading to it are lined by stratified cuboidal epithelium, whereas the smaller ducts leading to the lactiferous duct are lined by a simple columnar epithelium. Stellate myoepithelial cells located between the epithelium and the basal lamina also wrap around the developing acini and become functional during pregnancy.

Lactating (Active) Mammary Glands

During pregnancy, the terminal portions of the ducts branch and grow and develop secretory units known as acini.

Mammary glands are activated by elevated surges of **estrogen** and **progesterone** (as well as human somatomammotropin from the placenta) during pregnancy to become lactating glands to provide milk for the newborn. At this time, the terminal portions of the ducts branch and grow and the acini develop and mature (Fig. 20–18). As pregnancy progresses, the breasts enlarge as a result of hypertrophy of the glandular parenchyma and engorgement with **colostrum**, a protein-rich fluid, in preparation for the newborn. Within a few days after birth, when estrogen and progesterone secretions have subsided, **prolactin**, secreted by acidophils of the anterior pituitary gland, activates the secretion of milk, which replaces the colostrum.

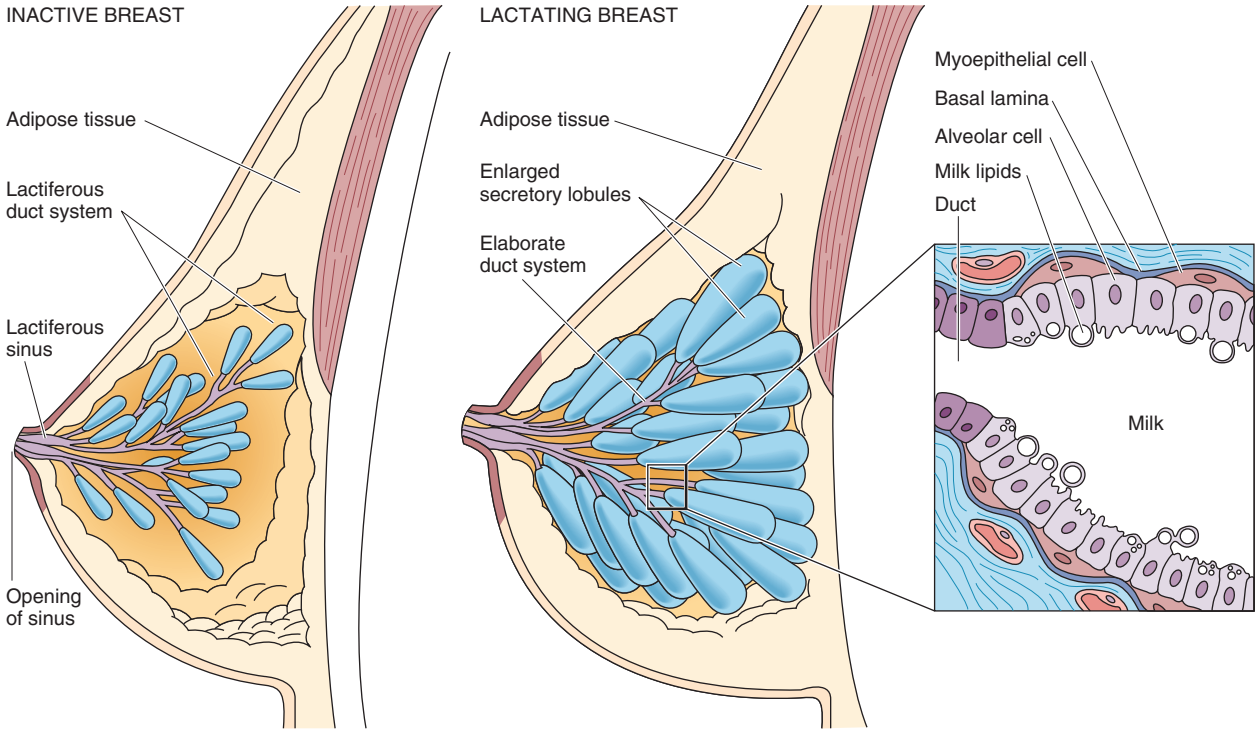


Figure 20–18 Diagram comparing the glandular differences between an inactive and a lactating breast. Observe the longitudinal section of a gland and duct of the active mammary gland.

The **acini** of the lactating (active) mammary glands are composed of cuboidal cells partially surrounded by a meshwork of myoepithelial cells. These secretory cells possess abundant RER and mitochondria, several Golgi complexes, many lipid droplets, and numerous vesicles (Fig. 20–19) containing caseins (milk proteins) and lactose. However, not all regions of the acinus are in the same stage of production because different acini display various degrees of preparation for synthesis of milk substances (Fig. 20–20).

The secretions of the acinar cells are of two kinds: lipids and proteins.

Lipids are stored as droplets within the cytoplasm. They are released from the secretory cells, possibly by the **apocrine** mode of exocytosis, whereby small droplets coalesce to form larger and larger droplets that move to the periphery of the cell. Once there, they project as cytoplasmic blebs into the lumen; eventually, these lipid droplets containing blebs are pinched off and become part of the secretory product. Each bleb then consists of a central lipid droplet surrounded by a narrow rim of cytoplasm and enclosed by a plasmalemma.

Proteins synthesized within these secretory cells are liberated from the cells by the **merocrine** mode of exocytosis in much the same manner as would be expected of other cells that synthesize and release proteins into the extracellular space.

Areola and Nipple

The circular, heavily pigmented skin in the center of the breast is the **areola**. It contains sweat glands and sebaceous glands at its margin as well as **areolar glands (of Montgomery)** that resemble both sweat and mammary glands. In the center of the areola is the **nipple**, a protuberance covered by stratified squamous keratinized epithelium containing the terminal openings of the lactiferous ducts. In fair-skinned individuals, a pinkish color is imparted to the nipple as a result of the color of blood in the rich vascular supply within the long dermal papillae that extend near its surface. During pregnancy, the color becomes darker because of increased pigmentation of the areola and the nipple.

The core of the nipple is composed of dense collagenous connective tissue with abundant elastic fibers connected to the surrounding skin or interlaced within the connective tissue and a rich component of smooth muscle cells. The wrinkling of the skin on the nipple results from the attachments of the elastic fibers. The abundant smooth muscle fibers are arranged in two ways: circularly around the nipple and radiating longitudinally along the long axis of the nipple. The

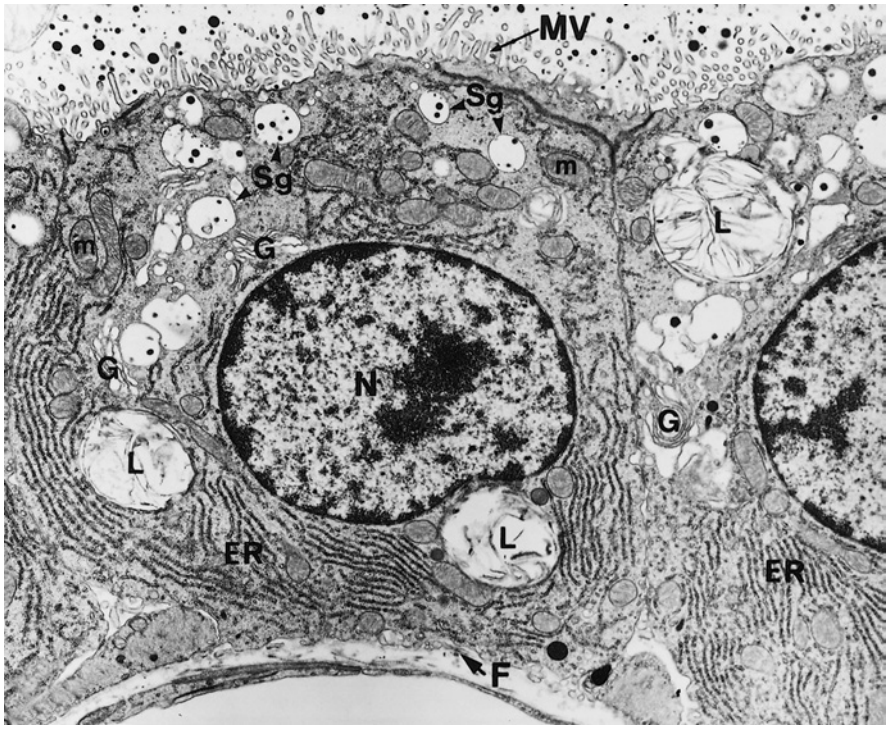


Figure 20–19 Electron micrograph of an acinar cell from the lactating mammary gland of the rat. Note the large lipid droplets (L), abundant rough endoplasmic reticulum (ER), and the Golgi apparatus (G). F, Folds of the basal plasmalemma; m, mitochondria; MV, microvilli; Sg, secretory granules (×9000). (From Clermont Y, Xia I, Rambourg A, et al. Structure of the Golgi apparatus in stimulated and non-stimulated acinar cells of mammary glands of the rat. *Anat Rec*. 1993;237:308-317. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

contraction of these muscle fibers is responsible for erection of the nipple.

Most of the sebaceous glands located around the lactiferous ducts open onto the surface or sides of the nipple, although some open into the lactiferous ducts just before those ducts open onto the surface.

Mammary Gland Secretions

Prolactin is responsible for the production of milk by the mammary glands; oxytocin is responsible for the milk ejection reflex.

Although the mammary gland is prepared to secrete milk even before birth, certain hormones prohibit this from occurring. However, when the placenta is detached in the adult female, **prolactin** from the anterior pituitary stimulates the production of milk, which reaches full capacity in a few days. Before that, for the first 2 or 3 days after birth, a protein-rich thick fluid called ***colostrum*** is secreted. This high-protein secretion, rich in vitamin A, sodium, and chloride, also contains lymphocytes and monocytes, minerals, lactalbumin, and antibodies (immunoglobulin A) to nourish and protect the newborn.

Milk, usually produced by the fourth day after parturition, is a fluid that contains minerals, electrolytes, carbohydrates (including lactose), immunoglobulins (mostly immunoglobulin A), proteins (including caseins), and lipids. Production of milk results from the stimuli of sight, touch, handling of the newborn, and anticipation of nursing, events that create a surge in **prolactin** release. Once initiated, milk production is continuous, with the milk being stored within the duct system.

Concomitant with the production of prolactin, **oxytocin** is released from the posterior lobe of the pituitary. Oxytocin initiates the **milk ejection reflex** by inducing contractions of the myoepithelial cells around the alveoli and the ducts, thus expelling the milk.

STEM CELLS

There are special cells, known as *stem cells*, that possess the ability to divide and form daughter cells that are identical to themselves **(symmetrical division)** and to form daughter cells that differentiate into somatic cells with specific characteristics (asymmetrical division).

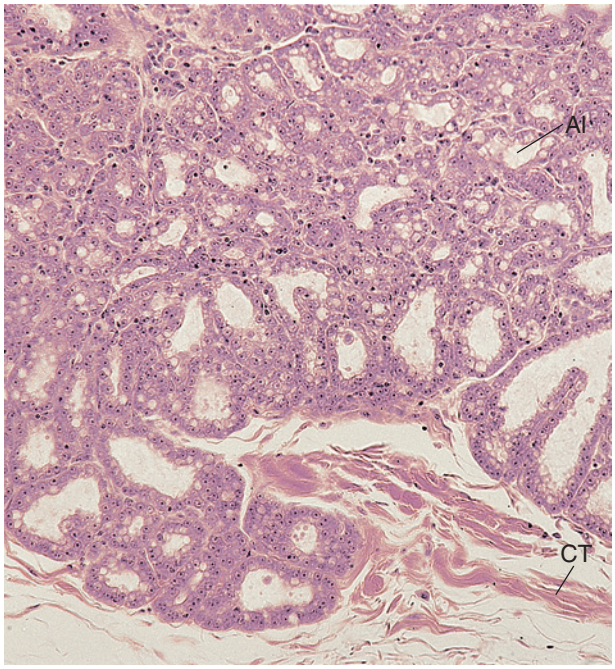


Figure 20–20 Light micrograph of the human mammary gland. Observe the crowded alveoli and note that various regions of the gland are in different stages of the secretory process (×132).

There are two basic types of stem cells, those that are present in the early embryo only, specifically the cells of the **morula** and the **inner cell mass (embryoblasts)** of the blastocyst, known as ***embryonic stem cells (ESCs)*** and those that remain in most adult tissues, known as ***adult stem cells (ASCs)***. These two categories are different because ESCs are **pluripotent**, that is, they can differentiate into any cell of the three germ layers (ectoderm, mesoderm, and endoderm), whereas adult stem cells have a limited differentiation capability. Examples of adult stem cells have been discussed in most chapters of this textbook, as in cells of the stratum basale of the epidermis, regenerative cells of the lining of the digestive tract, osteoprogenitor cells of bone, hematopoietic stem cells of the bone marrow, among many others.

Embryonic stem cells, therefore, may be harvested from blastocyts of in vitro fertilization, and, if cultured appropriately, these cells can divide symetrically an untold number of times without losing their undifferentiated state and without undergoing the senescence to which other types of cultured cells eventually succumb. This ability is attributed to the high concentration of the enzyme **telomerase** in these cells that protects them from losing the ability to divide. Given the proper signals, these cells can then undergo asymmetrical division and form any particular cell derivative of any of the three germ layers. These ESCs can be used in **stem cell therapy** to replace defective cells of the adult; for instance, they can be coaxed into forming beta cells of the islets of Langerhans, which can produce insulin in individuals with type I diabetes. However, ethical considerations prevent their use in human beings.

Adult stem cells function in replacing injured tissue (as pericytes of blood vessels) and also to replenish discarded cells, such as those of the blood. In the first decade of the 21st century, a major accomplishment was reported, namely that fully differentiated adult somatic cells can be reprogrammed to become pluripotential cells, and were named ***induced pluripotent stem cells (iPSC)***. These cells behave just as if they were ESCs,

CLINICAL CORRELATIONS

Mothers who cannot **breastfeed** their infants on a regular feeding schedule are inclined to experience poor lactation. This may motivate a decision to discontinue nursing altogether, with the result that the infant is deprived of the passive immunity conferred by ingesting antibodies from the mother.

Breast cancer, second only to lung cancer as one of the major causes of cancer-related death in women, may be of two different types: **ductal carcinoma** of the ductal cells and **lobular carcinoma** of the terminal ductules. Detection must be early, or the prognosis is poor because the carcinoma may **metastasize** to the axillary lymph nodes and from there to the lungs, bone, and brain. At the recommendation of the medical profession, early detection through self-examination and mammography has helped to reduce breast cancer mortality rates. In the year 2005, approximately 270,000 women and 1700 men were diagnosed with breast cancer in the United States, and approximately 40,000 women and 500 men died of breast cancer. There is an inverse relationship between the age of the woman and her risk of contracting the disease, in that, in 2005, one out of 2200 women younger than the age of 30 years contracted breast cancer, whereas 1 of 54 and 1 of 23 women contracted breast cancer under 50 and 60 years of age, respectively. Although breast cancer is more likely to occur at an older age, younger women tend to have more aggressive breast cancers. In younger women, a family history of breast cancer should prompt genetic analysis for the presence of mutations or deficiencies in the *BRCA1* and *BRCA2* genes (on chromosomes 17 and 11, respectively). If either gene is mutated, the patient should go for genetic counseling as well as for regular screening for the evidence of tumors. If focal calcifications, premalignant, or malignant lesions are suspected on the basis of mammography, needle biopsies should be performed to determine whether the lesion is suspicious.

that is, morphologically, they are identical to ESCs, possess the same high levels of telomerase, display the same surface markers as ESCs, and have the ability to be induced to differentiate into cells of all three germ layers. The major benefit of these cells is that the somatic cells can be harvested from the individual patient and reprogrammed to become iPSCs, and the patient's immune system will not mount an immune response against them. Unfortunately, currently, it is difficult to control iPSCs because only some of the reprogrammed cells form the requisite differentiated cell, and others can form teratomas and other tumors. It is hoped that continued research into stem cells will ameliorate these problems.

PATHOLOGICAL CONSIDERATIONS

See Figures 20–21 through 20–24.

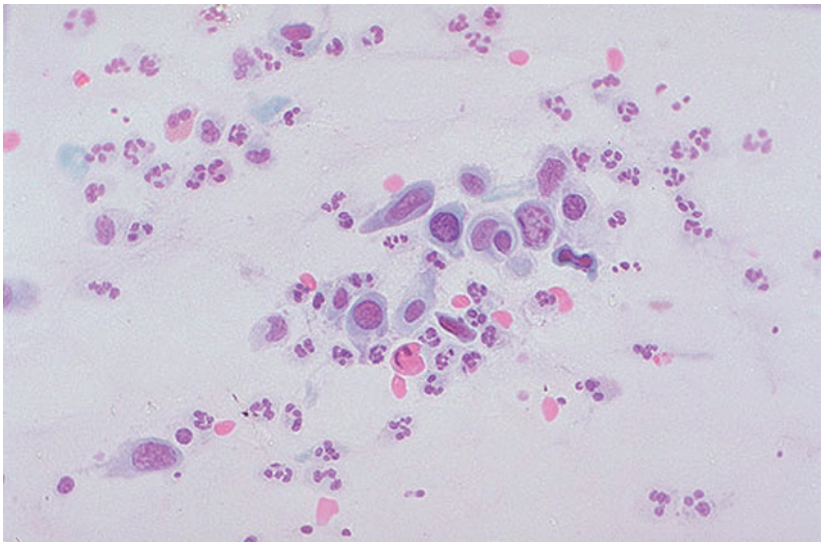


Figure 20–21 Photomicrograph of cervical squamous carcinoma present in a Pap smear. Note the presence of large, pleomorphic cells in the center of the field. The large number of inflammatory cells and erythrocytes indicate an aggressive, ulcerative, and invasive lesion. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:324.)

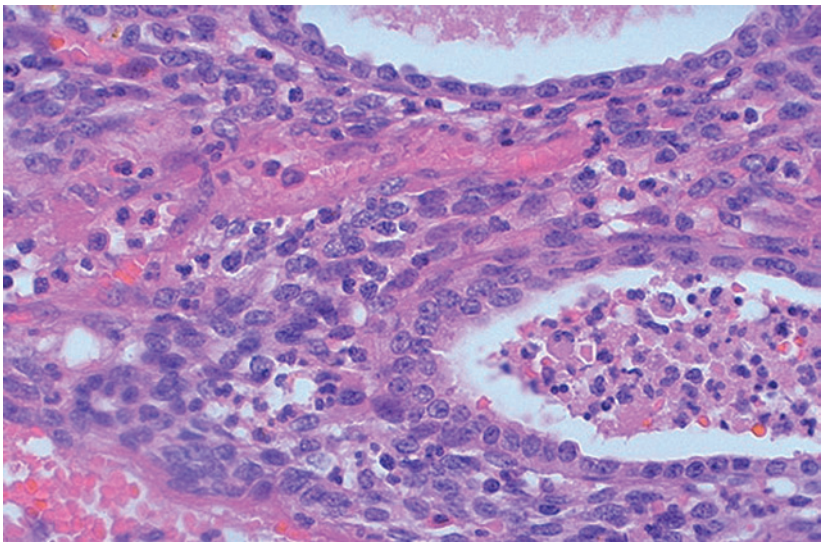


Figure 20–22 Photomicrograph of the uterus of a patient with acute endometriosis. This condition is evidenced by the presence of neutrophils scattered throughout the stroma and glands of the endometrium. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:333.)

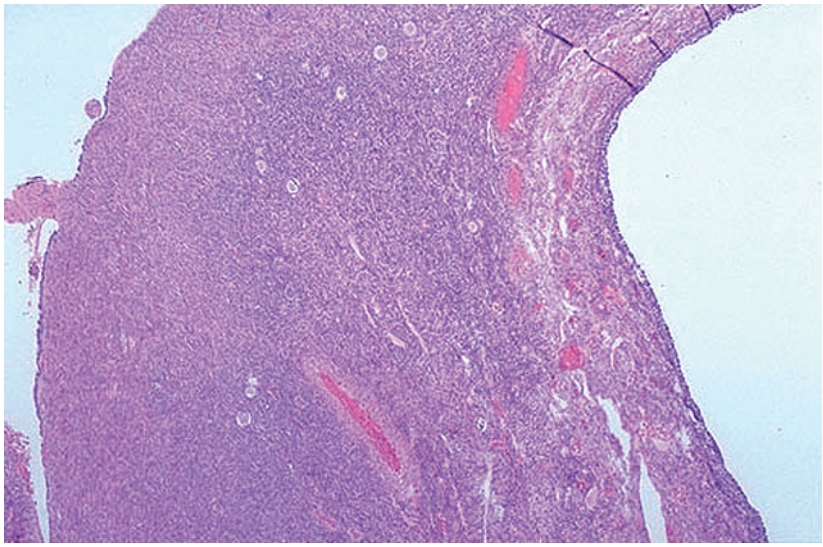


Figure 20–23 Photomicrograph of the ovary of a patient with polycystic ovarian disease. This condition is characterized by a very thick ovarian cortex *(left side of the field)* as well as the presence of numerous follicle cysts (one of which is displayed on the *right side of the field*). (Reprinted with permission from Klatt EC. *Robbins and Cotran: Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:341.)

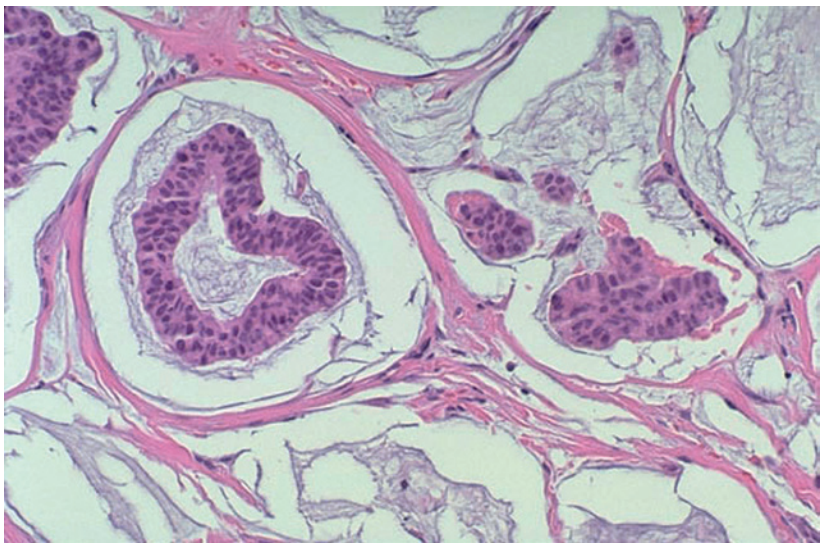
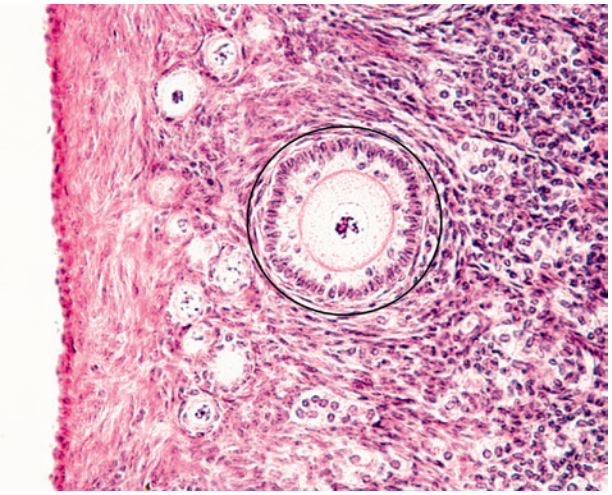


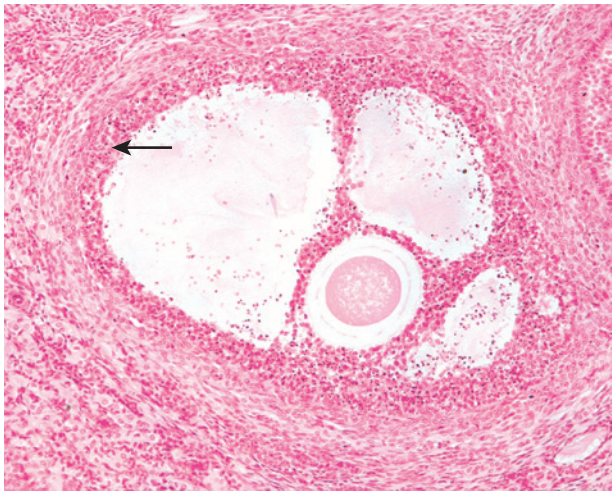
Figure 20–24 Photomicrograph of the breast of a patient with mucinous carcinoma of the breast (also known as *colloid carcinoma of the breast*). This condition is a form of ductal carcinoma, an invasive breast cancer, in which the malignant cells are surrounded by a large amount of mucin that they manufacture. Frequently, this type of tumor is associated with mutations in the *BRCA* gene. (Reprinted with permission from Klatt EC. *Robbins and Cotran: Atlas of Pathology*. 2nd ed. Philadelphia: Elsevier; 2010:377.)

REVIEW QUESTIONS

1. The primary oocyte is in which of the following phases of meiosis?
   a. Prophase I
   b. Prophase II
   c. Metaphase I
   d. Metaphase II
   e. Anaphase I
2. The oviduct has
   a. simple squamous epithelium lining it.
   b. simple cuboidal epithelium lining it.
   c. has stratified cuboidal epithelium lining it.
   d. has peg cells.
   e. has goblet cells.
3. Which of the following maintains the corpus luteum of pregnancy for several months?
   a. FSH (follicle stimulating hormone)
   b. LH (luteinizing hormone)
   c. GnRH (gonadotropin-releasing hormone)
   d. hCG (human chorionic gonadotropin)
   e. Anti-Mullerian hormone
4. The process of ovulation is initiated by which of the following hormones?
   a. Follicle-stimulating hormone
   b. Human chorionic gonadotropin
   c. Luteinizing hormone
   d. Oxytocin
   e. Inhibin
5. Which of the following cells possess processes that contact the cell membrane of the primary oocyte?
   a. Theca externa
   b. Corona radiata
   c. Granulosa lutein
   d. Theca interna
   e. Theca lutein
6. 

   The structure enclosed in the circle is
   a. primordial follicle.
   b. unilaminar primary follicle.
   c. multilaminar primary follicle.
   d. secondary follicle.
   e. mature follicle.
7. The epithelial lining of the uterus is
   a. simple squamous.
   b. simple cuboidal.
   c. simple columnar.
   d. stratified squamous.
   e. stratified cuboidal.
8. The primary oocyte remains arrested in which of the following stages of prophase I of meiosis?
   a. Leptotene
   b. Zygotene
   c. Pachytene
   d. Diplotene
   e. Diakinesis

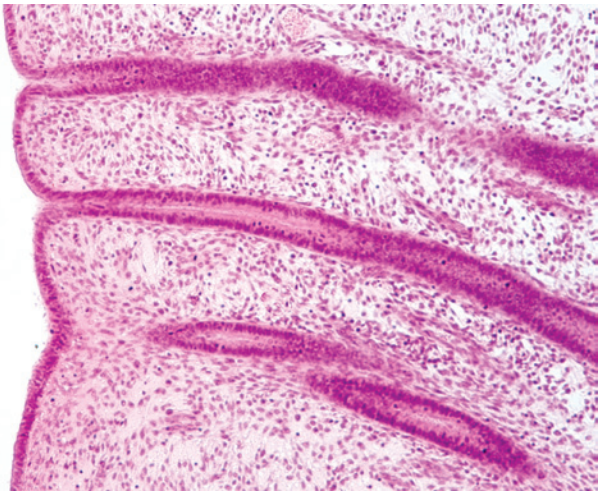
9.



The cells at the tip of the arrow possess which of the following?

a. Insulin-like growth factor receptors
b. Kit ligand receptors
c. Aromatase
d. Lipase
e. Hyalurinidase

10.



This photomicrograph represents which of the following?

a. Basalis layer of the proliferative (follicular) phase
b. Functionalis layer of the proliferative (follicular) phase
c. Basalis layer of the secretory (luteal) phase
d. Functionalis layer of the secretory (luteal) phase
e. Functionalis layer of the menstrual phase

ANSWERS

1. A. The primary oocyte is in the prophase I of meiosis until the oocyte is induced by the surge of luteinizing hormone to complete meiosis I and forms the first polar body and the secondary oocyte. Ovulation is the expulsion of the secondary oocyte from the ovary.
2. D. The oviduct is lined by a simple columnar, ciliated epithelium composed of ciliated cells that move the fertilized ovum toward the uterus and peg cells that manufacture and release a fructose-rich nutrient fluid that not only sustains the embryo in its first few days of development but also transports it into the uterine cavity. The oviduct lining does not have squamous cells, cuboidal cells, or goblet cells.
3. D. The corpus luteum of pregnancy is maintained by the placental hormone, human chorionic gonadotropin. Follicle stimulating hormone (FSH) functions in encouraging follicular development. Luteinizing hormone (LH) maintains the corpus luteum of menstruation. Gonadotropin-releasing hormone is manufactured in the hypothalamus and induces basophils cells of the pituitary to release FSH and LH. Antimullerian hormone is manufactured by Sertoli cells of the testis and inhibits, during early embryogenesis, the formation of the female reproductive tract, thus ensuring the development of a male embryo.
4. C. The surge of LH release functions in the induction of the primary oocyte to resume and finish its first meiotic division and to initiate the process of ovulation, whereby the secondary oocyte is released from the ovary. Follicle-stimulating hormone encourages the development of the ovarian follicle. Human chorionic gonadotropin, a placental hormone, maintains the corpus luteum of pregnancy. Oxytocyn stimulates smooth muscle contraction during the expulsion of the fetus from the uterus and also in the ejection of milk from the breast. Inhibin inhibits FSH release by the basophils of the pituitary gland.
5. B. Cells of the corona radiata, follicular cells that are adjacent the zona pellucida, extend their processes through this region to contact the cell membrane of the primary oocyte. Cells of the theca externa and cells of the theca interna do not contact the primary oocyte. Granulosa lutein cells and theca lutein cells compose the corpus luteum, and oocytes are not associated with that structure.
6. C. The structure in the circle is a multilaminar primary follicle, as is evidenced by the large size of the follicle, in comparison with the primordial follicles just to the left of the circle, as well as by the more than a single layer of cuboidal follicular cells. Primordial follicles are smaller structures in which the follicular cells are flat and are arranged in a single layer. Unilaminar primary follicles are smaller than multilaminar primary follicles but larger than primordial follicles, and they possess a single layer of cuboidal follicular cells. Secondary follicles have several layers of follicular cells as well as small accumulations of follicular fluid. Mature follicles (Graafian follicles) are very large, possess a cumulus oophorus, and display a fluid-filled antrum.
7. B. The epithelial lining of the uterus is composed of a simple columnar epithelium. This lining is continuous with the columnar cells that form the uterine glands.
8. D. The primary oocyte reaches the diplotene stage of the prophase of meiosis I and is arrested there until ovulation because the follicular cells surrounding oocyte release the signaling molecule oocyte maturation inhibitor (OMI). Leptotene, zygotene, and pachytene stages are not affected by OMI; therefore, meiosis progresses until it is stopped in the diplotene stage.
9. C. The arrow is pointing to granulosa cells (membrana granulosa) that are separated from the theca interna by a basement membrane. These theca interna cells produce the male sex hormone androstenedione, which enters the granulosa cells, where it is converted by the enzyme aromatase into the estrogen estradiol (estrogen). Granulosa cells manufacture and release kit ligand but do not express kit ligand receptors.
10. B. This is a photomicrograph of the functionalis layer of the proliferative (follicular) phase of the menstrual cycle because the epithelium lining the lumen of the uterus is intact. If this were the basalis layer, neither the lumen nor the intact epithelial lining would be visible. The glands are forming and contain no secretory product as they would in the secretory (luteal) phase. If this were the menstrual phase, the endometrium would be jagged, would not have an intact epithelium, and coagulated blood would be present.

Male Reproductive System

21

The male reproductive system consists of the two testes suspended in the scrotum, a system of intratesticular and extratesticular genital ducts, associated glands, and the male copulatory organ, the penis (Fig. 21–1). The testes are responsible for the formation of the male gametes, known as *spermatozoa*, as well as for the synthesis, storage, and release of the male sex hormone: testosterone.

The glands associated with the male reproductive tract are the paired **seminal vesicles,** the single **prostate gland,** and the two **bulbourethral glands (of Cowper)**. These glands form the noncellular portion of **semen** (spermatozoa suspended in the secretions of the accessory glands), which not only nourishes the spermatozoa, but also provides a fluid vehicle for their delivery into the female reproductive tract. The **penis** has a dual function; it delivers semen to the female reproductive tract during copulation and serves as the conduit of urine from the urinary bladder to outside of the body.

TESTES

The testes, located in the scrotum, are paired organs that produce spermatozoa and testosterone.

Each testis of a mature male is an oval organ approximately 4 cm long, 2 to 3 cm wide, and 3 cm thick. During embryogenesis, the testes develop retroperitoneally on the posterior wall of the abdominal cavity. As they descend into the scrotum, they carry with them a portion of the peritoneum. This peritoneal outpouching, the **tunica vaginalis,** forms a serous cavity that partially surrounds the anterolateral aspect of each testis, permitting it some degree of mobility within its compartment in the scrotum. In approximately 60% of males the left testis is suspended 1 to 2 cm lower in the scrotum than the right testis.

The wall of the scrotum houses smooth muscle elements, known as the ***dartos muscle***, which contributes to the regulation of the temperature within the scrotum. In cold temperature, the dartos muscle contracts, thereby not only releasing heat of contraction, but also brings the testes closer to the body wall. It is assisted in this mechanism by the paired **cremaster muscles**, skeletal muscles located in the inguinal canal, and the scrotum that wrap around the testes. These muscles contract and bring the testes closer to the body wall. The main function of the cremaster muscles is protection of the testes by moving them out of harm's way during sexual activity as well as when the individual is experiencing fear. Most of the time, the cremaster muscles act in an involuntary fashion, although they may be activated voluntarily when the abdominal muscles are contracted.

General Structure and Vascular Supply

Connective tissue septa divide the testis into lobuli testis, each of which houses one to four seminiferous tubules.

Each testis is surrounded by a capsule of dense, irregular collagenous connective tissue known as the ***tunica albuginea.*** Immediately deep to this layer is a highly vascularized loose connective tissue, the **tunica vasculosa,** which forms the vascular capsule of the testis. The posterior aspect of the tunica albuginea is somewhat thickened, forming the **mediastinum testis,** from which connective tissue septa radiate to subdivide each testis into approximately 250 pyramid-shaped intercommunicating compartments known as the ***lobuli testis*** (Fig. 21–2).

Each lobule has one to four blindly ending **seminiferous tubules,** which are surrounded by a richly innervated and highly vascularized loose connective tissue derived from the tunica vasculosa. Dispersed throughout this connective tissue are small conglomerations of endocrine cells, the **interstitial cells (of Leydig)** (see later section on interstitial cells), which are responsible for the synthesis of testosterone.

Spermatozoa are produced by the **seminiferous epithelium** of the seminiferous tubules. Spermatozoa enter short straight ducts, **tubuli recti,** that connect the open end of each seminiferous tubule to the **rete testis,** a system of labyrinthine spaces housed within the mediastinum testis. The spermatozoa leave the rete testis through 10 to 20 short tubules, the **ductuli efferentes,** which eventually fuse with the **ductus epididymis.**

The vascular supply of each testis arises from the abdominal aorta as the **testicular artery**, which descends with the testis into the scrotum accompanying the **ductus deferens (vas deferens).** The testicular artery forms several branches before it pierces the capsule of the testis to form the intratesticular vascular elements. The capillary beds of the testes are collected into several veins, the **pampiniform plexus of veins,** which are wrapped around the testicular artery. The artery, veins, and ductus deferens together form the **spermatic cord,** which passes through the inguinal

SUMMARY

The male reproductive system is composed of the paired testicles and their intratesticular system of genital ducts (tubuli recti and rete testis), the extratesticular genetic ducts (ductuli efferentes, epididymis, ductus deferens, and ejaculatory duct), accessory genital glands (the paired seminal vesicles, the single prostate gland, and the paired bulbourethral glands), and the penis. Each testis is composed of a vascular connective tissue that houses testosterone-producing interstitial cells of Leydig and surrounds coiled seminiferous tubules whose seminiferous epithelium is supported by Sertoli cells. The seminiferous epithelium is composed of spermatogenic cells in various stages of development. As spermatogenic cells develop, they undergo cell divisions that maintain their numbers by mitosis, whereas some cells mature into haploid spermatids by meiosis. Spermatids shed much of their cytoplasm and become transformed into spermatozoa. When ready, spermatozoa are released by Sertoli cells to enter the intratesticular genital ducts from which they proceed into the extratesticular ducts. During sexual activity, the spermatozoa, suspended in semen, a fluid manufactured by the accessory genital glands, is ejaculated through the urethra of the penis.

KEY WORDS

Accessory genital glands; bulbourethral glands; ductuli efferentes; ductus deferens; ejaculatory duct; epididymis; extratesticular ducts; interstitial cells of Leydig; intratesticular ducts; male reproductive system; prostate gland; rete testes; semen; seminal vesicles; seminiferous epithelium; seminiferous tubules; Sertoli cells; spermatids; spermatogenic cells; spermatozoa; testicles; testosterone; tubuli recti

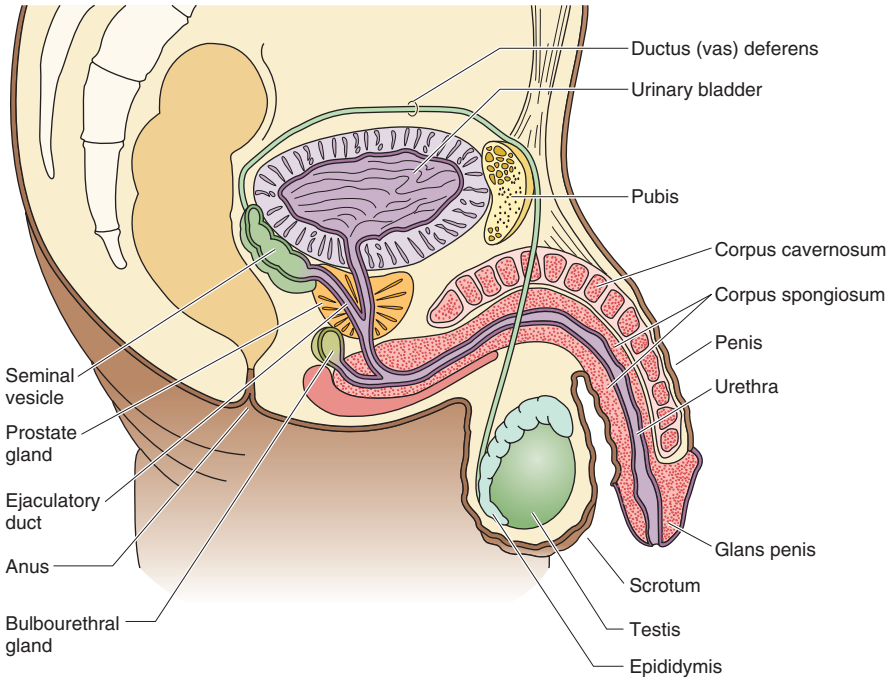


Figure 21–1 Schematic diagram of the male reproductive system.

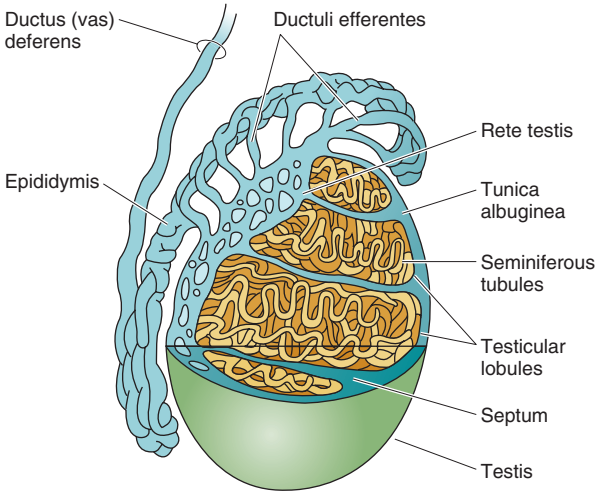


Figure 21–2 Schematic diagram of the testis and epididymis. Lobules and their contents are not drawn to scale.

canal, the passageway from the abdominal cavity to the scrotum.

Blood in the pampiniform plexus of veins, which is cooler than that in the testicular artery, acts to reduce the temperature of the arterial blood, thus forming a **countercurrent heat exchange system.** In this fashion, it helps keep the temperature of the testes a few degrees lower than that of the remainder of the body. At this cooler temperature (35° C), spermatozoa develop normally; whereas, at body temperature, spermatozoa that do develop are sterile. The testes are maintained at a cooler temperature in the scrotum thus aiding the cooling effect of the pampiniform plexus of veins.

Lymphatic drainage is accomplished by lymph vessels that accompany the testicular arteries to drain into the periaortic lymph nodes.

CLINICAL CORRELATIONS

1. Because hyperthermia has been identified as a factor in male infertility, it has been reported that males who work with laptop computers held on their laps for 1 hour of continuous use exhibited an increase in scrotal temperatures by as much as 2.8°C. Although these studies are not conclusive, it is suggested that boys and young men may wish to limit the use of computers on their laps.
2. Metastatic testicular cancers spread via the lymphatic drainage into the periaortic lymph nodes and from there to their various final destinations.

Seminiferous Tubules

Seminiferous tubules are composed of a thick seminiferous epithelium surrounded by a thin connective tissue, the tunica propria.

Seminiferous tubules are highly convoluted hollow tubules, 30 to 70 cm long and 150 to 250 µm in diameter, and are surrounded by extensive capillary beds. About 1000 seminiferous tubules are present in the two testes, for a total length of nearly 0.5 km (0.3 mile), dedicated to the production of spermatozoa. Approximately 85% to 90% of the volume of each testis is occupied by the seminiferous tubules.

The wall of the seminiferous tubule is composed of a slender connective tissue layer, the **tunica propria,** and a thick seminiferous epithelium. The tunica propria and the seminiferous epithelium are separated from each other by a well-developed **basal lamina.** The connective tissue comprises mostly interlaced, slender, type I collagen fiber bundles housing several layers of fibroblasts. In some animals, but not in humans, **myoid cells**, similar to smooth muscle, are also present; the cells impart contractility to the seminiferous tubules of the animals.

The seminiferous epithelium (or **germinal epithelium**) is several cell layers thick (Figs. 21–3 and 21–4) and is composed of two types of cells: Sertoli cells and spermatogenic cells (Fig. 21–5; see Fig. 21–4). The latter cells are in various stages of maturation.

Figure 21–3 Photomicrograph of the capsule of a monkey testis and cross-sectional profiles of seminiferous tubules (ST), blood vessel (BV), tunica albuginea (TA), tunica vasculosa (TV), lumen (L), seminiferous epithelium (SE), and septa (Se) (×132).

Sertoli Cells

Sertoli cells support, protect, and nourish spermatogenic cells; phagocytose cytoplasmic remnants of spermatids; secrete androgen-binding protein, hormones, and a nutritive medium; and establish the blood–testis barrier.

Sertoli cells are tall, columnar cells whose lateral cell membranes possess complex infoldings, which make it impossible to distinguish their lateral cell boundaries using light microscopy. These basolateral infoldings also envelop groups of spermatogenic cells. Their apical cell membranes are also highly folded and project into the lumina of the seminiferous tubules, which couch developing spermatozoa. These cells have a basally located, clear, oval nucleus with a large, centrally positioned nucleolus (see Fig. 21–5). The cytoplasm has been shown to house inclusion products known as ***crystalloids of Charcot-Böttcher,*** the composition and function of which are not known. The basal cell membrane of Sertoli cells possesses receptors for follicle-stimulating hormone (FSH) and for androgens.

Electron micrographs reveal that the cytoplasm of Sertoli cells is replete with profiles of smooth endoplasmic reticulum, but the amount of rough endoplasmic reticulum (RER) is limited. The cell also has numerous mitochondria, a well-developed Golgi apparatus, and numerous vesicles that belong to the endolysosomal complex. The cytoskeletal elements of Sertoli cells are also abundant, indicating that one of the functions of these cells is to provide structural support for the developing gametes.

The lateral cell membranes of adjacent Sertoli cells form occluding junctions with each other, thus subdividing the lumen of the seminiferous tubule into two isolated, concentric compartments (Fig. 21–6; see Fig. 21–5). The **basal compartment** is narrower, is located basal to the zonulae occludentes, and surrounds the wider **adluminal compartment.** Thus, the zonulae occludentes of the Sertoli cells establish a blood–testis barrier that isolates the adluminal compartment from connective tissue influences, thereby protecting the developing gametes from the immune system. Because spermatogenesis begins after puberty, the newly differentiating germ cells, which have a different chromosome number as well as express different surface membrane receptors and molecules, would be considered "foreign cells" by the immune system. Were it not for the isolation of germ cells from the connective tissue compartments by the zonulae occludentes of the Sertoli cells, an immune response would be mounted against them.

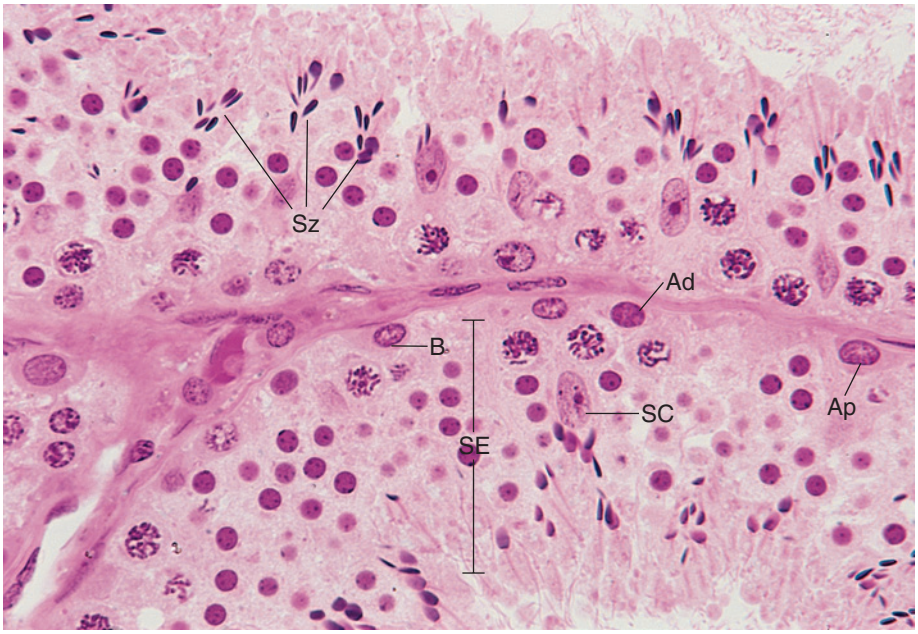


Figure 21–4 Seminiferous tubule. Seminiferous epithelium (SE), pale spermatogonia A (Ap), dark spermatogonia A (Ad), spermatogonia B (B), Sertoli cell (SC), and spermatozoa (Sz) (×540).

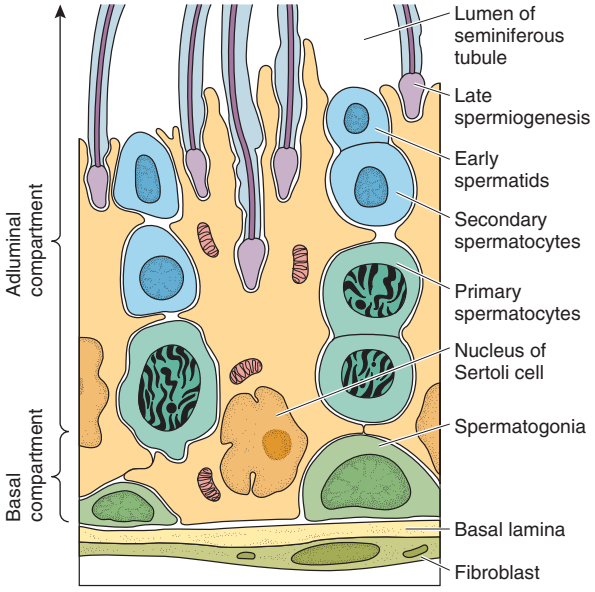


Figure 21–5 Schematic diagram of the seminiferous epithelium.

Sertoli cells perform the following functions:

- Physical and nutritional support of the developing germ cells
- Phagocytosis of cytoplasm eliminated during spermiogenesis
- Establishment of a blood–testis barrier by the formation of zonulae occludentes between adjacent Sertoli cells
- Synthesis and release of **androgen-binding protein (ABP),** a macromolecule that facilitates an increase in the concentration of testosterone in the seminiferous tubule by binding to it and preventing it from leaving the tubule
- Synthesis and release (during embryogenesis) of **antimüllerian hormone,** which suppresses the formation of the müllerian duct (precursor of the female reproductive system) and thus establishes the "maleness" of the developing embryo
- Synthesis and secretion of **inhibin,** a hormone that inhibits the release of FSH by the anterior pituitary
- Synthesis of **activin**, a hormone that facilitates the release of FSH from the anterior pituitary
- Secretion of a fructose-rich medium that nourishes and facilitates the transport of spermatozoa to the genital ducts
- Synthesis and secretion of **testicular transferrin,** an apoprotein that accepts iron from serum transferrin and conveys it to maturing gametes

Spermatogenic Cells

The process of spermatogenesis, whereby spermatogonia give rise to spermatozoa, is divided into three phases: spermatocytogenesis, meiosis, and spermiogenesis.

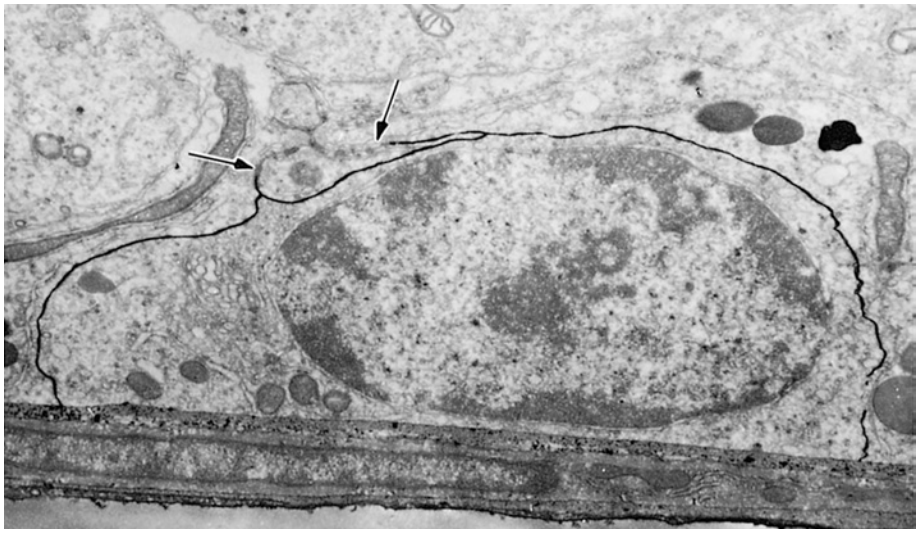


Figure 21–6 Electron micrograph of the basal compartment of the seminiferous epithelium (×15000). The testis has been perfused with an electron-dense tracer (lanthanum nitrate) to demonstrate that the occluding junctions *(arrows)* between adjacent Sertoli cells prevent the tracer from entering the adluminal compartment. (From Leeson TS, Leeson CR, Papparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

Most of the cells composing the thick seminiferous epithelium are **spermatogenic cells** in various stages of maturation (see Fig. 21–5). Some of these cells, **spermatogonia,** are located in the basal compartment, whereas most of the developing cells—**primary spermatocytes, secondary spermatocytes, spermatids,** and **spermatozoa**—occupy the adluminal compartment. Spermatogonia are diploid cells that undergo mitotic division to form more spermatogonia as well as primary spermatocytes, which migrate from the basal into the adluminal compartment. Primary spermatocytes enter the **first meiotic division** to form secondary spermatocytes, which undergo the **second meiotic division** to form **haploid** cells known as ***spermatids.*** These haploid cells are transformed into spermatozoa (mature sperm) through shedding of much of their cytoplasm, rearrangement of their organelles, and formation of flagella.

The various cell types that result from this process of cell maturation, called ***spermatogenesis,*** are diagrammed in Figure 21–7. The maturation process is divided into three phases:

- **Spermatocytogenesis**. Differentiation of spermatogonia into primary spermatocytes.
- **Meiosis**. Reduction division whereby diploid primary spermatocytes reduce their chromosome complement, forming haploid spermatids.
- **Spermiogenesis**. Transformation of spermatids into spermatozoa (sperm).

Differentiation of Spermatogonia

Spermatogonia (2n) are influenced by testosterone at puberty to enter the cell cycle.

Spermatogonia are small, diploid germ cells located in the basal compartment of the seminiferous tubules (see Figs. 21–5 and 21–7). These cells lie on the basal lamina and, subsequent to puberty, become influenced by testosterone to enter the cell cycle. There are three categories of spermatogonia:

- **Dark type A spermatogonia**. Small (12 μm in diameter), dome-shaped cells that have flattened, oval nuclei with abundant heterochromatin, imparting a dense appearance to the nucleus. Dark type A spermatogonia are **reserve cells** that have *not* entered the cell cycle but may do so. Their mitotic rate is slow, and they are fairly resistant to insults such as low levels of ionizing radiation. Once they undergo mitosis, they form additional dark type A spermatogonia as well as pale type A spermatogonia.
- **Pale type A spermatogonia**. Identical to the dark type A cells, but their nuclei have abundant euchromatin, giving them a pale appearance. These cells have only a few organelles, including mitochondria, a limited Golgi complex, some RER, and numerous free ribosomes. These cells are induced by testosterone to *proliferate* and give rise, by mitosis, to additional pale type A spermatogonia and to type B spermatogonia.

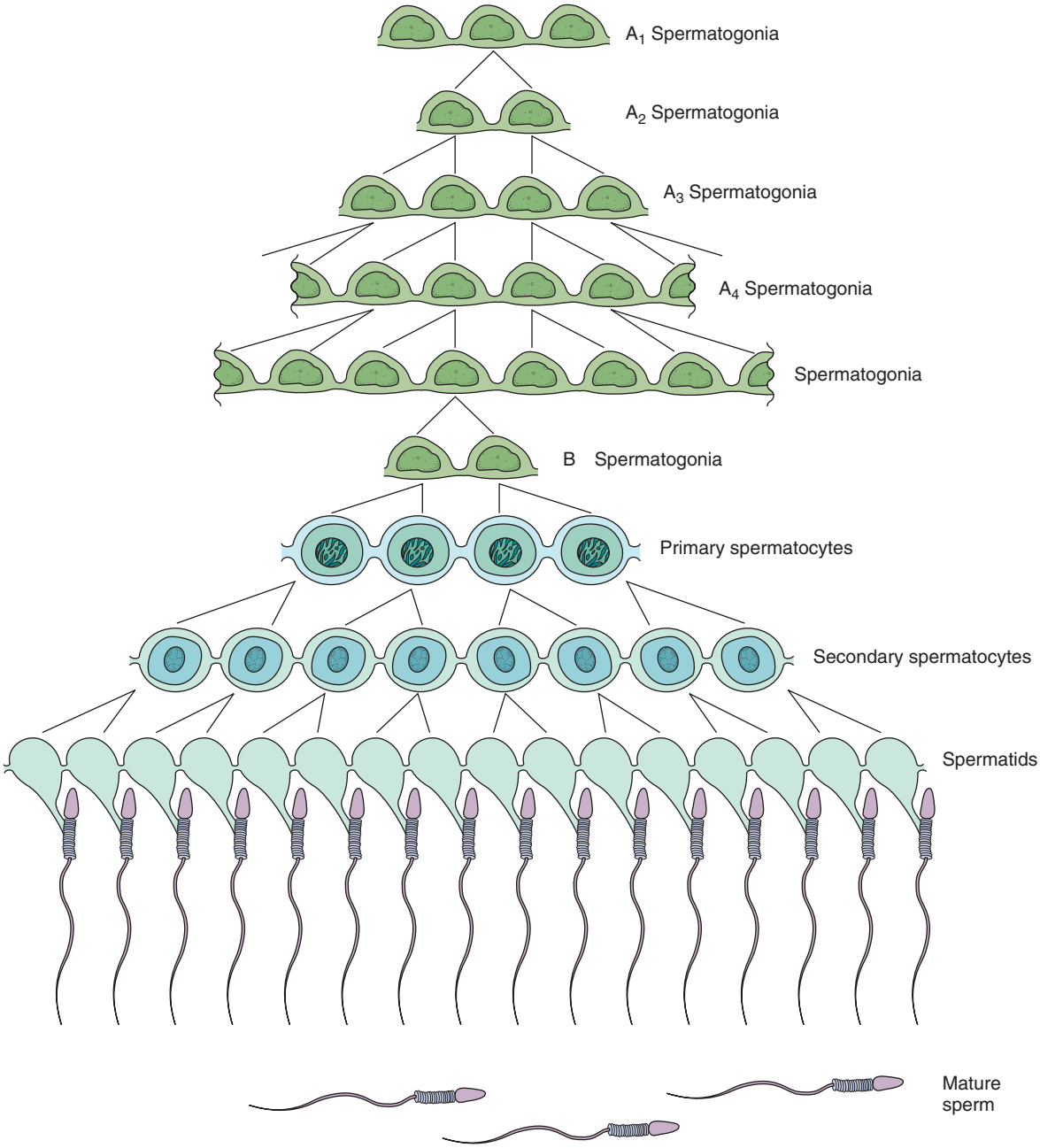


Figure 21–7 A schematic diagram of spermatogenesis, displaying the intercellular bridges that maintain the syncytium during differentiation and maturation. (Modified from Ren X-D, Russell L. Clonal development of interconnected germ cells in the rat and its relationship to the segmental and subsegmental organization of spermatogenesis. *Am J Anat*. 1991;192:127. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

- **Type B spermatogonia**. Resemble pale type A spermatogonia, but usually their nuclei are round rather than flattened. These cells also divide mitotically to give rise to primary spermatocytes. They are the most sensitive of the spermatogenic cells to the deleterious effects of ionizing radiation.

Meiotic Division of Spermatocytes

The first meiotic division of the primary spermatocyte, followed by the second meiotic division of the secondary spermatocyte, reduces the chromosome number and deoxyribonucleic acid (DNA) content to the haploid (n) state in the spermatids.

As soon as primary spermatocytes are formed, they migrate from the basal compartment into the adluminal compartment. As these cells migrate between adjacent Sertoli cells, they form zonulae occludentes with the Sertoli cells and thus help maintain the integrity of the blood–testis barrier. Primary spermatocytes are the largest cells of the seminiferous epithelium (see Fig. 21–5). They have large, vesicular-appearing nuclei whose chromosomes are in various stages of condensation. Shortly after their formation, primary spermatocytes duplicate their DNA to obtain a 4n DNA content; however, the chromosome number remains diploid (2n).

During the **first meiotic division,** the DNA content is halved (to 2n DNA) in each daughter cell, and the chromosome number is reduced to haploid (n). During the **second meiotic division,** the DNA content of each new daughter cell is reduced to haploid (1n DNA), whereas the chromosome number remains unaltered (haploid).

Prophase I of the first meiotic division lasts for as long as 22 days and involves four stages:

- Leptotene
- Zygotene
- Pachytene
- Diakinesis

The chromosomes of a primary spermatocyte begin to condense, forming long threads during **leptotene** and pairing with their homologues during **zygotene.** Further condensation yields short, thick chromosomes, recognizable as **tetrads**, during **pachytene.** The exchange of segments **(crossing over)** of homologous chromosomes occurs during **diakinesis;** this random genetic recombination results in the unique genome of each gamete and contributes to the variation of the gene pool.

During **metaphase I,** the paired homologous chromosomes line up at the equatorial plate. The members of each pair pull apart then migrate to opposite poles of the cell in **anaphase I,** and the daughter cells separate (although a cytoplasmic bridge remains), forming two secondary spermatocytes during **telophase I.**

Because the homologous chromosomes are segregated during anaphase, the X and Y chromosomes are sorted into separate secondary spermatocytes, eventually forming spermatozoa that carry either X or Y chromosomes. Thus, it is the spermatozoon that determines the chromosomal (genetic) sex of the future embryo.

Secondary spermatocytes are relatively small cells, and because they are present for less than 10 hours, they are not readily seen in the seminiferous epithelium. These cells, which contain 2n DNA, do not replicate their chromosomes; they quickly enter the second meiotic division, forming two haploid (1n DNA) spermatids.

During mitosis of spermatogonia and meiosis of spermatocytes, nuclear division **(karyokinesis)** is accompanied by a **modified cytokinesis.** As each cell divides to form two cells, a **cytoplasmic bridge** remains between them, holding the newly formed cells tethered to each other (see Fig. 21–7). Because this incomplete division occurs over a number of mitotic and meiotic events, it results in the formation of a **syncytium** of cells, a large number of spermatids that are connected to one another. This connection enables the spermatogenic cells to communicate with each other and thus to synchronize their activities.

CLINICAL CORRELATIONS

The most common abnormality due to nondisjunction of the XX homologues is known as ***Klinefelter syndrome.*** Individuals with this syndrome usually have XXY chromosomes (an extra X chromosome). They are typically infertile, are tall and thin, exhibit various degrees of masculine characteristics (including small testes), and are somewhat challenged mentally. Approximately equal numbers of the individuals with Klinefelter syndrome are due to nondisjunction of paternally and to maternally derived chromosomes.

Transformation of Spermatids (Spermiogenesis)

Spermatids discard much of their cytoplasm, rearrange their organelles, and form a flagellum to become transformed into spermatozoa; this process of transformation is known as spermiogenesis.

Spermatids are small, round haploid cells (8 μm in diameter). All of the spermatids that are the progeny of a single pale type A spermatogonium are connected to one another by cytoplasmic bridges. They form small clusters and occupy a position near the lumen of the seminiferous tubule. These cells have abundant RER, numerous mitochondria, and a well-developed Golgi complex. During their transformation into spermatozoa, they accumulate hydrolytic enzymes, rearrange and reduce the number of their organelles, form flagella and

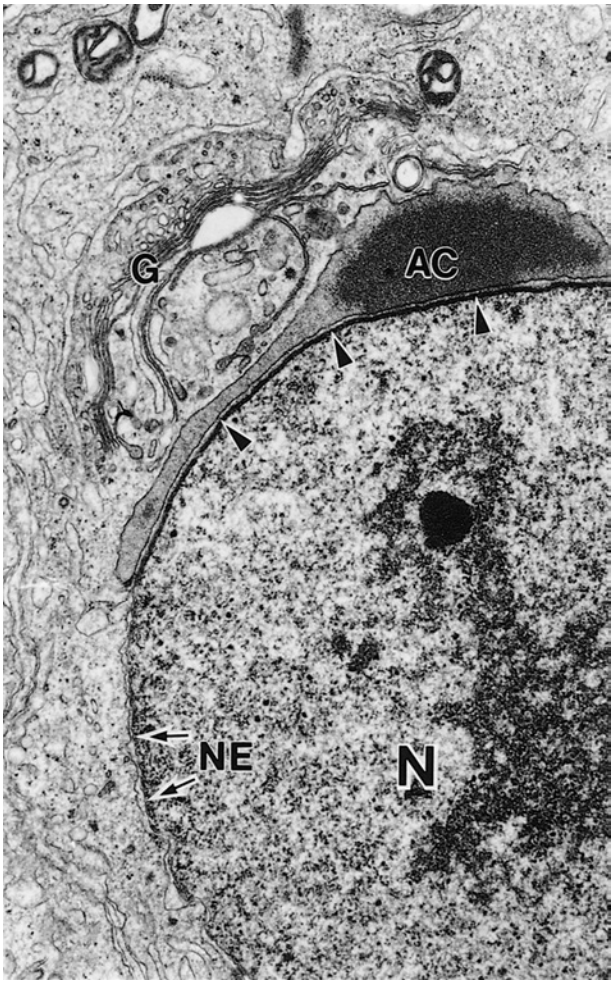


Figure 21–8 Electron micrograph of the cap stage of a rodent spermatid (×18000). AC, Acrosome; G, Golgi apparatus; N, nucleus; NE, nuclear envelope. (From Oshako S, Bunick D, Hess RA, et al. Characterization of a testis specific protein localized in the endoplasmic reticulum of spermatogenic cells. *Anat Rec.* 1994;238:335-348. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons.)

associated skeletal apparatus, and shed some of their cytoplasm. This process of **spermiogenesis** is subdivided into four phases (Figs. 21–8 and 21–9):

- Golgi phase
- Cap phase
- Acrosomal phase
- Maturation phase

Golgi Phase

During the **Golgi phase** of spermiogenesis, hydrolytic enzymes are formed on the RER, modified in the Golgi apparatus, and packaged by the ***trans* Golgi network** as small, membrane-bound **preacrosomal granules**. These small vesicles fuse with one another, forming an **acrosomal vesicle**. The hydrolytic enzymes in this vesicle are visualized with the electron microscope as an electron-dense material known as the ***acrosomal granule*.** The acrosomal vesicle comes into contact with and becomes bound to the nuclear envelope, thus establishing the anterior pole of the developing spermatozoon.

As the acrosomal vesicle is being formed, the centrioles leave the vicinity of the nucleus, and one of them participates in the formation of the **flagellar axoneme.** After the generation of the microtubules is initiated, the centrioles return to the vicinity of the nucleus to assist in the formation of the **connecting piece,** a structure that will surround the centrioles (see later in the description of the spermatozoon).

Cap Phase

During the **cap phase,** the acrosomal vesicle increases in size, and its membrane partially surrounds the nucleus (see Fig. 21–8). As this vesicle enlarges to its final size, it becomes known as the ***acrosome (acrosomal cap).***

Acrosomal Phase

The **acrosomal phase** is characterized by several alterations in the morphology of the spermatid. The nucleus becomes condensed, the cell elongates, and the mitochondria shift location.

The chromosomes become tightly condensed and tightly packaged. As the chromosomal volume decreases, the volume of the entire nucleus is also reduced. Additionally, the nucleus becomes flattened and assumes its specific morphology.

Microtubules assemble to form a cylindrical structure, the **manchette,** which aids in the elongation of the spermatid. As the elongating cytoplasm reaches the microtubules of the flagellar axoneme, the manchette microtubules disassemble. Their place is assumed by the **annulus,** an electron-dense, ring-like structure that delineates the junction of the spermatozoon's **middle piece** with its **principal piece** (see Fig. 21–9). A mitochondrial sheath forms around the axoneme of the middle piece of the tail of the spermatozoon.

During formation of the mitochondrial sheath and elongation of the spermatid, nine columns of **outer dense fibers** form around the axoneme. These dense fibers are attached to the connecting piece formed during the Golgi phase. After their establishment, the dense fibers become surrounded by ribs, a series of ring-like, dense structures known as the ***fibrous sheath*.**

Maturation Phase

The **maturation phase** is characterized by the shedding of spermatid cytoplasm. As the excess cytoplasm is released, the syncytium is disrupted and individual spermatozoa are liberated from the large

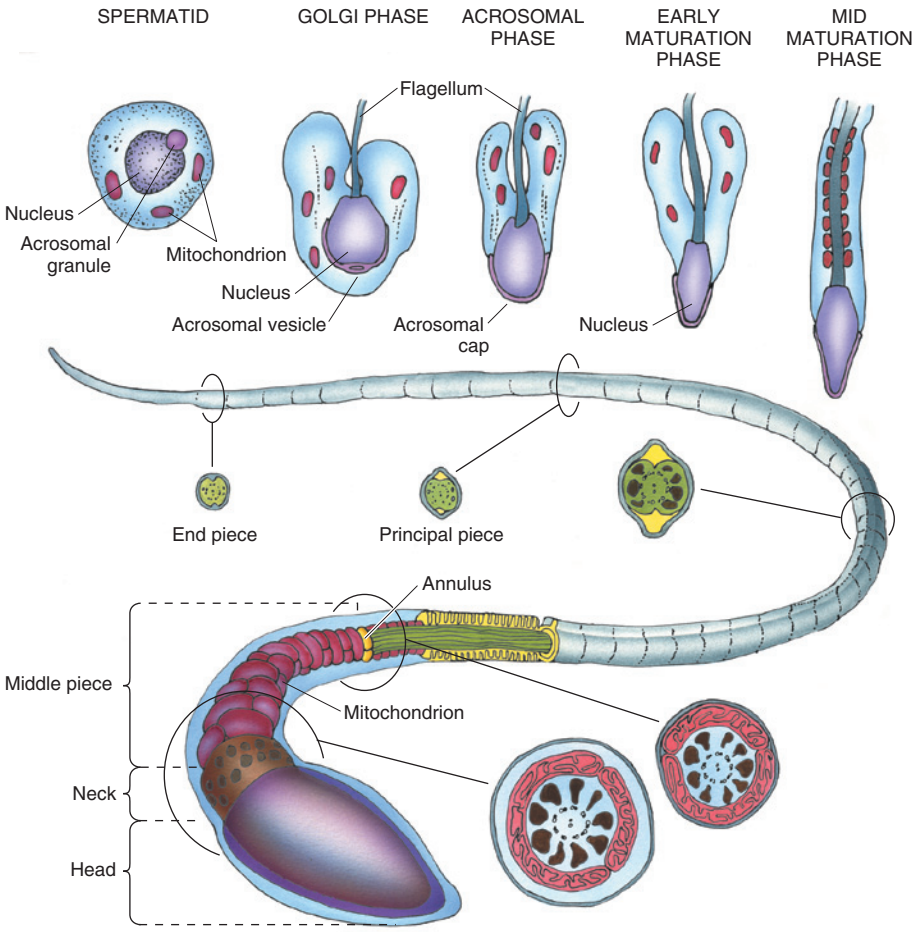


Figure 21–9 Schematic diagram of spermiogenesis and of a mature spermatozoon.

cellular mass. The cytoplasmic remnants are phagocytosed by Sertoli cells, and the disengaged spermatozoa are released into the lumen of the seminiferous tubule **(spermiation)**.

Note that the newly formed spermatozoa are **immotile** and cannot fertilize an oocyte. Spermatozoa gain motility while passing through the epididymis. Only after they enter the female reproductive system do spermatozoa become **capacitated** (i.e., become capable of fertilization).

Structure of Spermatozoa

Spermatozoa are composed of a head, housing the nucleus, and a tail that is divided into four regions: neck, middle piece, principal piece, and end piece.

The **spermatozoa (sperm),** produced by spermatogenesis, are long cells (~65 µm). Each spermatozoon is composed of a head, housing the nucleus, and a tail, which accounts for most of its length (Fig. 21–10; see Fig. 21–9).

Head of the Spermatozoon

The flattened head of the spermatozoon is about 5 µm long and is surrounded by plasmalemma (see Fig. 21–9). It is occupied by the condensed electron-dense nucleus, containing only 1 member of the 23 pairs of chromosomes (22 autosomes + the Y chromosome – or 22 autosomes + the X chromosome), and the **acrosome,** which partially surrounds the anterior aspect of the nucleus. The acrosome comes into contact with the cell membrane of the spermatozoon anteriorly. It houses various enzymes, including neuraminidase, hyaluronidase, acid phosphatase, aryl sulfatase, and a trypsin-like protease known as ***acrosin***.

Binding of a spermatozoon to the ZP3 molecule of the zona pellucida triggers the **acrosomal reaction**, the release of the acrosomal enzymes that digest a path

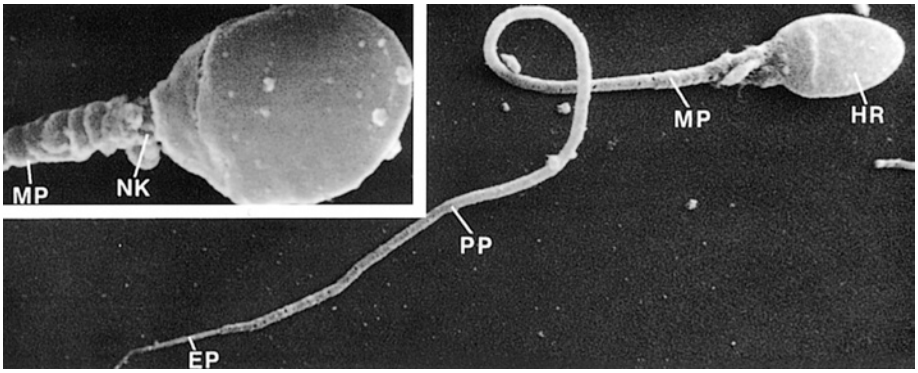


Figure 21–10 Scanning electron micrograph of human spermatozoa. The entire spermatozoon is shown: head region (HR), middle piece (MP), principal piece (PP), and end piece (EP) (×650). *Inset*, Head, neck (NK), and middle piece (MP) (×15130). (From Kessel RG. *Tissue and Organs: A Text Atlas of Scanning Electron Microscopy*. San Francisco: WH Freeman; 1979.)

for the sperm to reach the oocyte, thereby facilitating the process of fertilization. The acrosomal reaction as well as the process of fertilization is described in Chapter 20.

Tail of the Spermatozoon

The tail of the spermatozoon is subdivided into four regions: neck, middle piece, principal piece, and end piece (see Fig. 21–9). The plasmalemma of the head is continuous with the tail's plasma membrane.

The **neck** (~5 µm long) connects the head to the remainder of the tail. It is composed of the cylindrical arrangement of the nine columns of the **connecting piece** that encircles the two centrioles, one of which is usually fragmented. The posterior aspects of the columnar densities are continuous with the nine **outer dense fibers.**

The **middle piece** (~5 µm long) is located between the neck and the principal piece. It is characterized by the presence of the mitochondrial sheath, which encircles the **outer dense fibers** and the central-most **axoneme.** The middle piece stops at the **annulus,** a ring-like, dense structure to which the plasmalemma adheres, thus preventing the mitochondrial sheath from moving caudally into the tail. Also, two of the nine outer dense fibers terminate at the annulus; the remaining seven continue into the principal piece.

The **principal piece** (~45 µm long) is the longest segment of the tail and extends from the annulus to the end piece. The axoneme of the principal piece is continuous with that of the middle piece. Surrounding the axoneme are the seven outer dense fibers that are continuous with those of the middle piece and are surrounded, in turn, by the **fibrous sheath.** The principal piece tapers near its caudal extent, where both the outer dense fibers and the fibrous sheath terminate, and is continuous with the end piece.

The **end piece** (~5 µm long) is composed of the central axoneme surrounded by plasmalemma. The axoneme is disorganized in the last 0.5 to 1.0 µm, so that instead of the nine doublets and two singlets, 20 haphazardly arranged, individual microtubules are evident.

CLINICAL CORRELATIONS

Mutations in the gene that codes for the protein **beta defensin 126 (DEFB126)** has been demonstrated to reduce male fertility. Apparently, DEFB126 is necessary for the sperm to be able to navigate freely through the female genital tract because spermatozoa with mutated forms of this protein often are trapped in the mucous secretions lining the uterus and oviducts.

CYCLE OF THE SEMINIFEROUS EPITHELIUM

The seminiferous epithelium displays 16-day cycles; four cycles are required to complete spermatogenesis.

Because germ cells that arise from a single pale type A spermatogonium are connected by cytoplasmic bridges and constitute a syncytium, they can readily communicate with each other and synchronize their development. Careful examination of the human seminiferous epithelium reveals six possible characteristic associations of developing cell types, known as the ***six stages of spermatogenesis***, because they are undergoing transformations to form spermatozoa (Fig. 21–11). Each cross-sectional profile of a seminiferous tubule may be subdivided into three or more wedge-shaped areas, each displaying a different stage of spermatogenesis.

Studies following the fate of tritium-labeled thymidine (^{3}H-thymidine) injected into the testes of human

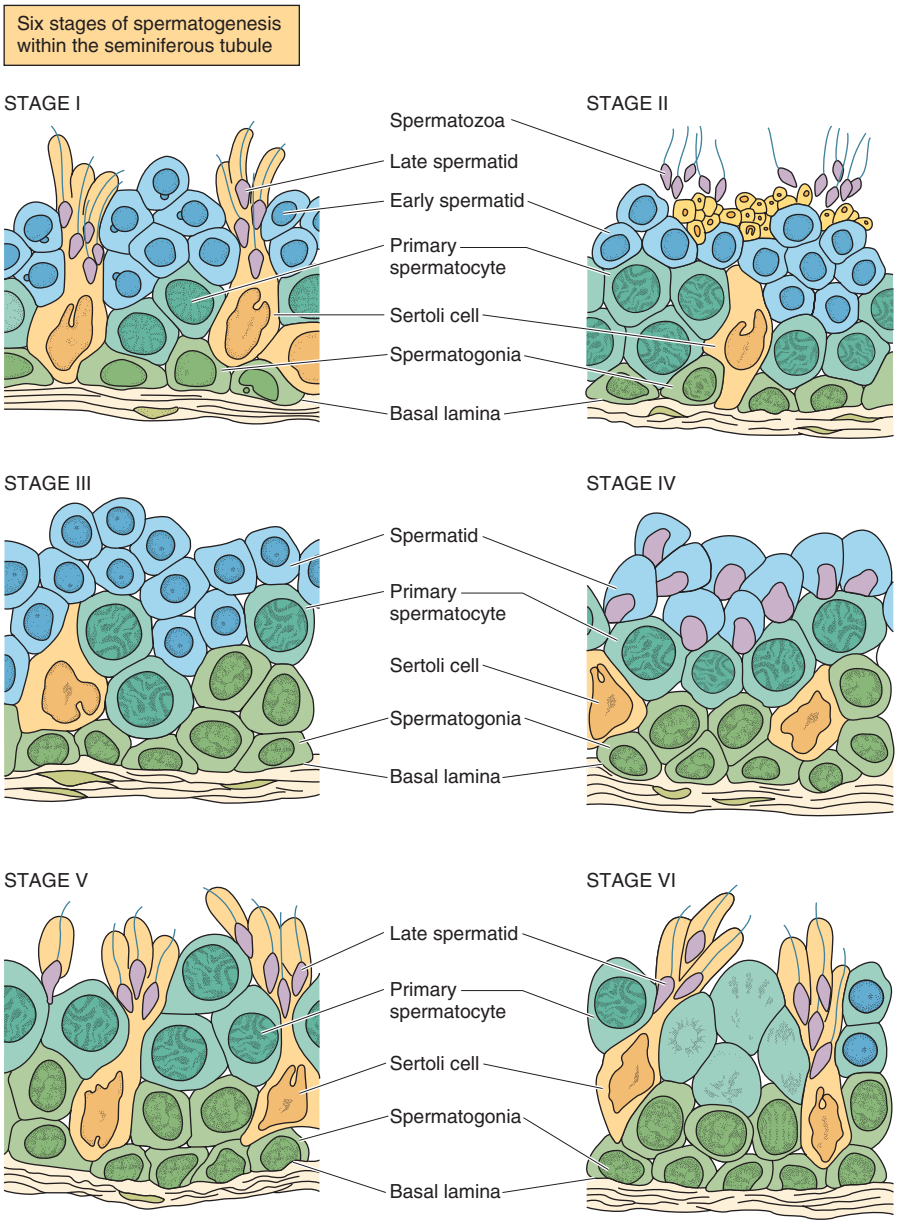


Figure 21–11 Schematic diagram of the six stages of spermatogenesis in the human seminiferous tubule. (From Clermont Y. The cycle of the seminiferous epithelium in man. *Am J Anat*. 1963;112:35-52. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

volunteers have demonstrated that radioactivity appears at 16-day intervals in the same stage of spermatogenesis. Each 16-day interval is known as a ***cycle of the seminiferous epithelium***, and the process of spermatogenesis requires the passage of four cycles, or 64 days. Examination of serial cross-sections of a single seminiferous tubule reveals that the same stage of the seminiferous epithelium continues to reappear at specific distances along the length of the tubule. The distance between two identical stages of the seminiferous epithelium is called the ***wave of the seminiferous epithelium.*** Thus, in humans, there are six repeating waves of the seminiferous epithelium, corresponding to the six stages.

Interstitial Cells of Leydig

The interstitial cells of Leydig, scattered among connective tissue elements of the tunica vasculosa, secrete testosterone.

The seminiferous tubules are embedded in the tunica vasculosa, a richly vascularized, loose connective tissue housing scattered fibroblasts, mast cells, and other cellular constituents normally present in loose connective tissue. Also dispersed throughout the tunica vasculosa are small collections of endocrine cells, the **interstitial cells (of Leydig),** which produce the hormones **testosterone** and **insulin-like factor 3 (INSL 3).** The former facilitates the spermatogenesis, and the latter facilitates the descent of the testes into the scrotum in fetal life. During puberty and in the adult, INSL 3 enhances the release of testosterone by Leydig cells and maintains the health of spermatogenic cells.

Interstitial cells of Leydig are polyhedral and are approximately 15 μm in diameter. They have a single nucleus, although, occasionally, they may be binucleate. They are typical steroid-producing cells that have mitochondria with tubular cristae, a large accumulation of smooth endoplasmic reticulum, and a well-developed Golgi apparatus (Fig. 21–12). These cells also house some RER and numerous lipid droplets, but they contain no secretory vesicles because testosterone is probably released as soon as its synthesis is complete. Lysosomes and peroxisomes are also evident, as are lipochrome pigments (especially in older men). The cytoplasm also contains crystallized proteins, the **crystals of Reinke,** a characteristic of human interstitial cells.

Histophysiology of the Testes

The principal functions of the testes are the production of spermatozoa and the synthesis and release of testosterone.

The two testes form about 120 million spermatozoa per day by a process that may be considered a holocrine type of secretion. Sertoli cells of the seminiferous epithelium also produce a fructose-rich fluid that acts to nourish and transport the newly formed spermatozoa from the lumen of the seminiferous tubule to the extratesticular genital ducts.

Luteinizing hormone (LH), a gonadotropin released from the anterior pituitary gland, binds to LH receptors on the Leydig cells, activating adenylate cyclase of these cells to form cyclic adenosine monophosphate (cAMP). Activation of protein kinases of the Leydig cells by cAMP induces inactive **cholesterol esterases** to become active and cleave free cholesterol from intracellular lipid droplets. The first step in the pathway of testosterone synthesis is also LH-sensitive because LH activates **cholesterol desmolase**, the enzyme that

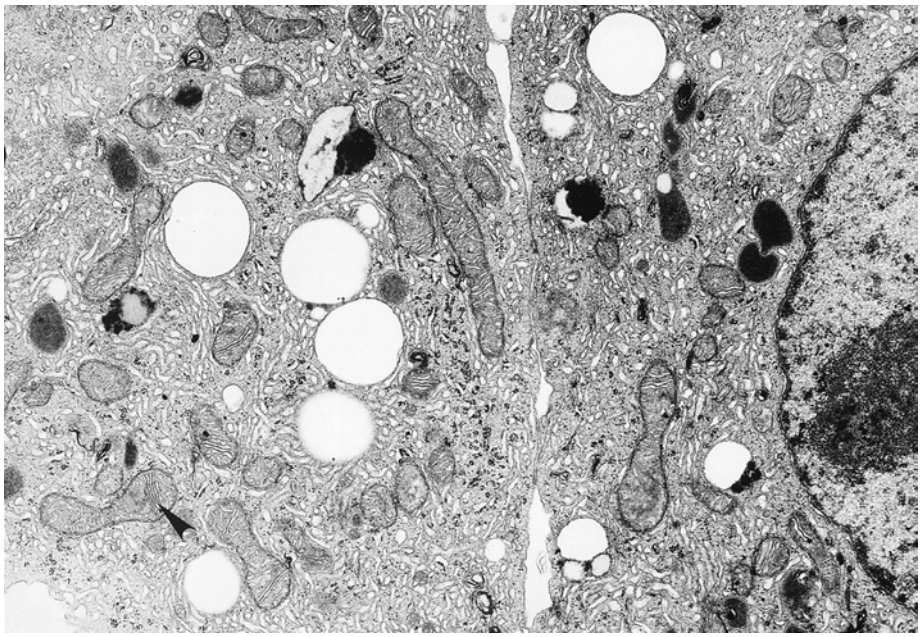


Figure 21–12 Low-magnification electron micrograph exhibits areas of two human Leydig cells (×18150). Mitochondria are relatively uniform in diameter, and even at low magnification, stacked lamellae are an evident form of the cristae *(arrowhead)*. (From Prince FP. Mitochondrial cristae diversity in human Leydig cells: a revised look at cristae morphology in these steroid-producing cells. *Anat Rec*. 1999;254:534-541. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

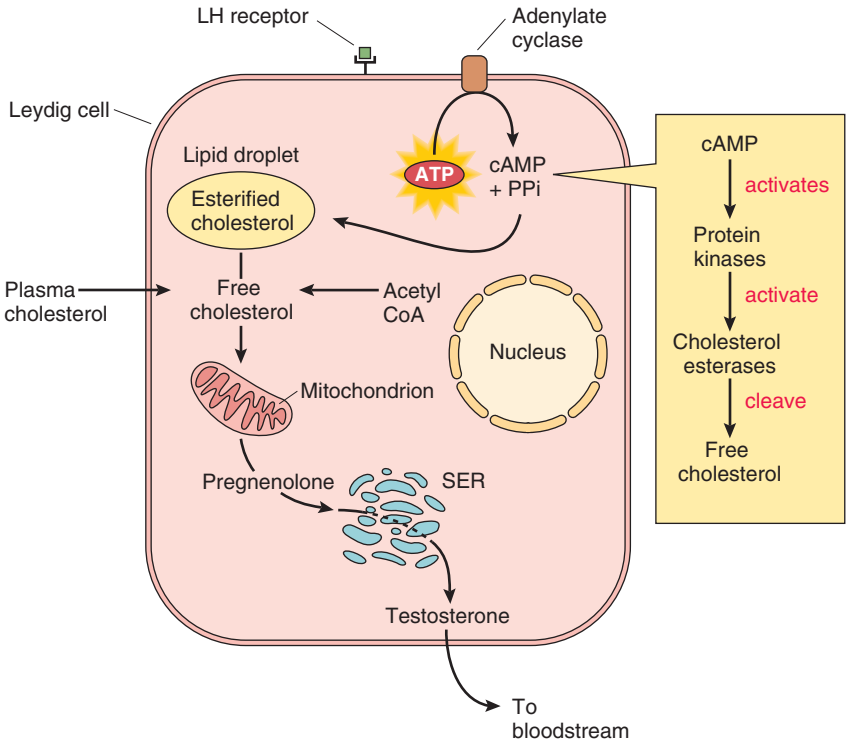


Figure 21–13 Schematic diagram of testosterone synthesis by the interstitial cells of Leydig. ATP, Adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CoA, coenzyme A; LH, luteinizing hormone; SER, smooth endoplasmic reticulum.

converts free cholesterol into pregnenolone. The various products of the synthetic pathway are shuttled between the smooth endoplasmic reticulum and mitochondria until **testosterone,** the male hormone, is formed and is ultimately released by these cells (Fig. 21–13).

Because blood testosterone levels are not sufficient to initiate and maintain spermatogenesis, FSH, another anterior pituitary gonadotropin, induces Sertoli cells to synthesize and release **ABP (androgen binding protein)** (Fig. 21–14). As its name implies, ABP binds testosterone, thereby preventing the hormone from leaving the region of the seminiferous tubule and elevating the testosterone levels in the local environment sufficiently to sustain spermatogenesis.

Release of LH is inhibited by increased levels of testosterone and dihydrotestosterone, whereas release of FSH is inhibited by the hormone **inhibin** and enhanced by the hormone **activin,** both produced by Sertoli cells (see Fig. 21–14). It is interesting to note that estrogens, female sex hormones, are also bound by ABP and thus can reduce the levels of spermatogenesis.

Germ cell loss due mostly to apoptosis during meiosis phase of spermatogenesis can be as high as 40%, and this number is increased after the individual reaches 40 years of age. It is presumed that this loss would be much greater were it not for the hormone **INSL 3**, produced by the interstitial cells of Leydig, which protects spermatogenic cells from apoptosis.

Testosterone is also required for the normal functioning of the seminal vesicles, prostate, and bulbourethral glands as well as for the appearance and maintenance of the male secondary sexual characteristics. The cells that require testosterone possess **5α-reductase**, the enzyme that converts testosterone to its more active form, **dihydrotestosterone**.

GENITAL DUCTS

The genital ducts may be subdivided into two categories: those located within the testes **(intratesticular)** and those located external to the testes **(extratesticular)** (Table 21–1).

Intratesticular Genital Ducts

The genital ducts located within the testis connect the seminiferous tubules to the epididymis. These intratesticular ducts are the tubuli recti and the rete testis (see Fig. 21–2).

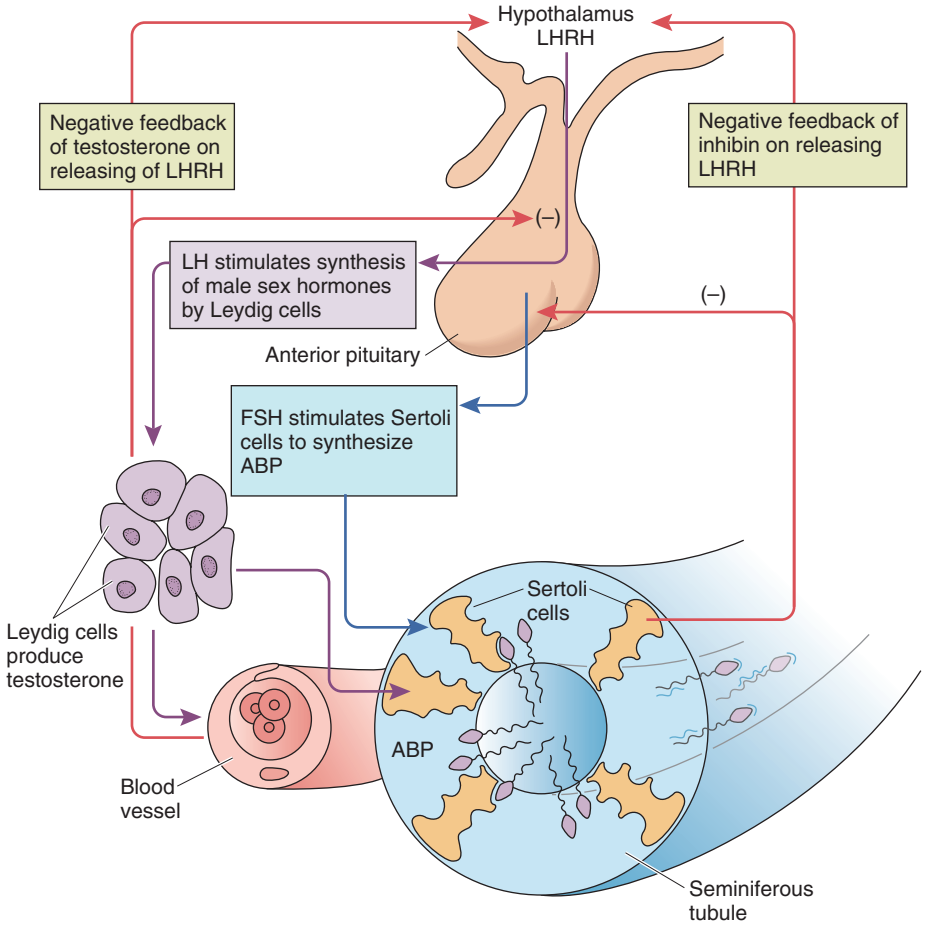


Figure 21–14 Schematic diagram of the hormonal control of spermatogenesis. ABP, Androgen-binding protein; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LHRH, luteinizing hormone–releasing hormone. (From Fawcett DW. *Bloom and Fawcett's A Textbook of Histology*. 10th ed. Philadelphia: WB Saunders; 1975.)

TABLE 21–1 Histological Features and Functions of Male Genital Ducts

Duct	Epithelial Lining	Supporting Tissues	Function
Tubuli recti	Sertoli cells in proximal half; simple cuboidal epithelium in distal half	Loose connective tissue	Convey spermatozoa from the seminiferous tubules to the rete testis
Rete testis	Simple cuboidal epithelium	Vascular connective tissue	Conveys spermatozoa from the tubuli recti to the ductuli efferentes
Ductuli efferentes	Patches of nonciliated cuboidal cells alternating with ciliated columnar cells	Thin loose connective tissue surrounded by a thin layer of circularly arranged smooth muscle cells	Convey spermatozoa from the rete testis to the ductus epididymis
Ductus epididymis	Pseudostratified epithelium composed of short basal cells and tall principal cells (with stereocilia)	Thin loose connective tissue surrounded by a layer of circularly arranged smooth muscle cells	Conveys spermatozoa from the ductuli efferentes to the ductus deferens
Ductus (vas) deferens	Stereociliated pseudostratified columnar epithelium	Loose fibroelastic connective tissue; thick three-layered smooth muscle coats; *inner* and *outer* longitudinal, *middle* circular	Delivers spermatozoa from tail of epididymis to ejaculatory duct
Ejaculatory duct	Simple columnar epithelium	Subepithelial connective tissue folded, giving lumen irregular appearance; no smooth muscle	Delivers spermatozoa and seminal fluid to prostatic urethra at colliculus seminalis

Tubuli Recti

The tubuli recti deliver spermatozoa from the seminiferous tubules into the rete testis.

The **tubuli recti** are short, straight tubules that are continuous with the seminiferous tubules and deliver the spermatozoa, formed by the seminiferous epithelium, to the rete testis. These short tubules are lined by Sertoli cells in their first half, near the seminiferous tubule, and by a simple cuboidal epithelium in their second half, near the rete testis. The cuboidal cells have short, stubby microvilli, and most possess a single flagellum.

Rete Testis

Immature spermatozoa pass from the tubuli recti into the rete testis, labyrinthine spaces lined by cuboidal epithelium.

The **rete testis** consists of labyrinthine spaces, lined by a simple cuboidal epithelium, within the mediastinum testis. These cuboidal cells, which resemble those of the tubuli recti, have numerous short microvilli with a single flagellum (Fig. 21–15).

Extratesticular Genital Ducts

The extratesticular genital ducts are the ductuli efferentes, ductus epididymis, ductus deferens, and ejaculatory duct.

The **extratesticular genital ducts** associated with each testis are the ductuli efferentes, ductus epididymis, the ductus (vas) deferens, and the ejaculatory duct (see Fig. 21–1). The epididymis secretes numerous factors that, in an unknown manner, facilitate maturation of spermatozoa. As noted previously, however, spermatozoa cannot fertilize a secondary oocyte until they undergo **capacitation,** a process triggered by secretions produced in the female genital tract (see Chapter 20).

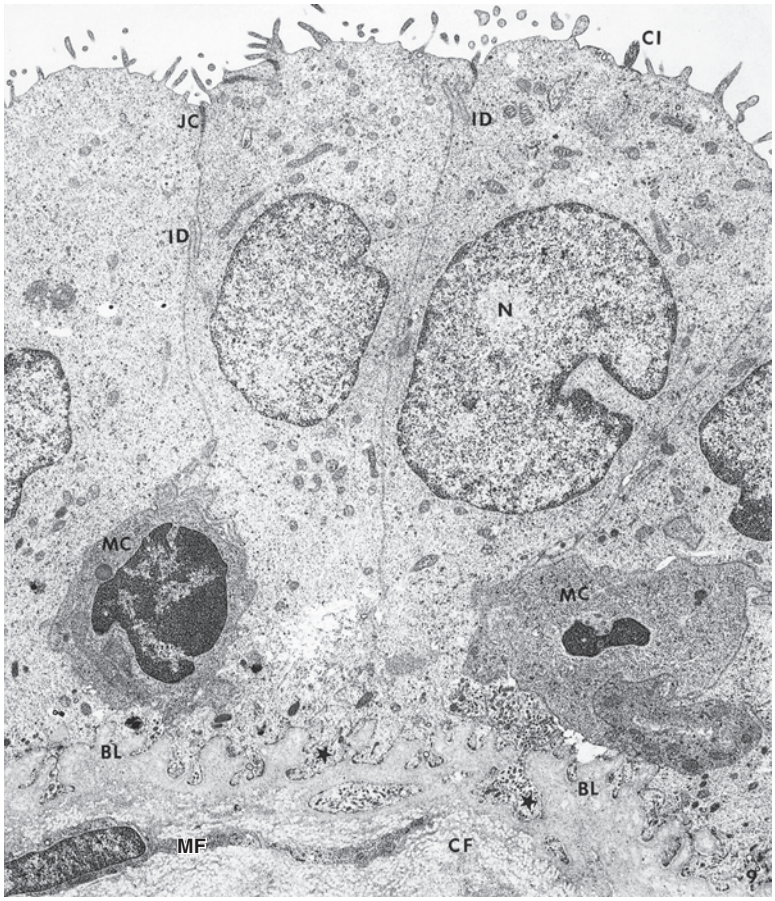


Figure 21–15 Electron micrograph of the epithelium of the bovine rete testis (×19900). BL, Basal lamina; CF, collagen fibers; CI, cilium; ID, interdigitation of the lateral plasmalemmae; JC, junctional complexes; MC, monocellular cell; MF, myofibroblast; N, nucleus. (From Hees H, Wrobel KH, Elmagd AA, Hees I. The mediastinum of the bovine testis. *Cell Tissue Res*. 1989;255:29-39.)

Epididymis

The ductuli efferentes and the ductus epididymis form the epididymis.

Each **epididymis** is formed by the ductuli efferentes and the ductus epididymis.

Ductuli Efferentes

The ductuli efferentes are interposed between the rete testis and the ductus epididymis.

The 10 to 20 **ductuli efferentes** are short tubules that drain spermatozoa from the rete testis and pierce the tunica albuginea of the testis to conduct the sperm to the ductus epididymis (see Fig. 21–2). Thus, the ductuli efferentes become confluent with the ductus epididymis at this point.

The simple epithelial lining of the lumen of each ductule consists of patches of **nonciliated cuboidal cells** alternating with regions of **ciliated columnar cells.** The successive clusters of short and tall epithelial cells impart a scalloped appearance to the lumina of the ductuli efferentes, a notable characteristic of these ductules. The cuboidal cells are richly endowed with lysosomes, and their apical plasmalemmae display numerous invaginations indicative of endocytosis. These cells are believed to **resorb** most of the luminal fluid elaborated by the Sertoli cells of the seminiferous tubule. The cilia of the columnar cells probably move the spermatozoa toward the epididymis.

The simple epithelium sits on a basal lamina that separates it from the thin, loose connective tissue wall of each ductule. The connective tissue is surrounded by a thin layer of smooth muscle whose cells are circularly arrayed and, by undergoing rhythmic contractions, assist in propelling their luminal contents into the epididymis.

Ductus Epididymis

The ductus epididymis is a thin, long highly convoluted tubule composed of three regions, the head, body, and tail.

The **ductus epididymis** is a thin, long (4 to 6 m in length), highly convoluted tubule that is folded into a space only 5 cm long on the posterior aspect of the testis (see Fig. 21–2). The epididymis may be subdivided into three regions: head, body, and tail. The head, formed by the union of the 10 to 20 ductuli efferentes, becomes highly coiled and continues as the equally highly coiled body. The distal portion of the tail, which stores spermatozoa for a short time, loses its convolutions as it becomes continuous with the ductus deferens.

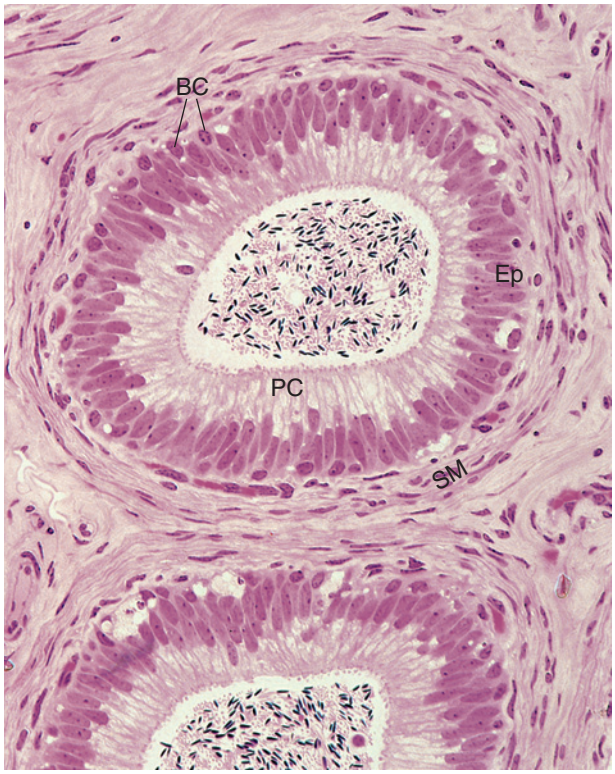


Figure 21–16 Photomicrograph of the epididymis in a monkey, showing smooth muscle (SM), principal cells (PC), epithelium (Ep), and basal cells (BC) (×270).

The lumen of the epididymis is lined by a **pseudostratified epithelium** composed of two cell types (Fig. 21–16):

- **Short basal cells**. Pyramidal to polyhedral. They have round nuclei in which large accumulations of heterochromatin impart a dense appearance to this structure. The sparse cytoplasm of these cells is relatively clear with a scarcity of organelles. It is believed that the basal cells function as stem cells, regenerating themselves as well as the principal cells as the need arises.
- **Tall principal cells**. They have irregular, oval nuclei with one or two large nucleoli. These nuclei are much paler than those of the basal cells and are located basally within the cell.

The cytoplasm of the principal cell houses abundant RER located between the nucleus and the basal plasmalemma. The cytoplasm also has a large, supranuclearly positioned Golgi complex, numerous profiles of apically located smooth endoplasmic reticulum, endolysosomes, and multivesicular bodies. The apical cell membranes of the principal cells display a profusion of pinocytotic and coated vesicles at the bases of the many

stereocilia that project into the lumen of the epididymis. These long, branched, cellular extensions are clusters of nonmotile microvilli that appear to form clumps as they adhere to one another.

The principal cells resorb the luminal fluid, which is endocytosed by pinocytotic vesicles and delivered to the endolysosomes for disposal. Additionally, these cells phagocytose remnants of cytoplasm that were not removed by Sertoli cells. Principal cells also manufacture **glycerophosphocholine,** a glycoprotein that inhibits spermatozoon capacitation, thus preventing the spermatozoon from fertilizing a secondary oocyte until the sperm enters the female genital tract. Additionally, the high level of **fertilization promoting peptide** (**FPP**) also prevents the spermatozoon from undergoing capacitation until it reaches the female reproductive tract where FPP levels become diluted.

The epithelium of the epididymis is separated by a basal lamina from the underlying loose connective tissue. A layer of circularly arranged smooth muscle cells surrounds the connective tissue layer. **Peristaltic contractions** of this layer help conduct the spermatozoa to the ductus deferens.

Ductus Deferens (Vas Deferens)

The ductus deferens (also known as the vas deferens*) is a muscular tube that conveys spermatozoa from the tail of the epididymis to the ejaculatory duct.*

Each **ductus deferens** (also known as the ***vas deferens***) is a thick-walled muscular tube with a small, irregular lumen that conveys spermatozoa from the tail of the epididymis to the ejaculatory duct (see Figs. 21–1 and 21–2).

The stereociliated **pseudostratified columnar epithelium** of the ductus deferens is similar to that of the epididymis, although the principal cells are shorter. A basal lamina separates the epithelium from the underlying loose fibroelastic connective tissue, which has numerous folds, thus making the lumen appear irregular. The thick smooth muscle coat surrounding the connective tissue is composed of three layers, inner and outer longitudinal layers with an intervening middle circular layer. The smooth muscle coat is invested by a thin layer of loose fibroelastic connective tissue.

CLINICAL CORRELATIONS

Because the ductus deferens has a muscular wall 1 mm thick, it is easily perceptible through the skin of the scrotum as a dense, rolling tubule. **Vasectomy** (surgical removal of part of the ductus deferens) is performed via a small slit through the scrotal sac, thus sterilizing the person.

The dilated terminus of each ductus deferens, known as the ***ampulla*,** has a highly folded, thickened epithelium. As the ampulla approaches the prostate gland, it is joined by the seminal vesicle. The continuation of the junction of the ampulla with the seminal vesicle is called the ***ejaculatory duct*.**

Ejaculatory Duct

The ampulla of the ductus deferens joins the seminal vesicle to form the ejaculatory duct, which then enters the prostate gland and opens in the prostatic urethra.

Each **ejaculatory duct** is a short, straight tubule that enters the substance of and is surrounded by the prostate gland (see Fig. 21–1). The ejaculatory duct ends as it pierces the posterior aspect of the prostatic urethra at the **colliculus seminalis**. The lumen of the ejaculatory duct is lined by a simple columnar epithelium. The subepithelial connective tissue is folded, a feature responsible for the irregular appearance of its lumen. The ejaculatory duct has no smooth muscle in its wall.

ACCESSORY GENITAL GLANDS

The male reproductive system has five **accessory glands**: the paired seminal vesicles, the single prostate gland, and the paired bulbourethral glands (see Fig. 21–1).

Seminal Vesicles

The paired seminal vesicles, located adjacent to the posterior wall of the prostate gland, secrete a viscous fluid that constitutes about 70% of the ejaculate.

The paired **seminal vesicles** are highly coiled tubular structures about 15 cm long. They are located between the posterior aspect of the neck of the bladder and the prostate gland and join the ampulla of the ductus deferens just above the prostate gland.

The mucosa of the seminal vesicles is highly convoluted, forming labyrinth-like cul-de-sacs that, in three dimensions, are observed to open into a central lumen. The lumen is lined by a **pseudostratified columnar epithelium** composed of short basal cells and low columnar cells (Fig. 21–17).

Each **columnar cell** has numerous short microvilli and a single flagellum projecting into the lumen of the gland. The cytoplasm of these cells displays RER, Golgi apparatus, numerous mitochondria, some lipid and **lipochrome pigment** droplets, and abundant secretory granules. The height of the cells varies directly with blood testosterone levels. The subepithelial connective tissue is **fibroelastic** and is surrounded by smooth muscle cells, arranged as an inner circular layer and an outer longitudinal layer. The **smooth muscle coat** is,

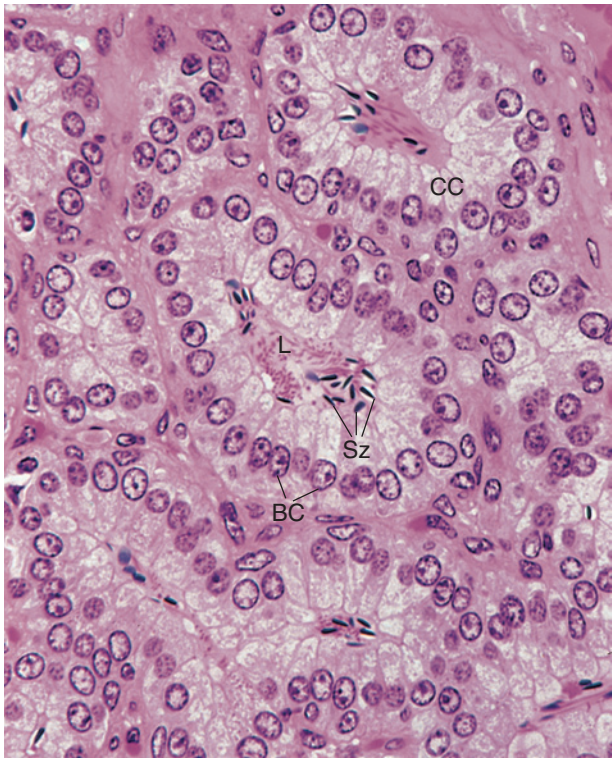


Figure 21–17 Photomicrograph of the monkey seminal vesicle, showing spermatozoa (Sz), lumen (L), basal cells (BC), and columnar cells (CC) (×270).

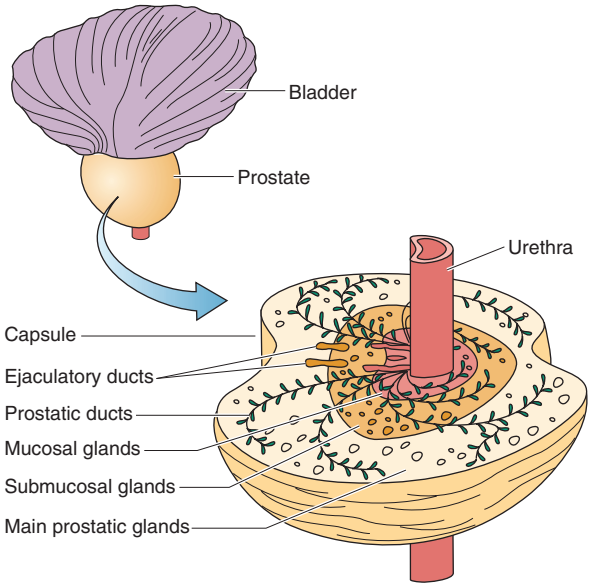


Figure 21–18 Schematic diagram of the human prostate gland illustrating the mucosal, submucosal, and main prostatic glands.

in turn, surrounded by a flimsy layer of fibroelastic connective tissue.

The seminal vesicles once were believed to store spermatozoa, some of which are always present in the lumen of this gland. It is now known that these glands produce a yellow, viscous, **fructose-rich seminal fluid** that constitutes 60% of the volume of semen. Although seminal fluid also contains amino acids, citrates, prostaglandins, and proteins, fructose is its principal constituent because it is the source of energy for spermatozoa. The characteristic pale yellow color of semen is due to the lipochrome pigment released by the seminal vesicles.

Prostate Gland

The prostate gland, surrounding a portion of the urethra, secretes acid phosphatase, fibrinolysin, and citric acid directly into the lumen of the urethra.

The **prostate gland,** the largest of the accessory glands, is pierced by the urethra and the ejaculatory ducts (Fig. 21–18). The slender **capsule** of the gland is composed of a richly vascularized, dense irregular collagenous connective tissue interspersed with **smooth muscle cells.** The connective tissue **stroma** of the gland is derived from the capsule and is, therefore, also enriched by smooth muscle fibers, in addition to their normal connective tissue cells.

The prostate gland, a conglomeration of 30 to 50 individual **compound tubuloacinar glands**, is arranged in three discrete, concentric layers:

- Mucosal
- Submucosal
- Main

Each tubuloacinar gland has its own duct that delivers the secretory product into the prostatic urethra.

The **mucosal glands** are closest to the urethra and thus are the shortest of the glands. The **submucosal glands** are peripheral to the mucosal glands and are consequently larger than the mucosal glands. The largest and most numerous of the glands are the peripheral-most **main glands,** which compose the bulk of the prostate.

The components of the prostate gland are lined by a **simple** to **pseudostratified columnar epithelium** (Fig. 21–19), the cells of which are well endowed with organelles responsible for the synthesis and packaging of proteins. Hence, these cells have an abundant RER, a large Golgi apparatus, numerous secretory granules (Fig. 21–20), and many lysosomes.

The lumina of the tubuloacinar glands frequently house round to oval **prostatic concretions (corpora amylacea),** composed of calcified glycoproteins, whose

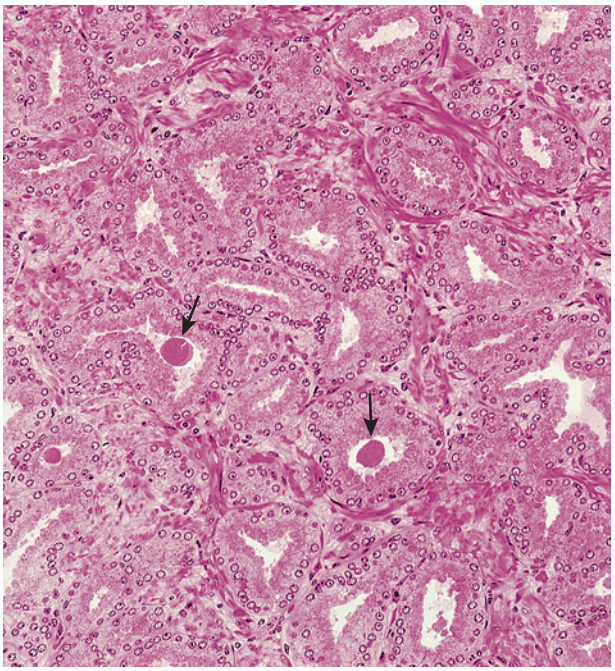


Figure 21–19 Photomicrograph of the prostate gland of a monkey (×132). *Arrows* represent prostatic concretion.

numbers increase with a person's age (see Fig. 21–19). The significance of these concretions is not understood.

The **prostatic secretion** constitutes approximately 30% of the semen. It is a serous, white fluid rich in lipids, proteolytic enzymes, acid phosphatase, fibrinolysin, and citric acid. The formation, synthesis, and release of the prostatic secretions are regulated by **dihydrotestosterone**, the active form of testosterone.

Bulbourethral Glands

The paired bulbourethral glands, located at the root of the penis, secrete a slippery lubricating solution directly into the urethra.

The **bulbourethral glands (Cowper glands)** are small (3 to 5 mm in diameter) and are located at the root of the penis, just at the beginning of the membranous urethra (see Fig. 21–1). Their fibroelastic capsule contains not only fibroblasts and smooth muscle cells, but also skeletal muscle fibers derived from the muscles of the urogenital diaphragm. Septa derived from the capsule divide each gland into several lobules. The epithelium of these compound tubuloacinar glands varies from **simple cuboidal** to **simple columnar.**

The secretion produced by the bulbourethral glands is a thick, slippery fluid containing galactose and sialic acid that probably plays a role in lubricating the lumen of the urethra. During the process of ejaculation, this viscous fluid precedes the remainder of the semen.

Histophysiology of the Accessory Genital Glands

The bulbourethral glands produce a viscous slippery fluid that lubricates the lining of the urethra. It is the first of the glandular secretions to be released subsequent to erection of the penis. Just before ejaculation, secretions from the prostate are discharged into the urethra, as are the spermatozoa from the ampulla of the ductus deferens. The prostatic secretions apparently help the spermatozoa achieve motility. The final secretions arise from the seminal vesicles, which are responsible for a significant increase in the volume of the semen. Their fructose-rich fluid is used by the spermatozoa for energy.

The ejaculate, known as ***semen,*** consists of secretions from the accessory glands and 200 to 400 million

CLINICAL CORRELATIONS

As men age, the prostatic stroma and mucosal and submucosal glands begin to enlarge, a condition known as ***benign prostatic hypertrophy (BPH).*** The enlarged prostate partially strangulates the lumen of the urethra, resulting in difficulties with urination. Approximately 40% of men 50 years of age are afflicted with this condition; the percentage increases to 95% in 80-year-old men. It has been suggested, based on some clinical studies of patients with BPH who ate 5 to 15 g of pumpkin seeds, that the oil in these seeds improved their symptoms by reducing both the frequency of nocturnal urination and urinary leaking.

The second most common form of cancer in men is **adenocarcinoma of the prostate.** It affects approximately 30% of men over 75 years of age. Frequently, the cancer cells enter the circulatory system and metastasize to bone. A simple blood test to detect **prostatic-specific antigen** has been developed that permits early detection of prostatic adenocarcinoma. Although tumor growth can be detected by digital palpation through the rectum, a biopsy is required for confirmation. Surgery or radiotherapy are the usual treatments, but they are not without possible side effects, such as impotence and incontinence.

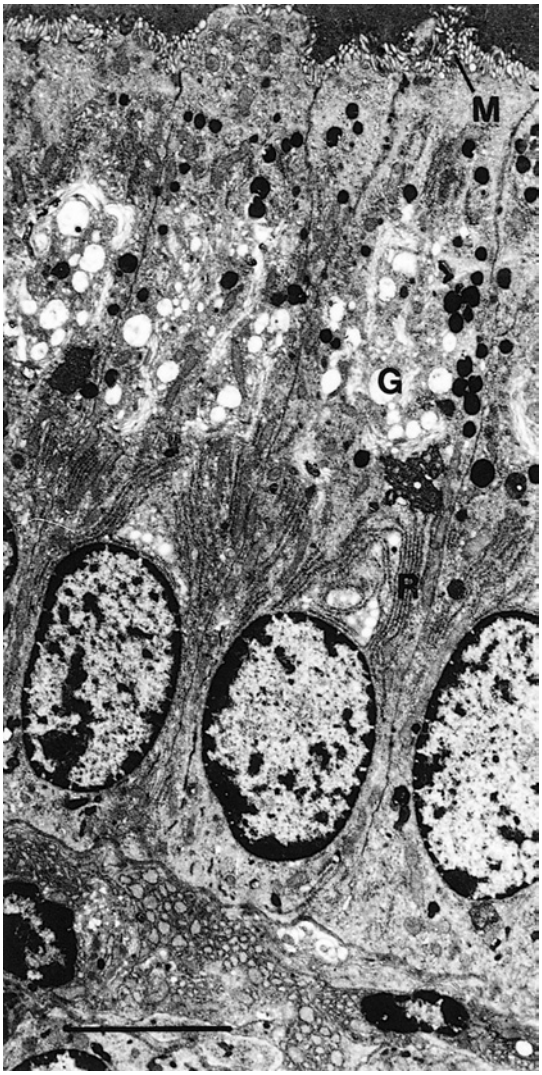


Figure 21–20 Electron micrograph of the prostate gland in the hamster. G, Golgi apparatus; M, microvilli; R, rough endoplasmic reticulum. Bar = 5 µm. (From Toma JG, Buzzell GR. Fine structure of the ventral and dorsal lobes of the prostate in a young adult Syrian hamster, *Mesocritetus auratus*. *Am J Anat*. 1988;181:132-140. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

spermatozoa. It is about 3.5 mL in volume in humans with a pH of about 7.5 due to the buffering action of the prostatic secretions.

PENIS

The penis functions as an excretory organ for urine and as the male copulatory organ for the deposition of spermatozoa into the female reproductive tract.

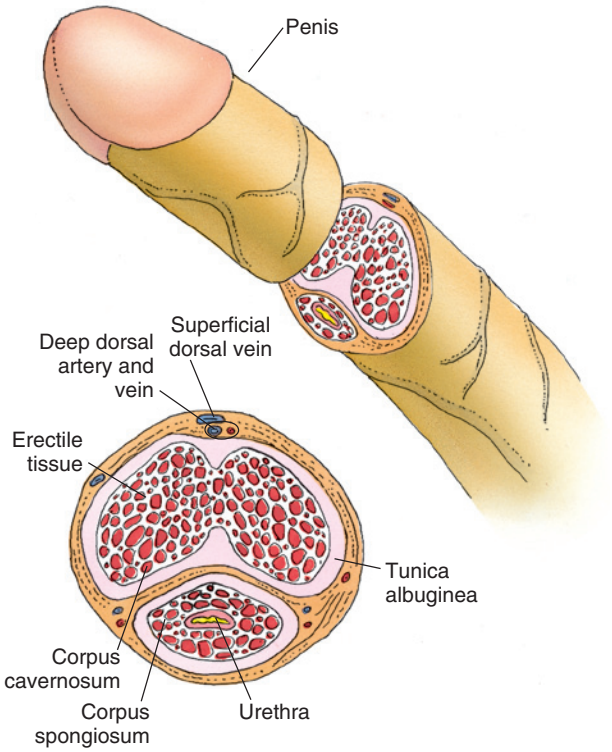


Figure 21–21 Schematic illustration of the penis in cross section.

The **penis** is composed of three columns of **erectile tissue,** each enclosed by its own dense, fibrous connective tissue capsule, the **tunica albuginea** (Fig. 21–21).

Two of the columns of erectile tissue, the **corpora cavernosa,** are positioned dorsally; their tunicae albugineae are discontinuous in places, permitting communication between their erectile tissues. The third column of erectile tissue, the **corpus spongiosum,** is positioned ventrally. Because the corpus spongiosum houses the penile portion of the urethra (see Chapter 19), it is also called the ***corpus cavernosum urethrae.*** The corpus spongiosum ends distally in an enlarged, bulbous portion, the **glans penis** (head of the penis). The tip of the glans penis is pierced by the end of the urethra as a vertical slit.

The three corpora are surrounded by a common loose connective tissue sheath, but no hypodermis, and are covered by thin skin. The skin of the proximal portion of the penis has coarse pubic hairs and numerous sweat and sebaceous glands. The distal portion of the penis is hairless and has only a few sweat glands. Skin continues distal to the glans penis to form a retractable sheath, the **prepuce,** which is lined by a mucous membrane, a moist, stratified squamous nonkeratinized epithelium. When an individual is circumcised, it is the prepuce that is removed.

CLINICAL CORRELATIONS

It has been reported that circumcision of adults alters the microbiome at the glans penis by decreasing the resident anaerobic population and replacing it with aerobic organisms. Whether this factor accounts for the 50% to 60% reduction in the HIV occurrence in the newly circumcised males is not known.

Structure of Erectile Tissue

Vascular spaces within the erectile tissues become engorged with blood, causing erection of the penis.

Erectile tissue of the penis contains numerous variably shaped, endothelially lined spaces that are separated from one another by trabeculae of connective tissue and smooth muscle cells. The vascular spaces of the corpora cavernosa are larger centrally and smaller peripherally, near the tunica albuginea. However, the vascular spaces of the corpus spongiosum are similar in size throughout its extent. The trabeculae of the corpus spongiosum contain more elastic fibers and fewer smooth muscle cells than those of the corpora cavernosa.

The erectile tissues of the corpora cavernosa receive blood from branches of the **deep** and **dorsal arteries of the penis** (see Fig. 21–22). These branches penetrate the walls of the trabeculae of the erectile tissue and form either capillary plexuses, which supply some blood flow into the vascular spaces, or coiled arteries **(helical arteries),** which are important sources of blood to the vascular spaces during erection of the penis.

Venous drainage occurs via three groups of veins, which are drained by the **deep dorsal vein** (see Fig. 21–22). The three groups of veins arise from the base of the glans penis, from the dorsal aspect of the corpora cavernosa, and from the ventral aspect of the corpora cavernosa and the corpus spongiosum. Additionally, some of the veins leave the erectile tissue at the root of the penis and drain into the plexus of veins that drain the prostate gland.

Mechanisms of Erection, Ejaculation, and Detumescence

Erection is controlled by the parasympathetic nervous system; it is a result of sexual, tactile, olfactory, visual, auditory, and/or psychological stimulation. Ejaculation is controlled by the sympathetic nervous system.

When the penis is flaccid, the vascular spaces of the erectile tissue contain little blood. In this condition, much of the arterial blood flow is diverted into arteriovenous anastomoses that connect the branches of the deep and dorsal arteries of the penis to veins that deliver their blood into the deep dorsal vein (Fig. 21–22A). Thus, the blood flow bypasses the vascular spaces of the erectile tissue.

Erection occurs when blood flow is shifted to the vascular spaces of the erectile tissues (the corpora cavernosa and, to a more limited extent, the corpus spongiosum), causing the penis to enlarge and become turgid (see Fig. 21–22B). During erection, the tunica albuginea surrounding the erectile tissues is stretched and decreases in thickness from 2 mm to 0.5 mm.

The shift in blood flow that leads to erection is controlled by the **parasympathetic nervous system** following sexual stimulation (e.g., pleasurable tactile, olfactory, visual, auditory, and/or psychological stimuli). The parasympathetic impulses trigger local release of **nitric oxide,** which causes relaxation of smooth muscles of the branches of the deep and dorsal arteries of the penis, increasing the flow of blood into the organ. Simultaneously, the arteriovenous anastomoses undergo constriction, diverting the flow of blood into the helical arteries of the erectile tissue. As these spaces become engorged with blood, the penis enlarges and becomes turgid, and erection ensues. The veins of the penis become compressed, and the blood is trapped in the vascular spaces of the erectile tissue, thus maintaining the penis in an erect condition (see Fig. 21–22).

Continued stimulation of the glans penis results in **ejaculation,** the forceful expulsion of **semen** from the male genital ducts. Each ejaculate, which has a volume of about 3.5 mL in humans, consists of secretions from the accessory genital glands and 200 to 400 million spermatozoa. Subsequent to erection, the bulbourethral glands release a viscous fluid that lubricates the lining of the urethra. Just before ejaculation, the prostate gland discharges its secretion into the urethra, and spermatozoa from the ampullae of the two ductus deferentes are released into the ejaculatory ducts. The prostatic secretion apparently helps the spermatozoa achieve motility. The final secretion added to semen is a fructose-rich fluid, released from the seminal vesicles, that provides energy to the spermatozoa. This secretion forms much of the volume of the ejaculate.

Ejaculation, unlike erection, is regulated by the **sympathetic nervous system.** These impulses trigger the following sequence of events:

1. Contraction of the smooth muscles of the genital ducts and accessory genital glands forces the semen into the urethra.
2. The sphincter muscle of the urinary bladder contracts, preventing the release of urine (or the entry of semen into the bladder).
3. The bulbospongiosus muscle, which surrounds the proximal end of the corpus spongiosum (bulb of the penis), undergoes powerful, rhythmic contractions, resulting in forceful expulsion of semen from the urethra.

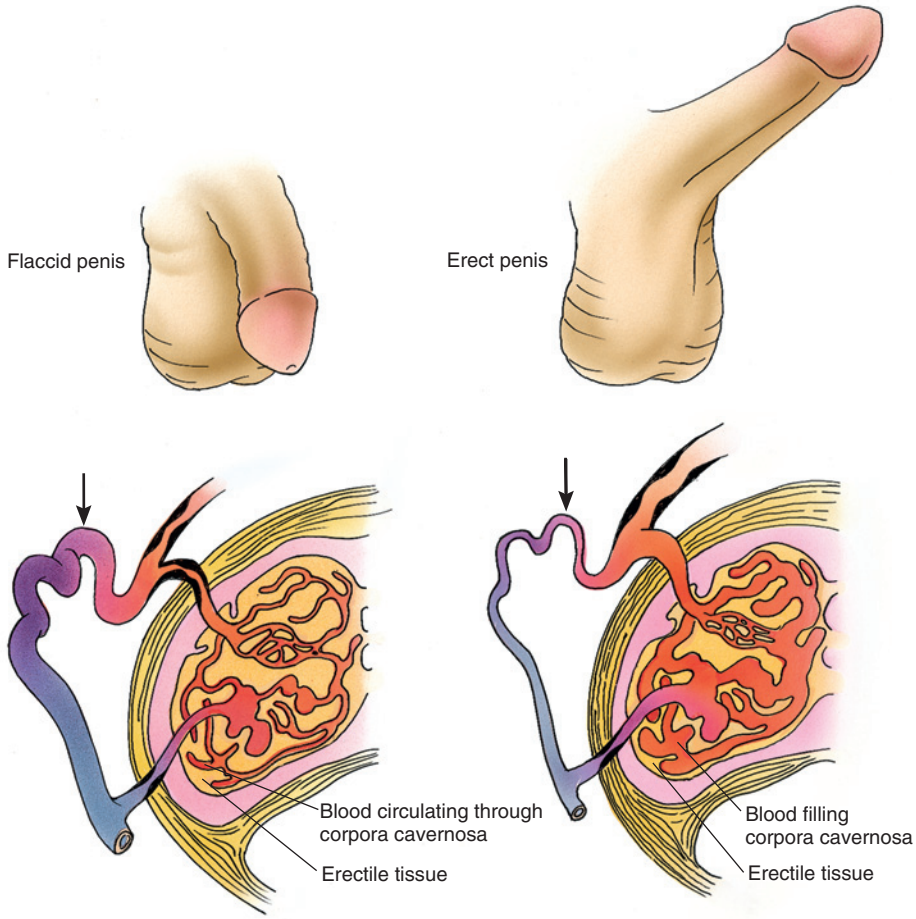


Figure 21–22 Schematic illustration of circulation in the flaccid and erect penis. The arteriovenous anastomosis *(arrow)* in the flaccid penis is wide, diverting blood flow into the venous drainage. In the erect penis, the arteriovenous anastomosis is constricted, and blood flow into the vascular spaces of the erectile tissue is increased, causing the penis to become turgid with blood. (From Conti G. *Acta Anat*. 1952;5:217.)

CLINICAL CORRELATIONS

1. A single ejaculate normally contains approximately 60 to 100 million spermatozoa per milliliter. A male whose sperm count is less than 20 million spermatozoa per milliliter of ejaculate is considered **sterile.** Interestingly, men who watch more than 20 hours of television per week have a 45% lower sperm count than men who do not watch television. Additionally, men who are more active and exercise have a 75% greater sperm count than do men who do not exercise. The reason for these findings is not known.
2. The inability to achieve erection is known as ***impotence.*** Temporary erectile dysfunction can result from psychological factors or drugs (e.g., alcohol), whereas permanent impotence can be caused by many factors, including lesions in certain regions of the brain and hypothalamus, as well as spinal cord injuries, autonomic innervation malfunction, stroke, Parkinson disease, diabetes, multiple sclerosis, and even psychological disorders.

CLINICAL CORRELATIONS

The neurotransmitter **nitric oxide (NO)** released by the endothelial cells of the sinusoids (i.e., vascular spaces of the erectile tissues) activate guanylate cyclase of smooth muscle cells to produce cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP), thus relaxing the smooth muscle cells. Relaxation of the smooth muscle cells permit the accumulation of blood in the sinusoids, and these enlarged vessels compress the small return venous channels that drain the sinusoids, resulting in the erection of the penis.

After ejaculation or when the parasympathetic impulses cease and cGMP levels dwindle, another enzyme, **phosphodiesterase (PDE),** destroys the cGMP, permitting smooth muscle contraction to occur again, the sinusoids begin to be drained of blood, and the erection is terminated.

Although **sildenafil (Viagra)** was originally developed as a treatment for heart failure, it was found to produce erections in many patients. Further study showed that the medication blocked phosphodiesterase from inhibiting cGMP degradation, thus leading to erection.

Ejaculation is followed by the cessation of parasympathetic impulses to the vascular supply of the penis. As a result, the arteriovenous shunt is reopened, blood flow through the deep and dorsal arteries of the penis is diminished, and the vascular spaces of the erectile tissues are slowly emptied of blood by the venous drainage. As the blood leaves these vascular spaces, the penis undergoes **detumescence** and becomes flaccid.

PATHOLOGICAL CONSIDERATIONS

See Figures 21–23 through 21–26.

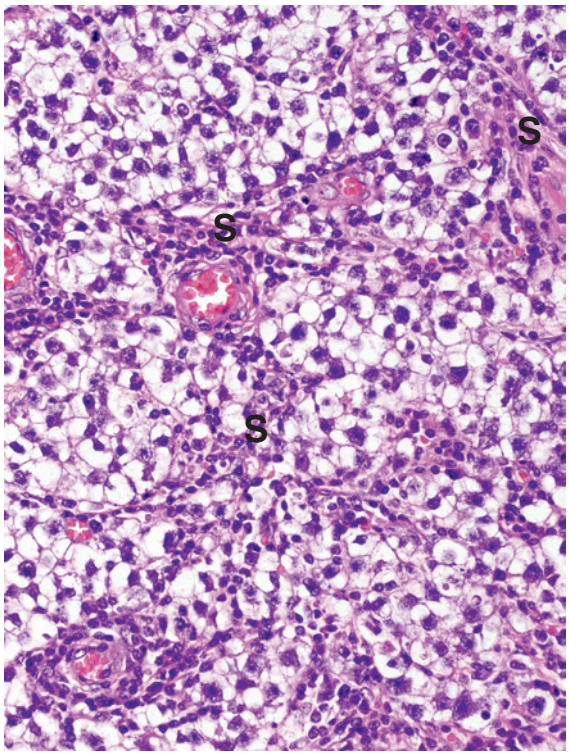


Figure 21–23 Photomicrograph of a patient with classical seminoma, the most common testicular tumor. Note the presence of clusters of numerous polygonal-shaped cells with clear cytoplasm. The clusters are separated from each other by slender connective tissue septa (S). (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:248.)

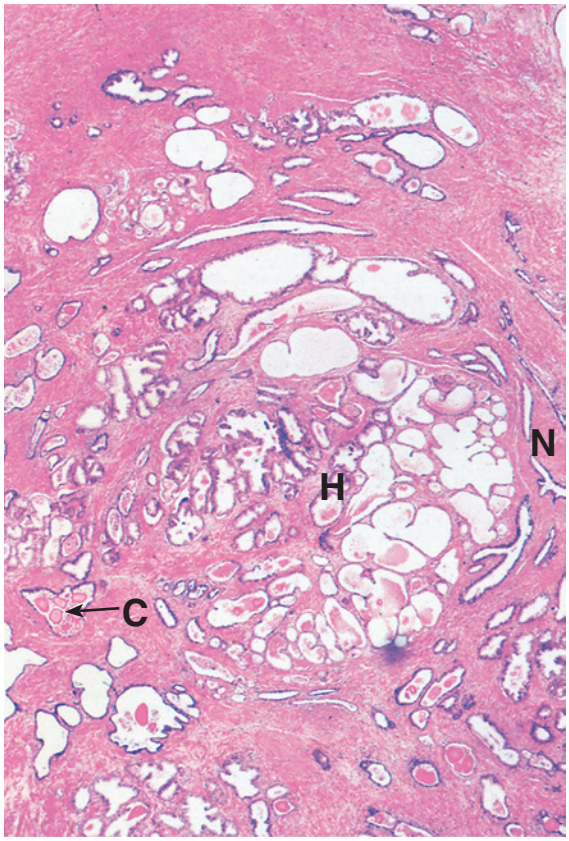


Figure 21–24 Photomicrograph of a patient with benign prostatic hyperplasia. Observe the hyperplastic prostatic tissue (H) compressing the normal tissue (N) at the periphery. Note the presence of corpora amylacea (C) throughout the tissue. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:251.)

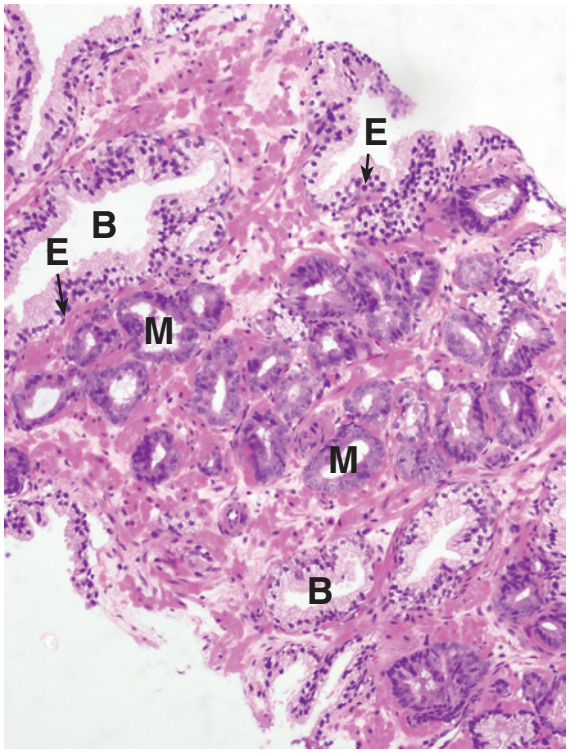


Figure 21–25 Photomicrograph of a patient with prostatic adenocarcinoma. This is a higher grade cancer and is characterized by the presence of malignant glandular tissue (M) interspersed among the benign glandular tissue (B). Note that the prominent basal cell layer (E) present in the benign glands is absent in the malignant tissue. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:252.)

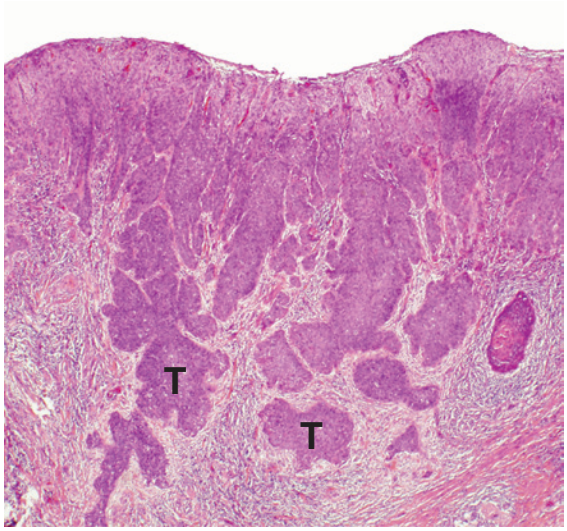
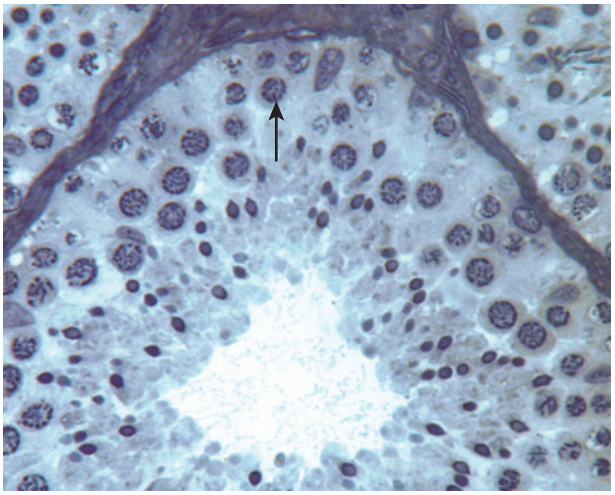
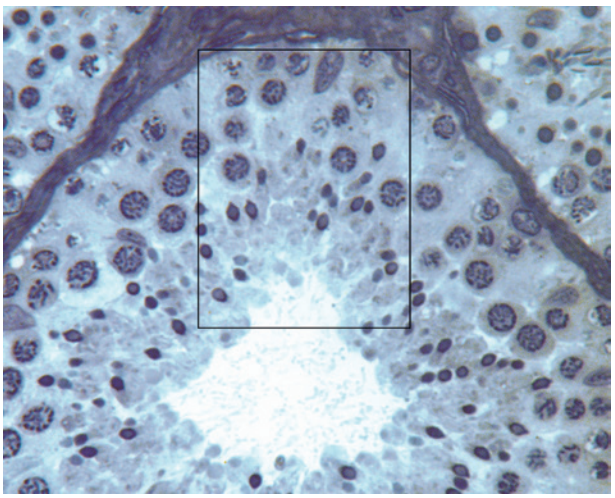


Figure 21–26 Photomicrograph of a patient with squamous cell carcinoma of the glans penis. Note that this keratinizing squamous cell carcinoma invaded the underlying connective tissue, thus presenting with islands of tumor cells (T) deep in the glans penis. (Reprinted with permission from Young B, et al. *Wheater's Basic Pathology*. 5th ed. Philadelphia: Elsevier; 2010:253.)

REVIEW QUESTIONS

1. A patient with Klinefelter syndrome has which of the following chromosomal complement?
   a. XY
   b. XXY
   c. XYY
   d. XXX
   e. X0
2. Which of the following cells manufacture androgen binding protein (ABP)?
   a. Spermatid
   b. Sertoli cell
   c. Peg cell
   d. Interstitial cell of Leydig
   e. Theca lutein cell
3. Cells that are responsible for the blood–testis barrier are known as
   a. Leydig cells.
   b. Sertoli cells.
   c. interstitial cells.
   d. granulosa cells.
   e. spermatogonia.
4. Corpora amylacea are located in which of the following?
   a. Bulbourethral glands
   b. Seminal vesicles
   c. Seminiferous tubules
   d. Prostate gland
   e. Ductus deferens
5. Which of the following cells is located in the adluminal compartment of the seminiferous tubule?
   a. Pale A spermatogonia A
   b. Dark A spermatogonia
   c. Spermatogonia B
   d. Spermatids
   e. Primary spermatocytes
6. Which of the following cells is located **only** in the basal compartment of the seminiferous tubule?
   a. Pale A spermatogonia A
   b. Primary spermatocytes
   c. Secondary spermatocytes
   d. Spermatids
   e. Sertoli cells
7. Which of the following cells manufacture testosterone?
   a. Spermatids
   b. Sertoli cells
   c. Peg cells
   d. Interstitial cells of Leydig
   e. Theca lutein cells
8. 

   Identify the cell at the tip of the arrow.
   a. Spermatogonia A
   b. Spermatogonia B
   c. Primary spermatocyte
   d. Secondary spermatocyte
   e. Spermatid
9. 

   The area enclosed by the rectangle is known as the
   a. basal compartment.
   b. adluminal compartment.
   c. seminiferous epithelium.
   d. zonula occludens.
   e. zonula adherens.

10. Which of the following statements is true concerning Sertoli cells?
    a. They possess luteinizing hormone receptors.
    b. They possess follicle-stimulating hormone receptors.
    c. They produce androgens.
    d. They possess the enzyme 5α-reductase.
    e. They produce insulin-like factor 3.

ANSWERS

1. B. A patient with Klinefelter syndrome has an extra X chromosome; therefore, his chromosomal complement is XXY. These patients are typically infertile, tall, thin people with some male characteristics (including small testes), and are somewhat challenged mentally. XY is the normal chromosomal complement. XYY patients are tall males and frequently have language deficiencies. XXX patients have the triple X syndrome; they are female patients with amenorrhea and mental deficiencies. XO patients are females who have Turner syndrome; their ovaries never develop, they have underdeveloped breasts, and they are mentally deficient.
2. B. Sertoli cells of the testis manufacture androgen binding protein. Spermatids are first cells of spermiogenesis. Peg cells are present in the oviduct, and they produce a fructose-rich fluid to nourish the early embryo and convey it to the uterus. Interstitial cells of Leydig manufacture androgens. Theca lutein cells are part of the corpus luteum and manufacture progesterone and androgens.
3. B. Sertoli cells of the testis form the blood–testis barrier and subdivide the lumen of the seminiferous tubule into and adluminal and a basal compartment. Leydig cells are outside of the seminiferous tubule and produce testosterone. Interstitial cells are present in the ovary and secrete estrogens. Granulosa cells are in the ovarian follicles. Spermatogonia are stem cells that occupy the basal compartment of the seminiferous tubule and give rise to spermatozoa.
4. D. Corpora amylacea, also known as *prostatic concretions*, are round to oval-calcified glycoproteins that occupy the lumina of the tubuloacinar glands of the prostate. These concretions increase in number with age. The bulbourethral glands, seminal vesicles, seminiferous tubules, and ductus deferens do not possess corpora amylacea.
5. D. Sertoli cells divide the lumen of the seminiferous tubule into two concentric compartments, the peripheral basal compartment and the centrally based adluminal compartment. Spermatids are located in the adluminal compartment, whereas pale A spermatogonia, dark A spermatogonia, spermatogonia B, and primary spermatocytes are all located in the basal compartment.
6. A. Pale A spermatogonia are located in the basal compartment only. Sertoli cells divide the lumen of the seminiferous tubule into two concentric compartments, the peripheral basal compartment and the centrally based adluminal compartment. Spermatids and secondary spermatocytes are located in the adluminal compartment only, whereas Sertoli cells are located in both compartments, and primary spermatocytes migrate from the basal compartment into the adluminal compartment.
7. D. Interstitial cells of Leydig manufacture testosterone. Spermatids are first cells of the spermiogenesis. Peg cells are present in the oviduct, and they produce a fructose-rich fluid to nourish the early embryo and convey it to the uterus. Theca lutein cells are part of the corpus luteum and manufacture progesterone and androgens.
8. C. The cell at the tip of the arrow is a primary spermatocyte, as evidenced by its large nucleus. Spermatogonia A and B are located adjacent the wall of the seminiferous tubule. Secondary spermatocytes are difficult to see because they very quickly finish their second meiotic division to become spermatids, small cells with round to oval nuclei on the left side of the arrow's shaft.
9. C. The area enclosed by the rectangle is known as the *seminiferous epithelium*. Both the basal compartment and the adluminal compartment of the seminiferous tubule are enclosed. Zonula occludens and zonula adherens refer to junctions that cells form with each other.
10. B. Sertoli cells possess FSH receptors, whereas interstitial cells of Leydig possess LH receptors, manufacture androgens and insulin-like factor 3, and possess the enzyme 5α-reductase.

Special Senses

22

Peripheral nerve terminals are of two structural types: (1) terminals of axons, which transmit impulses from the central nervous system (CNS) to skeletal and smooth muscles **(motor endings)** or to glands **(secretory endings)**, and (2) terminals of dendrites, called ***sensory endings*** or ***receptors***, which perceive various stimuli and transmit this sensory input to the CNS. These sensory receptors are classified into three types, depending on the source of the stimulus, and are components of the general or special somatic and visceral afferent pathways:

- Exteroceptors
- Proprioceptors
- Interoceptors

Exteroceptors, located near the body surface, are specialized to perceive stimuli from the external environment. These receptors, sensitive to temperature, touch, pressure, and pain, are components of the **general somatic afferent** pathways and are described in the first part of this chapter. Other exteroceptors, specialized for perceiving light (sense of vision) and sound (sense of hearing), are components of the **special somatic afferent** pathways (discussed later). Smell and taste stimuli are perceived by unique nerve endings in the viscera of the respiratory and digestive systems, respectively; these exteroceptors are classified as the **special visceral afferent** modality. Receptors for olfaction (sense of smell) are discussed in Chapter 15, and receptors of taste are discussed in Chapter 16.

Proprioceptors are specialized receptors located in joint capsules, tendons, and intrafusal fibers within muscles (see Chapter 8). These **general somatic afferent** receptors transmit sensory input to the CNS, which is translated into information that relates to an awareness of the body in space and in movement. Certain receptors of the **vestibular (balance) mechanism** (see later text), located within the inner ear, are specialized for receiving stimuli related to motion vectors within the head; this input is transmitted to the brain for processing into awareness of motion for corrective balancing.

Interoceptors are specialized receptors that perceive sensory information from within organs of the body; therefore, the modality serving this function is **general visceral afferent**.

SPECIALIZED PERIPHERAL RECEPTORS

Certain peripheral receptors, specialized to receive particular stimuli, include mechanoreceptors, thermoreceptors, and nociceptors.

The dendritic endings of certain sensory receptors, located in various regions of the body, including muscles, tendons, skin, fascia, and joint capsules, are specialized to receive particular stimuli. These adaptations help the dendrite respond to a particular stimulus. Thus, these receptors are classified into three types:

- **Mechanoreceptors**, which respond to touch (Figs. 22–1 to 22–3)
- **Thermoreceptors**, which respond to cold and warmth
- **Nociceptors (pain receptors)** and **pruriceptors (itch receptors)**, where the former respond to pain due to mechanical stress, extremes of temperature differences, and chemical substances, the latter respond to itch sensations

Although these specialized receptors generally are triggered only by a particular stimulus, any stimulus that is intense enough can trigger any receptor.

Mechanoreceptors

Mechanoreceptors respond to mechanical stimuli that may in some fashion alter the morphology either of the receptor or the tissues surrounding the receptor. Stimuli that trigger the mechanoreceptors are touch, stretch, vibrations, and pressure.

Nonencapsulated Mechanoreceptors

Nonencapsulated mechanoreceptors are simple unmyelinated receptors present in the skin, connective tissues, and surrounding hair follicles.

Peritrichial nerve endings, the simplest form of mechanoreceptors, are unmyelinated, lack Schwann cells, and are not covered by a connective tissue capsule. Such nerve endings are located in the epidermis of skin, especially in regions of great sensitivity, such as the face and the cornea of the eye, where they respond to stimuli

SUMMARY

The **special senses** are composed of **exteroceptors**, which are specialized to react to stimuli in the **external environment**, and these include **temperature**, **touch**, **pressure**, and **pain** as well as to **light** and **sound**; **proprioceptors**, which react to the body's **spatial orientation** as well as to **balance** and the **vectorial motion** of the head with respect to the rest of the body; and **interoceptors**, which react to sensory information arising within the **body organs**. The perception of light is relegated to the **retina** of the **eyes**, the reception of sound is relegated to the **cochlear apparatus of the ears**, and the perception of balance and vectorial motion belongs to the **vestibular apparatus of the ears**.

KEY WORDS

Balance; cochlear apparatus; ear; exteroceptors; eyes; interoceptors; light; pain; pressure; proprioceptors; retina; sound; special orientation; special senses; temperature; touch; vectorial motion; vestibular apparatus

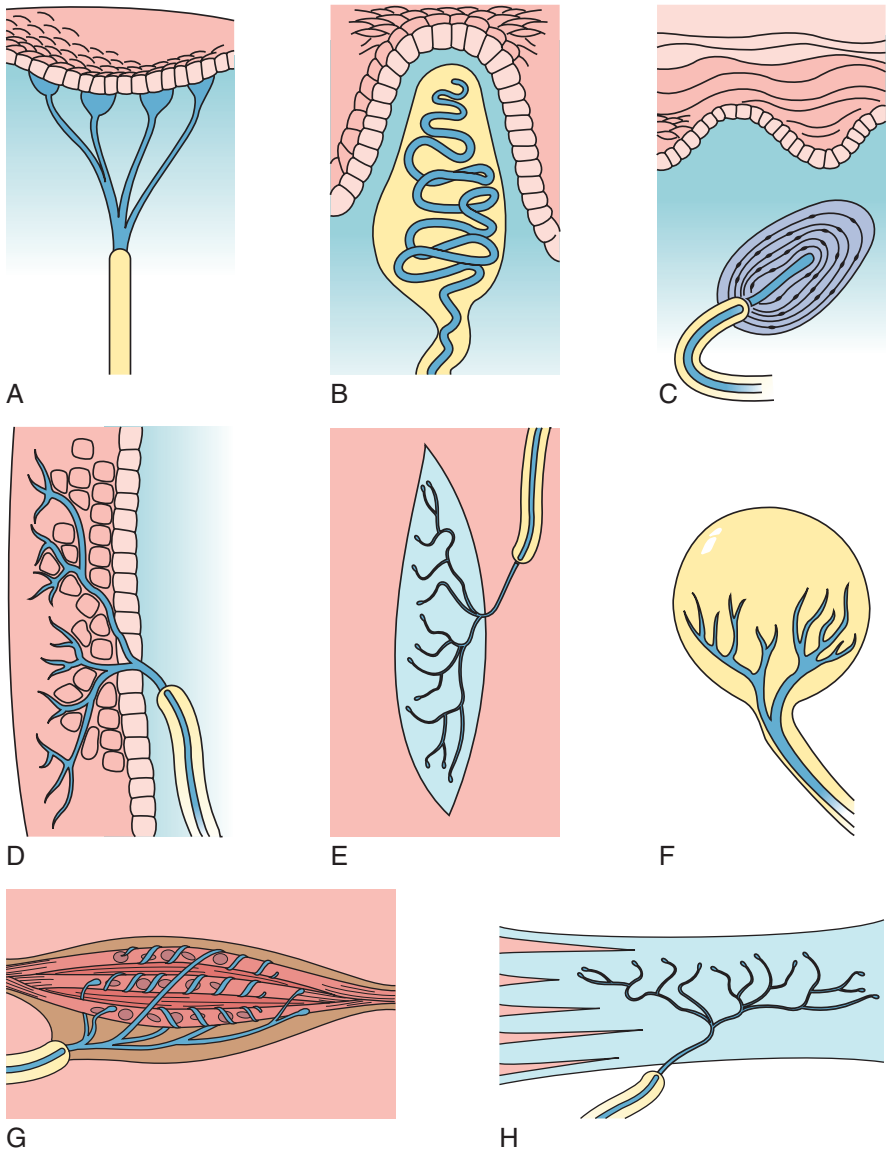


Figure 22–1 Diagram of various sensory receptors. **A,** Merkel disk. **B,** Meissner corpuscle. **C,** Pacinian corpuscle. **D,** Peritricial (naked) nerve endings. **E,** Ruffini corpuscle. **F,** Krause end bulb. **G,** Muscle spindle. **H,** Golgi tendon organ.

related to touch and pressure (see Fig. 22–1D). Additionally, peritrichial nerve endings are wrapped around the base and shaft of hair follicles and function in touch perception related to the deformation of the hairs. Moreover, some naked nerve endings function as nociceptors or as thermoreceptors.

Merkel disks are slightly more complex mechanoreceptors (see Fig. 22–1A). Specialized for perceiving discriminatory touch, these receptors are composed of an expanded unmyelinated nerve terminal associated with **Merkel cells**, specialized epithelial cells interspersed with keratinocytes in the stratum basale of the skin (see Fig. 14–1). These receptors are located mostly in nonhairy skin and regions of the body more sensitive to touch.

Encapsulated Mechanoreceptors

Encapsulated mechanoreceptors exhibit characteristic structures and are present in specific locations.

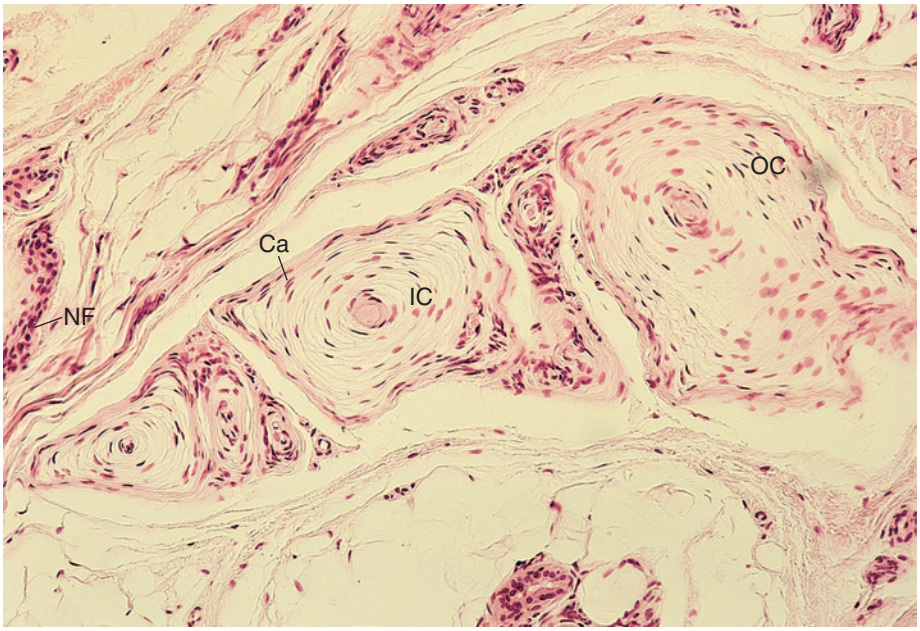


Figure 22–2 Pacinian corpuscles. Inner core (IC), outer core (OC), nerve fiber (NF), capsule (Ca) (×132).

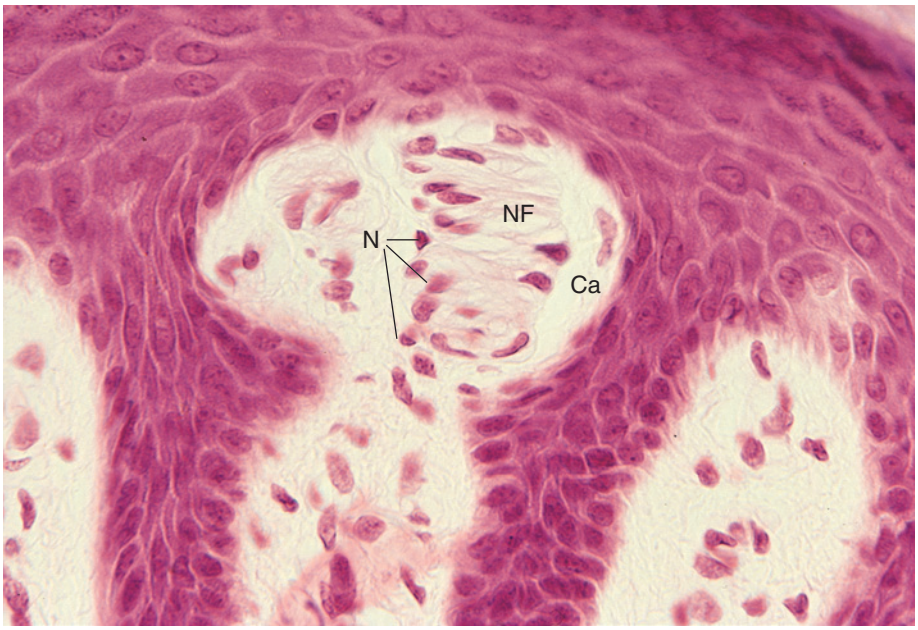


Figure 22–3 Meissner corpuscle. Nuclei (N), capsule (Ca), nerve fibers (NF) (×540).

Meissner corpuscles (see Fig. 22–3) are encapsulated mechanoreceptors specialized for **tactile discrimination**. These receptors are located in the dermal papillae of the glabrous (nonhairy) portion of the fingers and palms of the hands, where they account for about half of the tactile receptors. They also are located in the eyelids, lips, tongue, nipples, skin of the feet, and forearms. Meissner corpuscles, which measure 80 μm × 30 μm, are located in the dermal papillae with their long axes oriented perpendicular to the skin surface (see Fig. 22–1B). Each Meissner corpuscle is formed by three or four nerve terminals and their associated

Schwann cells, all of which are encapsulated by connective tissue. Contained within the capsule are stacks of epithelioid cells, possibly modified Schwann cells or fibroblasts, that serve to separate the branching nerve terminals. Meissner corpuscles respond to **fine touch** and are especially sensitive to **edges** and **points** and to **movements** of these objects.

Pacinian corpuscles, another example of the encapsulated mechanoreceptors, are located in the dermis and hypodermis in the digits of the hands and in the breasts as well as in connective tissue of the joints, periosteum, and the mesentery. These mechanoreceptors are specialized to perceive **pressure**, **touch**, and **vibration**. Pacinian corpuscles are large, ovoid receptors 1 to 2 mm long by 0.1 to 0.7 mm in diameter (see Fig. 22–1C and Fig. 22–2). Each receptor is composed of a single unmyelinated fiber that courses the entire length of the corpuscle. The **core** of the corpuscle contains the nonmyelinated nerve terminal and its Schwann cells, surrounded by approximately 60 layers of modified fibroblasts, each layer separated from the next by a small fluid-filled space. An additional group of 30 less dense modified fibroblasts surround the core and are, in turn, enveloped by connective tissue, forming the **capsule** around the core. The arrangement of the cells in the lamellae makes the histological section of a pacinian corpuscle resemble a sliced onion.

Ruffini endings (corpuscles) are encapsulated endings located in the dermis of the skin, nail beds, periodontal ligaments, and joint capsules. These large receptors, 1 mm long by 0.2 mm in diameter (see Fig. 22–1E), are composed of branched nonmyelinated terminals interspersed with collagen fibers and surrounded by four to five layers of modified fibroblasts. The connective tissue capsule surrounding each of these receptors is anchored at each end, increasing their sensibility to **stretching** and **pressure** in the skin and in the joint capsules.

Krause end bulbs are spherical, encapsulated nerve endings located in the papillary region of the dermis, joints, conjunctiva, peritoneum, genital regions, and subendothelial connective tissues of the oral and nasal cavities] (see Fig. 22–1F). Originally, they were thought to be receptors sensitive to cold, but present evidence does not support this concept. Their function is unknown.

Both **muscle spindles** and **Golgi tendon organs** are encapsulated mechanoreceptors involved in proprioception. **Muscle spindles** (see Fig. 22–1G) provide feedback concerning the changes in muscle length as well as the rate of alteration in the length of the muscle, and **Golgi tendon organs** (see Fig. 22–1H) monitor the tension as well as the rate at which the tension is being produced during movement. Information from these two sensory structures is processed mostly at the unconscious levels within the spinal cord; however, the information also reaches the cerebellum and even the cerebral cortex, so that the individual may sense muscle position. Golgi tendon organs and muscle spindles are discussed in Chapter 8.

Thermoreceptors

Thermoreceptors, which respond to temperature differences of about 2°C, are of three types: warmth receptors, cold receptors, and temperature-sensitive nociceptors.

Although specific receptors have not been identified for warmth, it is assumed that these receptors are naked endings of small nonmyelinated nerve fibers that respond to temperature increases (greater than 43°C). It has been reported recently that ion channels, known as ***transient receptor V1 channels*** (also known as ***capsaicin receptors***) open when the temperature of the tissue in their immediate vicinity rises to higher than 43°C or when substances such as capsaicin or acids are encountered and Na^+ ions can enter the cell, depolarizing it. The information relayed to the CNS is interpreted as burning pain. Cold receptors are derived from naked nerve endings of myelinated fibers that branch and penetrate the epidermis and respond to temperatures lower than 30°C. Because thermoreceptors are not activated by physical stimulation, they are believed to respond to differing rates of temperature-dependent biochemical reactions.

Nociceptors and Pruriceptors

Nociceptors are receptors sensitive to pain caused by mechanical stress, extremes of temperature, and cytokines, such as bradykinin, serotonin, and histamine.

Nociceptors are responsible for pain perception. These receptors are naked endings of myelinated nerve fibers that branch freely in the dermis before entering the epidermis. Nociceptors are divided into three groups: (1) those that respond to mechanical stress or damage; (2) those that respond to extremes in heat or cold; and (3) those that respond to chemical compounds such as bradykinin, serotonin, and histamine.

Nociception is dependent on opening of voltage-gated Na^+ ion channels. There are nine types of known voltage-gated Na^+ ion channels, and each type opens at slightly different voltages. Three of the nine channels react to painful stimuli, and these are known as $Na_V1.7$, $Na_V1.8$, $Na_V1.9$, where Na_V = voltage-gated sodium ion channel and 1.7 is the seventh; 1.8, the eighth; and 1.9, the ninth voltage-gated sodium ion channel.

Itching (pruriception) is a somatic sensation that may be due to local causes such as a tiny insect walking on one's arm or to generalized and systemic causes such as a form of dermatitis or even to cancer and organ failure. The mechanism of an itch is better known in

CLINICAL CORRELATIONS

Chronic neuropathic pain (usually in the hands and feet) is one of the most common illnesses in the world, affecting a large segment of the U.S. population. Many individuals with this condition present with mutations in the nociceptors ion channels (especially $Na_V1.7$) so that these ion channels are hyperactive and open even during conditions that would not be painful under normal circumstances. Normally, the resulting information is transmitted to the central nervous system (CNS), where interneurons intercept the information and inhibit its transmission (postsynaptic inhibition). The inhibition is due to the presence of a **chloride transporter** in the interneuron plasmalemma (also present in other neuron plasma membranes), known as ***potassium/chloride co-transporter 2 (KCC2)***. In a normal individual, when pain is detected, **microglia** of the spinal cord release a signaling molecule that acts on interneurons to reduce their KCC2 transporters, and the interneuron can no longer inhibit the pain signal from being transmitted to higher levels in the CNS. In patients with neuropathic pain, what normally would be a nonpainful stimulus will cause the diminution of KCC2 levels in the interneurons, and the stimulus, instead of being blocked by the interneuron, is permitted to be transmitted, erroneously resulting in pain sensations.

mice than in humans, but it is assumed that there may be a close correlation in the two species. It has been demonstrated in mice that itching sensation is conveyed by C fibers to the murine spinal cord where secondary neurons reside that are capable of expressing gastrin-releasing peptide receptors (GRPR). Apparently there are a number of GRPR types of neurons and they are activated by various types of G protein–coupled receptors that respond to a particular cause of the itching sensation. Moreover, there seems to be a relationship between pain and itching sensations because they share similar but not identical pathways, but they both involve receptors in the skin, spinal cord, and brain.

EYE

The bulb of the eye is composed of three tunics: fibrous, vascular, and neural.

The **eyes (eyeballs)**, approximately 24 mm in diameter, are located within the hollow bony orbits of the skull. They are the **photosensory organs** of the body. Light passes through the cornea, lens, and several refractory structures of the eyeballs; light is then focused by the lens on the light-sensitive portion of the neural tunic of the eye, the **retina**, which contains the photosensitive **rods** and **cones**. Through a series of several layers of nerve cells and supporting cells, the visual information is transmitted by the optic nerve to the brain for processing.

The eyes begin to develop from three different sources at about the fourth week of embryogenesis. Outgrowths of the forebrain, the future retina and optic nerve, are the first to be observed. As a result of continued growth of this structure, the surface ectoderm is induced to develop into the lens and some of the accessory structures of the anterior portion of the eye. Later in development, adjacent mesenchyme condenses to form the tunics and associated structures of the eyeballs.

The eye is composed of three tunics (coats) (Fig. 22–4):

- **Fibrous tunic**. Forming the tough outer coat of the eye
- **Vascular tunic**. Pigmented and vascular middle coat
- **Neural tunic**. **Retina**, composing the innermost coat

The fibrous tunic of the eye also receives insertions of the **extrinsic muscles** of the eye, which are responsible for coordinated movements of the eyes to gain access to various visual fields. Smooth muscles located within the eyeball accommodate focusing of the lens and control the aperture of the pupil. Located outside the eyeball, but still within the orbit, is the **lacrimal gland** (tear gland), which secretes **lacrimal fluid** (tears) that moistens the anterior surface of the eye. The lacrimal fluid moistens the eye and the inner surface of the eyelids by passing through the **conjunctiva**, a transparent membrane that covers and protects the anterior surface of the eye.

Tunica Fibrosa

The tunica fibrosa is composed of the sclera and the cornea.

The external fibrous tunic of the eye, the **tunica fibrosa**, is divided into the **sclera** and the **cornea** (see Fig. 22–4). The white, opaque **sclera** covers the posterior five sixths of the eyeball, whereas the colorless, transparent **cornea** covers the anterior one sixth of the eyeball.

Sclera

The white opaque sclera is composed of type I collagen fibers interlaced with elastic fibers.

The **sclera**, the white of the eye, is nearly devoid of blood vessels. It is a tough fibrous connective tissue layer that covers approximately 85% of the eyeball. It is about 1 mm thick posteriorly, thinning at the equator and then thickening again near its junction with the

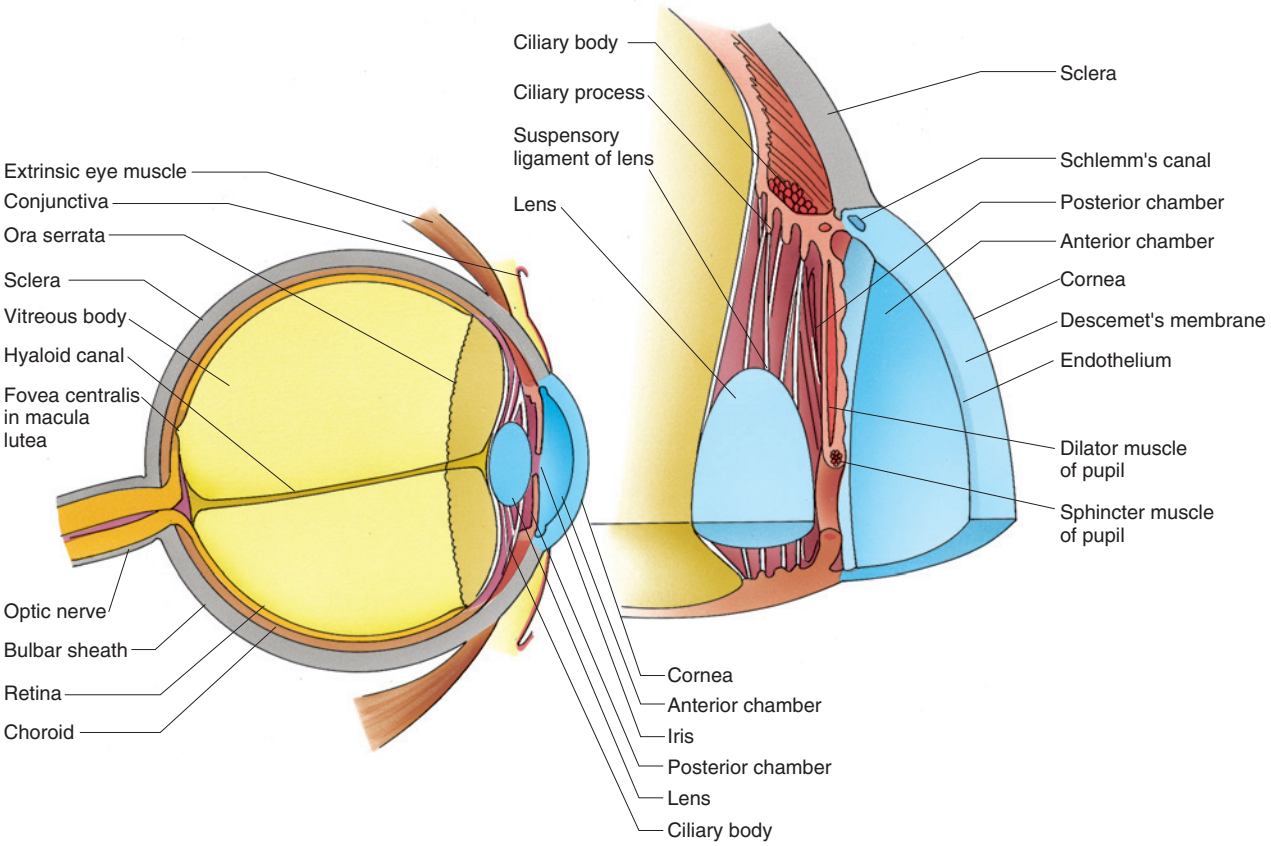


Figure 22–4 Diagram of the anatomy of the eye (orb).

cornea. It consists of interlacing type I collagen fiber bundles alternating with networks of elastic fibers; this arrangement gives form to the eyeball, which is maintained by intraocular pressure from the aqueous humor (located anterior to the lens) and the vitreous body (located posterior to the lens).

Fibroblasts of the sclera are elongated, flat cells. Melanocytes are located in deeper regions of the sclera. Tendons of the extraocular muscles insert into the dense connective tissue surface layer of the sclera, which is enveloped by the **capsule of Tenon**, a fascial sheath that covers the optic nerve and the eyeball as far anteriorly as the ciliary region. This sheath, which separates the eye from the periorbital fat, is connected to the sclera by a thin layer of loose connective tissue called the ***episclera***. The eyeball, along with its various parts and attached extraocular muscles, moves in unison within the periorbital fat–filled bony orbit.

Cornea

The cornea is the transparent bulging anterior one-sixth of the eyeball.

The **cornea** is the transparent, avascular, and highly innervated anterior portion of the fibrous tunic that bulges out anteriorly from the eyeball. It is slightly thicker than the sclera and is composed of six histologically distinct layers:

- Corneal epithelium
- Bowman membrane
- Stroma
- Pre-Descemet layer (Dua layer)
- Descemet membrane
- Corneal endothelium

The **corneal epithelium**, the continuation of the conjunctiva (a mucous membrane covering the anterior sclera and lining the internal surface of the eyelids), is a stratified, squamous, nonkeratinized epithelium, composed of five to seven layers of cells, that covers the anterior surface of the cornea. The larger superficial cells have microvilli and exhibit zonulae occludentes. The remaining cells constituting the corneal epithelium interdigitate with and form desmosomal contacts with one another. Their cytoplasm contains the usual array of organelles along with intermediate filaments. The corneal epithelium is highly innervated by numerous

free nerve endings. Mitotic figures are observed mostly near the periphery of the cornea with a turnover rate of approximately 7 days. Damage to the cornea is repaired rapidly as cells migrate to the defect to cover the injured region. Subsequently, mitotic activity replaces the cells that migrated to the wound. The corneal epithelium also functions in transferring water and ions from the stroma into the conjunctival sac.

Bowman membrane lies immediately deep to the corneal epithelium. Electron micrographs reveal it to be a fibrillar lamina, 6 to 30 µm thick, composed of type I collagen fibers arranged in an apparently random fashion. It is believed that Bowman membrane is synthesized by both the corneal epithelium and cells of the underlying stroma. Sensory nerve fibers pass through this structure to enter and terminate in the epithelium.

The transparent **stroma** is the thickest layer of the cornea, constituting about 90% of its thickness. It is composed of collagenous connective tissue, consisting mostly of type I collagen fibers that are arranged in 200 to 250 lamellae, each about 2 µm in thickness. The collagen fibers within each lamella are arranged parallel to one another, but fiber orientation shifts in adjacent lamellae. The collagen fibers are interspersed with thin elastic fibers, embedded in ground substance containing mostly chondroitin sulfate and keratan sulfate. Long, slender fibroblasts are also present among the collagen fiber bundles. During inflammation, lymphocytes and neutrophils are also present in the stroma. At the **limbus** (sclerocorneal junction) is a scleral sulcus whose inner aspect at the stroma is depressed and houses endothelium-lined spaces, known as the ***trabecular meshwork***, that lead to the canal of Schlemm. The **canal of Schlemm** is the site of outflow of the aqueous humor from the anterior chamber of the eye into the venous system.

Pre-Descemet layer (Dua layer) is a 15-µm, thin, tough collagenous membrane that is probably manufactured by the fibroblasts of the stroma. It has been suggested that the durability of this recently discovered layer protects the cornea from damage, whereas physical injury that breaches the integrity of pre-Descemet layer may result in **corneal hydrops** (infiltration of an aqueous fluid into the corneal stroma).

Descemet membrane is a thick basement membrane interposed between the stroma and the underlying endothelium. Although this membrane is thin (5 µm at birth) and homogeneous in younger persons, electron microscopy has demonstrated that it becomes thicker (17 µm) and has cross-striations and hexagonal fiber patterns in older adults.

The **corneal endothelium**, which lines the internal (posterior) surface of the cornea, is a **simple squamous epithelium**. It is responsible for synthesis of proteins that are necessary for secreting and maintaining Descemet membrane. These cells exhibit numerous pinocytotic vesicles, and their membranes have sodium pumps that transport sodium ions (Na^+) into the anterior chamber; these ions are passively followed by chloride ions (Cl^-) and water. Thus, excess fluid within the stroma is resorbed by the endothelium, keeping the stroma relatively dehydrated, a factor that contributes to maintaining the refractive quality of the cornea.

Tunica Vasculosa

The vascular middle tunic of the eye, the **tunica vasculosa (uvea)**, is composed of three parts: (1) the **choroid**, (2) the **ciliary body**, and (3) the **iris** (see Fig. 22–4).

Choroid

The choroid, the pigmented posterior portion of the middle vascular tunic, is loosely attached to the sclera and separated from the retina by Bruch membrane.

The **choroid**, the well-vascularized, pigmented layer of the posterior wall of the eyeball, is loosely attached to the tunica fibrosa. It is composed of loose connective tissue containing numerous fibroblasts and other connective tissue cells, and it is richly supplied with blood vessels. The black color of the choroid is due to the myriad of melanocytes present in it. Because of the abundance of small blood vessels in the inner surface of the choroid, that region is known as the ***choriocapillary layer*** and is responsible for providing nutrients to the retina. The choroid is separated from the retina by **Bruch membrane**, a 1- to 4-µm-thick membrane composed of a network of elastic fibers located in the central region and sandwiched on both sides by collagen fiber layers. The outer aspect of each collagen fiber layer is covered by a basal lamina that belongs to capillaries on one side and the **pigment epithelium** of the retina on the other side.

Ciliary Body

The ciliary body is a wedge-shaped portion of the choroid located in the lumen of the orb between the iris and the vitreous body and projecting toward the lens.

The **ciliary body**, the wedge-shaped extension of the choroid that rings the inner wall of the eye at the level of the lens, occupies the space between the ora serrata of the retina (the junction between and anterior and posterior portions of the retina) and the iris. One surface of the ciliary body abuts the sclera at the sclerocorneal junction, another surface abuts the vitreous body, whereas the medial surface projects toward the lens, forming short, finger-like projections known as the ***ciliary processes***.

The ciliary body is composed of loose connective tissue containing numerous elastic fibers, blood vessels,

and melanocytes. Its inner surface is lined by the **pars ciliaris of the retina**, a pigmented layer of the retina that is composed of two cell layers. The outer cell layer, which faces the lumen of the eyeball, is a nonpigmented columnar epithelium **(nonpigmented ciliary epithelium)**, whereas the inner cell layer is composed of a pigmented simple columnar epithelium **(pigmented ciliary epithelium)**, which is rich in melanin pigments.

The anterior third of the ciliary body has about 70 **ciliary processes**, which radiate out from a central core of connective tissue containing abundant fenestrated capillaries. Fibers, composed of fibrillin **(zonule fibers)**, radiate from the ciliary processes to insert into the lens capsule, forming the **suspensory ligaments of the lens**, which suspend the lens in place.

The ciliary processes are covered by the same two layers of epithelia that cover the ciliary body. The inner nonpigmented layer has many interdigitations and infoldings; its cells transport a protein-poor plasma filtrate, the **aqueous humor**, into the posterior chamber of the eye. The aqueous humor flows from the posterior chamber into the anterior chamber by passing through the **pupillary aperture** between the iris and the lens. The aqueous humor exits the anterior chamber by passing into the trabecular meshwork near the limbus and, finally, as stated above, into the canal of Schlemm, which leads directly into the venous system. The aqueous humor provides nutrients and oxygen for the lens and the cornea.

The bulk of the ciliary body is composed of three bundles of smooth muscle cells called the ***ciliary muscle***. One bundle, because of its orientation, stretches the choroid, thus altering the opening of the canal of Schlemm for drainage of the aqueous humor. The remaining two muscle bundles, attached at the scleral spur (the junction point of the sclera, cornea, and the ciliary body, just peripheral to the attachment of the iris), function in reducing tension on the zonulae. Contractions of this muscle, mediated by parasympathetic fibers of the oculomotor nerve (cranial nerve [CN] III), stretch the choroid body, thereby releasing tension on the suspensory ligaments of the lens. As a result, the lens becomes thicker and more convex. This action permits focusing on nearby objects, a process called ***accommodation***. Relaxation of all three muscle bundles increases tension on the zonule thereby flattening the lens enabling focusing on distant objects. Constant adjustments between various degrees of contraction and relaxation are required to permit focusing on objects among those that are distant, intermediate, and close up.

Iris

The iris, the colored anterior extension of the choroid, is a contractile diaphragm that controls the diameter of the pupillary aperture.

CLINICAL CORRELATIONS

Glaucoma is a condition resulting from prolonged increased intraocular pressure caused by the failure of drainage of the aqueous humor from the anterior chamber of the eye. It is one of the world's leading causes of blindness. In **chronic glaucoma**, the most common condition, the continued increasing pressure causes progressive damage to the eye, particularly to the retina; if left untreated, blindness results.

The **iris**, the anteriormost extension of the choroid, lies between the posterior and anterior chambers of the eye, completely covering the lens except at the **pupillary aperture (pupil)**. The iris is thickest in the middle, thinning both toward its junction with the ciliary body and at the rim of the pupil. The anterior surface consists of two concentric rings: the **pupillary zone**, lying nearest the pupil, and the wider **ciliary zone**. The anterior surface of the iris is irregular, with trenches extending into it; it also contains contraction furrows, which are easily distinguished when the pupil is dilated. An incomplete layer of pigmented cells and fibroblasts covers the anterior surface of the iris. Deep to this layer is a stroma of poorly vascularized connective tissue containing numerous fibroblasts and melanocytes, which gives way to a well-vascularized, loose connective tissue zone.

The posterior surface of the iris is smooth and is covered by the continuation of the two layers of retinal epithelium that cover the ciliary body. The surface facing the lens is composed of heavily pigmented cells, which block the light from passing through the iris except at the pupil. The epithelial cells facing the stroma of the iris have extensions that form the **dilator pupillae muscle**. Hence, this muscle is myoepithelial in nature. Another muscle, the **sphincter pupillae muscle**, is located in a concentric ring around the pupil. Contractions of these smooth muscles alter the diameter of the pupil. The diameter of the pupil changes inversely with the amount of light entering it. Thus, bright light causes constriction of pupillary diameter, whereas dim light dilates it. Pupil diameter is the result of the functions of the two intrinsic muscles contained within the iris. As their names imply, the dilator pupillae muscle, innervated by the sympathetic nervous system, dilates the pupil; whereas the sphincter pupillae muscle, innervated by parasympathetic fibers of the oculomotor nerve (CN III), constricts the pupil.

The abundant population of melanocytes in the epithelium and stroma of the iris not only blocks the passage of light into the eye (except at the pupil) but also imparts color to the eyes. The eyes are dark when the number of melanocytes is high, and they are blue when the number of melanocytes is low. Approximately

6000 to 10,000 years ago every person had brown eyes, but a mutation in the *HERC2* gene that appeared in a single female was transmitted down the generations. The mutation in this gene affects a neighboring gene, known as *OCA2*, which is responsible for the formation of melanin in the iris. The final result is that the pigment cells of the iris produce less melanin than in irises without the mutation; consequently, the irises of these individuals are blue.

Lens

The lens, the transparent biconvex disk located directly behind the pupil, focuses light rays on the retina.

The lens of the eye is a flexible, biconvex, transparent disk composed of epithelial cells and their secretory products. The lens consists of three parts: lens capsule, subcapsular epithelium, and lens fibers (see Fig. 22–4).

The **lens capsule** is a basal lamina, 10 to 20 µm thick, containing mostly type IV collagen and glycoprotein that covers the epithelial cells and envelops the entire lens. This elastic, transparent, homogeneous structure, which refracts light, is thickest anteriorly.

The **subcapsular epithelium** is located only on the anterior and lateral surfaces of the lens, immediately deep to the lens capsule (Fig. 22–5). It is composed of a single layer of cuboidal cells, which communicate with each other via gap junctions. The apices of these cells are directed toward the lens fibers and interdigitate with them, especially in the vicinity of the equator, where they are elongated and are columnar in shape.

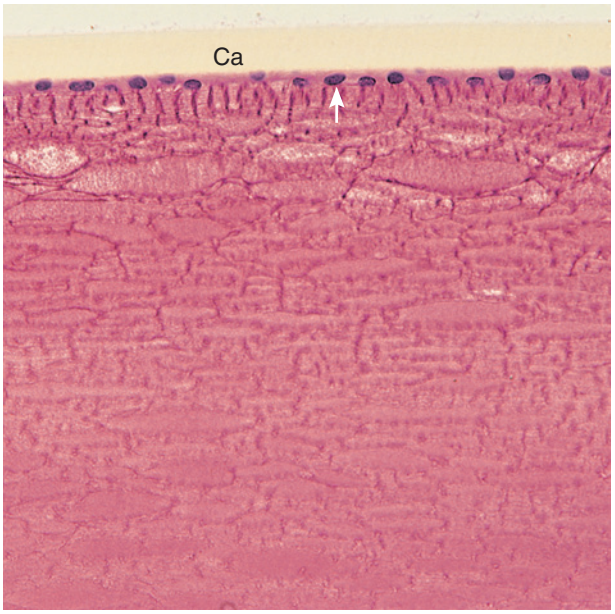


Figure 22–5 Light micrograph of the lens (×132). Note the simple cuboidal epithelium *(arrow)* on the anterior surface and the capsule (Ca) covering the epithelium.

The bulk of the lens is composed of approximately 2000 long cells known as ***lens fibers***. These cells lie immediately deep to the subcapsular epithelium and lens capsule (Fig. 22–6). The cells of the subcapsular epithelium give rise to these highly differentiated, hexagonal cells, the lens fibers, which lose their nuclei and organelles and continue elongating until they reach a length of 7 to 10 µm. This process of elongation, known as ***maturation***, continues throughout the life of the individual. Eventually, these long, hexagonal cells become filled with **crystallins**, which are lens proteins whose presence increases the refractory index of the lens fibers.

As indicated above, the lens maintains its location in the eyeball by the presence of the suspensory ligaments that are attached to the ciliary muscles. When these muscles are in the contracted state, there is little tension on the ligaments, and the lens is more convex so that near vision is sharper; whereas, during the relaxation of the ciliary muscles, the tension on the ligaments is increased, making the lens flatter and distant vision is sharp.

CLINICAL CORRELATIONS

Presbyopia is the inability of the eye to focus on near objects (accommodation) and is caused by an age-related decrease in the elasticity of the lens. As a result, the lens cannot become spherical for exact focusing. This condition can be corrected with eyeglasses.

Cataract is usually also an age-related condition in which the lens becomes opaque, thus impairing vision. This condition may be due to an accumulation of pigment or other substances as well as to excessive exposure to ultraviolet radiation. Although cataract does not usually respond to medication and eventually leads to blindness, the opaque lens may be excised and replaced with a corrective artificial lens.

Vitreous Body

The **vitreous body** is a transparent, refractile gel that fills the cavity of the eye **(vitreous cavity)** behind the lens. It is composed mostly (99%) of water containing a minute amount of electrolytes, collagen fibers, and hyaluronic acid. It adheres to the retina over its entire surface, especially at the ora serrata. Occasional macrophages and small cells called ***hyalocytes*** are observed at the periphery of the vitreous body; these are believed to synthesize collagen and hyaluronic acid. The fluid-filled **hyaloid canal**, a narrow channel that was occupied by the hyaloid artery in the fetus, extends through the entire vitreous body from the posterior aspect of the lens to the optic disk.

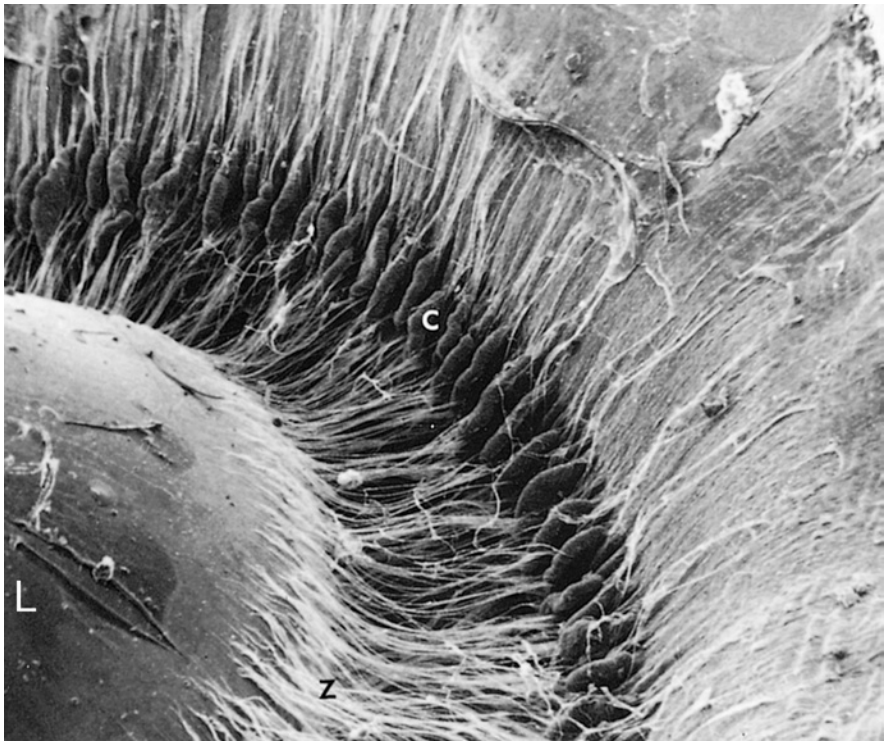


Figure 22–6 Scanning electron micrograph of the posterior surface of the lens (×28). C, Ciliary body; L, lens; Z, zonula fibers. (From Leeson TS, Leeson CR, Paparo AA. *Text/Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

CLINICAL CORRELATIONS

Eye floaters (vitreous opacities) specks, clouds, cob webs, etc., that individuals appear to see out in front of their eyes represent small debris that is floating in the vitreous body caused by its dehydration. These objects cast shadows on the retina that are translated by the brain as images in front of the eyes. Although most of the time these floaters will naturally resolve, some persons find that their lives are disrupted with the presence of floaters, especially when they are reading or driving. Specialized laser treatments can obliterate the floaters.

Retina (Neural Tunic)

The retina, composed of 10 layers, possesses specialized receptor cells, called rods *and* cones*, that are responsible for photoreception.*

The **retina**, the third and innermost tunic of the eye, is its neural portion, which contains the photoreceptor cells, known as *rods and cones* (Figs. 22–7 to 22–9; see Fig. 22–4). The rods are slender, about 2 to 5 µm in diameter, whereas the cones are wider, about 5 to 8 µm in diameter. The retina develops from the optic cup, an evagination of the diencephalon, which gives rise to the primary optic vesicle. Later in development, this structure invaginates to form a bilaminar secondary optic vesicle from which the retina develops, whereas the stalk of the optic cup becomes the optic nerve.

The retina is formed of an outer **pigmented layer** that develops from the outer wall of the optic cup. The neural portion of the retina develops from the inner layer of the optic cup and is called the ***retina proper***. The pigmented layer of the retina covers the entire internal surface of the eyeball, reflecting over the ciliary body and the posterior wall of the iris, whereas the retina proper stops at the ora serrata. The cells composing the retina proper constitute a highly differentiated extension of the brain.

The **optic disk**, located on the posterior wall of the eyeball, is the exit site of the optic nerve. Because it contains no photoreceptor cells, it is insensitive to light and is therefore called the ***blind spot*** of the retina. Approximately 2.5 mm lateral to the **optic disk** is a yellow-pigmented zone in the retinal wall called the ***macula lutea*** *(yellow spot)*. Located in the center of this spot is an oval depression, the **fovea centralis**, where visual acuity is greatest (see Fig. 22–4). The fovea

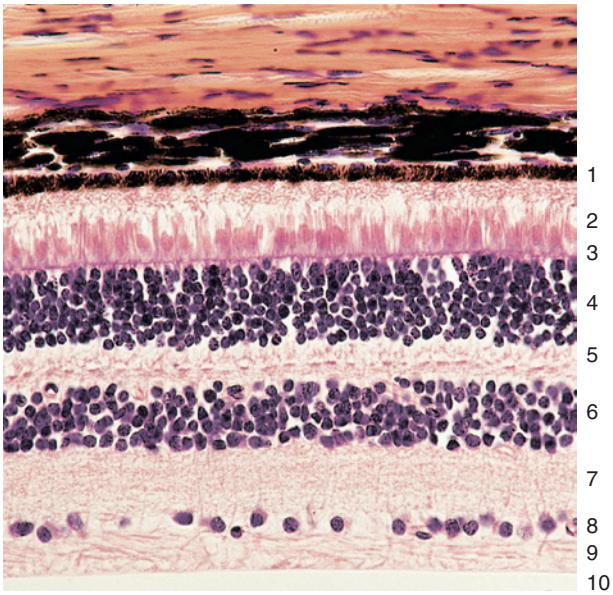


Figure 22–7 Light micrograph of the retina with its described 10 layers (×270). (1) pigment epithelium, (2) lamina of rods and cones, (3) external limiting membrane, (4) outer nuclear layer, (5) outer plexiform layer, (6) inner nuclear layer, (7) inner plexiform layer, (8) ganglion cell layer, (9) optic nerve fiber layer, (10) inner limiting membrane.

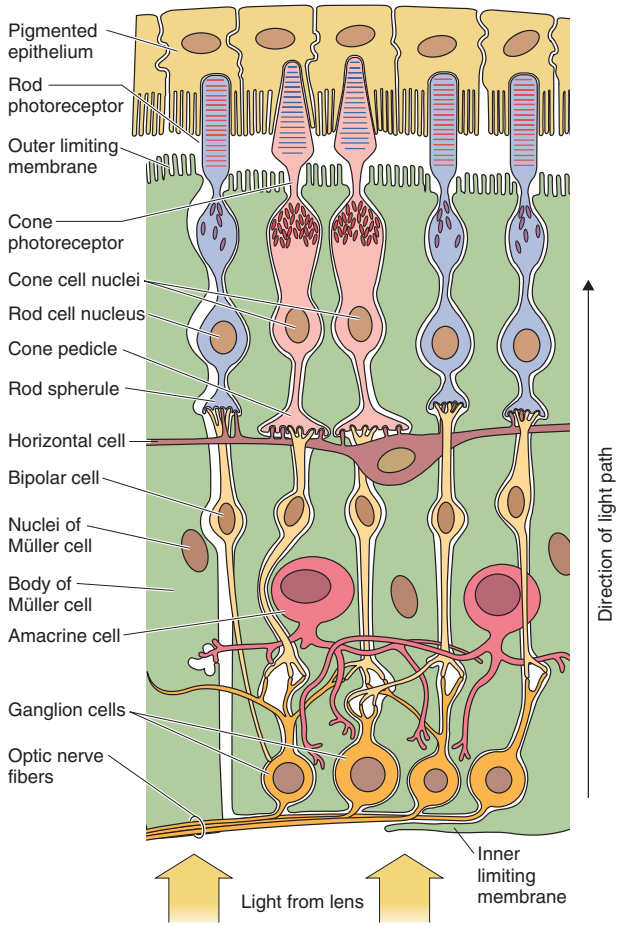


Figure 22–8 Diagram of the various layers of the retina. The space observed between the pigmented layer and the remainder of the retina is an artifact of development and does not exist in the adult, except during detachment of the retina.

is a specialized area of the retina containing almost entirely of cones, which are packed tightly as the other layers of the retina are pushed aside. These cones are somewhat different from other cones of the retina because they are narrower, only about 1.5 µm in diameter, and therefore more cones can fit in per unit area than elsewhere in the retina. Therefore, one may think of the configuration of cones in the fovea centralis as a region of high definition because there are more pixels per unit area. As distance from the fovea increases, the number of cones decreases and the number of rods increases.

The retina has a dual blood supply. Much of the internal layers of the retina, from the internal segments of the rods and cones through the optic nerve fiber layer, is supplied by the **central retinal artery**, which gains access to the eyeball via the center of the optic nerve and forms numerous branches on the surface of the internal aspect of the retina. The external layers of the retina that constitute the outer segments of the rods and cones and the pigment epithelium are served by the vascular supply of the **choroid**.

The portion of the retina that functions in photoreception lines (faces) the inner surface of the choroid layer from the optic disk to the ora serrata and is composed of 10 distinct layers (see Figs. 22–7 and 22–8). From outside, adjacent to the choroid, to inside, where the vitreous humor abuts the retina, there are 10 layers.

It should be noted that the light entering the eyeball reaches the tenth layer (inner limiting membrane) first and the first layer (pigment epithelium) last. These layers are as follows (Table 22–1):

1. Pigment epithelium
2. Layer of rods and cones
3. Outer limiting membrane
4. Outer nuclear layer
5. Outer plexiform layer
6. Inner nuclear layer
7. Inner plexiform layer
8. Ganglion cell layer
9. Optic nerve fiber layer
10. Inner limiting membrane

Although there are 10, more or less distinct, layers making up the retina, only 3 of these layers are composed of neurons that receive, integrate, and relay or transmit impulses to the brain for processing. These are

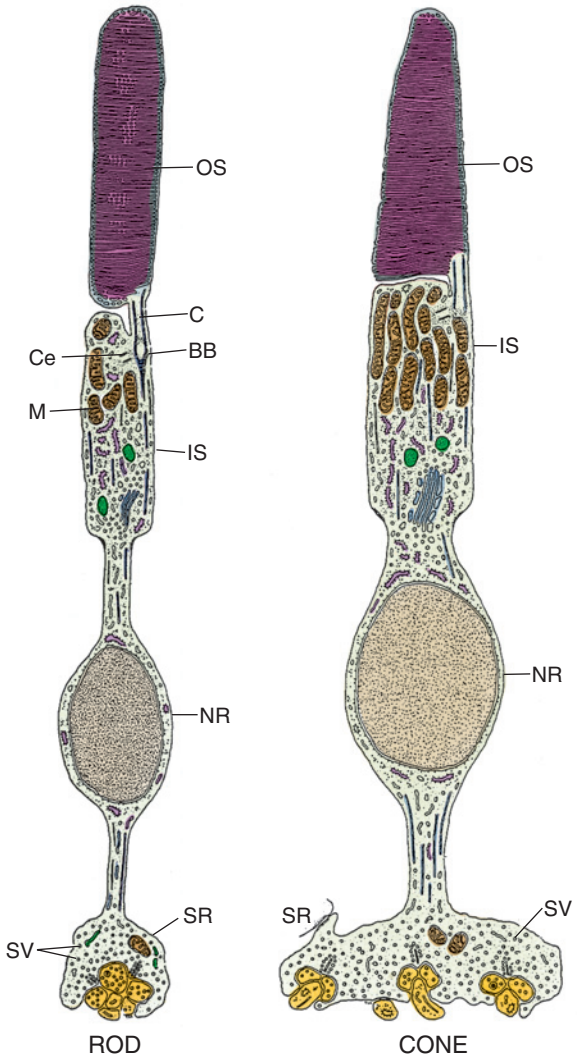


Figure 22–9 Diagram of the morphology of a rod **(A)** and cone **(B)**. OS, Outer segment; BB, basal body; C, connecting stalk; Ce, centriole; IS, inner segment; M, mitochondria; NR, nuclear region; SR, synaptic region; SV, synaptic vesicles. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

TABLE 22–1 Cells of the Retina and Their Functions

Cell	Function
Pigment cells	Absorption of light, thus preventing light reflection; phagocytosis of spent membranous disks from rods and cones; esterifying and storing vitamin A to release it to rods and cones when needed
Rods	Photosensitive cells responsible for monochromatic vision during low light conditions
Cones	Photosensitive cells responsible for color vision during bright light conditions
Bipolar neurons	Synapse with rods and cones and transmit information from these photoreceptor cells to dendrites of ganglion cells
Horizontal cells	Transmit information from rods and cones to bipolar cells and regulate the transmission of this information by bipolar cells via lateral inhibition
Amacrine cells	Act as interneurons controlling whether visual information should be transmitted to the visual center of the brain
Muller cells	Support the the cells of the retina physically and physiologically; act as if they were fiber optic cables by guiding various wavelength of light to the correct photoreceptor cells
Ganglion cells	Relay information from rods and cones to the visual center of the brain; a small fraction of ganglion cells responsible for providing information concerning daylight and night to the regions of the brain responsible for establishing the body's circadian rhythm; some of these cells are also control the pupillary reflex

the photoreceptor rods and cones, bipolar cells, and the ganglion cells.

Pigment Epithelium

The pigment epithelium, derived from the outer layer of the optic cup, is composed of cuboidal to columnar cells (14 µm wide and 10 to 14 µm tall) whose nuclei are located basally. The cells are attached to Bruch membrane, which is located between the choroid and the pigment cells. Mitochondria are especially abundant in the cytoplasm near the numerous cell invaginations with Bruch membrane, suggesting transport in this region. Desmosomes, zonulae occludentes, and zonulae adherentes are present on the lateral cell membranes, forming the blood–retina barrier. Moreover, gap junctions on the lateral cell membranes permit intercellular communication. The cell apices exhibit microvilli and sleeve-like structures that surround and isolate the tips of the individual photoreceptor cells.

The most distinctive feature of the pigment cells is their abundance of melanin granules, which these cells synthesize and store in their apical regions. The apical cytoplasm also contains residual bodies housing phagocytosed tips shed by the rods. Additionally, smooth endoplasmic reticulum, rough endoplasmic reticulum

(RER), and Golgi apparatus are abundant in the cytoplasm.

The pigmented epithelium has several functions. Pigmented epithelial cells absorb light after it has passed through and stimulated the photoreceptors, thus preventing reflections from the tunics, which would impair focus. These pigmented cells continually phagocytose spent membranous disks from the tips of the photoreceptor rods and cones. Pigment epithelial cells also play an active role in vision by esterifying vitamin A derivatives in their smooth endoplasmic reticulum and by storing vitamin A in their cytoplasm and releasing it to the rods and cones as needed.

CLINICAL CORRELATIONS

1. Individuals with albinism are unable to produce melanin pigment; therefore, the light that enters the eyeball is not absorbed by the pigment epithelium. Instead, the light is reflected throughout the eyeball. Consequently, the impinging light excites many more rods and cones than in a non-albino individual, reducing the ability of those with albinism to see clearly; usually, these individuals, instead of having a 20/20 vision, have 20/100 to 20/200 vision.
2. Because the sleeve-like extensions of the pigment epithelial cells merely surround the photoreceptor rod and cone tips, sudden hard jolts may disengage them, resulting in **detachment of the retina**, a common cause of partial blindness. The condition can be corrected surgically by "spot welding" the two structures back together. However, if this condition is left unattended, the rods and cones die because they will have lost the metabolic support normally provided by the pigment epithelium. Their death leaves a blind spot in the visual field corresponding to the area where the photoreceptors were lost.

Layer of Rods and Cones

The optical portion of the retina houses two distinct types of photoreceptor cells called ***rods*** and ***cones***. Both rods and cones are polarized cells whose apical portions, known as the ***outer segments***, are specialized dendrites. The outer segments of both rods and cones are surrounded by pigmented epithelial cells (see Fig. 22–8). The bases of the rod and cone cells form synapses with the underlying cells of the bipolar layer. There are approximately 100 to 120 million rods and only 3 million cones in each retina. Rods are specialized receptors for perceiving objects in dim light; whereas cones are specialized receptors for perceiving objects in bright light. Cones are further adapted for color vision, whereas rods perceive only light. Rods and cones are unevenly distributed in the retina, in that cones are highly concentrated in the fovea; thus, this is the area of the retina where high-acuity vision occurs.

Rods

Rods are the photoreceptors of the retina specialized for perceiving dim light.

Rods, which are activated in dim light only, are so sensitive that they can produce a signal from a single photon of light. However, they cannot mediate signals in bright light, and they cannot sense color.

Rods are elongated cells (50 µm long by 2 to 5 µm in diameter) oriented parallel to one another but perpendicular to the retina. These cells are composed of an **outer segment**, an **inner segment**, a **nuclear region**, and a **synaptic region** (see Fig. 22–9).

The **outer segment of the rod**, its dendritic end, presents close to a thousand flattened membranous lamellae oriented perpendicular to its long axis (Fig. 22–10; see Fig. 22–9). Each lamella represents an invagination of the plasmalemma, which is detached from the cell surface, thus forming a disk. Each disk is composed of two membranes separated from each other by an 8-nm space. The membranes contain a specific form of the light sensitive pigments **opsins**, known as ***rhodopsin (visual purple)***. Because the outer segment is longer in rods than in cones, rods contain more rhodopsin, respond more slowly than cones, and have the capacity to collectively summate the reception. The cell membrane of the outer segment houses **cyclic guanosine monophosphate (cGMP)–gated Na^+ ion channels** that are kept open by the cGMP that are bound to the gates if there are no photons impinging on the rod, that is, if it is dark. Therefore, in the dark, Na^+ ions may enter the outer segment of the rod.

The **inner segment of the rod** is separated from the outer segment by a constriction called the ***connecting stalk***. Passing through the connecting stalk and into the outer segment of the rod is a modified cilium (lacking the central singlet microtubules) that arises from a basal body located at the apical end of the inner segment. Congregated near the interface with the connecting stalk are abundant mitochondria and cytoplasmic glycogen granules, both necessary for the production of energy for the visual process. The cytoplasm basal to the mitochondria is rich in microtubules, polysomes, smooth endoplasmic reticulum, RER, and Golgi complexes. Proteins produced in the inner segment migrate to the outer segment, where they become incorporated into the disks. The disks gradually migrate to the apical end of the outer segment and are eventually shed into the sheaths of the pigment cells, where they will be phagocytosed. The length of time from protein incorporation, through migration, and finally to shedding is less than 2 weeks. The lateral cell membrane of the inner segment has adenosine triphosphate

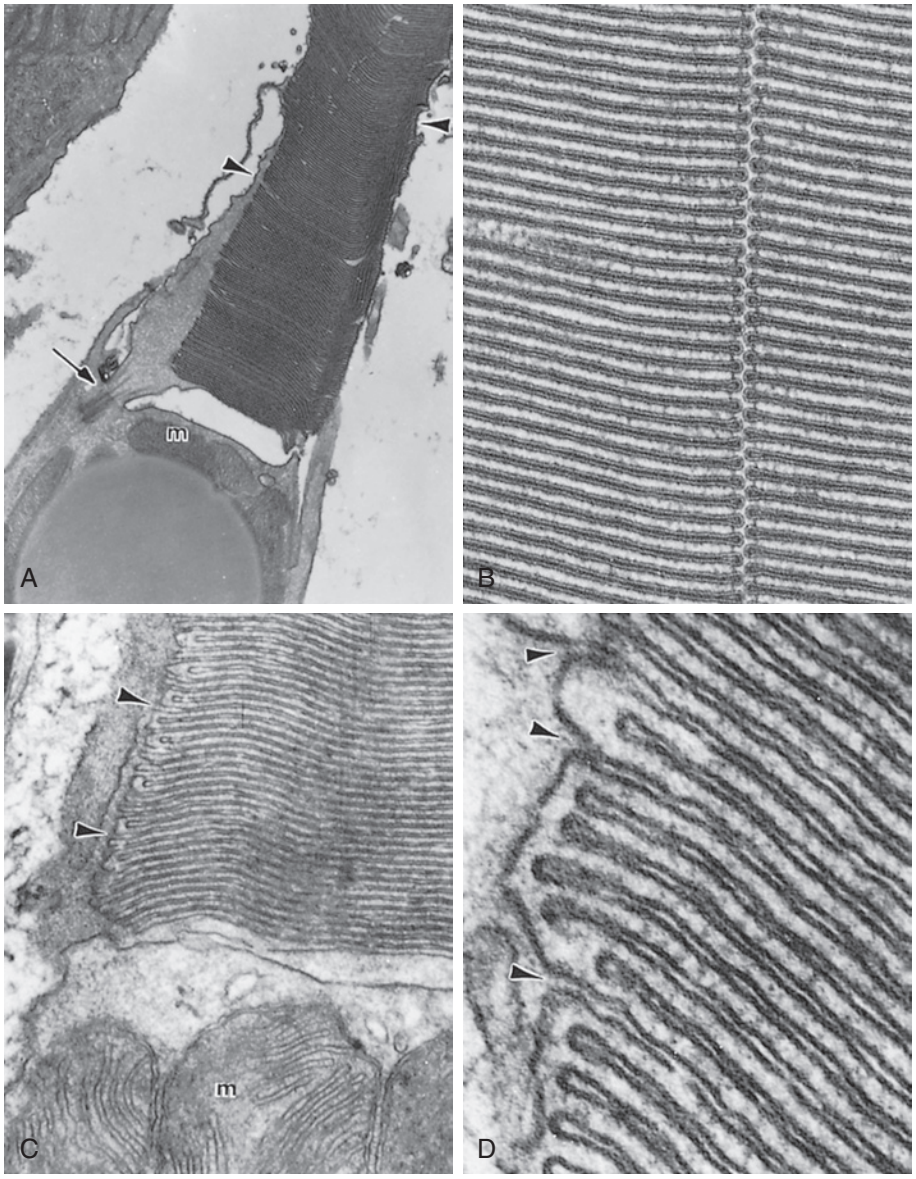


Figure 22–10 Electron micrographs of rods from the eye of a frog and cones from the eye of a squirrel. **A,** Disks in the outer segment and mitochondria (m) in the inner segment of the rod of a frog; *arrow* points to a cilium connecting the inner and outer segments (×16200). **B,** Higher magnification of the disks of the outer segment of the rod of a frog (×76500). **C,** Junction of the outer and inner segments of the cone of a squirrel (×28800). **D,** Higher magnification of the disks of the outer segment of a squirrel eye showing continuity of the lamellae with the plasmalemma *(arrowheads)* (×82800). (From Leeson TS, Leeson CR, Paparo AA. *Text-Atlas of Histology*. Philadelphia: WB Saunders; 1988.)

(ATP)–driven sodium-potassium pumps that delivers potassium into the rod and drives sodium out of the rod. Additionally, the lateral cell membrane of the inner segment also houses nongated K^+ channels through which K^+ ions can leave the rod. These ion channels operate the same way whether or not light is impinging on the rod and maintain the cell membrane potential at –40 millivolts. When light hits the rod, the cGMP-gated Na^+ ion channels of the outer segment become closed, sodium cannot enter the rod and cause the cytoplasmic

face of the rod cell membrane to become more negative (–70 to –80 millivolts) compared with its outer face, causing the rod to become **hyperpolarized**. *Therefore, it is the hyperpolarization of the rod plasmalemma rather than depolarization that causes the rod to fire.*

It was stated above that the rod membrane contains a light-sensitive pigment known as ***rhodopsin***, a light-sensitive molecule composed of a carotene-like pigment **11-*cis* retinal** and the protein **scotopsin**. When photons impinge on rhodopsin, within picoseconds the *cis*-retinal is transformed into **all-*trans* retinal**, changing its molecular conformation from a bent structure to a straight structure. This conformational alteration disrupts the bond between the retinal and scotopsin, and within milliseconds the rhodopsin molecule is transformed into **activated rhodopsin**, the molecule responsible for the electrical alterations within the rod. Activated rhodopsin is stable for 1 or 2 seconds and then decomposes, due to the activity of the enzyme **rhodopsin kinase**, into all-trans retinal and scotopsin. In order to replenish rhodopsin, the energy-requiring reaction occurs, in which the enzyme **retinal isomerase** converts all-*trans* retinal to 11-*cis* retinal. Once converted, it immediately binds to scotopsin to form rhodopsin. Interestingly, the same enzyme can convert **vitamin A (11-*cis* retinol)** to 11-*cis* retinal, thus ensuring an abundant supply of rhodopsin.

Activated rhodopsin acts on **transducin**, a G protein, by inducing it to activate the enzyme cGMP phosphodiesterase to convert cGMP to 5′-cGMP. The decrease in cGMP levels causes the sodium channels of the outer segment to close because cGMP is no longer bound to the gates, thereby preventing the entry of Na^+ ions into the rod, thus causing the rod cell membrane to become hyperpolarized. It is interesting to note that the impingement of a single photon on a rod has a tremendous effect by being able to influence the movements of millions of sodium ions in a single rod.

As indicated above the signal from rods is not induced by depolarization, as it is in most cells; rather, light-induced hyperpolarization causes the signal to be transmitted through the various cell layers to the ganglion cells, where the signal generates an action potential along the axons to the brain.

The **nuclear region** of rods houses the nucleus as well as many of the organelles of the cell and the **synaptic region** display short cytoplasmic processes that form synapses with bipolar and horizontal cells. The synaptic vesicles housed in the synaptic region contain the neurotransmitter substance **glutamate**, which they release into the synaptic cleft when the rods become hyperpolarized.

Rods are most sensitive to greenish-blue light whose wavelength is about 505 nm and completely insensitive to red light and beyond (640 nm).

CLINICAL CORRELATIONS

A severe lack of vitamin A causes **night blindness** because the amount of available rhodopsin is greatly reduced. Because vitamin A is stored in great supply by the liver, it takes as long as 1 year of vitamin A deficiency in the individual's diet before night blindness becomes evident. Fortunately, night blindness caused by a vitamin A deficiency can be reversed rapidly by a vitamin A injection.

Cones

Cones are specialized photoreceptors of the retina for perceiving bright light and color.

Although the morphology and mode of function of the cones is similar to that of rods, cones are activated in bright light and produce greater visual acuity compared with rods. There are three types of cones, L cones (long wavelength), M cones (medium wavelength), and S cones (short wavelength), each containing a different variety of the photopigment **opsin**, known as ***photopsin*** and responding to different wavelengths of light. Thus, each variety of photopsin has a maximum sensitivity to one of three colors of the spectrum—red, green, and blue—and the difference resides in the opsins rather than in the 11-*cis* retinal (see Tables 22–1 and Table 22–2).

Cones are elongated cells (60 μm long × 5 to 8 μm in diameter), being longer and narrower at the fovea centralis (70 μm in length × 1.5 μm in diameter). Their structure is similar to that of rods with the following few exceptions (Fig. 22–11; see Figs. 22–9B and 22–10):

- Their apical terminal (outer segment) is shaped more like a cone than a rod.
- The disks of cones, although composed of lamellae of the plasmalemma, are attached to the plasma membrane, unlike the lamellae of the rods, which are separated from the plasma membrane.
- Protein produced in the inner segment of cones is inserted into the disks throughout the entire outer segment; in the rods, it is concentrated in the most distal region of the outer segment.
- Unlike rods, cones are sensitive to color and provide greater visual acuity.
- Recycling of the cone photopigment does not require the retina pigment cells for the processing.
- Cones are not nearly as sensitive (as much as 100 times less sensitive) to light than rods, but they are still able to discriminate color at relatively poorly lit conditions.
- The synaptic vesicles housed in the synaptic region of cones, just as in rods, contain the neurotransmitter substance **glutamate**, which they release into the synaptic cleft when they become hyperpolarized.

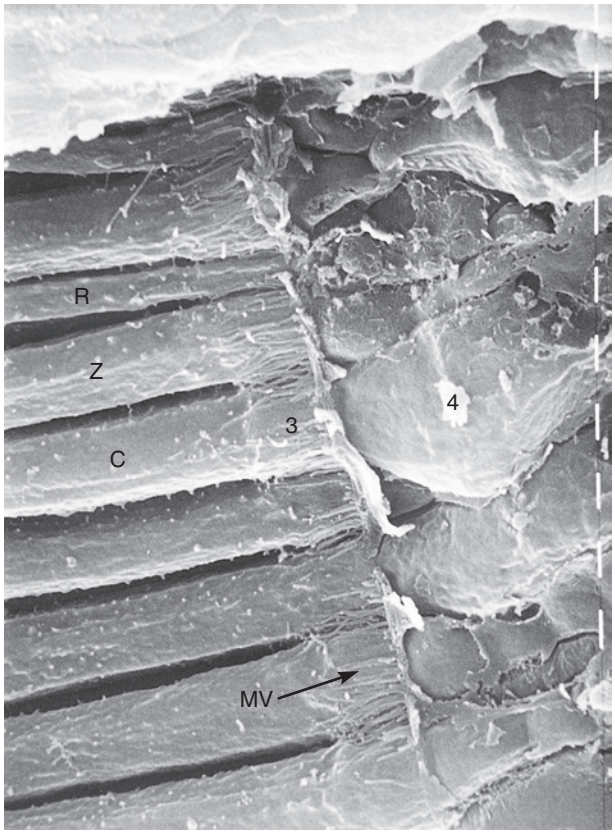


Figure 22–11 Scanning electron micrograph of the retina in a monkey in a displaying cones (C) and a few rods (R) (×5800). 3, External limiting membrane; 4, outer nuclear layer; MV, microvilli belonging to the Müller cells; Z, inner segments. (From Borwein B, Borwein D, Medeiros J, McGowan J. The ultrastructure of monkey foveal photoreceptors, with special reference to the structure, shape, size, and spacing of the foveal cones. *Am J Anat*. 1980;159:125-146. Reprinted with permission from John Wiley & Sons, Inc.)

The mechanism of photoreception in cones is very similar to that in rods, but cones react to different wavelengths than do the rods (Table 22–2).

TABLE 22–2 Peak Light Absorption by Opsins in Rods and Cones

Rod/Cones	Wavelength of Light (nm)
Rods	505
Blue sensitive pigment in cones (S cones)	445
Green sensitive pigment in cones (M cones)	535
Red sensitive pigment in cones (L cones)	570

L, Long wavelength; M, medium wavelength; S, short wavelength.

CLINICAL CORRELATIONS

Each type of cone can distinguish approximately 100 shades of color. Most mammals have two types of cones, are said to be **dichromatic**; therefore, they distinguish 10,000 different colors only. Because humans have three types of cones, people are said to be **trichromatic** and are able to distinguish 1 million different colors. It has been reported that some people possess four types of cones, tetrachromats, who can distinguish 100 million different colors.

External (Outer) Limiting Membrane

Although the term ***external limiting membrane*** is still used in descriptions of the layers of the retina, this structure is not a membrane. Instead, electron micrographs have revealed this "layer" to be a region of zonulae adherens between Müller cells (modified neuroglial cells) and the photoreceptors. Distal to this, microvilli of the Müller cells project into the interstices between the inner segments of the rods and cones.

Outer Nuclear Layer

The **outer nuclear layer** consists of a zone occupied mainly by the nuclei of the rods and cones. In histological sections, the nuclei of rods are smaller, more rounded, and more darkly stained than the nuclei of cones.

Outer Plexiform Layer

Axodendritic synapses between the photoreceptor cells and dendrites of bipolar and horizontal cells are located in the **outer plexiform layer**. There are two types of synapses in this layer: (1) *flat*, which display the usual synaptic histology, and (2) *invaginated*. Invaginated synapses are unique, in that they consist of a dendrite of a single bipolar cell and a dendrite from each of two horizontal cells, thus making a **triad**. Located within this invaginated synaptic region is a ribbon-like lamella **(synaptic ribbon)** containing neurotransmitter substance. It is believed that this structure captures and assists in distributing the neurotransmitter glutamate.

Inner Nuclear Layer

The nuclei of bipolar, horizontal, amacrine, and Müller cells compose the **inner nuclear layer**.

Bipolar neurons are interposed between photoreceptor cells and ganglion cells. These neurons may be contacted by many rods (10 near the macula to as many as 100 contacts near the ora serrata), thus permitting summation of the signals, which is especially useful in low light intensity. Cones, however, do not converge, at least not those near the fovea; instead, each cone

synapses with several bipolar cells, thus further enhancing visual acuity. Axons of the bipolar cells synapse with dendrites of the ganglion cells. There are two types of bipolar neurons, those that become **depolarized** upon being stimulated by the glutamine released by rods or cones and those that become **hyperpolarized** when stimulated by glutamine released by rods or cones. It is believed that the two types of cells assist in enhancing visual contrast (see the following paragraphs).

Horizontal cells located in this layer synapse with the synaptic junctions between the photoreceptor cells and the bipolar cells. As their name implies, these cells transmit information in a horizontal plane only (i.e., in the outer plexiform layer) from rods and cones to bipolar cells. These cells enhance visual contrast by permitting the transmission of impulses from bipolar cells that have been excited in the immediate vicinity of cones and rods that are hyperpolarized but inhibit the transmission of impulses by bipolar cells peripheral to the area of light impingement, a mechanism known as ***lateral inhibition***.

Amacrine cells are located at the inner limits of this layer. Their dendrites all exit from one area of the cell and terminate on synaptic complexes between bipolar cells and ganglion cells. They also synapse on **interproximal cells** that are interspersed with bipolar cell bodies. There are many different types of amacrine cells, some that synapse with bipolar cells and ganglion cells, or with bipolar cells and other amacrine cells. Some amacrine cells respond when a visual signal begins and others when a visual signal ends; therefore, they respond to changes in light intensity, whereas still other amacrine cells respond to an object moving across the visual field. Basically, amacrine cells appear to function as interneurons that control whether or not a visual information should be transmitted to the visual center of the brain.

Müller cells are neuroglial cells that extend between the vitreous body and the inner segments of the rods and cones. It is here that Müller cells end by forming zonulae adherens with the photoreceptor cells represented by the external limiting membrane. Microvilli extend from the apical surface. Thus, Müller cells function as supporting cells for the neural retina. This support is twofold, namely physical/physiological and as light conducting.

- **Physical or physiological support**. Involves maintaining the proper relationships among the cells of the retina, regulating the extracellular ion levels, neurotransmitter levels, and insulating the various cellular components of the retina
- **Light conducting support**. Involves the separation of the various wavelengths of light and guiding of the impinging photons of correct wavelength to the rods and cones that are designed for their maximal reception. Therefore, Muller cells appear to act as if they were wavelength-specific fiber optic cables.

Inner Plexiform Layer

The processes of amacrine, bipolar, and ganglion cells are intermingled in the inner plexiform layer. **Axodendritic synapses** between the axons of bipolar cells and the dendrites of ganglion cells and amacrine cells also are located here. As in the outer plexiform layer, there are two types of synapses in this layer: *flat* and *invaginated.* Invaginated synapses consist of an axon of a single bipolar cell and two dendrites of either amacrine cells or ganglion cells or one dendrite from each of the two different cells, thus making a **dyad**. Also located within this synapse is a shortened version of the **synaptic ribbon**, which contains neurotransmitter substances.

Ganglion Cell Layer

Cell bodies of large multipolar neurons of the ganglion cells, up to 30 μm in diameter, are located in the **ganglion cell layer**. The axons of these neurons pass to the brain. Hyperpolarization of the rods and cones indirectly activates these ganglion cells, which then generate an action potential that is passed by their axons to the brain via the visual relay system. There are four types of ganglion cells: W cells, X cells, Y cells dedicated to vision, and the photosensitive retinal glial cells (PSRGCs) dedicated to relay information to the pineal gland, informing that structure whether it is day or night and regulating the pupillary reflex.

- **W ganglion cells**. Smallest, with a cell body that is approximately 10 μm in diameter and slowest with a conduction velocity of 8 m/sec. The amacrine and bipolar cells that synapse with W cells convey information mostly from rods and they are responsible for much of the night vision.
- **X ganglion cells**. Comprise most of the ganglion cells and are 10 to 15 μm in diameter with a conduction velocity of 15 m/sec. These cells are responsible for most of the color vision because they receive information from amacrine and bipolar cells that synapse with cones. Each X cell receives information from a small region of the retina, therefore, they provide information that is specific for location of the visual event.
- **Y ganglion cells**. Larger and relay information faster than W or X cells, reaching 30 μm in diameter and a conduction velocity of at least 50 m/sec. They receive information from bipolar and amacrine cells from large areas of the visual field. They are responsible for monitoring changes in the entire visual field but do not provide precise information concerning the location of visual event.
- **Photosensitive retinal glial cells**. **PSRGCs** constitute a very small fraction, approximately 2% to 3%, of the ganglion cells of the retina. These cells possess a photopigment, similar to the opsins of the rods and cones, known as ***melanopsin***. These PSRGCs project to the suprachiasmatic nucleus and indirectly to the pineal gland, regions responsible for the

establishment of the body's circadian rhythm. When PSRGCs are exposed to light, they send information to these regions indicating that presence of light; when they do not send information, it indicates that it is dark. Interestingly, PSRGCs also receive input from amacrine cells and bipolar cells that synapse with rods and cones, providing a redundancy, thus ensuring that regions of the brain that establish the circadian rhythm receive pertinent information about daylight and dark (see the section concerning the pineal gland in Chapter 13). These cells are also responsible for the pupillary reflex, whereby the pupil opens or closes depending on the amount of light impinging on the retina.

Optic Nerve Fiber Layer

Nerve fibers are formed of unmyelinated axons of the ganglion cells in the **optic nerve fiber layer**. These axons become myelinated as the nerve pierces the sclera.

Inner Limiting Membrane

Basal laminae of the Müller cells compose the **inner limiting membrane**.

Image Processing in the Retina

For a considerable period of time, it was believed that photoreceptor cells (rods and cones) of the retina convey raw data to the brain for processing. In that concept, the retina was believed to act as a digital camera sending pixels to the brain, and the neurons of the visual cortex interpreted the pixels as visual images that are associated with pictures of the outside world. In the past decade, this view has been greatly modified, demonstrating that a considerable amount of preprocessing of these pixels occurs in the retina, and the information that is conveyed to the visual cortex is a much more elaborate representation of the external world than previously thought. It appears that the retina builds a series of primitive videos that it sends to the visual cortex. Some of these videos consist only of the periphery of the images focused on the retina, whereas other videos provide data concerning silhouettes and essential features; still other tracts focus on the direction and duration of motion across the visual field. It is these videos that are provided to the visual cortex, whose neurons "compile" and "edit" the information to construct a comprehensive image of the actual scene that the individual perceives.

Accessory Structures of the Eye

Conjunctiva

Conjunctiva is the mucous membrane lining the eyelids and reflecting onto the sclera of the anterior surface of the eye.

A transparent mucous membrane, known as the ***conjunctiva***, lines the inner surface of the eyelids **(palpebral conjunctiva)** and covers the sclera of the anterior portion of the eye **(bulbar conjunctiva)**. The conjunctiva is composed of a stratified columnar epithelium that contains goblet cells overlying a basal lamina and a lamina propria composed of loose connective tissue. Secretions of the goblet cells become a part of the **tear film**, which aids in lubricating and protecting the epithelium of the anterior aspect of the eye. At the corneoscleral junction, where the cornea begins, the conjunctiva continues as the stratified squamous **corneal epithelium** and is devoid of goblet cells.

CLINICAL CORRELATIONS

Conjunctivitis is an inflammation of the conjunctiva usually associated with hyperemia and a discharge. It may be caused by a number of bacterial agents, viruses, allergens, and parasitic organisms. Some forms of conjunctivitis are extremely contagious, are damaging to the eye, and may cause blindness if untreated.

Eyelids

Eyelids, covered externally by skin and internally by the conjunctiva, provide a protective barrier for the anterior surface of the eye.

The eyelids are formed as folds of skin that cover the anterior surface of the developing eye. Accordingly, stratified squamous epithelium of skin covers their external surface; at the **palpebral fissure**, palpebral conjunctiva covers their inner surface. The eyelids are supported by a framework of **tarsal plates** composed of dense, fibrous connective tissue. Sweat glands are located in the skin of the eyelids, as are fine hairs and sebaceous glands. The dermis of the eyelids is generally thinner than in most skin, contains numerous elastic fibers, and is without fat. The margins of the eyelids contain **eyelashes** arranged in rows of three or four, but they are without arrector pili muscles.

Modified sweat glands, called ***glands of Moll***, form a simple spiral before opening into the eyelash follicles. **Meibomian glands**, modified sebaceous glands located in the tarsus of each lid, open on the free edge of the lids. The oily substance secreted by these glands becomes incorporated into the tear film and impedes evaporation of the tears. Other smaller modified sebaceous glands, the **glands of Zeis**, are associated with the eyelashes and secrete their product into the eyelash follicles.

Lacrimal Apparatus

The lacrimal apparatus keeps the anterior surface of the eye lubricated with tears, thus preventing dehydration of the cornea.

The lacrimal apparatus consists of

- The **lacrimal gland**, which secretes the **lacrimal fluid** (tears)
- The **lacrimal canaliculi**, which carry the lacrimal fluid away from the surface of the eye
- The **lacrimal sac**, a dilated portion of the duct system
- The **nasolacrimal duct**, which delivers the lacrimal fluid to the nasal cavity

The **lacrimal gland** lies in the lacrimal fossa located in the superolateral aspect of the orbit. It lies outside the conjunctival sac, although it communicates with the sac via 6 to 12 secretory ducts, which open into the sac at the lateral portion of the superior conjunctival fornix. The gland is a serous, compound tubuloacinar gland that resembles the parotid gland. Myoepithelial cells completely surround its secretory acini.

Lacrimal fluid (tear) is composed mostly of water. This sterile fluid, containing the antibacterial agent **lysozyme**, passes through the secretory ducts to enter the conjunctival sac. The upper eyelids, by blinking, wash the tears over the anterior portion of the sclera and cornea, thus keeping them moist and protected from dehydration. The lacrimal fluid is wiped in a medial direction and enters the **lacrimal punctum**, an aperture located in each of the medial margins of the upper and lower eyelids. The punctum of each eyelid leads directly to **lacrimal canaliculi**, which join into a common conduit that leads to the lacrimal sac. The walls of the lacrimal canaliculi are lined by stratified squamous epithelium.

The **lacrimal sac** is the dilated superior portion of the nasolacrimal duct. It is lined by pseudostratified ciliated columnar epithelium.

The inferior continuation of the lacrimal sac is the **nasolacrimal duct**, also lined by pseudostratified ciliated columnar epithelium. This duct carries the lacrimal fluid into the inferior meatus located in the floor of the nasal cavity.

CLINICAL CORRELATIONS

Dry eye syndrome (DES), a condition in which the eye does not have enough moisture to keep it comfortable, is the most common eye disease, affecting approximately 6% of the population, but it may affect fully one third of older individuals. The eyes of these individuals feel tired, scratchy, and uncomfortable. The three principal causes of DES are not enough tear production, as in Sjorgren syndrome, or other diseases, postmenopausal conditions in women, and the production of tears that evaporate too quickly. Recently, a fourth possible cause has been added, namely, spending long periods of time staring at computer monitors, tablets, or smartphone screens. It has been noted that individuals who stare at these devices blink less frequently than when not occupied in those endeavors. Their tears dry up before the blinking of the eyelids can spread the tears across the cornea. If untreated, dry eyes can cause corneal ulcerations, corneal scarring, and, occasionally, perforation of the cornea, leading to possible loss of vision.

EAR (VESTIBULOCOCHLEAR APPARATUS)

The ear, the organ of hearing and balance, is composed of three regions: the outer ear, the middle ear, and the inner ear.

The ear, the organ of hearing as well as the organ of equilibrium or balance, is divisible into three parts: (1) the external ear, (2) the middle ear (tympanic cavity), and (3) the inner ear (Fig. 22–12).

Sound waves received by the **external ear** are translated into mechanical vibrations by the tympanic membrane (ear drum). These vibrations then are amplified by the bony ossicles in the **middle ear (tympanic cavity)** and transferred to the fluid medium of the **inner ear** at the oval window. The inner ear, a perilymph-filled bony labyrinth in which is suspended a membranous labyrinth, regulates hearing (the cochlear portion) and maintains balance (the vestibular portion). Sensory input into the entire vestibulocochlear apparatus is transmitted to the brain by the two divisions of the vestibulocochlear nerve (CN VIII).

External Ear

The external ear is composed of the auricle, the external auditory meatus, and the tympanic membrane.

The **external ear** is composed of the auricle (pinna), the external auditory meatus, and the tympanic membrane (see Fig. 22–12). The **auricle** develops from parts of the first and second branchial arches. Its general shape, size, and specific contours are usually distinctive for each person, with familial similarities. The pinna is composed of an irregularly shaped plate of elastic cartilage covered by thin skin that adheres tightly to the cartilage. The cartilage of the pinna is continuous with the cartilage lining the cartilaginous portion of the external auditory meatus.

The **external auditory meatus** is the canal that extends from the pinna into the temporal bone to the external surface of the tympanic membrane. Its superficial portion is composed of elastic cartilage, which is continuous with the cartilage of the pinna. Temporal bone replaces the cartilage as support within the inner two thirds of the canal. The external auditory meatus is covered with skin containing hair follicles, sebaceous glands, and modified sweat glands known as

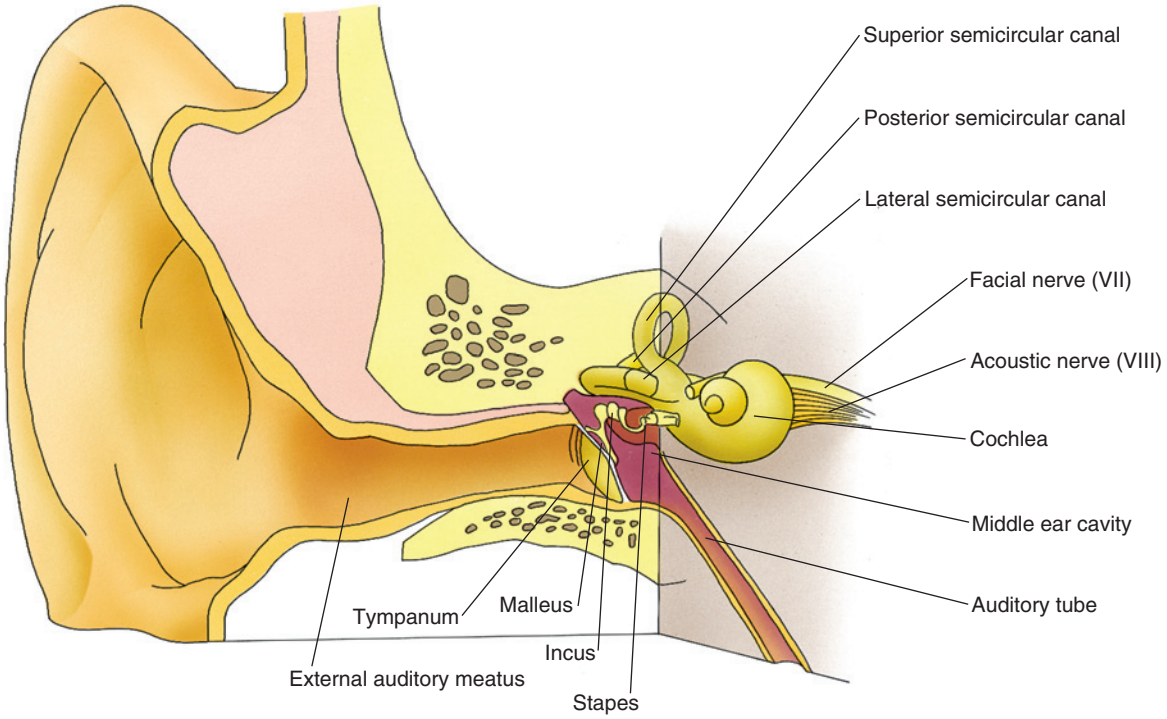


Figure 22–12 Diagram of the anatomy of the ear.

ceruminous glands, which produce a waxy material called ***cerumen*** (earwax). Hair and the sticky wax help prevent objects from penetrating deep into the meatus.

CLINICAL CORRELATIONS

Earwax, produced by the ceruminous glands of most of the world's population, has a sticky consistency. However, a large percent of individuals of East Asian descent have a desiccated, crumbling earwax that does not form a self-sticking substance. The formation of the dry variety of earwax is due to a mutation that occurred approximately 25,000 to 30,000 years ago in the *ABCC11* gene (ATP-binding cassette subfamily C member 11). Interestingly, people with this mutation also have less odorous armpits than the rest of the human population.

The **tympanic membrane** covers the deepest end of the external auditory meatus. It represents the closing plate between the first pharyngeal groove and the first pharyngeal pouch, where ectoderm, mesoderm, and endoderm are in close proximity. The external surface of the tympanic membrane is covered by a thin epidermis derived from ectoderm, whereas its internal surface is composed of a simple squamous to cuboidal epithelium derived from endoderm. A thin layer of mesodermal elements, including collagen fibers, elastic fibers, and fibroblasts, is interposed between the two epithelial layers of the tympanic membrane. This membrane receives sound waves transmitted to it by air through the external auditory meatus, which cause it to vibrate. In this fashion, the sound waves are converted into mechanical energy that is transmitted to the bony ossicles in the middle ear.

Middle Ear

The middle ear (tympanic cavity) houses the three bony ossicles: the malleus, the incus, and the stapes.

The **middle ear**, or **tympanic cavity**, is an air-filled space located in the petrous portion of the temporal bone. This space communicates posteriorly with the mastoid air cells and anteriorly, via the **auditory tube (eustachian tube)**, with the pharynx (see Fig. 22–12). The three bony ossicles are housed in this space, spanning the distance between the tympanic membrane and the membrane at the oval window.

The superior portion of the tympanic cavity is lined by simple squamous epithelium, whereas the remainder of the cavity is lined by a pseudostratified ciliated columnar epithelium, that is, continuous with the internal lining of the tympanic membrane and with the epithelium in the vicinity of the auditory tube. According to recent investigations, the pseudostratified ciliated

epithelium is derived from endoderm, whereas the simple squamous epithelium is of neural crest origin and this region of the tympanic cavity is less resistant to microbial infections than is the region near the auditory tube and near the internal lining of the eardrum. The lamina propria over the bony wall adheres to it tightly and does not contain glands, but the lamina propria overlying the cartilaginous portion contains many mucous glands whose ducts open into the lumen of the tympanic cavity. Additionally, goblet cells and lymphoid tissue are found in the vicinity of the pharyngeal opening. In its deepest two thirds, the bony housing of the tympanic cavity gives way to cartilage as it approaches the auditory tube.

During swallowing, blowing the nose, and yawning, the orifice of the auditory tube at the pharynx opens, permitting an equalization of air pressure in the tympanic cavity with that in the external auditory meatus, which is located on the opposite side of the tympanic membrane. This is why swallowing, blowing one's nose, or yawning relieves the "ear pressure" during rapid ascent or descent when one is flying in an airplane or riding in an elevator of a very tall building.

Located within the medial wall of the tympanic cavity are two openings, the **oval window** and the **round window**, which connect the middle ear cavity to the inner ear. These two windows are formed by membrane-covered voids in the **bony wall**. The bony ossicles, the **malleus**, **incus**, and **stapes** are articulated in series by synovial joints lined with simple squamous epithelium. The malleus is attached to the tympanic membrane, with the incus interposed between it and the stapes, which in turn is attached to the oval window. Two small skeletal muscles, the **tensor tympani** and the **stapedius**, modulate movements of the tympanic membrane and the bony ossicles to prevent damage from very loud sounds. Vibrations of the tympanic membrane set the ossicles into motion, and because of their lever action, the oscillations are magnified to vibrate the membrane of the oval window, thus setting the fluid medium of the cochlear division of the inner ear into motion. Essentially, the force of movement due to the lever action is increased by a factor of about 1.3. Additionally, the surface area of the tympanic membrane (55 mm^2) is 17 times greater than the footprint of the stapes (3.2 mm^2), and these two factors (17 times 1.3) provide a 22-fold increase between the force that impinges on the tympanic membrane versus the force acting on the fluid on the other side of the oval window, thus amplifying by a factor of 22 the energy of the original sound waves entering the external auditory meatus (the external ear canal).

Inner Ear

The **inner ear** is composed of the **bony labyrinth**, an irregular, hollowed-out cavity located within the petrous portion of the temporal bone, and the **membranous labyrinth**, which is suspended within the bony labyrinth (Fig. 22–13).

TABLE 22–3 Sodium and Potassium Concentrations of Perilymph, Endolymph, Extracellular Fluid, and Cytosol

Fluid	Sodium (mmol/L)	Potassium (mmol/L)
Perilymph	140.0	7
Endolymph	1.3	157
Extracellular fluid	140.0	5
Cytosol	12.0	140

Bony Labyrinth

The bony labyrinth has three components: the semicircular canals, the vestibule, and the cochlea.

The **bony labyrinth**, composed of the semicircular canals, vestibule, and the cochlea, is lined with endosteum and is separated from the membranous labyrinth by the **perilymphatic space**. This space is filled with a clear fluid similar in composition to the extracellular fluid, called the ***perilymph*** (Table 22–3), within which the membranous labyrinth is suspended.

The three **semicircular canals (superior**, **posterior**, and **lateral)** are oriented at 90 degrees to one another (see Fig. 22–13). One end of each canal is enlarged; this expanded region is called the ***ampulla***. All three semicircular canals arise from and return to the vestibule, but one end of each of two of the canals shares an opening to the vestibule; consequently, there are only five, rather than six, orifices to the vestibule. Suspended within the canals are the **semicircular ducts**, which are regionally named continuations of the membranous labyrinth.

The **vestibule** is the central portion of the bony labyrinth located between the anteriorly placed cochlea and the posteriorly placed semicircular canals. Its lateral wall contains the **oval window (fenestra vestibuli)**, covered by a membrane to which the footplate of the stapes is attached, and the **round window (fenestra cochleae)**, covered only by a membrane. The vestibule also houses specialized regions of the membranous labyrinth (the **utricle** and the **saccule**).

The **cochlea** arises as a hollow bony spiral that turns upon itself, like a snail shell, two and one-half times around a central bony column, the **modiolus**. The modiolus projects into the spiraled cochlea with a shelf of bone called the ***osseous spiral lamina***, through which traverse blood vessels and the **spiral ganglion**, the cochlear division of the vestibulocochlear nerve.

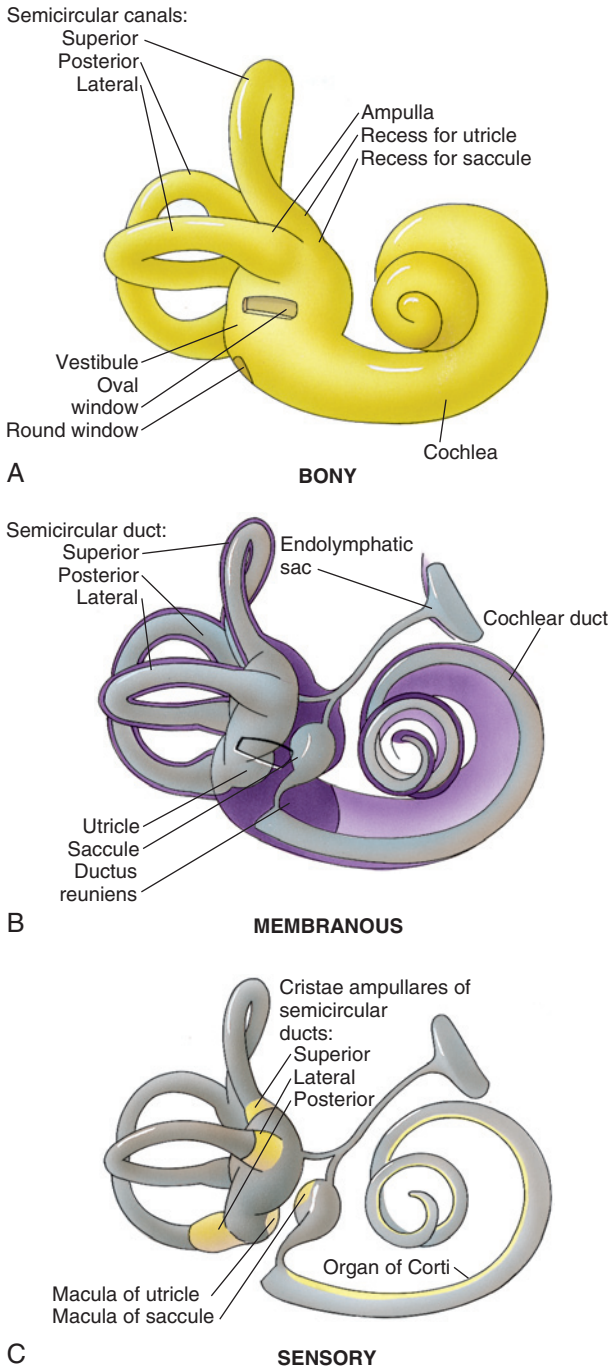


Figure 22–13 Diagram of the cochlea of the inner ear. **A,** Anatomy of bony labyrinth. **B,** Anatomy of the membranous labyrinth. **C,** Sensory labyrinth.

Membranous Labyrinth

The membranous labyrinth is filled with endolymph and possesses the following specialized areas: the saccule and utricle, the semicircular ducts, and the cochlear duct.

The **membranous labyrinth** is composed of an epithelium derived from the embryonic ectoderm, which invades the developing temporal bone and gives rise to two small sacs, the **saccule** and **utricle**, as well as to the **semicircular ducts** and the **cochlear duct** (see Fig. 22–13). Circulating through the entire membranous labyrinth is **endolymph**, a viscous fluid that resembles the cytosol (intracellular fluid) in its ionic composition (i.e., it is sodium poor but potassium rich; see Table 22–3).

Thin strands of connective tissue arising from the endosteum of the bony labyrinth pass through the perilymph to be inserted into the membranous labyrinth. In addition to anchoring the membranous labyrinth to the bony labyrinth, these connective tissue strands carry blood vessels that nourish the epithelia of the membranous labyrinth.

Saccule and Utricle

The saccule and utricle, sac-like structures lying in the vestibule, contain neuroepithelial cells that are specialized to sense position of the head and linear movement.

The **saccule** and the **utricle** are connected to each other by a small duct, the **ductus utriculosaccularis**. Additionally, small ducts from each join to form the **endolymphatic duct**, whose dilated blind end is known as the ***endolymphatic sac***. Another small duct, the **ductus reuniens**, joins the saccule with the duct of the cochlea (see Fig. 22–13).

The walls of the saccule and utricle are composed of a thin outer vascular layer of connective tissue and an inner layer of simple squamous to low cuboidal epithelium. Specialized regions of the saccule and utricle act as receptors for sensing orientation of the head relative to gravity and acceleration, respectively. These receptors are called the ***macula of the saccule*** and the ***macula of the utricle***.

The maculae of the saccule and utricle are located so that they are perpendicular to each other (i.e., the macula of the saccule is located predominantly in the wall, thus detecting linear vertical acceleration, whereas the macula of the utricle is located mostly in the floor, thus detecting linear horizontal acceleration). The epithelium of the nonreceptor regions of the saccule and utricle is composed of light and dark cells. **Light cells** have a few microvilli, and their cytoplasm contains a few pinocytotic vesicles, ribosomes, and only a small number of mitochondria. The cytoplasm of the **dark cells**, however, contains an abundance of coated vesicles,

smooth vesicles, and lipid droplets as well as numerous elongated mitochondria located in compartments formed by infoldings of the basal plasma membrane. Nuclei of the dark cells are irregular in shape and are often located apically. It is believed that light cells play a role in absorption and that dark cells control endolymph composition by transporting K^+ ions into the endolymph using potassium ion channels in the apical plasmalemmae of the dark cells.

CLINICAL CORRELATIONS

The **potassium ion channels** of dark cells that transfer K^+ ions from the dark cell into the endolymph, are composed of a regulatory protein (KCNE1) and channel proteins (KCNQ1). Certain mutations in the gene coding for the protein KCNE1 disturb the flow of K^+ ions into the endolymph, altering its ionic composition. Consequently, the entire epithelial lining of superior aspect of the ampullae, utricle, and saccule become damaged, resulting in the breakdown of the vestibular apparatus.

The maculae are thickened areas of the epithelium, 2 to 3 mm in diameter. They are composed of two types of **neuroepithelial cells**, called ***type I*** and ***type II hair cells***, as well as of supporting cells that sit on a basal lamina (Figs. 22–14 and 22–15). Nerve fibers from the vestibular division of the vestibulocochlear nerve supply the neuroepithelial cells.

Each type I or type II hair cell has a single kinocilium and 50 to 100 stereocilia arranged in rows according to length, with the longest (10 μm) being nearest the kinocilium.

Type I hair cells are plump cells with a rounded base that narrows toward the neck (see Fig. 22–15). Their cytoplasm contains occasional RER, a supranuclear Golgi complex, and numerous small vesicles. Each stereocilium, which is anchored in a dense terminal web, is a long microvillus with a core of many actin filaments cross-linked by **fimbrin**. The filamentous core imparts rigidity to the stereocilia, so that bending can occur only in the neck region, near their site of origin from the apical plasma membrane.

Type II hair cells are similar to type I hair cells with regard to the stereocilia and kinocilium, but

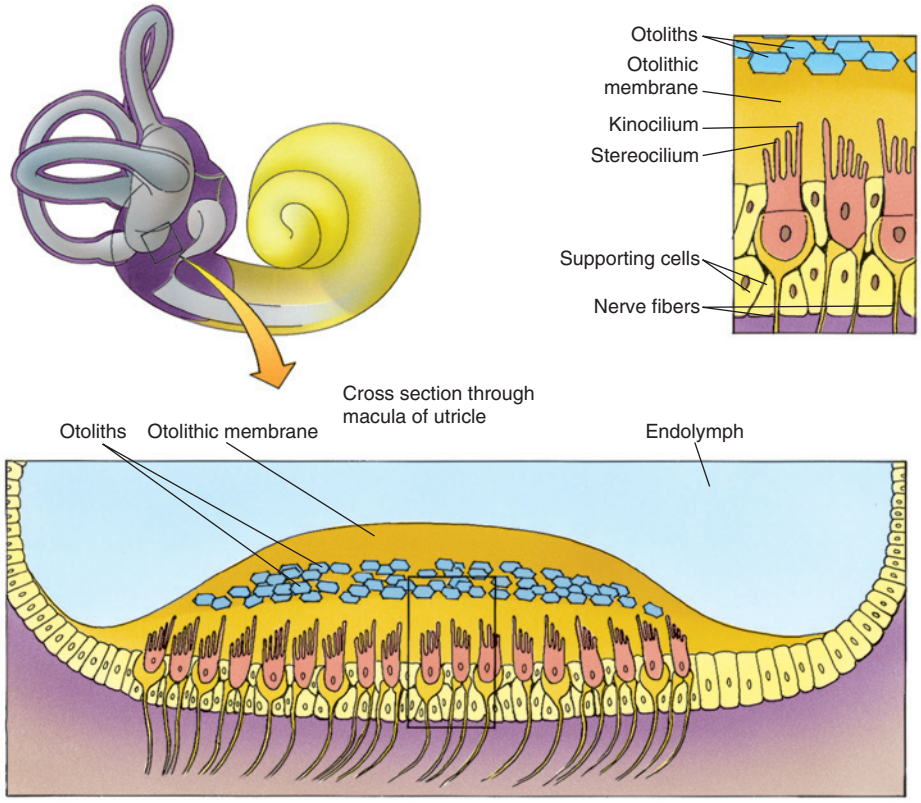


Figure 22–14 Diagram of hair cells and supporting cells in the macula of the utricle.

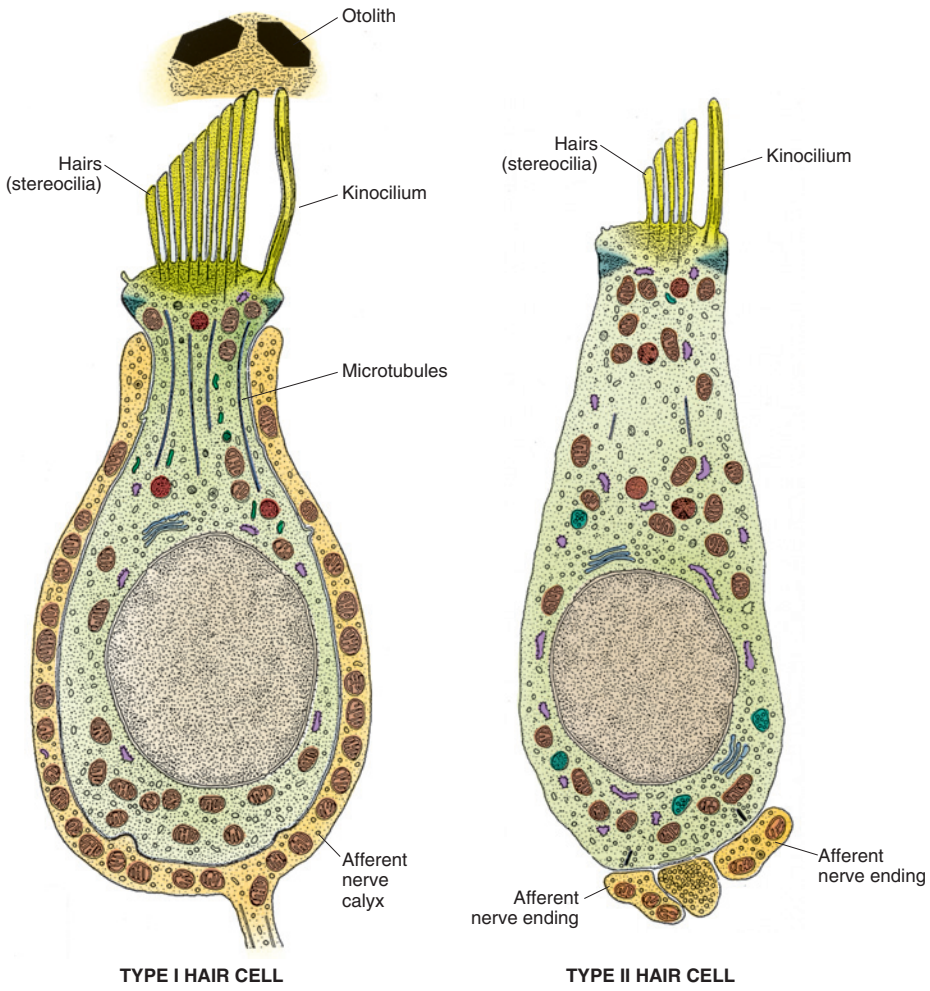


Figure 22–15 Diagram demonstrating the morphology of type I and type II neuroepithelial (hair) cells of the maculae of the saccule and utricle. The otolithic membrane is not shown with the type II hair cell. (From Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure*. Philadelphia: WB Saunders; 1971.)

their shape is more columnar, and their cytoplasm contains a larger Golgi complex and more vesicles (see Fig. 22–15).

Supporting cells of the maculae, which are interposed between both types of hair cells, have a few microvilli. Thick junctional complexes bind these cells to each other and to the hair cells. They exhibit a well-developed Golgi complex and secretory granules, suggesting that they may help maintain the hair cells and that they may contribute to the production of endolymph.

Innervation of the hair cells is derived from the vestibular division of the vestibulocochlear nerve. The rounded bases of the type I hair cells are almost entirely surrounded by a cup-shaped afferent nerve fiber. Type II hair cells exhibit many afferent fibers synapsing on the basal area of the cell. Structures resembling **synaptic ribbons** are present near the bases of type I and type II hair cells. The synaptic ribbons of the type II hair cells probably function in synapses with efferent nerves, which are thought to be responsible for increasing the efficiency of synaptic release.

The stereocilia of the neuroepithelial hair cells are covered by and embedded in a thick, gelatinous, glycoprotein mass, the **otolithic membrane**. The surface region of this membrane contains small calcium carbonate crystals known as ***otoliths*** or ***otoconia*** (see Figs. 22–14 and 22–15).

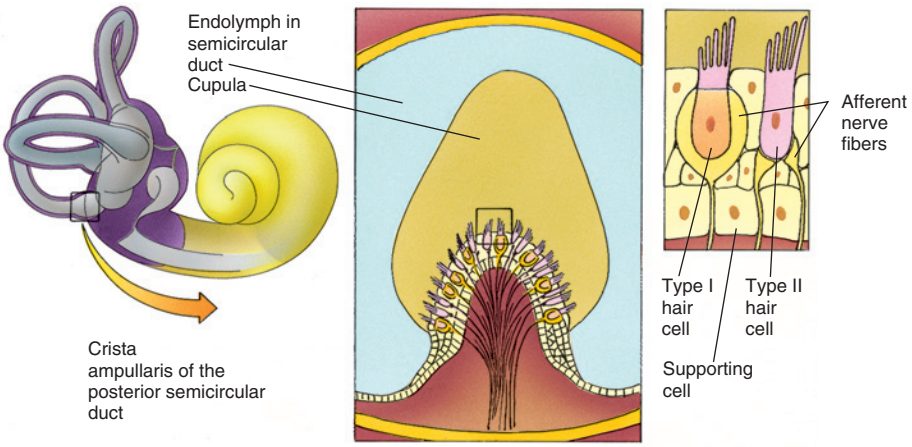


Figure 22–16 Diagram of the hair cells and supporting cells in one of the cristae ampullares of the semicircular canals.

Semicircular Ducts

Each of the three semicircular ducts contains an expanded region, the ampulla, where specialized receptors (neuroepithelial hair cells) sense linear and angular movement.

Each **semicircular duct**, a continuation of the membranous labyrinth arising from the utricle, is housed within its semicircular canal and thus conforms to its shape. Each of the three ducts is dilated at its lateral end (near the utricle). These expanded regions, called the ***ampullae***, contain the **cristae ampullares**, which are specialized receptor areas. Each crista ampullaris is composed of a ridge whose free surface is covered by sensory epithelium consisting of **neuroepithelial hair cells** and **supporting cells** (Fig. 22–16). The supporting cells sit on the basal lamina, whereas the hair cells do not; rather, the hair cells are cradled between the supporting cells. The neuroepithelial cells, also known as ***type I*** and ***type II hair cells***, exhibit the same morphology as the hair cells of the maculae. The **cupula**, a gelatinous glycoprotein mass overlying the crista ampullaris, is similar to the otolithic membrane in structure and function, but it is cone shaped and does not contain otoliths. The functional mechanism of the vestibular apparatus is described in the section titled Vestibular Function.

Scala Media (Cochlear Duct) and Organ of Corti

The scala media (cochlear duct) and its organ of Corti are responsible for the mechanism of hearing.

The **endolymph** filled **scala media (cochlear duct)**, a diverticulum of the saccule, is another regionally named portion of the membranous labyrinth. It is a wedge-shaped receptor organ housed in the bony cochlea and is surrounded by perilymph containing compartments that are separated from it by two membranes (Figs. 22–17 and 22–18). The roof of the **scala media** is the **vestibular (Reissner) membrane**, whereas the floor of the scala media is the **basilar membrane**. The perilymph-filled compartment lying above the vestibular membrane is called the ***scala vestibuli***, whereas the perilymph-filled compartment lying below the basilar membrane is the **scala tympani**. These two perilymph containing compartments communicate at the **helicotrema**, near the apex of the cochlea.

The **vestibular membrane** is composed of two layers of squamous epithelial cells separated from each other by a basal lamina. The inner layer is the lining cells of the scala media, and the outer layer is the lining cells of the scala vestibuli. Numerous tight junctions seal both layers of cells, thus ensuring a high ionic gradient across the membrane. The **basilar membrane**, extending from the spiral lamina at the modiolus to the lateral wall, supports the organ of Corti and is composed of two zones: the zona arcuata and the zona pectinata. The **zona arcuata** is thinner, lies more medial, and supports the organ of Corti. The **zona pectinata** is similar to a fibrous meshwork containing a few fibroblasts.

The lateral wall of the cochlear duct, extending between the vestibular membrane and the spiral prominence, is covered by a pseudostratified epithelium called the ***stria vascularis***. Unlike most epithelia, it contains an *intraepithelial plexus of capillaries.* The stria vascularis is composed of three cell types, marginal, basal, and intermediate cells. Dark-staining **marginal cells** have abundant microvilli on their free surfaces. Their dense cytoplasm contains numerous mitochondria and small vesicles. Labyrinthine, narrow cell processes containing elongated mitochondria are abundant on the basilar portion of the cells.

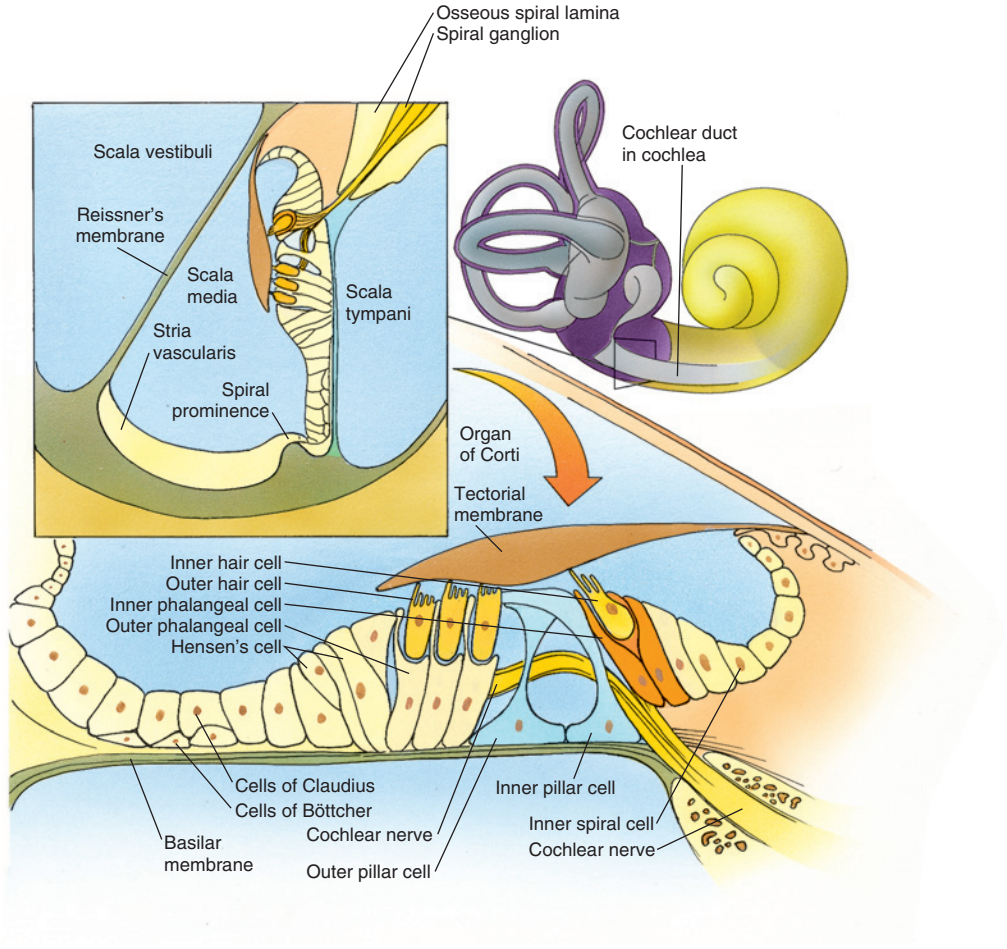


Figure 22–17 Diagram of the organ of Corti.

Light-staining **basal cells** and **intermediate cells** have less dense cytoplasm containing only a few mitochondria. Both have cytoplasmic processes that radiate out from the cell surfaces to interdigitate with the cell processes of the marginal cells and with other intermediate cells. Moreover, intermediate cells possess pigment granules in their cytoplasm. Basal cells also have cellular processes that ascend around the bases of the marginal cells, forming cup-like structures that isolate and support the marginal cells. **Intraepithelial capillaries** are positioned in such a fashion that they are surrounded by basal processes of the marginal cells and the ascending processes of the basal and intermediate cells.

The stria vascularis manufactures endolymph and delivers it into the scala media, and its **marginal cells** are responsible for delivering K^+ ions into the endolymph. The basal cells line the basal aspect of the stria vascularis and form a cellular membrane between the stria vascularis and the spiral ligament. The function of the intermediate cells is not known.

The **spiral prominence** is also located on the inferior portion of the lateral wall of the cochlear duct. It is a small protuberance that juts out from the periosteum of the cochlea into the cochlear duct throughout its entire length. The basal cells of the stria vascularis are continuous with the vascular layer of cells covering the prominence. Inferiorly, these cells are reflected into the spiral sulcus, where they become cuboidal. Other cells of this layer continue onto the basilar lamina as the **cells of Claudius**, which overlie the smaller **cells of Böttcher** (see Fig. 22–17). The latter cells are located only in the basilar turns of the cochlea. Cells of Claudius are believed to be supporting cells, whereas cells of Böttcher have been shown to possess high levels of **calmodulin** and **nitric oxide synthase**, suggesting that these cells function in the control of calcium ion levels and in the processes of secretion and absorption.

At the narrowest portion of the cochlear duct, where the vestibular and basilar membranes meet, periosteum

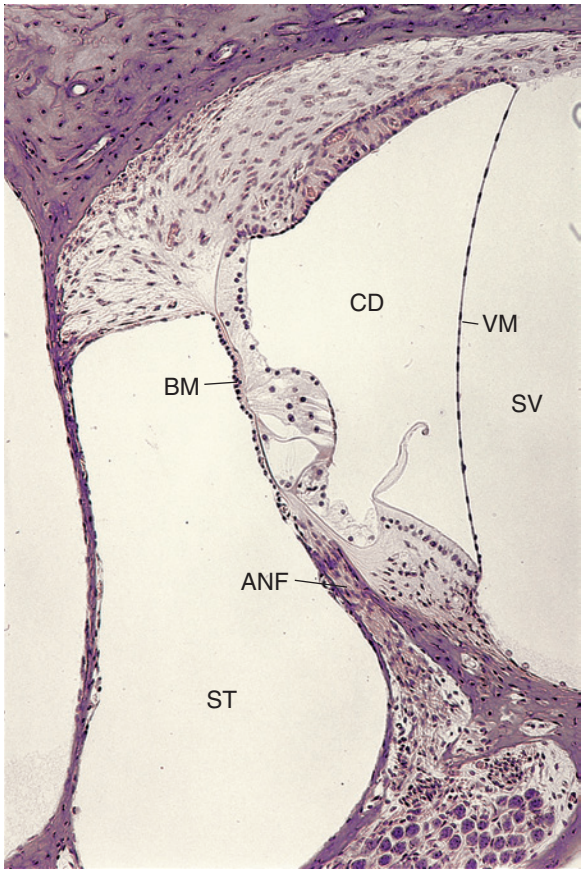


Figure 22–18 Light micrograph of the organ of Corti sitting on the basilar membrane (BM) within the cochlea (×180). The cochlear duct (CD), containing endolymph, is limited by the vestibular membrane (VM) and the basilar membrane (BM). The scala vestibuli (SV) and the scala tympani (ST) contain perilymph. Observe the spiral ganglion and the vestibulocochlear (acoustic) nerve fibers (ANF) coming from the hair cells of the organ of Corti.

covering the spiral lamina bulges out into the scala media, forming the **limbus of the spiral lamina (spiral limbus; limbus)**. Part of the limbus projects over the **internal spiral sulcus (tunnel)**. The upper portion of the limbus is the **vestibular lip**, and the lower portion is called the ***tympanic lip*** of the limbus, a continuation of the basilar membrane. Numerous perforations in the tympanic lip accommodate branches of the cochlear division of the vestibulocochlear nerve (acoustic nerve). **Interdental cells** located within the body of the spiral limbus secrete the **tectorial membrane**, a proteoglycan-rich gelatinous mass containing numerous fine keratin-like filaments, that overlies the organ of Corti. Stereocilia of specialized receptor hair cells of the organ of Corti are embedded in the tectorial membrane (see Fig. 22–17).

TABLE 22–4 Supporting Cells of the Organ of Corti

Cell	Function
Inner and outer pillar cells	Form the inner tunnel; support hair cells; support tectorial and basilar membranes
Outer phalangeal cells (outer Dieter cells)	Support the basilar portion of outer hair cells; support bundles of nerve fibers; form the space of Nuel
Inner phalangeal cells (inner Dieter cells)	Support and completely surround hair cells
Border cells	Support and define the inner aspect of the organ of Corti
Cells of Hensen	Support and define the outer aspect of the organ of Corti
Cells of Claudius	Support cells of Hensen cells and the outer aspect of the organ of Corti
Cells of Böttcher	Possess high levels of **calmodulin** and **nitric oxide synthase**, suggesting that these cells function in the control of calcium ion levels and in the processes of secretion and absorption

The **organ of Corti**, the specialized receptor organ for hearing, lies on the basilar membrane and is composed of neuroepithelial hair cells and several types of supporting cells. Although the supporting cells of the organ of Corti have different characteristics, they all originate on the basilar membrane and contain bundles of microtubules and microfilaments, and their apical surfaces are all interconnected at the free surface of the organ of Corti. Supporting cells include **pillar cells**, **phalangeal cells** (also known as ***Dieter cells***), **border cells**, and **cells of Hensen** (see Figs. 22–17 and 22–18).

Supporting Cells of the Organ of Corti

The supporting cells of the organ of Corti are the inner and outer pillar cells, the inner and outer phalangeal cells, the border cells, the cells of Hensen, and the cells of Böttcher.

Inner and **outer pillar cells** (Table 22–4) are tall cells with wide bases and apical ends; thus, they are shaped like an elongated "I." They are attached to the basilar membrane, and each one arises from a broad base. The central portions of both inner and outer pillar cells are deflected to form the walls of the **inner tunnel**, where the inner pillar cells form the medial wall of the tunnel and the outer pillar cells form the lateral wall of the

tunnel. At their apices, both inner and outer pillar cells are again in contact with each other. Their cytoplasm contains bundles of microfilaments and microtubules. Inner pillar cells outnumber outer pillar cells, with three inner pillar cells usually abutting two outer pillar cells. The pillar cells support not only the hair cells of the organ of Corti but also the tectorial and basilar membranes.

Outer phalangeal cells (outer Dieter cells) are tall columnar cells that are attached to the basilar membrane. Their apical portions are cup shaped to support the basilar portions of the outer hair cells along with bundles of efferent and afferent nerve fibers, which pass between them on their way to the hair cells. Because their cup-shaped apices cradle the hair cells, the outer phalangeal cells do not reach the free surface of the organ of Corti. However, originating from the lateral aspect of each of these cells is a small **phalangeal process** that extends to the reticular lamina. Microtubules and microfilaments within the phalangeal process add to its rigidity. The distal, flattened end of the phalangeal process is in contact with its cradled hair cell and an adjacent hair cell. There is a fluid-filled gap around unsupported regions of the outer hair cells. This space is called the ***space of Nuel***, and it communicates with the inner tunnel.

Inner phalangeal cells (inner Dieter cells) are located deep to the inner pillar cells; unlike the outer phalangeal cells, they completely surround the inner hair cells they support.

Border cells delineate the inner border of the organ of Corti. They are slender cells that support the inner aspects of the organ of Corti.

Cells of Hensen define the outer border of the organ of Corti. These tall cells are located between the outer phalangeal cells and the shorter cells of Claudius, which rest on the underlying **cells of Böttcher**.

All of these cells support the outer aspects of the organ of Corti (see Fig. 22–17).

Neuroepithelial Cells (Hair Cells) of the Organ of Corti

There are two types of neuroepithelial cells in the organ of Corti: inner hair cells and outer hair cells.

Neuroepithelial hair cells are specialized for transducing impulses for the organ of hearing. Depending on their locations, these cells are called ***inner hair cells*** and ***outer hair cells***.

Inner hair cells, a single row of approximately 3500 cells, each about 12 μm in diameter, are supported by inner phalangeal cells. These inner hair cells extend the inner limit of the entire length of the organ of Corti. Inner hair cells are short and exhibit a centrally located nucleus, numerous mitochondria (especially beneath the terminal web), RER and smooth endoplasmic reticulum, and small vesicles. The basal aspect of these cells also contains microtubules. Their apical surface contains 50 to 60 stereocilia arranged in a "V" shape. The core of the stereocilia contains microfilaments, cross-linked with fimbrin, as in the type I hair cells of the vestibular labyrinth. The microfilaments of the stereocilia merge with those of the terminal web. Although a kinocilium is not present in inner hair cells, a basal body and centriole are both evident in the apical region of these cells. The basal aspects of these cells synapse with afferent fibers of the cochlear division of the vestibulocochlear nerve.

Outer hair cells, supported by outer phalangeal cells, are located near the outer limit of the organ of Corti and are arranged in rows of three (or four) along the entire length of this organ (see Fig. 22–17). There are approximately 3500 outer hair cells, each about 8 μm in diameter. They are elongated cylindrical cells whose nuclei are located near their bases. Their cytoplasm contains abundant RER, and their mitochondria are located basally. The cytoplasm of those cells just beneath the lateral walls contains a **cortical lattice**, composed of 5- to 7-nm filaments cross-linked by thinner filaments, that appears to support the cell and resist deformation.

Cochlear nerve fibers synapse on the lateral and basilar portion of the hair cells, with a large preponderance terminating on the *inner* and only about 5% to 10% terminating on the outer hair cells. Extending from the apical surface of the outer hair cells are as many as 100 stereocilia organized in the shape of the letter "W." These stereocilia vary in length and are arranged in ordered gradation. Just as inner hair cells, outer hair cells do not have a kinocilium but do have a basal body.

Vestibular Functions

The vestibular function is the sense of position in space and during movement.

The sense of position in space and during movement is essential to activate and deactivate certain muscles that function in accommodating the body for balance. The sensory mechanism for this function is the **vestibular apparatus**, which is located in the inner ear. This apparatus comprises the utricle, saccule, and semicircular ducts.

Stereocilia of neuroepithelial hair cells located in the ampullae of the utricle and saccule are embedded in the otolithic membrane. **Linear movements** of the head cause displacement of the endolymph, which disturbs the positioning of the otoliths within the otolithic membrane and, consequently, the membrane itself, thereby bending the stereocilia of the hair cells. Movements of the stereocilia are transduced into action potentials, which are conducted by synapses to the vestibular division of the vestibulocochlear nerve for transmittal to the brain.

Circular movements of the head are sensed by receptor sites in the semicircular ducts housed within the semicircular canals. Stereocilia of the neuroepithelial hair cells of the cristae ampullares are embedded in the cupula. Movements of the endolymph within the semicircular ducts disturb the orientation of the cupula, which subsequently distorts the stereocilia of the hair cells. This mechanical stimulus is transduced to an electrical impulse, which is transferred by synapse to branches of the vestibular division of the vestibulocochlear nerve for transmission to the brain.

Information concerning the linear and circular movements of the head, recognized by receptors of the inner ear, is transmitted to the brain via the vestibular division of the vestibulocochlear nerve. There, it is interpreted, and adjustments to the balance are initiated by activation of specific muscle masses responsible for posture.

CLINICAL CORRELATIONS

Ménière disease is a disorder with hearing loss resulting from excess fluid accumulation in the endolymphatic duct. Other symptoms include vertigo, tinnitus, nausea, and vomiting. Some drugs can relieve vertigo and nausea. But in severe cases, the vestibular division nerve may have to be severed. In extreme cases, the semicircular canals and cochlea are surgically removed. Interestingly, tinnitus has affected people even in ancient times, as indicated by information noted on clay tablets preserved from the Assyrian empire and from papyrus scrolls from ancient Egypt.

Cochlear Functions

The cochlea functions in the perception of sound.

Sound waves collected by the external ear pass into the external auditory meatus and are received by the tympanic membrane, which is set into motion. The tympanic membrane converts sound waves into mechanical energy. Vibrations of the tympanic membrane set the malleus, and consequently the remaining two ossicles, into motion.

As indicated above, because of a mechanical advantage rendered by the articulations of the three bony ossicles and the difference between the surface area of the tympanic membrane and the foot of the stapes, the mechanical energy is amplified about 22 times when it reaches the membrane of the fenestra vestibuli (oval window). Movements of the oval window membrane initiate pressure waves in the perilymph within the scala vestibuli. Because fluid (in this instance, perilymph) is incompressible, the wave is passed through the scala vestibuli, through the helicotrema, and into the scala tympani and is dissipated via the membrane covering the round window. The pressure wave in the perilymph of the scala tympani causes the basilar membrane to vibrate.

Because the **organ of Corti** is firmly attached to the **basilar membrane**, a rocking motion within the basilar membrane is translated into a shearing motion on the stereocilia of the hair cells that are embedded in the overlying rigid tectorial membrane. When the shearing force produces a deflection of the stereocilia toward the taller stereocilia, the cell becomes depolarized, thus generating an impulse that is transmitted via the afferent nerve fibers of the cochlear division of the vestibulocochlear nerve (Fig. 22–19).

The differences in sound frequency or pitch are distinguished because regions of the basilar membrane, which becomes longer with each turn of the cochlea, vibrate at different frequencies relative to its width. Therefore, *low-frequency sounds* are near the apex of the cochlea (in the vicinity of the helicotrema), whereas

CLINICAL CORRELATIONS

1. **Conductive deafness** may be caused by any condition that impedes the conduction of the sound waves from the external ear through the middle ear and into the organ of Corti of the inner ear. Conditions that can lead to conductive deafness include the presence of foreign bodies, **otitis media**, and **otosclerosis** (fixation of the footplate of the stapes in the oval window).
2. **Otitis media** is a common infection of the middle ear cavity in young children. It usually develops from a respiratory infection that involves the auditory tube. Fluid buildup in the middle ear cavity dampens the tympanic membrane, thus constricting the movements of the bony ossicles. Antibiotics are the usual treatment.
3. **Nerve deafness** usually results from a disease process that interrupts transmission of the nerve impulse. The interruption may be located anywhere in the cochlear division of the acoustic nerve, from the organ of Corti to the brain. Disease processes that can lead to nerve deafness include rubella, tumors of the nerve, and nerve degeneration.
4. Neuroepithelial hair cells of the organ of Corti possess **nicotinic acetylcholine receptors (nAChRs)** that decrease the sensitivity of hair cells to the volume of sound. At high decibels, as apparent in certain music concerts and at many clubs, nAChRs cause hair cells to be desensitized and thus protect the individual from becoming deaf after long-term exposure to music at high volume. Interestingly, people living in exceptionally quiet areas of the world, such as the Mabaan people of South Sudan (near Ethiopia), have remarkably good hearing even into old age.

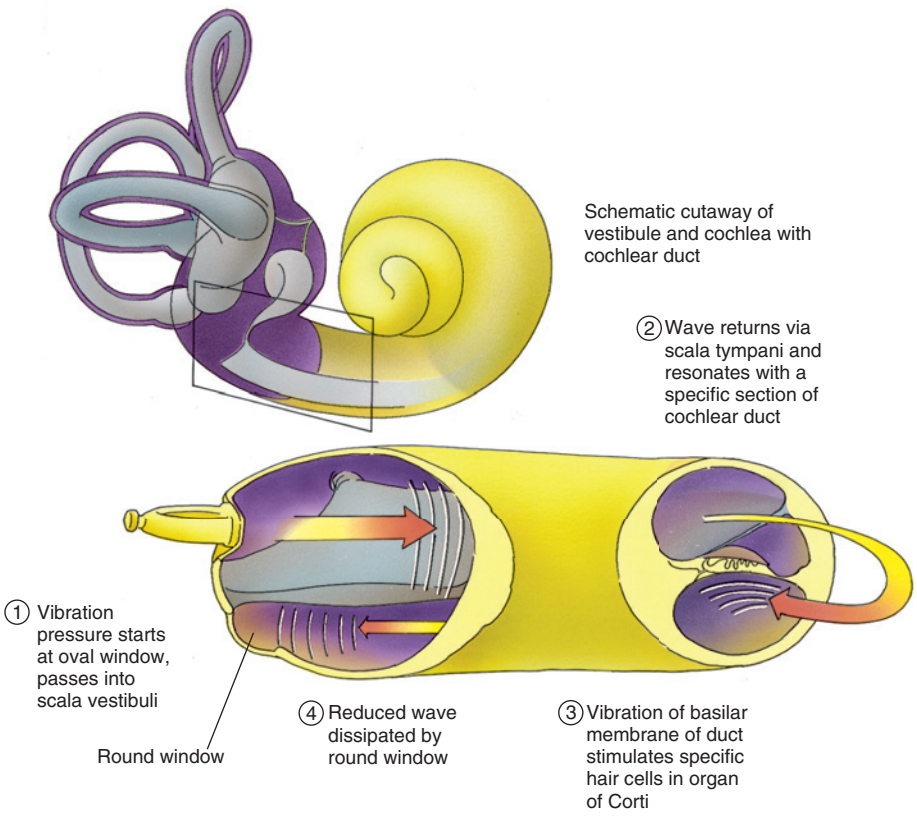


Figure 22–19 Schematic diagram of how vibrations of the footplate of the stapes move the membrane on the oval window. This action produces a pressure in the perilymph, located in the scala vestibuli. At the helicotrema, where the scala vestibuli and scala tympani communicate, the pressure wave within the perilymph of the scala tympani sets the basilar membrane and the organ of Corti, sitting on it, into motion. This causes a shearing motion on the hair cells of the basilar membrane, which is transduced into an electric current and, in turn, is transmitted by a synapse to the cochlear division of the vestibulocochlear nerve for conduction to the brain for processing.

high-frequency sounds are near the base of the cochlea (in the region of the oval window). Evidence suggests that outer hair cells contain the necessary machinery to react rapidly to efferent input, causing them to vary the length of their stereocilia and consequently altering the shear force between the tectorial membrane and the basilar membrane, thus "tuning" the basilar membrane. This action then alters the response of the sound-detecting inner hair cells, affecting their reaction to different frequencies.

PATHOLOGICAL CONSIDERATIONS

See Figures 22–20 through 22–22.

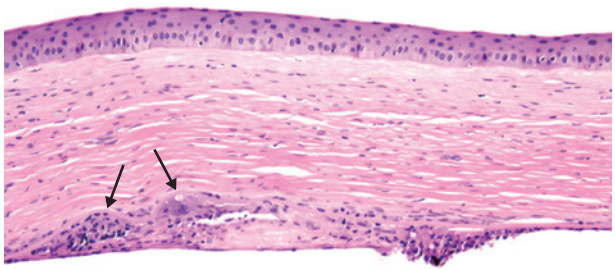


Figure 22–20 Photomicrograph of the cornea of a patient with chronic herpes simplex keratitis. Observe that the number of fibroblasts is greatly increased when compared with a healthy cornea; also note the presence of the granulomatous reaction in Descemet membrane *(arrows)*, a characteristic of this condition. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:1325.)

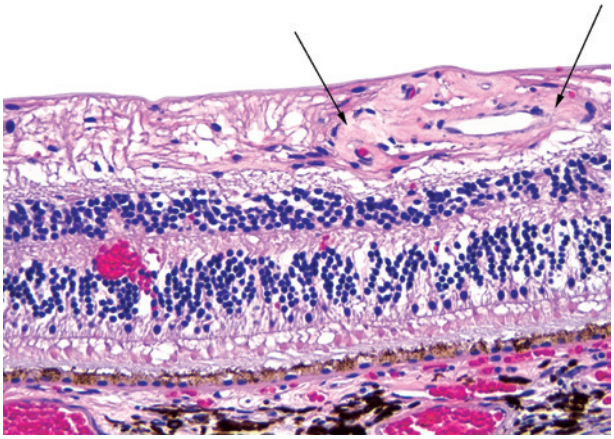


Figure 22–21 Photomicrograph of the retina of a patient with diabetes mellitus. Observe the presence of tangled vascular elements deep to the internal limiting membrane *(between the two arrows)*, a characteristic of intraretinal angiogenesis. Note also the hemorrhage within the outer plexiform layer of the retina. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:1337.)

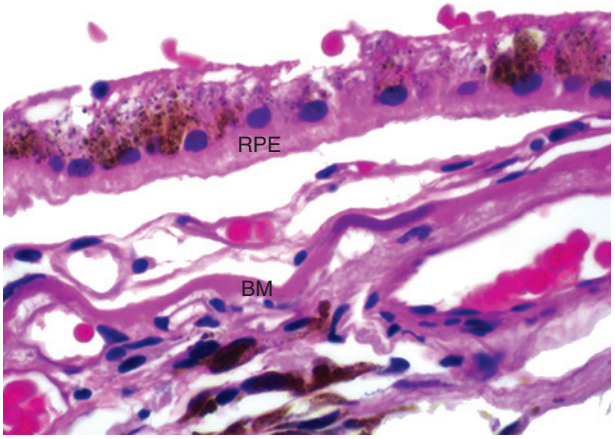
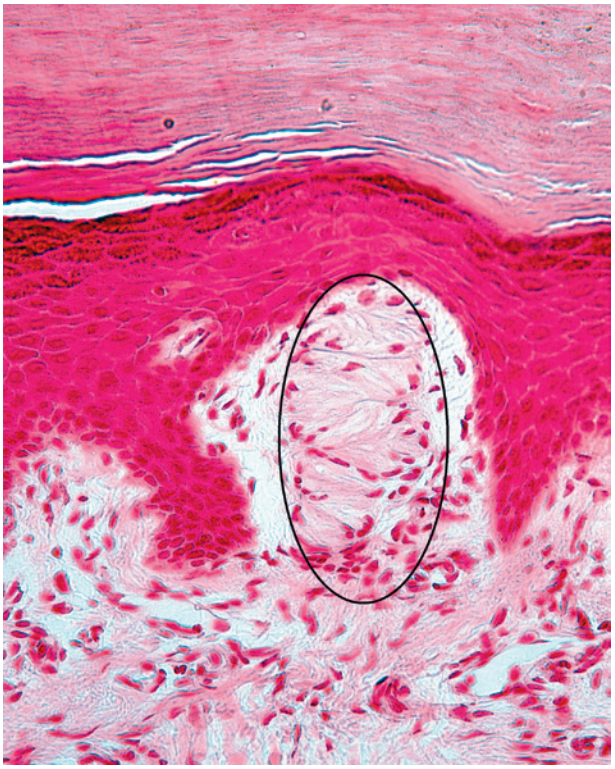


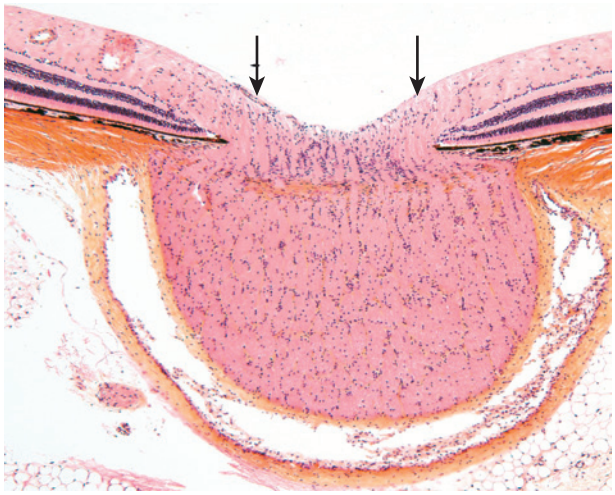
Figure 22–22 Photomicrograph of the choroid of a patient with "wet" age-related macular degeneration. Observe the presence of a vascular membrane interposed between the retinal pigment epithelium (RPE) and Bruch membrane (BM). Observe the dense color of BM to the right of the label, indicative of local calcification. (Reprinted with permission from Kumar V, et al. *Robbins and Cotran: Pathological Basis of Disease*. 9th ed. Philadelphia: Elsevier; 2015:1338.)

REVIEW QUESTIONS

1. Ion channels, known as *transient receptor V1 channels*, are associated with which of the following sensations?
   a. Pressure
   b. Stretch
   c. Cold
   d. Heat
   e. Itch
2. Ruffini endings respond to which of the following sensations?
   a. Pain
   b. Stretch
   c. Cold
   d. Vibration
   e. Fine touch
3. A patient sees her ophthalmologist, who determines that she has glaucoma. This condition is caused by
   a. increased pressure in the posterior chamber of the eye.
   b. increase in the volume of the vitreous humor.
   c. decreased pressure in the posterior chamber of the eye.
   d. increased pressure in the anterior chamber of the eye.
   e. decreased pressure in the anterior chamber of the eye.
4. A patient with Meniere disease has
   a. loss of the central visual field.
   b. loss of peripheral visual field.
   c. inability to focus on near objects.
   d. inability to focus on far objects.
   e. vertigo.
5. The perilymph passes from the scala vestibuli to the scala tympani through which of the following opening?
   a. Round window
   b. Oval window
   c. Helicotrema
   d. Inner tunnel
   e. Outer tunnel
6. A patient complains to her ophthalmologist that she cannot see the stars on a clear night and needs assistance walking in the dark. The ophthalmologist indicates that the patient is suffering from night blindness and suggests that the patient should receive an injection of
   a. Vitamin A.
   b. Vitamin B_{12}.
   c. Vitamin C.
   d. Vitamin D_3.
   e. Vitamin E.
7. 

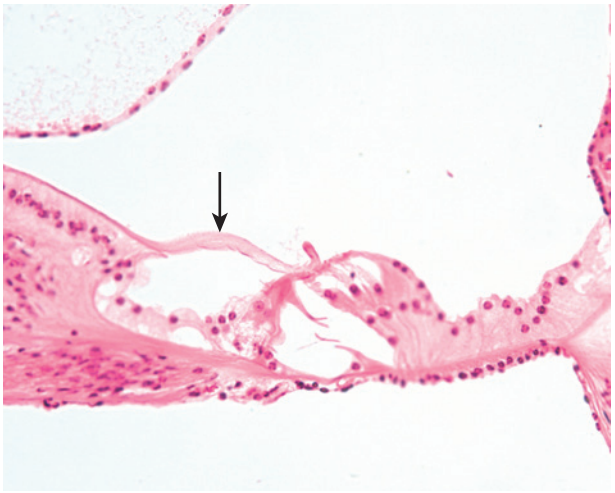
   The structure in the oval area is known as a _____ and responds to _____
   a. Meissner corpuscle; fine touch
   b. Meissner corpuscle; vibration
   c. Pacinian corpuscle; pressure
   d. Pacinian corpuscle; touch
   e. Merkel disk; pain

8.



The region between the two arrows
a. is the macula lutea.
b. is the fovea centralis.
c. is the optic disk.
d. is the ora serrata.
e. houses cones only.

9.



The structure indicated by the arrow
a. is the vestibular membrane.
b. is the tectorial membrane.
c. is secreted by interdental cells.
d. is attached to cells of Claudius.
e. is attached to cells of Hensen.
f. has microvilli.

10. A patient complains about her loss of hearing. Her otolaryngologist determines that the patient has nerve deafness and attributes the condition to
a. otitis media.
b. otosclerosis.
c. wax buildup in the ear canal.
d. constriction of the ear canal.
e. rubella.

ANWERS

1. D. Transient receptor VI channels, also known as *capsaicin receptors*, become activated if the temperature in their immediate vicinity exceeds 43°C. Receptors that possess these channels do not respond to any other stimuli.
2. B. Ruffini endings respond to pressure and stretch stimuli. Pain stimuli are sensed by nociceptors; cold is sensed by thermoreceptors; Pacinian corpuscles respond to vibration; and fine touch is sensed by Meissner corpuscles.
3. D. Glaucoma is a condition caused by increased pressure in the anterior chamber of the eye because of decreased drainage of the aqueous humor through the canal of Schlemm. It is one of the world's leading causes of blindness. Glaucoma is not caused by the volume or pressure changes in the posterior chamber of the eye or by its contents, the vitreous humor.
4. E. A patient with Meniere disease has vertigo, hearing loss, tinnitus, nausea, and vomiting. The disorder is due to the accumulation of excess fluid in the endolymphatic duct. The patient's central and peripheral visual fields are not affected, and the patient can focus on both near and far objects.
5. C. The helicotrema is an opening near the apex of the cochlea, which connects the scala vestibule with the scala tympani, permitting the perilymph access between the two compartments. The round window and the oval window both connect the middle ear cavity to the inner ear, but each has a membrane covering it. The inner and outer tunnels are in the organ of Corti and do not connect to the scala vestibuli or to the scala tympani.
6. A. A severe lack of vitamin A causes night blindness because the amount of available rhodopsin is greatly reduced. Because vitamin A is stored in great supply by the liver, it takes as long as 1 year of vitamin A deficiency in the individual's diet before night blindness becomes evident. Fortunately, night blindness caused by a vitamin A deficiency can be reversed rapidly by vitamin A injection. A lack of vitamin B_{12} is responsible for pernicious anemia. Vitamin C is necessary for healthy connective tissue; vitamin D assists in the intestinal absorption of various elements, including calcium, zinc, and phosphorus; and vitamin E has important functions in cell signaling and acting as an antioxidant.
7. A. The structure in the oval region is a Meissner corpuscle that responds to fine touch and is sensitive to edges and points. Pacinian corpuscles are located deep in the dermis and in the hypodermis and are sensitive to pressure, touch, and vibration. Merkel disks are located in the stratum basale of the epidermis and are sensitive to touch not sensitive to pain.
8. C. The region between the two arrows is known as the *optic disk*, the blind spot on the retina, where the axons gather and leave the eyeball as they form the optic nerve. The macula lutea is a yellow spot on the back of the eye, and the central region of the macula lutea is the fovea centralis, the region of greatest visual acuity, where the rods are replaced by specialized cones. The ora serrata is the edge of the retina where it meets the ciliary body. The fovea centralis is the only region of the retina that houses cones only.
9. C. The arrow points to the tectorial membrane, which is secreted by interdental cells of the organ of Corti. The vestibular membrane is the roof of the scala media. The tectorial membrane is not attached either to the cells of Claudius or to the cells of Hensen. Because the tectorial membrane is a proteoglycan-rich gelatinous mass containing keratin filaments, it has no cellular components, and it cannot have microvilli.
10. E. Rubella is one of the causes of nerve deafness, a condition that is caused by the interruption of nerve transmission. Otitis media is an inflammation of the middle ear that results in conductive deafness. Otosclerosis (in which the footplate of the stapes is affixed to the oval window) and wax buildup in the ear canal are both attributors to conductive deafness. Constriction of the ear canal, unless it is completely obstructed, does not cause impaired hearing.

This page intentionally left blank

Index

Page numbers followed by "*f*" indicate figures, "*t*" indicate tables, and "*b*" indicate boxes.

A

A bands
  in cardiac muscle, 200*f*
  in skeletal muscle, 180, 183*f*–184*f*
A-kinase, 24–25
A-site, 26
Abluminal plasmalemma, of capillaries, 299
ABO blood group system, 257*t*
Absorption
  by colon, 463
  by small intestine, 462–463
Accessional teeth, 426
Accessory genital glands, 560*f*, 575–578
  histophysiology of, 577–578
Accessory structures, of eye, 600–601
Accommodation, 590, 591*b*
Acellular cementum, 423
Acetoacetic acid, 491
Acetyl coenzyme A (acetyl CoA), 40, 44, 491
Acetyl groups, 64–69
Acetylcholine
  in autonomic nervous system, 237
  in gallbladder, 495
  in HCl secretion, 451
  in lacrimal gland, 208
  as neurotransmitter, 232*t*, 237
  pancreatic, 476, 478
  in signaling, 23
  suprarenal release of, 368
Acetylcholinesterase, 192
Acid-base balance, 499
Acid hydrolases, 39
Acidic keratins, 51*t*
Acidophilic components, 2
Acidophils, in pituitary gland, 350–352, 350*f*–351*f*
Acidosis, 492*b*
Acinar cells, pancreatic, 476, 477*f*–479*f*
Acinar glands, 121
Acinic cell carcinoma, 476*b*
Acinus(i)
  of lactating mammary glands, 554
  of liver, 485, 485*f*
  pancreatic, 476, 477*f*–478*f*
  of Rappaport, 485
  of salivary gland, 471, 472*f*–473*f*
  in sebaceous glands, 386
Acne, 386*b*
Acoustic nerve, 602*f*
Acquired immunodeficiency syndrome (AIDS), 312*t*, 327*b*
Acromegaly, 173*b*
Acrosin, 548, 567
Acrosomal cap, 566, 566*f*–567*f*
Acrosomal granule, 566, 567*f*
Acrosomal phase, of spermiogenesis, 566, 567*f*
Acrosomal reaction, 567–568
Acrosomal vesicle, 566, 567*f*
Acrosome, 566–568, 566*f*
Acrosome reaction, 548
α-Actin, 47
β-Actin, 47
γ-Actin, 47
Actin-binding proteins, 49*t*
Actin filaments
  in cytokinesis, 74
  in cytoplasm, 46
  in eccrine sweat glands, 383
  in epithelial microvilli, 104, 107*f*–108*f*
  in skeletal muscle, 186*f*
  as vesicle pathway, 35
Actin monomer, 48*f*
Actin ring, of osteoclast, 159
α-Actinin, 49*t*, 50*f*
  in epithelium, 113
  in skeletal muscle, 188, 188*t*
  in smooth muscle, 204–205
Action potential, mechanism of, 227, 227*f*
Activated cells, in immune response, 315
Activated macrophages, 138
Activation, of lymphoid cells, 324–325
Activation gate, 226
Active catalytic subunits, of A-kinase, 24–25
Active sites, of synapse, 192
Active transport, 20, 20*f*, 22
  primary, by Na^+-K^+ pump, 22
  secondary, by coupled carrier proteins, 22
Activin
  in male reproductive system, 562, 571
  ovarian, 534–535, 537*f*, 538*t*
Acute cystitis, 526*b*–527*b*
Acute myeloblastic leukemia, 281*b*
Acute myelogenous leukemia, 76*b*
Acute pancreatitis, 478*b*, 496*f*
Adaptin, 35
Adaptin complex, 35
Adaptive immune system, 311, 314–315
Addison disease, 366*b*, 379*b*
Adducin, 255
Adenine, 63
Adenocarcinoma, 119*b*
  of prostate, 577*b*, 582*f*
Adenohypophysis, 346–353
Adenoid, 343
Adenoma
  benign pleomorphic, 476*b*
  pituitary, 355*b*
Adenosine diphosphate (ADP)
  in active transport, 22
  in muscle contraction, 190*f*
Adenosine monophosphate (AMP)-activated protein kinase, 145
Adenosine triphosphate (ATP), 232*t*
  in active transport, 22
  in chromaffin cells, 367–368
  in mitochondria, 44
  in muscle contraction, 189, 190*f*
  in signaling, 23
Adenylate cyclase, 488
  in fat release, 144
  in mast cells, 134
  in signaling, 23
Adenylyl cyclase, 481
Adhesion molecules, and capillaries, 300
Adipocytes, 46
Adipokines, 131, 144
  produced by white adipose tissue, 144, 147*t*
Adiponectin, 145, 147*t*
Adipose cells (adipocytes), 131–132, 131*f*–132*f*
  brown (multilocular), 132, 133*f*, 146–147
  storage and release of fat by, 144, 145*f*
  tumors of, 147*b*
  white (unilocular), 143–144
Adipose tissue
  in connective tissue, 143–144
  histogenesis of, 147
Adipose triglyceride lipase (ATL), 144, 481
Aditus, laryngeal, 400–401
Adluminal compartment, of seminiferous tubules, 561, 562*f*
Adluminal plasmalemma, of capillaries, 299
ADP. *see* Adenosine diphosphate (ADP)
ADP/ATP exchange proteins, 44
Adrenal glands. *see* Suprarenal (adrenal) glands
Adrenocorticosteroids, effect on thymus, 331

Adrenocorticotropic hormone (ACTH, corticotropin), 349*t*–350*t*, 352, 356*t*, 361, 364, 366*b*
Adult hemoglobin (HbA), 254
Adult stem cells (ASCs), 556–557
Adventitia
  of alimentary canal, 436, 436*f*, 449*t*–450*t*
  of esophagus, 438, 449*t*–450*t*
  of large intestine, 449*t*–450*t*
  of small intestine, 449*t*–450*t*
  of stomach, 449*t*–450*t*
  of trachea, 402*f*, 403
  of urinary bladder, 525
  of uterus, 530*f*, 544
  of vagina, 552
Adventitial reticular cells, of bone marrow, 272, 272*b*
Aerobic energy system, 191
Afferent component, of peripheral nervous system, 211
Afferent fibers, of somatic nervous system, 234
Afferent glomerular arteriole, 516
Afferent lymphatic vessels, 342
Afferent neurons, 219
Afferent pathways
  somatic, 583
  visceral, 583
Aggrecans, 82–83, 82*f*, 84*f*
  in bone, 155
  in cartilage, 152
Aging, nucleolus and, 70*b*
Agranulocytes, 264
Alar sheath, 106–108
Albinism, 379*b*
Albinos, 595*b*
Albumin, in blood, 252*t*
Albuminuria, 508*b*
Alcoholics, liver disorders in, 490*b*
Aldosterone, 356*t*, 364
  and distal convoluted tubule, 512
  in urine formation, 521
Alimentary canal, 435–468. *see also* Digestive system
  defined, 435
  diffuse neuroendocrine system cells and hormones of, 444–446, 447*t*, 448*f*
  esophagus in, 437–439, 449*t*–450*t*
  histological layers of, 435–436, 436*f*, 449*t*–450*t*
  innervation of, 436–437
  large intestine in, 449*t*–450*t*, 463–468
  pathological considerations of, 468*f*–470*f*
  small intestine in, 449*t*–450*t*, 452–463
  stomach in, 439–452, 449*t*–450*t*
Alkaline phosphatase, 158*b*
All-or-none law, 189, 192
Alport syndrome, 504*b*
Alveolar bone proper, 428
Alveolar capillary network, 405*f*, 409*f*
Alveolar cells, 408–410, 411*f*–413*f*
  of mammary glands, 554, 556*f*
Alveolar ducts, 396*t*, 407–408, 408*f*–410*f*
Alveolar elastin network, 405*f*
Alveolar glands, 121
Alveolar macrophages, 411*f*, 412, 412*b*, 413*f*
Alveolar pore (of Kohn), 408, 410*f*–411*f*
Alveolar sacs, 396*t*, 407–408, 409*f*
Alveolus(i)
  of lung, 396*t*, 408–415, 409*f*–411*f*
  of tooth, 421, 421*f*, 428
Amacrine cells, 593*f*, 594*t*, 599
Ameloblasts, 422, 426, 426*f*
D-Amino acid oxidase, 40
Amino acid(s), 316
  derivatives of, in hormones, 345
  sulfation and phosphorylation of, 33*f*
Amino sugar, 81, 83*t*
Aminoacyl tRNAs, 26
γ-Aminobutyric acid (GABA)
  functions of, 232*t*
  in Huntington chorea, 231*b*
Amino-terminal regions, 319–320
Amniotic cavity, 547*f*
Amphipathic molecules, 16
Ampulla
  of ductus deferens, 575
  of inner ear, 603, 607
  of oviduct, 541
  of Vater, 494
Amylase
  pancreatic, 477
  salivary, 419, 471, 474
Amylin, 481
Amyloid β, accumulation of, 248*f*
Anabolic glucocorticoids, 365
Anagen phase, of hair growth, 388–389
Anal canal, 449*t*–450*t*, 467
Anal columns, 467
Anal glands, 467
Anal mucosa, 467
Anal sinuses, 467
Anal sphincter muscle, 467
Anal valves, 467
Anamnestic response, 315
Anaphase
  meiotic, 76*f*–77*f*, 77
    in spermatocytes, 565
  mitotic, 72*f*–73*f*, 74
Anaphylactic reaction, 134–135
Anaphylactic shock, 137*b*, 263*b*
Anaphylaxis
  slow-reacting substance of, 263
  systemic, 137*b*
Anaplastic large cell lymphoma, 344*f*
Anastomosis, arteriovenous, 298
Anastomosis, arteriovenous, in penis, 580*f*
Anchoring fibrils, in dermis, 381
Anchoring filaments, lymphatic, 306, 306*f*
Anchoring junctions, 109, 113
Anchoring plaques, 93*f*
Anchoring villi, 551
Androgen(s), 356*t*, 365
  in follicular development, 535
  weak, 366
Androgen-binding protein (ABP), 562, 571, 572*f*
Androstenedione, 356*t*, 365–366, 534–535
Anemia
  iron deficiency, 280*b*
  pernicious, 442*b*
  sickle cell, 255*b*
Anencephaly, 212*b*
Aneuploidy, 63*b*
Aneurysm, 291*b*
Angina pectoris, 306*b*
Angiotensin
  in blood pressure regulation, 294
  in mineralocorticoid hormones, 364
Angiotensin-converting enzyme (ACE), 521
  angiotensin II produced by, 294
  in juxtaglomerular cells, 513
  in tunica intima, 288
Angiotensin I, 521
  in juxtaglomerular cells, 513
Angiotensin II, 503–504, 521, 521*t*
  in chronic essential hypertension, 521*b*
  in juxtaglomerular cells, 513
Angiotensinogen, 521
  in blood pressure regulation, 294
Ankyrin, 255, 256*f*
Annulate lamella, 45
Annulus(i)
  of annulate lamella, 45
  of spermatozoon, 566, 567*f*, 568
Annulus fibrosus, 153–154
Anode, in electron microscopy, 3*f*, 9
Anosmin-1, 353*b*
Anterior chamber, of eye, 588*f*
Anterograde intraciliary transport, 106–108
Anterograde reaction, to nerve injury, 245–247
Anterograde transport, 34
  axonal, 217
Antibody(ies), 315
  constant regions (Fc regions) of, 36
  diagram of, 316*f*
  membrane-bound, 315
  reaginic, 318*t*
  secretory, 318*t*
Antibody-dependent cellular cytotoxicity, 312, 312*t*

Antibody labeling, direct and indirect, 5, 5*f*–6*f*
Anticodon, 26, 67
Antidiuresis, 520*f*
Antidiuretic hormone (ADH), 349*t*–350*t*, 353–354
  in blood pressure regulation, 294
  in urine formation, 513, 515, 521
Antigen(s)
  antibodies against, 265
  lipid, 323
  thymic-independent, 319
Antigenic determinant, 315
Antigenicity, 315*b*
Antigen-presenting cells (APCs), 312*t*, 324
  Langerhans cells as, 378
  macrophages as, 138, 263
Antimicrobial peptides, 312
Anti-Müllerian hormone (AMH), 530*b*, 562
Antiport transport, 20*f*, 22
Antral follicles, 531*f*, 533*t*, 535, 535*f*, 536*t*
Antrum, 531*f*, 535
Anus, 467
Aorta, 289, 308*f*, 363
  aneurysm of, 291*b*
Aortic bodies, 293
Aortic valves, 303*b*
Apical canaliculi, 508–509
Apical domain, of epithelium, 104–108, 105*f*–108*f*
Apical foramen, of tooth, 421, 421*f*
Apical light zone, of lymphoid nodule, 333
Apocrine glands, 120, 120*f*
  mammary, 120
  sweat, 384–385
Apoptosis, 78–79, 78*t*, 79*f*
  of hemopoietic cells, 277
  mitochondrial, 79
  of myometrial muscle, 543–544
Apoptotic bodies, 78, 79*f*
Appendices epiploicae, 463
Appendicitis, 468*b*
Appendix, 449*t*–450*t*, 467–468
Appetite, regulation of, 466–467
Appetite control
  hormonal factors of, 467
  neuronal factors of, 467
Appositional growth, of hyaline cartilage, 149
Appositional stage, of odontogenesis, 426–427, 426*f*–427*f*
APUD cells. *see* Diffuse neuroendocrine system (DNES) cells
Aquaporins, 21–22, 104, 521, 522*t*
Aqueous humor, 590
Arachidonic acid, in mast cell mediator synthesis, 135
Arachnoid layer, 241–242
Arachnoid trabecular cells, 241
Arachnoid villi, 241
Arcuate arteries
  of endometrium, 543, 543*f*
  of kidney, 500*f*–501*f*, 516
  of myometrium, 543, 543*f*
Arcuate vein, 500*f*–501*f*, 516
Area cribrosa, 499
Areola, 554–555
Areolar connective tissue, 141
Areolar glands, 554
Argentaffine and argyrophilic cells. *see* Diffuse neuroendocrine system (DNES) cells
Aromatase, 534–535, 539
Arrector pili muscles, 374, 374*f*, 381, 388
Arterial blood pressure, regulation of, 293–294
Arteries, 289–294. *see also specific artery*
  classification of, 289–293, 289*t*
  defined, 287
  diagram of, 288*f*
  elastic (conducting), 290, 290*f*
  muscular (distributing), 290, 291*f*
  specialized sensory structures in, 293
Arteriolae rectae, 516, 517*f*
Arterioles, 289*t*, 291–293, 292*f*
  glomerular, 503, 503*f*
    afferent, 516
    efferent, 516, 521
  hepatic, 484
  sheathed, 335
  of splenic pulp, 335
  terminal, 298
Arteriosclerosis, 294*b*
Arteriovenous anastomosis (AVA), 298
  in penis, 580*f*
Aryl sulfatase, mast cell release of, 132–133, 136*t*
Aspartate, 231
Aspirin, and ulcer, 453*b*
Asthma, 137*b*, 407*b*
Astral microtubules, 74
Astrocytes, 219–221, 220*f*–221*f*
Astrocytomas, 218*b*
Asymmetric synapses, 231
Atherosclerosis, 294*b*
ATP. *see* Adenosine triphosphate (ATP)
ATP-binding cassette transporters (ABC-Transporters), 22–23, 23*b*
ATP-binding cassettes, 22–23
ATP synthase, 43*f*, 44
Atresia, of oogonia, 529
Atretic follicles, 540
Atrial natriuretic peptide (ANP), 202, 304, 305*f*
Atriopeptin, 304
Atrioventricular bundle, 304
Atrioventricular valves, 303
Atrium
  alveolar, 407–408
  of heart, 303, 303*f*
Attachment plaques, 114
Auditory meatus, external, 601–602, 602*f*
Auditory tube, 400*b*, 602, 602*f*
Auerbach plexus
  myenteric, 238, 435–436, 458
  of parasympathetic nervous system, 212*b*
Auricle, 601, 602*f*
Autocrine signaling, 23, 119
Autografts, 173*b*
Autoimmune diseases, 316*b*
Autologous transplant, 275*b*
Autonomic fibers, of periodontal ligament, 428
Autonomic ganglia, 238–239, 239*f*
Autonomic innervation, of salivary glands, 474
Autonomic system, 211, 235–238, 236*f*
Autophagosomes, 39
Autoradiography, 6–7, 7*f*–8*f*
Autosomes, 59–60
Axoaxonic synapses, 228
Axodendritic synapses, 228, 240*f*, 598–599
Axolemma, 216–217
Axon(s), 216–218
  defined, 216–218
  hypothalamic, 347
  myelinated, 217, 217*f*–218*f*
  of olfactory cells, 397
  unmyelinated, 218*f*
Axon hillock, 213, 216–217
Axon reaction, 245–247, 246*f*
Axon terminals, 192, 213
Axonal transport, 217, 217*b*
Axonemal transport, 106–108
Axoneme, 105–108
  flagellar, 566, 567*f*, 568
Axoplasm, 217
Axosomatic synapses, 228
Azures, 251
Azurophilic granules
  of basophils, 262
  of eosinophils, 261
  of neutrophils, 259

B

B antigens, 257*t*
B cell lymphoma, 344*f*
B-cell receptors, 317
B lymphocytes (B cells)
  effector cells of, 265
  functions of, 264–265
  immunocompetent, 317
  of innate immune systems, 317–319
  memory cells of, 265
  in small intestine, 459–460, 460*f*

B-type natriuretic peptide (BNP), 202, 304*b*
Balance mechanism, 583
BALT (bronchus-associated lymphoid tissue), 312*t*, 342, 416
Band 3 proteins, 254, 256*f*
Band 4.1 proteins, 255, 256*f*
Barbiturates, tolerance to, 493*b*
Bardet-Biedl syndrome protein complex (BBSome complex), 108
Baroreceptors, 293
Barr body, 62, 258
Barrett syndrome, 439*b*
Bartholin glands, 553
Basal body, of cilium, 106
Basal cell(s), 608
  of ductus epididymis, 574, 574*f*
  of nasal cavity, 397–399, 398*f*
  of taste buds, 431, 432*f*
  of trachea, 402, 402*f*
Basal cell carcinoma, 383*b*
Basal compartment, of seminiferous tubules, 561, 562*f*–563*f*
Basal lamina
  of capillary, 296*f*
  of extracellular matrix, 93–95, 93*f*–95*f*
  of glomerulus, 504, 505*f*–506*f*
  of kidney, 501, 508*b*
  of renal corpuscle, 503*f*
  of seminiferous tubules, 561
  of spleen, 338–339
  of thyroid, 355–356
Basal light zone, of lymphoid nodule, 333
Basal surface specializations, of epithelium, 116–118
Basal zone, of osteoclast, 159
Basalis layer, of endometrium, 543, 543*f*
Basement membrane, of skin, 374*f*, 375
Basic keratins, 51*t*
Basilar membrane, 607, 611
Basket cells, of salivary gland, 472, 472*f*
Basolateral domain, of epithelium, 108–118
Basophil(s), 275*f*
  features of, 258*t*
  functions of, 139, 262–263
  granules, 262
  in pituitary gland, 350*f*, 352
  structure of, 252*f*
Basophilic components, 2
Basophilic erythroblast, 275*f*, 277*f*, 280*t*
Basophilic metamyelocyte, 275*f*
Basophilic myelocyte, 275*f*
Basophilic series, 283*f*
Basophilic stab cell, 275*f*
BBSome complex (Bardet-Biedl syndrome protein complex), 108
Bell stage, of odontogenesis, 426–427, 426*f*–427*f*
Bellini, ducts of, 499, 501, 515
Benign fibroma, 218
Benign pleomorphic adenoma, 476*b*
Benign prostatic hyperplasia, 582*f*
Benign prostatic hypertrophy, 577*b*
Beta defensin 126 (DEFB126), 568*b*
Beta oxidation, 40
Betaglycan, 82
Bicarbonate ions (HCO_3-)
  in gas exchange, 414
  in HCl production, 452
  production of, by colon, 464
Bicuspid valve, 303
Bile, 471, 481, 488
  heme in, 340
  manufacture of, 490, 492*f*
  primary, 488
  storage, concentration, and release of, 494
Bile acids, 490
Bile canaliculi, 483*f*, 485–487
Bile duct, 486
  common, 436*f*, 476–477, 494
Bile salts, 490, 490*b*
Bilirubin, 340, 490, 492*f*
  conjugated, 490
  free, 490
Bilirubin glucuronide, 490, 492*f*
Biogenic amines, 231
Bipolar neurons, 215*f*, 218
  in retina, 593*f*, 594*t*, 598–599
Birbeck granules, 378
Bladder, 499, 524–525, 524*f*–525*f*, 525*b*
Bladder cancer, 76*b*, 525*b*, 527*f*
Blastocele, 547*f*
Blastocyst, 547*f*, 548
Bleeding disorders, 271*b*
Blood, 251–285
  circulating, 252*f*
  coagulation of, 251
  defined, 251–271
  formed element of, 253–271
    agranulocytes as, 264
    basophils as, 262–263
    eosinophils as, 261
    erythrocytes as, 253–257, 253*f*–254*f*
    formation of. *see* Hemopoiesis
    leukocytes as, 257–265
    lymphocytes as, 264, 264*f*
    macrophages as, 263
    monocytes as, 263
    neutrophils as, 257–261
    platelets as, 265–271
  pathological considerations of, 285
  plasma of, 251–252
  proteins in, 252*t*, 492–493
Blood-brain barrier, 221
Blood cells
  formation of. *see* Hemopoiesis
  red. *see* Erythrocyte(s)
  white. *see* Leukocyte(s)
Blood clot, 267, 268*f*
Blood coagulation pathways, diagram of, 270*t*
Blood-CSF barrier, 244
Blood-gas barrier, 411*f*, 414
Blood group system, 257*t*
Blood islands, 272
Blood pressure, 499, 505–508
  arterial, 293–294
Blood supply. *see* Vascular supply
Blood-thymus barrier, 331
Blood vessels
  arteries as, 287, 289–294
  capillaries as, 295–300, 295*f*
  general structure of, 287–289
  nerve supply to, 288–289
  tunics of, 287–288
  veins as, 300–302
Bolus, 419
Bone(s), 154–175. *see also* Osteoblasts; Osteoclast(s)
  calcification of, 170
  calcium level maintenance by, 158
  cancellous (spongy), 161, 162*f*
  cells of, 155–161
  classification of, 161
  compact, 161, 162*f*, 163
    haversian canal systems of, 162*f*, 163–164, 164*f*
    lamellar systems of, 162*f*, 163–164
    outer and inner circumferential lamellae of, 163
  decalcified sections of, 154–155
  defined, 154
  flat, 161
  formation of
    alkaline phosphatase in, 158*b*, 170
    endochondral, 165–170, 166*f*
    intramembranous, 164–165, 165*f*–166*f*
  gross observation of, 161–163, 162*f*
  ground sections of, 154–155
  growth of
    in length, 168–170
    in width, 170
  histogenesis of, 164–170
  hormonal effects on, 160–161, 172–174
  hyaline cartilage model of, 166–167
  inorganic component of, 155
  irregular, 161
  in joints, 174–175, 175*f*
  long, 161
  matrix of, 155–161
  microscopic observations of, 163–164
  nutritional effects on, 174–175, 174*t*
  organic component of, 155
  osteoblasts of, 155–156, 157*f*
  osteoclasts of, 158–161
  osteocytes of, 154*f*, 156–158, 158*b*
  osteoprogenitor cells of, 154*f*, 155

Bone(s) *(Continued)*
pathological considerations for, 175, 175*f*–177*f*
primary (immature), 163
remodeling of, 158*b*, 171
repair of, 171–172, 172*f*, 173*b*
resorption of, mechanism of, 159–160, 161*f*
secondary (mature), 163
sesamoid, 161
short, 161
structure of, 161–174
Bone graft, 173*b*
Bone-lining cells, 156
Bone marrow, 271–285, 271*f*, 273*f*, 275*b*
B lymphocytes and, 264
defined, 154, 271
in hemopoiesis, 271
postnatal, 272–285
prenatal, 272
stem cells, progenitor cells, and precursor cells in, 273–277, 275*b*, 275*f*
red, 271–272, 272*b*
transplantation of, 281*b*
yellow, 271–272, 272*b*
Bone morphogenic protein-15, 534
Bone morphogenic proteins, in odontogenesis, 425
Bone sialoprotein, 155–156
Bony labyrinth, of ear, 603, 603*t*, 604*f*
Bony ossicles, 602*f*
"Bony union," 173*b*
Bony wall, of ears, 603
Border cell(s), of organ of Corti, 609–610, 609*t*
Border cell layer, of meninges, 241
Botox Cosmetic, 194*b*
Böttcher cells, 608, 608*f*, 610
Botulism, 194*b*
Bouin fluid, 1
Bouton terminal, 228
Bowman capsule, 500*f*–503*f*, 502, 508
Bowman (olfactory) glands, 397, 397*f*–398*f*, 399
Bowman membrane, 589
Bowman space, 502, 503*f*, 508, 513*f*
Bradykinin(s)
capillary permeability and, 299–300
in inflammatory response, 136
mast cell release of, 132–133, 136*t*
salivary glands and, 474
Brain. *see also* Central nervous system (CNS); Nervous system
Alzheimer disease and, 248*f*
blood-brain barrier, 221
cerebellar cortex of, 244
cerebral cortex of, 244
cerebrospinal fluid in, 243
choroid plexus of, 243
development of, 211
Brain *(Continued)*
HIV-induced encephalitis and, 249*f*
plasticity, 247
prion diseases and, 248*f*
swelling of, 244*b*
vasomotor center in, 293
"Brain sand," 369
Breast(s)
areola and nipple of, 554–555
cancer of, 556*b*, 558*f*
mammary glands of, 553–555, 554*f*–556*f*
nipple of, 553
Breast-feeding, 556*b*
Breathing, 395, 415
Bridge tetrasaccharides, 82
Broad ligament, of uterus, 529, 530*f*
Bronchial arteries, 416
Bronchial tree, 403–407, 405*f*
Bronchial veins, 416
Bronchioles
primary, 396*t*, 404
respiratory, 396*t*, 407, 408*f*
terminal, 396*t*, 407
Bronchopulmonary segment, 404, 415
Bronchus(i)
lobar, 404
primary (extrapulmonary), 396*t*, 404
secondary and tertiary (intrapulmonary), 396*t*, 404
Bronchus-associated lymphoid tissue (BALT), 312*t*, 342, 416
Brown adipose tissue, 131, 133*f*, 146–147
Bruch membrane, 589, 594
Brunner glands, 456*f*, 457–458
Brush border
of proximal tubule, 104
of renal corpuscle, 503*f*
Brush cells, 494, 495*f*
of respiratory epithelium, 402–403
Bud stage, of odontogenesis, 424, 425*f*
Buffy coat, 251
Bulb, of eye, 587
Bulbar conjunctiva, 600
Bulbar sheath, of eye, 588*f*
Bulbourethral gland, 559, 560*f*, 577
Bullous pemphigoid antigen 180 (BP180), 117
Bundle of His, 303*f*, 304
Bursa of Fabricius, 285, 317
Burst-forming units-erythrocyte (BFU-E), 277

C

C-kinase, 25
C protein, 188*t*
C-reactive protein (CRP), 294*b*
C-rings, tracheal, 401, 403
Cachexia, 146*b*
Cadherins, 113
Cajal, interstitial cells of, 435
Calcification, zone of, 169
Calcitonin
in bone resorption, 160–161
functions of, 355, 356*t*
in parafollicular cells, 359
parathyroid hormone and, 361
Calcitriol, 499, 518
Calcium (Ca^{2+})
binding, 207*t*
blood levels of, 360
control of, in muscles, 207*t*
parathyroid hormone and, 360
in primary follicles, 533
as second messenger, 24, 345–346
in signaling, 25
Calcium-calmodulin-dependent protein kinases (CaM-kinases), 25
Calcium hydroxyapatite
in cementum, 423
in dentin, 422
in enamel, 422
Calcium pumps, 191, 201
Calcium-sodium channels, 201
Caldesmon, 204–205
Calmodulin, 608
in signaling, 25
in smooth muscle, 205
Calmodulin-calcium complex, 206
Calponin, 204, 206
Calsequestrin, 191
Caltrectin, 52
Calvaria, 163
Calyx(ces), renal, 499, 500*f*–501*f*, 523
cAMP. *see* Cyclic adenosine monophosphate (cAMP)
cAMP response elements (CREs), 24–25
Camurati-Engelmann disease, 158*b*
Canal of Schlemm, 588*f*, 589
Canaliculi, 162*f*, 163
apical, 508–509
bile, 483*f*, 485–487
in cementum, 423
in eccrine sweat glands, 383
lacrimal, 601
pancreatic, 477*f*
of parietal cells, 443, 445*f*
Canals of Hering, 486
Cancellous (spongy) bone, 161, 162*f*
Cancer. *see also specific type*
benign pleomorphic adenoma as, 476*b*
of bladder, 76*b*, 525*b*, 527*f*
of breast, 556*b*, 558*f*
cervical carcinoma as, 544*b*, 557*f*
dermatologic, 383*b*
gastric carcinoma as, 453*b*
meningioma as, 242*b*
neurological, 218*b*
pancreatic, 478*b*
prostatic, 577

Cancer chemotherapy, and cell cycle, 76*b*
Cap phase, of spermiogenesis, 566, 566*f*–567*f*
Cap stage, of odontogenesis, 424–426, 425*f*
Cap Z, in skeletal muscle, 188, 188*t*
Capacitation, of spermatozoa, 541, 567, 573
Capillaries, 295–300, 295*f*
  classification of, 297–298, 298*f*
  continuous, 179, 295*f*, 297, 298*f*
    of blood-brain barrier, 242
    of interalveolar septa, 408, 414
    of lungs, 415–416
    of pia mater, 242
    of thymus, 331
  defined, 287
  general structure of, 295–297
  histophysiology of, 299–300
  lymphatic, 306–307, 306*f*
  regulation of blood flow into, 298–299
  renal, 502–504, 504*f*–506*f*
  sinusoidal (discontinuous), 298*f*
Capillary bed, regulation of blood flow into, 298–299, 299*f*
Capillary plexus
  hypophyseal, 347, 349*f*
  peribiliary, 484
Capping proteins, 47, 49*t*
Capsaicin receptors, 586
Capsule, 175
  Bowman, 500*f*–503*f*, 502, 508
  of gland, 121–122
  Glisson, 481
  of kidney, 499, 500*f*–501*f*
  of lens, 591
  of liver (Glisson), 481
  of lymph nodes, 331–332
  of Meissner corpuscle, 584, 585*f*
  of Pacinian corpuscle, 585*f*, 586
  of palatine tonsils, 342
  of prostate gland, 576, 576*f*
  of spleen, 335
  of Tenon, 588
Capsule cells, 238
Carbaminohemoglobin, 254
Carbamylhemoglobin, 254
Carbohydrate synthesis, in liver, 491–493, 492*f*
Carbon dioxide
  exchange of, 414–415
  poisoning due to, 255*b*
  release of, 409*f*
  uptake of, 409*f*
Carbon monoxide, as neurotransmitter, 231
Carbon monoxyhemoglobin, 255*b*
Carbonic anhydrase, 254, 414, 452, 478
Carboxypeptidase, 478
Carcinoma(s), 119*b*
  adenocarcinoma as, of prostate, 577*b*, 582*f*
  basal cell, 383*b*
  cervical, 544*b*, 557*f*
  ductal, 556*b*, 558*f*
  gastric, 453*b*
  invasive, 544*b*
  lobular, 556*b*
  renal cell, 527*f*
  in situ, 544*b*
  squamous cell, 383*b*
    of glans penis, 582*f*
Cardia, 440, 446–451, 449*t*–450*t*
Cardiac arrhythmias, 117*b*
Cardiac muscle, 198–202
  cells of, 198–202, 199*f*
  characteristics of, 181*f*, 207*t*
  contraction of, 198, 207*t*
  intercalated disks of, 198–200, 200*f*–201*f*
  organelles of, 200–202, 202*f*
  regeneration of, 207
Cardiac skeleton, 305
Cardiac stomach, 446–451, 449*t*–450*t*
Cardiodilatin, 304
Cardiolipin, 44
Cardionatrin, 304
Cardiovascular system
  arteries in, 287, 289–294
  capillaries in, 295–300, 295*f*
  veins in, 287, 300–302
Cargo, 106–108
Cargo receptors, 36
Cargo vesicles, 33
Caries, 422*b*
Carotid artery, left common, 289
Carotid body, 293
Carotid sinus, 293
Carpopedal spasms, 361*b*
Carrier-mediated diffusion, 20*f*
Carrier proteins, 22–23
Cartilage, 149–154, 150*f*, 151*t*
  articular, 150
  elastic, 150*f*, 151*t*, 153, 153*f*
  epiphyseal plate of, 150
  extracellular matrix of, 149
  fibrocartilage as, 150*f*, 151*t*, 153–154, 153*f*
  hyaline, 149–152, 151*t*
    cells in, 150–151, 151*f*
    degeneration and regeneration of, 152*b*
    histogenesis and growth of, 149–150
    histophysiology of, 152
    hormone and vitamin effects on, 152*t*
    matrix of, 150*f*, 151–152
Cartilage rings (C-rings), 401, 403
Carvajal syndrome, 114*b*
Cas proteins, 69*b*
Caspases, 78
Catabolic glucocorticoids, 365
Catagen phase, of hair growth, 388–389
Catalase, 40, 261
Catalytic receptors, 346
Cataract, 591*b*
Catecholamines, 356*t*, 366, 368
Catenins, 113
Cathode, in electron microscopy, 3*f*, 9
Caveolae, 205
Caveolin, 33
Cavities, 422*b*
CD markers, 319
CD molecules (CD markers), 319
CD2 associated protein, 505
CD8 markers, 319
Cecum, 463
Cell(s). *see also* Cytoplasm; *specific cells*
  of Böttcher, 608, 608*f*, 610
  characteristics of, 13, 14*f*–15*f*
  of Claudius, 608
  defined, 13
  of Hensen, 609–610, 609*t*
  protein synthetic and packaging machinery of, 25–35
Cell adhesion molecules (CAMs), 109
Cell adhesive glycoproteins, in extracellular matrix, 84–85
Cell body, neuronal, 213–215, 216*f*
  somatic, 234
Cell coat, 19
Cell cycle, 70–78, 70*f*
  anaphase of
    meiotic, 76*f*–77*f*, 77
    mitotic, 72*f*, 74
  cancer chemotherapy and, 76*b*
  cytokinesis in, 74–75, 75*f*
  defined, 70
  G_1 phase of, 71
  G_2 phase of, 71–72
  interphase of, 71–72
  meiosis in, 76–78, 76*f*–77*f*, 78*b*
  metaphase of
    meiotic, 76*f*–77*f*, 77
    mitotic, 74
  mitosis in, 72–74, 72*f*–73*f*
  prometaphase of, 73*f*, 74
  prophase of
    meiotic, 76–77, 76*f*–77*f*
    mitotic, 72–74
  S phase of, 71
  telophase of
    meiotic, 76*f*–77*f*, 77
    mitotic, 74
Cell death, programmed. *see* Apoptosis
Cell-free zone, of pulp, 423
Cell-mediated cytotoxicity, antibody dependent, 317–318
Cell-mediated immune response, 314

Cell membrane, 13–15, 16*f*
carrier proteins in, 22–23
cell signaling in, 23–25
cell-surface receptors in, 24–25
channel proteins in, 20–22
of erythrocyte, 255–257, 256*f*
fluid mosaic model of, 17, 18*f*
functions of, 13
glycocalyx of, 19
integral proteins in, 16, 18*f*
ion channels in, 20
membrane transport proteins in, 19–23, 20*f*
molecular composition of, 15–25, 18*f*–19*f*
multipass proteins in, 16–17
peripheral proteins in, 16, 18*f*
signaling molecules in, 16–17, 23–24
Cell-rich zone, of pulp, 423
Cell signaling, 23–25
Cell-surface fibronectin, 84
Cell-surface receptors, 24–25, 345
α Cells, 478–479, 479*f*, 480*t*, 481, 513
β-Cells, 478–481, 479*f*, 480*t*, 482*b*, 482*t*, 513
δ Cells, 478–479, 480*t*, 481
ε-Cells, 478–479, 480*t*, 481
Cellular cementum, 423
Cementing line, 154*f*, 163
Cementoblasts, 423, 427
Cementocytes, 423
Cementum, 421*f*–422*f*, 423, 423*b*, 427
Central artery, of spleen, 335
Central canal, 239–240
Central channel, 299, 299*f*
Central domain, 49–50
Central longitudinal vein, 272
Central memory T cells, 321
Central nervous system (CNS), 239–244. *see also* Brain; Nervous system
blood-brain barrier in, 221
cerebellar cortex in, 244, 245*f*
cerebral cortex in, 244
cerebrospinal fluid in, 243–244
choroid plexus in, 243–244, 243*f*
congenital malformations of, 212*b*
defined, 211
meninges in, 240–242, 241*f*
regeneration in, 247, 247*b*
Central plug, in nuclear pore complex, 56
Central retinal artery, 593
Central sheath, of cilia, 105
Central vein, of liver, 484, 484*f*
Centrin, 52
Centrioles, 16*f*, 48*f*, 51–53
in mitosis, 72
Centroacinar cells, 476–478, 477*f*–478*f*
Centroblasts, of lymphoid nodule, 333
Centrocytes, 333
Centromere, 72
Centrosome, 34, 50–53, 72
Cephalic phase, of HCl secretion, 451
Cerebellar cortex, 244, 245*f*
Cerebellar islands, 244
Cerebral cortex, 244
Cerebrospinal fluid (CSF), 243–244, 244*t*
blood-CSF barrier and, 244
ependymal cells and, 223
vs. serum, 244*t*
Cerumen, 601–602
Ceruminous glands, 384, 601–602, 602*f*
Cervical carcinoma, 544*b*, 557*f*
Cervical ganglia, 236*f*
Cervical glands, 544
Cervical loop, of tooth, 424
Cervix
of tooth, 421, 421*f*
uterine, 530*f*, 544
α-chain
synthesis of, 88–89, 89*f*
in tropocollagen, 86–87, 87*f*
Chain-terminator site, 65
Channel proteins, 20–22
Charcot-Böttcher
crystal of, 46
crystalloids of, 561
Checkpoints, 71
Chemical synapses, 227–228
Chemiosmotic theory, 45
Chemokines, 312
Chemoreceptors, 293
Chemotherapy, demyelination due to, 225*b*
Chiasmata, 77
Chief cells
of parathyroid, 360
of stomach, 441*f*–442*f*, 443–444, 446*f*
Chloride channel, 406*b*
Chloride ion (Cl^-), in HCl production, 452
Chloride shift, 254, 414
Chloride transporter, 587*b*
Choana, 395
Cholangiocytes, 486
Cholangioles, 486
Cholecystokinin, 447*t*, 476, 478, 495
Cholelithiasis, 496*b*
Cholera, pancreatic, 482*b*
Cholera toxin, 460*b*
Cholesterol
in cell membrane, 16, 18*f*
corticosteroid synthesis from, 361, 364
Cholesterol desmolase, 570–571
Cholesterol esterase, 570–571, 571*f*
pancreatic, 477
Cholesterol stones, 496*b*, 497*f*
Choline acetyl transferase, 192–193
Cholinergic splanchnic nerves, 366
Chondrification centers, 149
Chondroblasts, 149–150, 150*b*, 151*f*
Chondrocytes
defined, 149
in endochondral bone formation, 166–167
of hyaline cartilage, 151, 151*f*
Chondrogenic cells, 149–150, 151*f*
Chondroitin 4-sulfate, in extracellular matrix, 81–82, 83*t*
Chondroitin 6-sulfate, in extracellular matrix, 81–82, 83*t*
Chondroitin sulfate, mast cell release of, 133–134, 136*t*
Chondronectin, 85, 152
Choriocapillary layer of choroid, 589
Chorion, 550*f*, 551
Chorion frondosum, 550*f*, 551
Chorion laeve, 551
Chorionic cavity, 547*f*, 550*f*
Chorionic plate, 550
Chorionic somatomammotropin, 551
Chorionic thyrotropin, 538*t*, 551
Chorionic villi, 550–551, 550*f*–551*f*
Choroid, of eye, 588*f*, 589
Choroid plexus, 223
Chromaffin cells, 366–368, 367*f*
Chromatids, sister, 72, 72*f*
Chromatin, 58–70, 61*f*
defined, 55
nucleolus-associated, 69
packaging of, 59
sex, 62–63
Chromatin assembly factor 1, 58
Chromatolysis, 247
Chromogranins, 367–368
Chromophils, 350–352
Chromophobes, 350*f*, 352–353
Chromosomes, 59–64, 62*f*
abnormalities of, 63*b*, 78*b*, 565*b*
methylation of, 64*b*
Chronic essential hypertension, 521*b*
Chronic glaucoma, 590*b*
Chronic neuropathic pain, 587*b*
Chyle, 462
Chylomicrons, 144, 252*t*, 462, 491
Chyme, 439, 451
Chymotrypsin, 477
Cilia
in epithelium, 105–108, 109*f*–110*f*
of larynx, 401
motile, 105–108
olfactory, 397, 397*f*–398*f*
primary, 108
Ciliary body, 588*f*, 592*f*
Ciliary dynein arm, 105–106
Ciliary ganglion, 236*f*, 239*f*
Ciliary muscle, 590
Ciliary processes, 588*f*, 589
Ciliary zone, 590

Ciliated cells
columnar
of paranasal sinuses, 400
of trachea, 402, 403*f*
of ductuli efferentes, 574
of endometrium, 542, 542*f*
of oviduct, 541
Ciliopathies, 108*b*
Circular deoxyribonucleic acid (cDNA), 44
Circular movements, 611
Circulation. *see* Cardiovascular system; Lymphatic vascular system; Vascular supply
Circulatory system, 287–307
blood vessel structure in, 287–289
heart in, 287
pathological considerations of, 307
pulmonary circuit in, 287
systemic circuit in, 287
Circumanal glands, 467
Circumcision, 579*b*
Circumvallate papillae, 430, 430*f*
Cirrhosis, 490*b*, 497*f*
cis Golgi network, 32, 32*f*
Cistern, of endoplasmic reticulum, 26
Cisterna(e)
of Golgi apparatus, 30
hypolemmal, 213
perinuclear, 55, 58*f*
of sarcoplasmic reticulum, 184*f*
Cisterna chyli, 307
Cisternal maturation, 35
Clara cells, 404–406, 406*f*
Class II-associated invariant protein (CLIP), 312*t*, 324
Class switching, 317
Classical liver lobule, 484–485, 485*f*
Clathrin, 33, 35*f*
Clathrin basket, 34–35
Clathrin-coated vesicle, 34–35, 35*f*, 37*f*
Clathrin-mediated endocytosis, 230
Clathrin triskelions, 33*f*, 34–35
Claudins, 113
Claudius cells, 608
Clear cells
of eccrine sweat gland, 383, 385*f*
of gallbladder, 494, 495*f*
parafollicular, of thyroid, 355, 357*f*, 359–360
Clear zone, of osteoclast, 159
Clearing, in tissue preparation, 1
Clefts of Schmidt-Lanterman, 223
Clitoris, 553
Clonal anergy, 315
Clonal deletion, 315
Clonal expansion, 315
Clone, 264
Closed circulation theory, of spleen, 336, 337*f*
Clostridium botulinum, 230*b*
Clostridium difficile, 464*b*–465*b*
Clot formation, 268*f*, 269
Clotting proteins, 252*t*
Club cells, 404–406, 406*f*
Club hairs, 388–389
Cluster of differentiation protein 151, 117
Coagulation, 251
Coagulation factors, 265
Coated vesicles, 33
Coatomer I (COP I), 33, 33*f*
Coatomer II (COP II), 33, 33*f*
Cochlea, 602*f*, 603, 604*f*
Cochlear duct, 604, 604*f*, 607–610, 608*f*–609*f*
Coding RNA, 64
Coding segments, of DNA, 63–64
Codons, 26, 63–64
start, 27, 65
stop, 28, 65
Cofilin, 49*t*
Cohesions, 74
Colchicine, 50*b*, 63, 76*b*
Collagen
in bone, 155
in cementum, 423
in dentin, 422
type VIII, 92
Collagen fibers
in connective tissue, 129*f*
degradation of, 91*f*
in dermis, 380
fibril-forming, 85–86
in periodontal ligament, 427
structure and function of, 85–90, 85*f*, 86*t*, 87*f*–89*f*
synthesis of, 88–90, 89*f*
types of, 86*t*, 129*f*
white fibers as, 85–86
Collagen-like proteins, 88
Collateral branches, of axons, 216
Collateral ganglia, 238
Collecting tubules, 501, 513–515, 514*f*–515*f*, 523*t*
cortical, 509*f*, 513
loss of water and urea from filtrate in, 521–522
medullary, 513–515, 515*f*
papillary, 499, 515
structure of, 500*f*–501*f*, 523*t*
Collecting veins, of liver, 484
Colliculus seminalis, 575
Colloid, in thyroid, 355, 357*f*
Colloid osmotic pressure, 251–252, 505–508
Colon, 449*t*–450*t*, 463–467, 464*b*–466*b*, 464*f*–466*f*
Colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM), 273
Colony forming unit-lymphocyte (CFU-Ly), 273
Colony stimulating factors, 312, 312*t*, 538*t*
Colostrum, 553, 555
Columnar epithelium
pseudostratified, 100*t*, 101*f*, 102–103, 104*f*
simple, 100, 100*t*, 101*f*–102*f*
stratified, 100*t*, 101*f*, 102
Columns of Bertin, 499
Coma, hepatic, 492*b*
Committed precursors, 273
Common bile duct, 436*f*, 476–477, 494
Common hepatic duct, 494
Communicating junctions. *see* Gap junctions
Compact bone, 161, 162*f*, 163
haversian canal systems of, 162*f*, 163–164, 164*f*
lamellar systems of, 162*f*, 163–164
outer and inner circumferential lamellae of, 163
Complement proteins
in innate immune system, 311
in phagocytosis, 36
in plasma, 252*t*
Composite bodies, 411
Compound microscope, 3, 3*f*
Compound tubuloacinar glands, of mammary gland, 553
Concentric cell layers, in tunica media, 288
Condensed mitochondria, 45
Condenser lens, 3, 3*f*
Condensing vesicles, 35
Condensins, 74
Conducting arteries, 290, 290*f*
Conducting portion, of respiratory system, 395–407, 396*t*
Conduction, of nerve pulse
continuous, 234
saltatory, 227
Conduction system, of heart, 303
Conduction velocity, 234, 234*t*
Conductive deafness, 611*b*
Cones, of retina, 587, 592, 593*f*–594*f*, 594*t*, 597–598, 598*b*, 598*f*
layers of, 595–598
Confocal microscopy, 7, 8*f*
Congestive heart failure, 304*b*
Conjugated bilirubin, 490
Conjunctiva, 587, 588*f*, 600
Conjunctivitis, 600*b*
Connecting piece, of spermatozoon, 566, 568
Connecting stalk, of rods, 595–597
Connecting tubule, 513
Connective tissue, 127–148
adiponectin in, 145
adipose, 143–144
adipose cells in, 131–132, 131*f*–132*f*
cellular components of, 127–139

Connective tissue *(Continued)*
classification of, 141–147, 141*t*
defined, 127
dense, 141–143
embryonic, 141
extracellular matrix of, 127
fibers in, 127
fibroblasts as, 129–130, 130*b*, 130*f*
fixed cells of, 129–139
functions of, 127
interleukin-6 in, 146–147
leptin in, 144–145
leukocytes in, 139
loose (areolar), 141
macrophages in, 136–139, 137*f*
mast cells in, 132–136, 134*f*
mesenchymal, 141
mucous, 141
of muscles, 207*t*
myofibroblasts in, 130
origins of cells of, 128*f*
pathological considerations of, 148, 148*f*
pericytes in, 130–131
plasma cells in, 139, 139*f*–140*f*
proper, 141–147
resistin in, 146
reticular, 143, 143*f*
retinol-binding protein-4, 146
specialized, 127
transient cells of, 139
tumor necrosis factor-α in, 146
Connective tissue investments, of peripheral nerves, 233, 233*f*
Connexin 37, 534
Connexin 43, 534
Connexins, 114–115
gene mutations of, 117*b*
Connexons, 111*f*, 114–115, 116*f*
Constant (Fc) regions, of antibodies, 36
Constant regions, of T-cell receptors, 319–320
Constitutive secretory pathway
of glands, 119
of Golgi apparatus, 34
transport along, 35
Contact sites, in mitochondrial membranes, 44
Continuous capillaries. *see* Capillaries, continuous
Continuous conduction, 234
Contractile bundles, 47
Contractile ring, 74
Contraction(s)
of cardiac muscle, 198
peristaltic, 575
of skeletal muscle, 189–191, 190*f*
of small intestine, 460–461
of smooth muscle, 205–206, 206*f*, 207*t*
of uterus, 544
Core
of Pacinian corpuscles, 585*f*, 586
of pulp, 423
Core particle, 41
Core promoter, 65
Corium. *see* Dermis
Cornea, of eye, 588–589, 588*f*
Corneal hydrops, 589
Corona
of lymphoid nodules, 333
of spleen, 333
Corona radiata, 531*f*, 535
Coronary arteries, 294
Coronary heart disease, 306*b*
Coronary vessels, atherosclerosis of, 306*b*, 307*f*
Corpora amylacea, 576–577
Corpora arenacea, 369
Corpus albicans, 531*f*, 540
Corpus cavernosum, 578*f*
Corpus cavernosum urethrae, 578
Corpus hemorrhagicum, 537
Corpus luteum, 531*f*, 537–540, 539*f*, 546
degeneration of, 540
of menstruation, 540
of pregnancy, 540
Corpus spongiosum, 578*f*
Cortex
cerebellar, 244, 245*f*
cerebral, 244
gray matter in, 239–240
of hair shaft, 388, 388*f*
of lymph nodes, 332–333, 333*f*
ovarian, 531–540, 531*f*, 533*f*, 558*f*
at onset of puberty, 531–532
renal, 499, 500*f*–502*f*
of suprarenal glands, 356*t*, 361, 362*f*, 365–366
of thymus, 328–330, 329*t*
Cortical arch, 500
Cortical arteries, 363
Cortical collecting tubules, 509*f*, 513
Cortical columns (of Bertin), 499
Cortical granules, 532
Cortical labyrinth, 500
Cortical lattice, 610
Cortical nephrons, 500*f*–501*f*, 502, 516
Cortical plates, of dental alveolus, 428
Cortical reaction, 548
fast component of, 548
slow component of, 548
Cortical sex cords, 529
Cortical sinuses, of lymph nodes, 332
Corticosteroids, 361
effect on thymus, 331
Corticosterone, 356*t*, 361, 365
Corticotrophs, 352, 366
Corticotropin-releasing hormone (CRH), 347, 349*t*–350*t*, 366
Cortisol, 356*t*, 361, 365
Cortisone, effect on cartilage, 152*t*
Costameres, 182–183
Cotranslation, 29
Cough reflex, 401*b*
Countercurrent exchange system
heat, in testes, 560
and vasa recta, 522, 522*f*, 523*t*
Countercurrent multiplier system, and Henle loop, 519–520, 520*f*
Coupled transport, 20*f*, 22
Cowper's gland, 559, 560*f*, 577
Cranial nerves, 234
Craniosacral outflow, 237
CRE-binding protein (CREB), 24–25
Creatine phosphate, in muscle contraction, 191
Cremaster muscle, in testes, 559
Cretinism, 359*b*
Cristae, 43, 43*f*
Cristae ampullares, 607
Cross section, 4*f*
Crossing over, of chromosomes, 565
Crown, of tooth, 421, 421*f*
CRSPR-associated proteins, 69*b*
Crypts
of Lieberkühn
of colon, 463, 464*f*–466*f*
of rectum and anal canal, 467
of small intestine, 453, 454*f*–455*f*, 455–456
of palatine tonsils, 342
αβ–Crystallin, 182
Crystalloids of Charcot-Böttcher, 561
Crystals
of Charcot-Böttcher, 46
in cytoplasm, 46, 47*f*
of Reinke, 46, 570
Cuboidal epithelial cells, in hepatic ducts, 486
Cuboidal epithelium
simple, 100, 100*t*, 101*f*
stratified, 100*t*, 101, 101*f*, 103*f*
Cumulus oophorus, 531*f*, 535, 536*t*
Cupula, 607
CURL endosomes, 37*f*
Cushing disease, 366*b*
Cuticle
enamel, 422
of hair, 387–388, 388*f*
of nail, 390, 390*f*
Cycle adenosine monophosphate (cAMP)-dependent protein kinase, 201
Cyclic adenosine monophosphate (cAMP), 345–346, 474
and fat release, 144
in islet of Langerhans, 481
in mast cells, 134
as second messenger, 24–25
in signaling, 21, 23

Cyclic adenosine monophosphate (cAMP) phosphodiesterases, 25
Cyclic adenosine monophosphate (cAMP) response elements (CREs), 24–25
Cyclic guanosine 3',5' monophosphate, 345–346
Cyclic guanosine monophosphate (cGMP), 21
Cyclic guanosine monophosphate (cGMP)-gated Na^+ ion channels, 595
Cyclin B, in ovulation, 536
Cyclin-dependent kinases (CDKs), 71
  in ovulation, 536
Cyclins, 71
Cysteine proteases, 78
Cystic duct, 493–494
Cystic fibrosis (CF), 406*b*
Cystic fibrosis transmembrane conductance regulator (CFTR) protein, 23*b*, 406*b*
Cystitis, acute, 526*b*–527*b*
Cytochemistry, 5
Cytochrome b-c_1 complex, 44
Cytochrome oxidase complex, 44
Cytokeratins, 378
Cytokines
  antigen-presenting cell release of, 324, 325*t*
  in class (isotope) switching, 317
  mast cell release of, 132–133
  as signaling molecules, 119, 312
  T cell release of, 265
Cytokinesis, 74–75, 75*f*
  modified, 565
Cytomorphosis, of keratinocytes, 374
Cytoplasm, 13–53
  annulate lamella in, 45
  cell membrane and, 13–15
  centrioles in, 51–53
  centrosome in, 51–53
  cytoskeleton and, 13, 46–53
  defined, 13
  endocytosis, endosomes and lysosomes in, 36–39
  endoplasmic reticulum in, 26, 27*f*
  Golgi apparatus in, 30–32, 31*f*–33*f*
  inclusions in, 13, 45–46, 47*f*
  mitochondria in, 42–45, 43*f*
  of neuron, 213
  organelles and, 13–45
  pathological considerations and, 53, 53*f*–54*f*
  peroxisomes in, 40–41, 42*f*
  polyribosomes in, 26
  proteasomes in, 41–42
  ribosomes in, 25–26
Cytoplasmic bridge, 564*f*, 565
Cytoplasmic ring, 56, 60*f*
Cytosine, 63
Cytoskeleton, 46–53
  defined, 13
  of erythrocyte, 256*f*
  intermediate filaments of, 48–50, 48*f*, 50*b*, 51*t*
  microtubule-associated proteins of, 50–51, 52*f*
  microtubules of, 50–53, 50*b*, 52*f*
  of neurons, 215
  thin filaments of, 46–48, 48*f*–50*f*
Cytosol
  defined, 13
  protein synthesis in, 27–29, 29*f*
Cytosolic proteins, synthesis of, 27–29
Cytotoxic T lymphocytes (CTLs), 312*t*, 322–323
Cytotoxicity, antibody-dependent cell-mediated, 317–318
Cytotrophoblasts, 549

D

D cells, 478–479, 480*t*, 481
D_1 cells, 478–479, 480*t*, 481
Dark cells, 513
  of eccrine sweat gland, 383, 385*f*
  of saccule, 604–605
  of taste buds, 431
Dark zone, of lymphoid nodule, 333
Dartos muscle, in testes, 559
Daughter cells, 70
Deafness
  conductive, 611*b*
  nerve, 611*b*
  nonsyndromic, 117*b*
Death inducing signaling complex, 79
Decidua, 550–551, 550*f*
Decidua basalis, 550*f*, 551
Decidua capsularis, 550, 550*f*
Decidua parietalis, 550*f*, 551
Decidual cells, 548, 551
Deciduous teeth, 421, 426
Decorin, 82
Deep dorsal artery and vein, of penis, 578*f*, 579
Default pathway, to Golgi apparatus, 30, 34
Defensins, 312, 456
Degranulation, in mast cells, 135
Dehydration, in tissue preparation, 1
Dehydroepiandrosterone, 356*t*, 365–366
Deiodinated tyrosine (DIT), 358
Demarcation channels, 284
Demyelination, 225*b*
Dendrites, 212, 214*f*
Dendritic cells
  epidermal, 378
  follicular, 333
  interdigitating, 338
  in renal interstitium, 515
Dendrodendritic synapse, 228
Dense bars, 192
Dense bodies, in smooth muscle, 203–205
Dense connective tissue, 141–143
  irregular, 142, 142*f*
  regular collagenous, 142, 142*f*–143*f*
  regular elastic, 142–143
Dense tubular system, of platelets, 266, 266*f*, 266*t*
Dental lamina, 424, 425*f*
Dental papilla, 424–426
Dental sac, 425–426
Denticles, 424
Dentin, 421*f*–422*f*, 422–423, 423*b*
Dentinal tubules, 423
Dentinoenamel junction (DEJ), 422*f*, 426
Deoxycorticosterone, 356*t*, 364
Deoxyribonucleic acid (DNA)
  chromatin and, 58
  circular, 44
  linker, 58
  in nucleus, 55
  in protein synthesis, 26
  structure of, 63
  transcription of, 65–66, 66*f*
Depolarization, 182
  wave of, 227
Dermal ridges (papillae), 373, 376*f*, 381–382, 386
Dermatan sulfate, 81–82, 83*t*, 381
Dermatoglyphs, 373, 382
Dermis, 380–382
  epidermal interface with, 382
  papillary layer of, 375*t*, 376*f*, 381
  reticular layer of, 374*f*, 375*t*, 381
  structure of, 373, 374*f*
Descemet membrane, 589
Desmin
  in capillaries, 295
  in cytoskeleton, 51*t*
  in skeletal muscle, 182
  in smooth muscle, 204–205
Desmogleins, 111*f*, 114
Desmoplakins, 114, 114*b*
Desmosine cross-links, 91–92
Desmosomes, 111*f*, 114, 115*f*
  in cardiac muscle, 200*f*
  of skin, 375
Desquamation, 377
Detoxification of drugs, in liver, 493
Detrusor muscle, in urinary bladder, 525
Detumescence, of penis, 579–581, 580*b*, 580*f*
Diabetes insipidus, 355*b*
  congenital nephrogenic, 522*b*
Diabetes mellitus, 482*b*, 482*t*, 613*f*
Diabetic kidney disease, 508*b*
Diacylglycerol, in signaling, 24
Diakinesis, 77
  in spermatogenesis, 565
Diapedesis, 257

Diaphragma sellae, 346
Diaphysis, 161–162
Diarrhea, 464*b*–465*b*
Diarthroses, 174, 175*f*
Diastole, 201, 294
Dicer, 67–68
Dichromatic cone, 598*b*
Dieter cells, 609, 609*t*
  inner, 610
  outer, 610
Dieulafoy lesion, 448*b*
Diffuse lymphoid system, 311
Diffuse neuroendocrine system (DNES) cells, 476
  of colon, 463
  paracrine and endocrine hormone production by, 123, 125*f*
  of respiratory epithelium, 401, 403
  of small intestine, 447*t*, 454–455, 460
  of stomach, 441*f*, 444–446, 447*t*, 448*f*
Diffusion
  carrier-mediated, 20*f*
  facilitated, 20
  ion channel-mediated, 20*f*
DiGeorge syndrome, 331*b*
Digestive system
  alimentary canal in. *see* Alimentary canal
  esophagus in, 437–439, 438*f*, 439*b*, 449*t*–450*t*
  gallbladder in, 493–495, 494*f*
  gingiva in, 421, 421*f*, 428–429
  large intestine in, 449*t*–450*t*, 463–468
  lips in, 419–420
  liver in, 481–495, 483*f*
  oral cavity in, 419–433
  oral mucosa in, 419–433, 420*t*
  palate in, 429
  pancreas in, 476–481, 477*f*–478*f*, 478*b*
  pathological considerations, 496, 496*f*–497*f*
  salivary glands in, 471–476, 472*f*–473*f*
  small intestine in, 449*t*–450*t*, 452–463
  stomach in, 439–452
  teeth in, 421–433, 421*f*
  tongue in, 429–433, 430*f*
Digital imaging techniques, 4
Dihydrotestosterone, 571, 577
Dihydroxyphenylalanine, 213
Dilator pupillae muscle, 588*f*, 590
Dimers, 88, 318*t*
Dipalmitoyl phosphatidylcholine, 411
Dipeptidases, 461
Diploë, 163
Diploid cells, 63, 76
Diplotene, 77
Direct immunocytochemistry, 5, 5*f*–6*f*
Disaccharidases, 461
Discontinuous (sinusoidal) capillaries, 298*f*
Discrimination, tactile, 584
Disk
  intervertebral, ruptured, 154*b*
  Merkel, 584, 584*f*
  optic, 592–593
  Z, in skeletal muscle, 183*f*–184*f*, 186*f*
Disse, perisinusoidal space of, 485–486, 487*f*–488*f*
Distal ring, of nuclear basket, 56, 60*f*
Distal tubule, 502, 510–512, 513*f*
  convoluted, 500*f*–501*f*, 512, 512*f*, 523*t*
Distributing arterioles, of liver, 484
Distributing veins, of liver, 484
Diuresis, 520*f*
Diversity, of adaptive immune system, 314
DNA. *see* Deoxyribonucleic acid (DNA)
DNase, pancreatic, 477
Docking protein, 26, 29–30
Dome-shaped cells, of urinary bladder, 524
Dominant follicle, 537, 546
Dopamine
  functions of, 232*t*
  neurotransmitter, 213
Dorsal artery and vein, of penis, 578*f*
Dorsal horns, 239–240
Dorsal root ganglia, 211, 238
Doublets, of microtubules, 105, 397
Down syndrome, 63*b*, 78*b*
Drugs
  detoxification of, in liver, 493
  tolerance to, 493*b*
Dry eye syndrome (DES), 601*b*
Dua layer, of cornea, 589
Duct(s)
  alveolar, 396*t*, 407–408, 408*f*–410*f*
  of Bellini, 499, 501, 515
  bile, common, 436*f*, 476–477, 494
  cochlear, 604, 604*f*, 607–610, 609*f*
  cystic, 493–494
  of eccrine sweat glands, 382, 384, 384*f*–385*f*
  ejaculatory, 560*f*, 572*t*
  endolymphatic, 604
  extrahepatic, 494, 496*t*
  genital, 571–575, 572*t*
  hepatic, 486, 494
  lactiferous, 553, 554*f*
  lymphatic, 307
  nasolacrimal, 601
  pancreatic, 476–478, 477*f*
  of salivary glands, 472–473, 472*f*
  semicircular, 603–604, 607, 607*f*
  thoracic, 307
Ductal carcinoma, 556*b*
Ductules, terminal, of mammary gland, 553
Ductuli efferentes, 559–560, 560*f*, 572*t*, 574
Ductus deferens, 559–560, 560*f*, 572*t*
Ductus epididymis, 572*t*, 574–575, 574*f*
Ductus reuniens, 604, 604*f*
Ductus utriculosaccularis, 604, 604*f*
Duodenal glands, 456*f*, 457–458
Duodenal papilla (of Vater), 458
Duodenum, 449*t*–450*t*, 456, 458. *see also* Small intestine
Dura mater, 163, 240–241, 241*f*
Dust cells, 138, 412, 412*b*, 413*f*
Dyad, in retina, 599
Dynamin, 36
Dynamin-related protein 1, 45
Dynein
  in axonal transport, 218
  of cilia, 105–106, 106*b*
  as microtubule motor protein, 51
  and minus end of MTOC, 34
Dynein-2, 106–108
Dystrobrevin, 185
Dystroglycan complex, 183
Dystroglycans, 93–96
Dystrophin, 96, 183–185
Dystrophin-associated glycoprotein complex, 182–183

E

E-cadherins, 113
E-face
  of cell membrane, 17–19, 19*f*
  of microvillar membrane, 112–113, 113*f*
E-site, 26
Ear(s), 601–612, 602*f*
  cochlea of, 602*f*, 603, 604*f*
  cochlear duct of, 604, 604*f*, 607–610, 608*f*–609*f*
  cochlear functions in, 611–612, 612*f*
  disorders of, 602*b*, 611*b*
  external, 601–602, 602*f*
  inner, 601, 603–612, 604*f*–605*f*
  membranous labyrinth of, 603–610, 604*f*
  middle, 601–603, 602*f*
  organ of Corti of, 607–611, 608*f*–609*f*, 609*t*
  saccule and utricle of, 603–606, 604*f*–605*f*
  semicircular ducts of, 603–604, 607, 607*f*
  vestibular functions of, 610–611
Earwax, 602*b*
Eccrine sweat gland, 374*f*, 382–384, 384*f*–385*f*
Ectoderm, 373
Ectomesenchyme, in odontogenesis, 424
Edema, 142*b*, 299–300
Effector cells, 265
Effector memory T cells, 321
Effector organ, 235
Effector T cells, 321–322

Efferent components
of autonomic nervous system, 234
of peripheral nervous system, 211
of somatic nervous system, 234
Efferent fibers, 233
Efferent glomerular arteriole, 516, 521
Efferent lymphatic vessels, 332
Efferent neurons. *see* Motor neurons
Ehlers-Danlos syndrome, 90*b*, 291*b*
Ejaculate, 579, 580*b*
Ejaculation, 579–581, 580*b*, 580*f*
Ejaculatory duct, 560*f*, 572*t*, 575
Elastase, 477
Elastic arteries, 290, 290*f*
Elastic cartilage, 150*f*, 151*t*, 153, 153*f*
Elastic fibers, 91–92, 91*f*–93*f*
Elastic fibers, in dermis, 381
Elastic fibers, of trachea, 403
Elastic lamina
external, of muscular arteries, 291
internal
of arterioles, 291
of elastic arteries, 290
of tunica intima, 288
Elastic lamina, of trachea, 403
Elastin, in extracellular matrix, 91–92
Elastin, of tunica intima, 288
Electrical potential (voltage) difference, 21
Electrical synapses, 227
Electrochemical gradient, 44
Electron microscopy, 3*f*, 7–9
Electron transport chain, 44
Eleidin, 377
Embedding, 2
Embryoblasts, 548, 555–556
Embryonic stem cells (ESC), 556
Emerin, 55
Emphysema, 412*b*
Emulsification, of lipids, 461, 462*f*
En passant type of synapse, 206
Enamel, 421*f*, 422, 426–427
Enamel cuticle, primary, 422
Enamel epithelium, 424, 425*f*
Enamel knot, 425
Enamel organ, 424, 425*f*
Enamel rods, 422
Enamelins, 422
End bulbs, 213, 215*f*
Krause, 381, 586
End piece, of spermatozoon, 568, 568*f*
Endocardium, 303
Endochondral bone formation, 149, 165–170, 166*f*, 167*t*
Endocrine cells, 345
Endocrine glands, 122–123, 346
defined, 345
pancreas as, 479*f*, 480*t*, 482*b*, 482*t*
parathyroid, 355*f*, 356*t*, 357*f*, 360–361
pineal, 356*t*, 368–369, 369*f*
Endocrine glands *(Continued)*
pituitary, 346–354
suprarenal (adrenal), 356*t*, 361–368, 362*f*
thyroid, 354–360, 356*t*
Endocrine signaling, 23, 119
Endocrine substance, of DNES cells, 445–446
Endocrine system, 345–370
defined, 345
hormones in, 345–346
pathological considerations of, 370*f*–372*f*
Endocytosis, 36–39
receptor-mediated, 36, 37*f*–38*f*
Endocytotic vesicles, 39*f*
Endogenous proteins, 324
Endolymph, 604, 607
Endolymphatic duct, 604
Endolymphatic sac, 604
Endometriosis, 544*b*, 557*f*
Endometrium, 530*f*, 542–543, 543*f*
in luteal phase, 546–547, 546*f*
in menstrual cycle, 545
Endomitosis, 281
Endomysium, 179
Endoneurium, 233
Endoplasmic reticulum (ER), 16*f*, 26
Golgi apparatus and, 30, 31*f*, 33*f*
hormone synthesis in, 364
rough, 16*f*, 26
in chondrogenic cells, 150
collagen synthesis on, 88
of neuron, 213
outer nuclear membrane and, 55
protein synthesis on, 29–30, 30*f*
smooth, 16*f*, 26, 27*f*
of hepatocytes, 489
hormone synthesis in, 364
of neuron, 213
of prostate, 576, 578*f*
transitional, 30, 33*f*
Endoplasmic reticulum/Golgi intermediate compartment (ERGIC), 32, 33*f*
Endorphins, 232*t*
Endosomal carrier vesicles, 38–39
Endosomal compartment, 37, 261
Endosomes, 36–39, 37*f*
early, 36, 39*f*
late, 33*f*, 36
recycling, 37–38
Endosteum, 154
Endothelial cells, of capillaries, 296, 297*f*
Endothelial growth factor, 538*t*
glomerular, 504
Endothelin
in clot formation, 267–269
secretion of, by capillaries, 300
Endothelium
corneal, 589
of endocardium, 303
of eye, 588*f*
Enkephalins, 232*t*, 367–368
Entactin, 84, 93–95
Enteric nervous system, 238, 435–437
Enteritis, 464*b*–465*b*
Enteroendocrine cell
of small intestine, 447*t*, 454–455, 460
of stomach, 441*f*, 444–446, 447*t*, 448*f*
Enteroglucagon, 447*t*
Enterohepatic recirculation, of bile salts, 490
Enzyme-linked receptors, 24
Eosin stain, 2, 2*t*, 11*f*
Eosinophil(s), 261, 262*f*, 275*f*
features of, 258*t*
functions of, 139, 261
granules of, 261
increase or decrease in, 262*b*
structure of, 252*f*
Eosinophil chemotactic factor (ECF), 132–133, 136, 136*t*
Eosinophil-derived neurotoxin, 261
Eosinophilic metamyelocyte, 275*f*
Eosinophilic myelocyte, 275*f*
Eosinophilic series, 282*f*
Eosinophilic stab cell, 275*f*
Ependymal cells, 223
Epicardium, 304–305
Epidermal growth factor (EGF), 382, 458, 533
in odontogenesis, 425
Epidermal melanin unit, 378
Epidermal ridges, 373, 376*f*
Epidermis, 373–380, 375*t*
dermal interface with, 382
keratinocytes in, 374
Langerhans cell in, 374*f*, 378
melanocytes in, 378–380, 380*f*
Merkel cells in, 378
non-keratinocytes in, 378–380
stratum basale (stratum germinativum) of, 374–375, 374*f*, 375*t*, 376*f*, 383*b*
stratum corneum of, 374, 374*f*, 375*t*, 377–380
stratum granulosum of, 374, 374*f*, 375*t*, 376–377
stratum lucidum of, 374, 374*f*, 375*t*, 377
stratum spinosum of, 374–376, 374*f*, 375*t*, 376*f*–377*f*
thick skin in, 374, 374*f*, 375*t*, 376*f*
thin skin in, 374, 374*f*
Epidermolysis bullosa, 90*b*
Epididymis, 559–560, 560*f*, 572*t*, 574, 574*f*
Epidural space, 241
Epigenetic marks, 68

Epigenetics, 64–69
Epiglottis, 430*f*
Epilepsy, 212*b*
Epimysium, 179
Epinephrine
  functions of, 232*t*, 356*t*
  production of, 361, 367–368
Epiphyseal plates, 150, 161–162
Epiphyses, 161–162
Episclera, 588
Epithelial reticular cells, 320
Epithelial tissue, 99–119
Epithelioid cells, 138, 233
Epithelioid precursor cells, 147
Epithelium, 99–125
  adenocarcinoma of, 119*b*
  of alimentary canal, 435, 449*t*–450*t*
  of anal canal, 467
  apical domain of, 104–108, 105*f*–108*f*
  basal surface specializations of, 116–118
  basolateral domain of, 108–118
  of bulbourethral glands, 577
  carcinoma of, 119*b*
  cell renewal in, 118–119
  cell-surface specializations of, 103–118
  of cervix, 544
  of choroid, 589
  cilia of, 105–108, 109*f*–110*f*. *see also* Ciliated cells
  classification of, 99–103, 100*t*, 101*f*
  corneal, 588–589, 600
  defined, 99
  desmosomes in, 111*f*, 114, 115*f*
  of ductuli efferentes, 572*t*, 574
  of ductus deferens, 572*t*, 575
  of ejaculatory duct, 572*t*, 575
  enamel, 424, 425*f*
  of endometrium, 542, 543*f*
  of epididymis, 572*t*, 574*f*, 575
  of esophagus, 437, 449*t*–450*t*
  of female urethra, 526
  flagella in, 108
  focal adhesions in, 118
  of gallbladder, 494, 495*f*
  gap junctions in, 111*f*, 114–116, 116*f*, 117*b*
  germinal, 530, 561, 561*f*–563*f*
  of gingiva, 421*f*, 428–429
  hemidesmosomes in, 117–118, 118*f*
  of Henle loop, 514*f*
  junctional complexes in, 109, 111*f*–112*f*
  of large intestine, 449*t*–450*t*
  of larynx, 401
  lateral membrane specializations of, 109–116
  mesothelial, 530, 530*f*, 532
  metaplasia of, 119*b*
  microvilli in, 104–105, 105*f*–108*f*
Epithelium *(Continued)*
  olfactory, 397, 398*f*
  oral, in odontogenesis, 424, 425*f*
  of oral mucosa, 419
  of oviduct, 541, 542*f*
  of paranasal sinuses, 400
  pathological considerations for, 125*f*–126*f*
  pigment
    of ciliary body, 589–590
    of retina, 589, 592, 594–595
  plasma membrane infoldings in, 117
  polarity of, 103–118
  of prostate gland, 576, 577*f*
  pseudostratified columnar, 101*f*, 102–103, 104*f*
  of renal calyces, 523
  respiratory, 395–397, 401–403, 402*f*–403*f*
  of rete testis, 572*t*, 573, 573*f*
  of seminal vesicles, 575, 576*f*
  seminiferous, 559, 561, 561*f*–563*f*
  simple, 99, 101*f*
    columnar, 100, 101*f*–102*f*
    cuboidal, 100, 101*f*
    squamous, 99–100, 100*t*, 101*f*–102*f*
  of small intestine, 449*t*–450*t*, 453–455
  of stomach, 440, 442*f*–443*f*, 449*t*–450*t*
  stratified, 99, 100*t*, 101*f*
    columnar, 101*f*, 102
    cuboidal, 101, 101*f*, 103*f*
    squamous
      keratinized, 100*t*, 101, 101*f*, 103*f*
      nonkeratinized, 100–101, 100*t*, 101*f*, 103*f*
  subcapsular, 591, 591*f*
  of trachea, 401, 403*f*
  transitional, 101*f*, 102
  of tubuli recti, 572*t*, 573
  of tympanic cavity, 602–603
  of ureter, 524
  of urinary bladder, 524, 527*f*
  of vagina, 552
  zonulae adherentes in, 111*f*–112*f*, 113–114
  zonulae occludentes in, 111–113, 111*f*–113*f*
Epitope(s), 41, 263
  on major histocompatibility complex molecules, 318–319, 323–324
Epitope-MHC complex, 334
Eponychium (cuticle), 390, 390*f*
Equatorial division (meiosis II), 76–78, 76*f*–77*f*
Erbin molecules, 117
Erectile bodies, 553
Erectile dysfunction, 580*b*
Erectile tissue, 578, 578*f*
  structure of, 579
Erection, 579–581, 580*b*, 580*f*
Erythroblastosis fetalis, 257*b*
Erythroblasts, 272, 275*f*, 277*f*, 280*t*
Erythrocyte(s), 251, 275*f*, 280*t*
  cell membrane, 255–257, 256*f*
  defects in, 256*b*
  structure of, 252*f*
Erythrokeratodermia variabilis, 117*b*
Erythropoiesis, 277–280, 277*f*, 279*f*
Erythropoietic series, cells of, 280*t*
Erythropoietin, 276*t*, 277, 277*b*, 499, 516
Esophageal cardiac glands, 438, 449*t*–450*t*
Esophageal varices, 303*b*
Esophagus, 437–439, 438*f*, 439*b*, 449*t*–450*t*
Estradiol, 535
  effect on cartilage, 152*t*
  in female reproductive system, 537*f*, 538*t*
  in follicular development, 534–535
  in mammary development, 553
Estradiol receptors, 546
Estrogen
  in follicular development, 535
  in lactation, 553
  in menstrual cycle, 545, 545*f*
  in placental development, 551
Euchromatin, 57*f*–58*f*, 58
Eustachian tube, 602, 602*f*
Excitatory neurotransmitters, 21
Excitatory postsynaptic potential, 228
Excitatory responses, 231
Excretory passages, of urinary system, 523–526
Exhalation, 415
Exocrine glands, 119–122, 120*f*
  classification of, 119
  multicellular, 121–122, 123*f*–125*f*
  pancreas as, 476–478, 477*f*–478*f*
  unicellular, 121, 121*f*–122*f*
Exocytosis, 34
  apocrine mode of, 554
Exogenous proteins, 324
Exons, 65–66
Exophthalmos, 359*b*
Exportins, 57–58
External capsule, 197*f*
External ear, 601–602, 602*f*
External lamina, 93
External (outer) limiting membrane, of retina, 593*f*, 598
Externum, of eosinophil, 261
Exteroceptors, 583
Extracellular fluid, 251–252
Extracellular matrix (ECM), 81–97
  basal lamina of, 93–95, 93*f*–95*f*
  basement membrane of, 93–95, 93*f*
  of cartilage, 149
  defined, 81
  dystroglycans in, 95–96

Extracellular matrix (ECM) *(Continued)*
fibers of, 85–92
collagen, 85–90, 85*f*, 86*t*, 87*f*–89*f*, 91*f*
elastic, 91–92, 91*f*–93*f*
fluid flow in, 81, 82*f*
glycoproteins (cell adhesive glycoproteins) in, 84–85
glycosaminoglycans in, 81–82, 83*t*
ground substance of, 81–85, 82*f*
integrins in, 95–96
lamina reticularis of, 93*f*–94*f*, 95
pathological considerations for, 96, 96*f*–97*f*
proteoglycans in, 82–83, 84*f*
Extrafusal fibers, 195, 197*f*
Extrahepatic ducts, 494, 496*t*
Extrapulmonary bronchi, 396*t*, 404
Extrapulmonary conducting division, 396*t*
Extratesticular genital ducts, 572*t*, 573–575
Extrinsic components, of enteric nervous system, 238
Extrinsic muscles
of eye, 587, 588*f*
of larynx, 400
of tongue, 429
Extrinsic pathway, caspases and, 78
Eye(s), 587–601, 588*f*
accessory structures of, 588*f*, 600–601
bulb of, 587
choroid of, 588*f*, 589
ciliary body of, 589–590, 592*f*
conjunctiva of, 587, 588*f*, 600
cornea of, 588–589, 588*f*
disorders of, 590*b*–592*b*, 597*b*, 600*b*–601*b*
extrinsic muscles of, 587, 588*f*
floaters in, 592*b*
iris of, 590–591
lacrimal apparatus of, 600–601
lacrimal gland of, 587, 601
lens of, 588*f*, 591, 591*f*–592*f*
retina of, 587, 588*f*, 592–600, 593*f*–594*f*, 594*t*
sclera of, 587–588, 588*f*
tunica fibrosa of, 587–589
tunica vasculosa of, 589–591
vitreous body of, 588*f*, 591
Eyeballs, 587
Eyelashes, 600
Eyelids, 600

F

F-actin, 46, 50*f*, 186*f*, 189
Fab fragment, 316
Facial expression, muscles of, 381
Facial nerve, 602*f*
Facilitated diffusion, 20
Fallopian tubes, 530*f*, 537, 540–541, 541*f*–542*f*
Fascia adherens
in cardiac muscle, 200*f*
of epithelium, 114
Fasciae occludentes, of capillaries, 297
Fascicles
muscle, 179, 181*f*
peripheral nerve, 232, 232*f*
Fasciculus longitudinalis, 494, 496*t*
Fast sodium channels, 201
Fat(s)
absorption and processing of, 462, 462*f*
formation of
primary, 147
secondary, 147
Fat cells. *see* Adipose cells (adipocytes)
Fat storing cells, of liver, 486, 487*f*
Fatty acid(s), 491
absorption and processing of, 462, 462*f*
in adipocytes, 144
in hormones, 345
Fatty acyl tails, in cell membrane, 15–16
Fatty stool, 490*b*
Fc (constant) regions, of antibodies, 36
Fc fragment, 316
Fc receptors
of antibodies, 316
FcεRI, on basophils, 262
on macrophages and neutrophils, 36
on mast cells, 134–135
Feces, 463
water loss in, 499
Feedback mechanism
for hormones, 346
negative, for glucocorticoids, 366
Female pronucleus, 548
Female reproductive system, 529–557, 530*f*
cervix in, 530*f*, 544
external genitalia in, 552–553
fertilization in, 547–548, 547*f*, 549*f*
implantation in, 548–549
major hormones involved in, 537*f*, 538*t*
mammary glands in, 553–555, 554*f*–556*f*
menstrual cycle in, 544–547, 545*f*
ovaries in, 529–555, 530*b*, 530*f*
oviducts (Fallopian tubes) in, 530*f*, 537, 540–541, 541*f*–542*f*
pathological considerations in, 557, 557*f*–558*f*
placenta in, 549–551, 550*f*–551*f*, 552*b*
uterus in, 541–544
vagina in, 552
Fenestra cochleae, 602*f*, 603
Fenestra vestibuli, 602*f*, 603
Fenestrated capillaries, 298
glomerular, 503–504, 504*f*–506*f*, 513*f*
Fenestrated membranes, 290, 290*f*
Fertilin, 548
Fertilization, 547–548, 547*f*, 549*f*
Fertilization promoting peptide (FPP), 548, 575
Fetal hemoglobin (HbF), 254
Fibrillin-1, 92
Fibrin, 269
Fibroblast(s), 129–130, 130*b*, 130*f*, 207
active, 129–130
differentiation of, 130*b*
fibronectin produced by, 84
inactive, 130
in periodontal ligament, 427
in renal interstitium, 515
Fibroblast growth factor, 538*t*
Fibroblast growth factor-4 (FGF-4), in odontogenesis, 425
Fibroblast growth factor-9, 530*b*
Fibroblastic reticular cell conduits, 332
Fibrocartilage, 150*f*, 151*t*, 153–154, 153*f*
Fibrocytes, 129
Fibroelastic, 575–576
Fibronectin, 504
in basal lamina, 95
cell-surface, 84
in cytoskeleton, 48, 50*f*
in extracellular matrix, 84
Fibronectin receptors, in extracellular matrix, 84
Fibrous astrocytes, 221, 221*f*
Fibrous outer coat, of ureter, 524
Fibrous sheath, of spermatozoon, 566, 568
Fibrous subunit, of intermediate filaments, 48*f*
Fibrous tunic, of eye, 587, 588*f*
Fibulin-5, 92
Filaggrin, 50, 382
Filamin, 49*t*
Filensin, 51*t*
Filiform papillae, 429–430, 430*f*
Filopodia, 411*f*
Filtrate
loss of water and urea from, 521–522
monitoring of, in juxtaglomerular apparatus, 520–521
Filtration
glomerular, 505–508
in renal corpuscle, 518
Filtration barrier, 503, 518
Filtration force, 518
Filtration slits, 504–505, 506*f*
Fimbriae, 530*f*, 540–541
Fimbrin, 48, 49*t*, 108*f*, 605
Fine touch, 584
Fingerprints, 373, 382
First polar body, 536
Fissure, palpebral, 600
Fixation, 1
Fixatives, for transmission electron microscopy, 9

Fixed cells, of connective tissue, 127, 129–139
Flagella, in epithelium, 108
Flagellar axoneme, 566, 567*f*, 568
Flatus, 463–464
Flavin adenine dinucleotide ($FADH_2$), 44
Floaters, in eye, 592*b*
Fluid flow, in extracellular matrix, 81, 82*f*
Fluid mosaic model, of cell membrane, 17, 18*f*
Fluorescent-labeled antibody, 5, 6*f*
Fluoride, 422*b*
5-Fluorouracil, 76*b*
FMR protein, 63*b*
Focal adhesions, in epithelium, 118
Focal contacts, 48
Foliate papillae, 430
Follicle(s)
  of hair, 374, 374*f*, 381, 386–388, 387*f*–389*f*
  ovarian. *see* Ovarian follicles
  of thyroid, 355, 357*f*
Follicle-stimulating hormone (FSH), 349*t*–350*t*, 352–353
  in follicular development and ovulation, 532, 535, 537*f*, 538*t*, 545*f*
  in male reproductive system, 571, 572*f*
Follicular cells
  dendritic, 333
  of endocrine glands, 123
  of ovary, 529, 531*f*, 532–533, 533*t*
  of thyroid, 355–357, 355*f*, 357*f*
Follicular phase, of menstrual cycle, 545*f*, 546
Folliculostellate cells, 353
Folliostatin (folliculostatin), 535, 537*f*
Foramen cecum, 429, 430*f*
Foreign-body giant cells, 138, 263
Forkhead box L2, 533
Forkhead box O3, 533
Formalin, 1
Formed elements, of blood. *see* Blood
Formin, 49*t*
Fovea centralis, 588*f*, 592–593
Foveolae, 440
Fracture face, 11
Fragile X syndrome, 63*b*
Freckles, 383*b*
Free bilirubin, 490
Free villi, 551
Freeze-fracture technique, 9–11, 10*f*
Fructose-rich seminal fluid, 576
Functional syncytium, 198–200
Functionalis layer, of endometrium, 543, 543*f*
  necrosis of, 545
Fundic glands, 440, 440*t*, 441*f*, 449*t*–450*t*
Fundic stomach, 440, 441*f*, 449*t*–450*t*
Fundus, of uterus, 542–544
Fungiform papilla, 430, 430*f*–431*f*
Fusiform precursor cells, 147
Fusiform smooth muscle fibers, 203

G

G-actin, 46, 188*t*, 189
G bands, 63
G cells, 478–479, 480*t*, 481
G-protein-gated ion channels, 21
G-protein-linked receptors, 24–25, 25*f*
G proteins, 24
  G_o, cell signaling via, 25
  G_s and G_i, cell signaling via, 24, 25*f*
  hormone receptor complexes and, 346
  signaling via, 25
G_0 (outside, stable) phase, of cell cycle, 70–71
G_1 (gap 1) phase, of cell cycle, 70*f*, 71
G_2 phase, of cell cycle, 70*f*, 71–72
Gallbladder, 493–495
  histophysiology of, 494–495
  structure of, 494, 494*f*–495*f*
Gallstones, 496*b*
GALT (gut-associated lymphoid tissue), 312*t*, 340–342, 341*f*
Ganglion(ia)
  autonomic, 238–239, 239*f*
  sensory, 212, 212*f*
  spiral, 603
Ganglion cell layer, of retina, 593*f*, 594*t*, 599–600
Ganglion cells, of suprarenal gland, 366
Gangliosidoses, 41*t*
Gap 1 phase (G_1) phase, of cell cycle, 70*f*, 71
Gap junction plaques, 117*b*
Gap junctions, 23
  of bone, 156
  of capillaries, 296–297
  of cardiac muscle, 200*f*
  of epithelium, 111*f*, 114–116, 116*f*, 117*b*
  of salivary glands, 474
Gap regions, in tropocollagen, 87*f*, 90
Gas(es)
  in colon, 463–464
  as neuromodulators, 231
Gas exchange, 414–415
Gas transport, 395
Gastric carcinoma, 453*b*
Gastric contents, emptying of, 451
Gastric glands, 440, 440*t*, 441*f*, 449*t*–450*t*
Gastric inhibitory peptide (GIP), 447*t*, 452
Gastric intrinsic factor, 442, 442*b*
Gastric lipase, 439, 451
Gastric phase, of HCl secretion, 451
Gastric pits, 440, 441*f*
Gastrin, 447*t*, 451, 480*t*, 481
Gastroenteric reflex, 460–461
Gastroesophageal sphincters, 439
Gastrointestinal peptides, 231
Gastrointestinal tract. *see* Alimentary canal; Digestive system
GATA3 transcription factor, 276*t*
Gated channels, 21. *see also* Voltage-gated channels
Gated ion-channel receptors, 227–228
Gates, 21
Gaucher's disease, 41*t*
Gel-like networks, 48
Gelsolin, 48, 49*t*
Genes, 63–64
Genioglossus muscle, 430*f*
Geniohyoid muscle, 430*f*
Genital ducts, 571–575, 572*t*
Genital glands, accessory, 560*f*, 575–578
  histophysiology of, 577–578
Genome, 59–60, 63–64
Germ cells, primitive, 529
Germinal centers, of lymphoid nodules, 333
Germinal epithelium, 530, 561, 561*f*–563*f*
Germinal vesicle, 533
Ghrelin, 439, 481
Giant cells, foreign-body, 263
Giemsa stain, 2*t*, 12*f*
Gingiva, 421, 421*f*, 428–429
Gingival epithelium, 421*f*, 428–429
Gingival sulcus, 421*f*
Gland(s), 99–125
  accessory genital, 560*f*, 575–578
  acinar, 121
  anal, 467
  apocrine, 120, 120*f*
    mammary, 120
    sweat, 384–385
  areolar, 554
  Bartholin, 553
  Bowman (olfactory), 397, 397*f*–398*f*, 399
  Brunner (duodenal), 456*f*, 457–458
  bulbourethral, 559, 560*f*, 577
  ceruminous (wax), 384
  cervical, 544
  circumanal, 467
  compound tubuloacinar, 553
    prostate, 576, 576*f*
  Cowper's, 559, 560*f*, 577
  diffuse neuroendocrine system as, 123, 125*f*
  ductless, 345
  duodenal, 456*f*, 457–458
  endocrine. *see* Endocrine glands
  esophageal cardiac, 438, 449*t*–450*t*
  exocrine, 119–122, 120*f*
  gallbladder as, 493–495, 494*f*–495*f*, 496*t*

Gland(s) *(Continued)*
gastric, 440, 440*t*, 441*f*, 449*t*–450*t*
holocrine, 120, 120*f*
interstitial, 535, 540
lacrimal, 587, 601
of large intestine, 449*t*–450*t*
of Littre, 526
liver as, 481–495, 483*f*
mammary, 553–555
Meibomian, 600
merocrine, 120, 120*f*
of Moll, 384, 600
mucous, 119
of urinary bladder, 525
pancreas as, 476–481, 477*f*–479*f*, 478*b*, 480*t*
parathyroid, 355*f*, 356*t*, 360–361
parotid, 474, 476*b*
pineal, 356*t*, 368–369, 369*f*
pituitary, 346–354
prostate as, 560*f*, 576–577, 576*f*–578*f*
salivary, 419, 471–476
sebaceous, 374, 374*f*, 381, 385–386, 386*b*, 386*f*
serous, 430*f*–431*f*
simple coiled tubular, 382
of skin, 373, 382–386
of small intestine, 449*t*–450*t*, 460
sublingual, 472*f*–473*f*, 475
submandibular, 475–476, 475*f*, 476*b*
suprarenal (adrenal), 356*t*, 361–368, 362*f*
sweat, 374*f*, 382–384, 384*f*–385*f*
thyroid, 354–360, 355*f*, 356*t*, 359*b*
tubular, 121
of endometrium, 542, 543*f*, 546
tubuloacinar (tubuloalveolar), 121
compound
of mammary gland, 553
of prostate, 576, 576*f*
salivary, 471
uterine, 543*f*
vestibular, 553
of von Ebner, 430
of Zeis, 600
Glans clitoridis, 553
Glans penis, 560*f*, 578, 578*f*
Glassy membrane, 386, 387*f*–388*f*
Glaucoma, 590*b*
Glial fibrillary acidic protein (GFAP), 51*t*
Glial scar, 247
Glicentin, 447*t*
Glisson capsule, 481
Globulins, 252*t*
α-Globulins, 252*t*
β-Globulins, 252*t*
γ-Globulins, 252*t*
Glomerular arterioles, 503, 503*f*
afferent, 516
efferent, 516, 521
Glomerular capillaries, 503, 504*f*–505*f*, 513*f*
Glomerular endothelial growth factor, 504
Glomerular filtration rate (GFR), 505–508, 518
Glomerular ultrafiltrate, 505–509
Glomerulonephritis, 508*b*, 527*f*
Glomerulus(i)
of nephron, 502–504, 504*f*–505*f*
of olfactory bulb, 399–400, 400*b*
renal, 298
Glomus(era), 299
Glomus cells, 293
Glucagon, 447*t*, 480*t*, 481
Glucocorticoid(s), 356*t*, 364–366, 365*t*
and mammary gland development, 553
receptors for, on adipose cells, 143–144
Gluconeogenesis, 481, 491, 499
Glucose-1-phosphate (G-1-P), 481
Glucose-6-phosphatase deficiency, 46*t*
Glucose transporter protein-4 (GLUT-4), 480
Glucuronide, bilirubin, 490, 492*f*
Glucuronyltransferase, 490
Glutamate
in cones, 597
as neurotransmitter, 231
in rods, 597
Glutamic acid, 232*t*
Glutaraldehyde, as fixative, 9
Glutathione peroxidase, 261
Gluten, 463*b*
Gluten enteropathy, 463*b*
Glycerol, 144, 491
absorption and processing of, 462*f*
in cell membrane, 16
Glycerophosphocholine, 575
Glycine, 87, 232*t*
as neurotransmitter, 231
Glycocalyx, 19
in small intestine, 454
Glycogen
in clear cells, 383
in cytoplasm, 45–46
in hepatocytes, 490, 491*f*
in vagina, 552
Glycogen-lactic acid system, 191
Glycogen storage disorders, 46*b*, 46*t*
Glycogenolysis, 491, 492*f*
Glycogenosis, 41*t*
Glycolipids, in cell membrane, 16, 18*f*
Glycolysis, 191
Glycophorin A, 255
Glycophorin C, 256*f*
Glycoprotein(s)
associated with nuclear pore complex, 56
Glycoprotein(s) *(Continued)*
cell adhesive, in extracellular matrix, 84–85
in cell membrane, 18*f*
as cell-surface receptors, 24
in extracellular matrix, 84–85
Glycoproteinosis, 41*t*
Glycosaminoglycans (GAGs), in extracellular matrix, 81–82, 83*t*
of bone, 155
of cartilage, 149
Glycosphingolipids, in cell membrane, 16
Glycosylation, terminal, 33*f*
Goblet cells, 15*f*, 121, 121*f*–122*f*
of colon, 463, 464*f*–465*f*
of respiratory epithelium, 401–402, 402*f*
of small intestine, 454, 454*f*, 456*f*
Goiter, 359*b*
Golgi apparatus, 16*f*, 30–32, 31*f*–33*f*
alternative concept of, 35
of prostate, 576, 578*f*
proteoglycan synthesis on, 82
vesicles associated with, 34
Golgi complex, 213
Golgi intermediate compartment, 31*f*, 32
Golgi network
cis, 32, 32*f*
trans, 32, 33*f*
collagen synthesis in, 88, 89*f*
sorting in, 34–35
in spermiogenesis, 566
Golgi phase, of spermiogenesis, 566, 566*f*–567*f*
Golgi protein H3 (GOLPH3), 30
Golgi stack, 30
Golgi tendon organs, 193–198, 198*b*, 584*f*, 586
Gonadal ridges, 529
Gonadotrophs, 352
Gonadotropic hormones, 529
Gonadotropin-releasing hormone (GnRH)
in female reproductive system, 531, 537*f*
in puberty, 531
Graafian follicles, 531*f*, 533*t*, 535–536
Granular layer
of cerebral cortex, 244, 245*f*
external, of cerebral cortex, 245*f*
Granulation tissue, in bone repair, 171–172
Granule(s). *see also* Secretory granules
Birbeck (vermiform), 378
of eosinophils, 261
of juxtaglomerular cells, 513, 514*f*
keratohyalin, 376
membrane-coating, 375, 382
of neutrophils, 259

Granule(s) *(Continued)*
of platelets, 266, 266*t*, 267*f*
trichohyalin, 388
Granule cells, of cerebral cortex, 244
α-Granules, of platelets, 266, 266*t*
δ-Granules, of platelets, 266, 266*t*
λ-Granules, of platelets, 266, 266*t*
Granulocytes
basophils as, 262–263
eosinophils as, 261
neutrophils as, 257–261
Granulocytopoiesis, 280–281, 281*f*
Granulomere, 265, 266*t*
Granulosa cells, 531*f*, 532, 533*t*, 535, 536*t*
Granulosa-lutein cells, 531*f*, 539, 539*f*, 546
Graves disease, 316*b*, 359*b*
Gray matter, 217, 239
Great alveolar cells, 410, 412*f*–413*f*
Grooves
in dermal ridges, 382
of nails, 390
Ground-substance, 81–85, 82*f*
of connective tissue, 127, 141
Growth differentiation factor-9, 534
Growth factor(s), 546
epidermal, 382, 458
in odontogenesis, 425
fibroblast, in odontogenesis, 425
hemopoietic, 274–277, 276*t*
insulin-like, 349*t*–350*t*, 351–352
in keratinocytes, 382
platelet-derived, 294*b*
transforming, 382
Growth hormone. *see* Somatotropin
Guanine, 63
Guanosine 3',5' monophosphate, cyclic, 345–346
Guanosine diphosphate (GDP), 24
Guanosine triphosphate (GTP), 24
Guillain-Barré syndrome, 225*b*, 415*b*
Gums (gingiva), 421, 421*f*, 428–429
Gut-associated lymphoid tissue (GALT), 312*t*, 340–342, 341*f*
Gyri, 244

H

H band, in skeletal muscle, 180, 183*f*
Hair(s), 374*f*, 386–390
arrector pili muscles of, 374, 374*f*, 381, 388
club, 388–389
growth phases of, 388–389
histophysiology of, 388–390, 390*f*
matrix of, 387
types of, 386
Hair bulb, 386
Hair cells
inner, 610
outer, 610
Hair cells *(Continued)*
in saccule and utricle, 605, 605*f*–606*f*
innervation of, 606
type I, 605, 606*f*
type II, 605–606, 606*f*
in semicircular ducts, 607, 607*f*
Hair follicle, 374, 374*f*, 381, 386–388, 387*f*–389*f*
Hair root, 374*f*, 386
sheath of, 387–388, 388*f*
Hair shaft, 374*f*, 387–388, 389*f*
Halitosis, 419*b*
Haploid cells, 63, 76
in spermatogenesis, 562, 564*f*
Hard palate, 429
Hartmann's pouch, 493–494
Hassall corpuscles, 330–331, 330*f*
Haustra coli, 463
Haversian canal systems (osteon), 162*f*, 163–164, 164*f*
Hay fever, 137*b*
Head, of spermatozoon, 567–568, 568*f*
Heart, 287, 303–305. *see also* Cardiac; Cardio- *entries*
Heart failure cells, 412*b*
Heart wall, layers of, 303–305
Heat shock protein 70, 44
Heavy chains, of immunoglobulin, 316
Heavy meromyosin, 186*f*, 189
Helical arteries
of endometrium, 543, 543*f*
in penis, 579
Helicobacter pylori, 451, 453*b*
Helicotrema, 607
Hematemesis, 448*b*
Hematocrit, 251
Hematopoiesis. *see* Hemopoiesis
Hematoxylin and eosin stain, 2, 2*t*, 11*f*
Heme, 254, 255*b*, 414
Hemichannels, 114–115
Hemidesmosomes, 93*f*
in epithelium, 117–118, 118*f*
of skin, 375
Hemodynamics, 499
Hemoglobin, 46, 254–255, 255*b*, 414
Hemolytic jaundice, 492*b*
Hemophilia, 271*b*
Hemopoiesis, 271
cells of, 274*t*
hemopoietic growth factors (colony stimulating factors) in, 274–277, 276*t*
postnatal, 272–285
prenatal, 272
spleen, 335–340
stem cells, progenitor cells, and precursor cells in, 273–277, 275*b*, 275*f*
Hemopoietic cell, islands of, 272
Hemopoietic compartment, 272
Hemopoietic cords, 272
islands, 272
Hemopoietic growth factors (colony stimulating factors), 274–277, 276*t*
Hemopoietic stem cells, 273, 275*b*
Hemorrhage, of pulp, 424*b*
Hemorrhagic discharge (menses), 545
Hemorrhoidal plexus, 467
Hemorrhoids, 303*b*, 468*b*
Henle layer, 387, 388*f*
Henle loop, 500*f*–501*f*, 502, 508
countercurrent multiplier system and, 519–520, 520*f*
thick limb of, 509*f*
ascending, 509*f*, 511, 519, 523*t*
descending, 508
thin limbs of, 510–511, 511*t*, 514*f*, 523*t*
ascending, 509*f*, 510, 519, 523*t*
descending, 510–511, 519, 520*f*, 523*t*
Hensen cells, 609–610, 609*t*
Heparan sulfate, 504
in basal lamina, 95
in extracellular matrix, 81–82, 83*t*
Heparin
characteristics of, 81–82, 83*t*
mast cell release of, 132–134, 136*t*
Heparin-like molecule, and clot formation, 267
Hepatic acinus, 485, 485*f*
Hepatic arteries, 483–484, 483*f*
Hepatic coma, 492*b*
Hepatic ducts, 486, 494
Hepatic phase, of prenatal hemopoiesis, 272
Hepatic sinusoids, 483*f*–484*f*, 484–486, 489*f*
Hepatic veins, 483–484, 483*f*
Hepatocytes, 486
carbohydrate synthesis in, 491–493, 492*f*
function of, 481
inclusions of, 488–490, 489*f*, 491*f*
lateral domains of, 487–488, 488*f*
lipids in, 46
microvilli of, 485, 488
organelles of, 488–490, 489*f*
protein synthesis in, 489, 489*f*, 491–493, 492*f*
sinusoidal domain of, 488, 488*f*
structure of, 487*f*
Hepatocytes (limiting) plates, 483*f*–484*f*, 484–486
Hereditary spherocytosis, 256*b*
Hering, canals of, 486
Hernia, hiatal, 439*b*
Herpes simplex keratitis, 612*f*
Herring bodies, 354, 354*f*
Hertwig epithelial root sheath (HERS), 427

Heterochromatin, 58
Heterogeneous nuclear ribonucleoprotein particles (hnRNPs), 65–66, 69
Heterogeneous nucleation, 170
Heterografts, 173*b*
Hiatal hernia, 439*b*
High endothelial venules (HEV), 332
Hilar cells, 540
Hilum
of kidney, 499
of lung, 415
of lymph node, 331–332
of spleen, 335
Hinge region, of antibody, 316
Hirschsprung disease, 212*b*
Histamine
and capillary permeability, 299–300
gastric production of, 447*t*, 451
in hay fever, 137*b*
in HCl secretion, 451
inactivation of, by eosinophils, 261
in inflammatory response, 136
mast cell release of, 132–133, 136*t*
Histochemistry, 5
Histodifferentiation, in odontogenesis, 426
Histology
defined, 1
introduction to, 1–11
Histones, in nucleosomes, 58
Hodgkin disease, 265*b*
Holocrine glands, 120, 120*f*
Homeobox genes, 273
Homografts, 173*b*
Horizontal cells
in retina, 593*f*, 594*t*, 599
Hormone(s), 122, 345–346
adrenocorticotropic, 349*t*–350*t*, 352, 356*t*, 361, 364, 366*b*
of alimentary canal, 447*t*
antidiuretic, 231, 349*t*–350*t*, 353–354
in blood pressure regulation, 294
in urine formation, 513, 515, 521
antimüllerian, 562
corticotropin-releasing, 347, 349*t*–350*t*, 366
defined, 345
degradation of, in liver, 493
in female reproductive system, 537*f*, 538*t*
follicle-stimulating, 349*t*–350*t*, 352–353
in follicular development and ovulation, 532, 535, 537*f*, 538*t*, 545*f*
in male reproductive system, 571, 572*f*
gonadotropic, 529, 537*f*
hypothalamic-releasing, 231
hypothalamic secretory, 347
Hormone(s) *(Continued)*
inhibiting, 347
interstitial cell-stimulating, 349*t*–350*t*, 352
lipotropic, 352
luteinizing, 349*t*–350*t*, 352–353
in male reproductive system, 570–571, 571*f*–572*f*
in ovulation, 529–530, 536, 537*f*, 538*t*
luteinizing hormone receptors, 534–535
luteinizing hormone-releasing, 347, 349*t*–350*t*, 531, 532*t*, 537*f*
in male reproductive system, 571*f*–572*f*
neurohormones as, 231
parathyroid, 356*t*, 360–361
pituitary, 347, 349*t*–350*t*
prolactin-releasing, 347, 349*t*–350*t*
in signaling, 23
in smooth endoplasmic reticulum, 364
somatotropin. *see* Somatotropin
somatotropin-releasing, 347, 349*t*–350*t*, 351–352
steroid, 345, 363
suprarenal, 356*t*
thyroid, 352, 356*t*, 357–359, 358*f*
thyroid-stimulating, 349*t*–350*t*, 352, 355, 356*t*, 358
thyroid-stimulating hormone-releasing, 347, 349*t*–350*t*, 352
thyrotropin-releasing, 231
Hormone-receptor complex, 345–346
Hormone-sensitive lipase, 144, 481
Horny cells, 377
Horseradish peroxidase, 218
Howship lacunae, 156*f*, 159
Human chorionic gonadotropin (hCG), 540, 551
in female reproductive system, 538*t*
Human chorionic somatomammotropin, in female reproductive system, 538*t*
Human Genome Project, 64
Human immunodeficiency virus (HIV), 327*b*
Human leukocyte antigen (HLA), 263
Human placental lactogen, in female reproductive system, 538*t*
Humorally mediated immune system, 265
Hunter's syndrome, 41*t*
Huntington chorea, 231*b*
Hurler's syndrome, 41*t*
Huxley layer, 387, 388*f*
Huxley's sliding filament theory, 187
Hyaline cartilage. *see* Cartilage, hyaline
Hyalocytes, 591
Hyaloid canal, 588*f*, 591
Hyalomere, 265, 266*t*
Hyaluronic acid
in extracellular matrix, 81–82, 83*t*
in synovial fluid, 175
Hyaluronidase, 83*b*
Hydration shell, of bone matrix, 155
Hydrocephalus, 244*b*
Hydrochloric acid (HCl), 451–452, 452*f*
Hydrocortisone, 365
effect on cartilage, 152*t*
Hydrogen (H^+), in HCl production, 452
Hydrogen peroxide (H_2O_2), 40, 261
Hydrogen sulfide, 463
Hydrophilic head, of phospholipid molecule, 15–16
Hydrophobic ligands, 23
Hydrophobic tail, of phospholipid molecule, 15–16
Hydropic degeneration, 53*f*
Hydrops, corneal, 589
Hydroxyapatite crystals
in bone, 155
in enamel, 422
Hydroxylysine, 87
Hydroxyproline, 87
Hymen, 553
Hyoid bone, 430*f*
Hyperactivity, of spermatozoa, 548
Hyperadrenocorticism, 366*b*
Hyperallergic person, 137*b*
Hypercellular obesity, 146*b*
Hyperparathyroidism, 361*b*
Hyperplasia, of myometrium, 543–544
Hyperpolarization, 182
Hypertension, chronic essential, 521*b*
Hyperthyroidism, 359*b*
Hypertrophic obesity, 146*b*
Hypertrophy
cardiac, 202*b*
of myometrium, 543–544
of smooth muscle, 207
zone of, 169
Hypervitaminosis A
bone effects of, 174*t*
cartilage effects of, 152*t*
Hypochlorous acid, 260*f*, 263
Hypodermis, 373, 374*f*, 380
Hypolemmal cisternae, 213
Hyponychium, 391
Hypoparathyroidism, 361*b*
Hypophyseal arteries, 347, 349*f*
Hypophyseal portal veins, 347, 349*f*
Hypophyseal stalk, 348*f*
Hypophysis. *see* Pituitary gland
Hypothalamic-releasing hormones, 231
Hypothalamic secretory hormones, 347
Hypothalamohypophysial tract, 349*f*, 353–354
Hypothalamus, 348*f*, 355*b*
Hypothyroidism, 359*b*
Hypovitaminosis A, cartilage effects of, 152*t*

Hypovitaminosis C, cartilage effects of, 152*t*

I

I bands
in cardiac muscle, 200*f*
in skeletal muscle, 180, 183*f*–184*f*
Ibuprofen, and ulcers, 453*b*
IgA dimer-IgA dimer receptor molecule complex, 471
IgA dimer receptor molecules, 471
Ikaros family of transmission factors, 276*t*
Ileocecal valve, 463
Ileum, 449*t*–450*t*, 458. *see also* Small intestine
Image processing, in retina, 600
Immediate hypersensitivity reaction, 134–135
Immotile spermatozoa, 567
Immune function, hepatocytes and, 493
Immune response
antigen-presenting cells in, 311, 324
cell-mediated, 314
Langerhans cells in, 378
primary, 315
secondary, 315
Immune system, 311–343
adaptive, 311
antigens in. *see* Antigen(s)
B lymphocytes in. *see* B lymphocytes (B cells)
bronchus-associated lymphoid tissue in, 342
cells of, 317–324
clonal selection and expansion in, 315–317
cytokines in. *see* Cytokines
gut-associated lymphoid tissue in, 312*t*, 340–342, 341*f*
humorally mediated, 265
immunogens in, 315
immunoglobulins in, 316–317. *see also specific immunoglobulin*
immunological tolerance in, 315
innate, 311–313
lymph nodes in, 331–335, 331*b*
major histocompatibility molecules in, 323–324
mucosa-associated lymphoid tissue in, 340–343
natural killer cells, 311, 323
overview of, 311–327, 312*t*
pathological considerations of, 343
spleen in, 335–340
T lymphocytes in. *see* T lymphocytes (T cells)
thymus in, 328–331, 329*f*–330*f*, 329*t*
toll-like receptors in, 311, 312*t*, 313*b*, 314*t*
tonsils in, 342–343
Immunocompetent B lymphocytes, 285
Immunocytochemistry, 5–6, 5*f*–6*f*
Immunogenicity, 315*b*
Immunogens, 315
Immunoglobulin(s), 316–317
classes of, 317
human, properties of, 318*t*
surface, 315
Immunoglobulin A (IgA)
hepatocytes and, 493
secretory, 419, 459–460, 474
in small intestine, 459–460, 460*f*
Immunoglobulin E (IgE) receptors (FcεRI), 262
Immunological activity, of lamina propria, 459–460, 460*f*–461*f*
Immunological memory, 315, 321
Immunological tolerance, 315
Implantation, 548–549
Importins, 57–58
Impotence, 580*b*
Impulse(s)
generation and conduction of, 224–232
transmission of, neuromuscular junctions, 192–193, 193*f*–196*f*
Inactivation gate, 226
Inactive position, of voltage-gated channels, 21
Incisures of Schmidt-Lanterman, 223
Inclusions
in cytoplasm, 13, 45–46, 47*f*
in hepatocytes, 488–490, 489*f*, 491*f*
in neurons, 213–215
Incontinence, urinary, 526*b*
Incus, 602*f*, 603
Indifferent gonads, 529
Indirect immunocytochemistry, 5, 5*f*–6*f*
Indole, 463
Induced pluripotent stem cells (iPSC), 556–557
Inducible T reg cells, 323
Induction, in drug tolerance, 493*b*
Infertility, male, 560*b*
Inflammatory response
edema related to, 142*b*
sequence of events in, 136
Infundibulum, of oviduct, 530*f*, 537, 540–541
Inhalation, 415
Inhibin
in female reproductive system, 535, 537, 537*f*, 538*t*
in male reproductive system, 562, 571
Inhibiting hormones (inhibiting factors), 347
Inhibitory neurotransmitters, 21
Inhibitory output, of Purkinje cells, 244
Inhibitory postsynaptic potential, 228
Inhibitory responses, 231
Initiator tRNA, 27
Inlet arterioles, of liver, 484
Inlet venules, of liver, 484
Innate immune system, 311–313
natural killer (NK) cells in, 312–313, 323
Inner dense plaque, 114
Inner ear, 601, 603–612, 604*f*–605*f*
Inner leaflet, of cell membrane, 13–15, 18*f*
Inner limiting membrane, of retina, 593*f*, 600
Inner membrane, mitochondrial, 43*f*, 44
Inner mitochondrial membrane complex, 44
Inner nuclear layer, of retina, 593*f*, 598–599
Inner nuclear membrane, 55, 57*f*–58*f*, 60*f*
Inner plexiform layer, of retina, 593*f*, 599
Inner table, of calvaria, 163
Inner tunnel, 609
Innervation. *see* Nerve supply
Inositol triphosphate (IP_3), in signaling, 24
Insensible water loss, 499
INSL 3, 571
Insulin, 479–480, 480*t*, 482*b*, 482*t*
sensitivity, 145
Insulin-like growth factors, 349*t*–350*t*, 351–352, 533, 538*t*
Insulin receptors, 143–144
Integral proteins
in cell membrane, 16, 18*f*
in rough endoplasmic reticulum, 26
Integrins
in cytoplasm, 48, 50*f*
in epithelium, 111*f*
in extracellular matrix, 84, 93–96
$\alpha_3\beta_1$ integrins, 504–505
Integument, 373–391
hair in, 374*f*, 386–390
nails in, 390–391, 390*f*–391*f*
pathological considerations of, 391*f*–393*f*
sebaceous glands in, 374, 374*f*, 381, 385–386, 386*b*, 386*f*
skin in. *see* Skin
sweat glands in, 374*f*, 382–384, 384*f*–385*f*
Interalveolar septa, 408, 409*f*, 411*f*, 414
Intercalated cells, of cortical collecting tubules, 513
Intercalated disks, 198–200, 200*f*–201*f*
Intercalated ducts
of pancreas, 476–478, 477*f*
salivary, 472, 472*f*
Intercellular adhesion molecule type 1 (ICAM-1), 259

Intercellular bridges, 375
Interchromatin granules, 69
Intercristal space, 43
Interdigitating dendritic cells, 338
Interdigitation, 504–505
Interfascicular oligodendrocytes, 221, 222*f*
Interferon(s), 312
Interferon-γ, 540
  effect on bone, 173
  as hemopoietic growth factor, 276*t*
  in innate immune system, 313
  secretion of, by microglial cells, 221
Interleukin(s) (IL)
  cytokines as, 312
  mast cell release of, 132–133
Interleukin-1 (IL-1)
  bone effects of, 159
  in hemopoiesis, 274–277, 276*t*
  keratinocytes and, 382
  neutrophils and, 259
Interleukin-2 (IL-2), in hemopoiesis, 274–277, 276*t*
Interleukin-3 (IL-3), in hemopoiesis, 274–277, 276*t*
Interleukin-4 (IL-4), in hemopoiesis, 274–277, 276*t*
Interleukin-5 (IL-5)
  and eosinophils, 261, 274–277
  in hemopoiesis, 274–277, 276*t*
Interleukin-6 (IL-6), 146–147, 147*t*
  bone effects of, 159
  in hemopoiesis, 274–277, 276*t*
Interleukin-7 (IL-7), in hemopoiesis, 274–277, 276*t*
Interleukin-8 (IL-8), in hemopoiesis, 274–277, 276*t*
Interleukin-9 (IL-9), in hemopoiesis, 274–277, 276*t*
Interleukin-10 (IL-10), in hemopoiesis, 274–277, 276*t*
Interleukin-12 (IL-12), in hemopoiesis, 274–277, 276*t*
Interleukin-15 (IL-15), in hemopoiesis, 274–277, 276*t*
Interlobar arteries, of kidney, 500*f*–501*f*, 516
Interlobar veins, 516
Interlobular arteries, of kidney, 500*f*–501*f*, 503, 516
Interlobular ducts
  of pancreas, 476–477
  salivary, 472, 472*f*
Interlobular veins, 516
Intermediate cells, 456
  of cochlear duct, 608
  of parathyroid, 360
  of taste buds, 431
Intermediate compartment, of Golgi apparatus, 31*f*, 32
Intermediate filaments, 55
  in cytoskeleton, 48–50, 48*f*, 50*b*, 51*t*
  in epithelium, 104, 108*f*, 114
  in skin, 375
Intermembrane space, mitochondrial, 43–44, 43*f*
Internal capsule, 197*f*
Internal spiral sulcus, 608
Interneurons, 219
Internodal segments, of Schwann cells, 223
Internum, of eosinophil, 261
Internuncial neurons, 239–240
Interoceptors, 583
Interpapillary peg, 382
Interphase, of cell cycle, 70*f*, 71–72, 73*f*
Interplaque regions, of urinary bladder, 524
Interplexiform cells, 599
Interstitial cell-stimulating hormone (ICSH), 349*t*–350*t*, 352
Interstitial cells
  of Cajal, 435
  dendritic, of renal interstitium, 515
  of Leydig, 559, 569–570, 570*f*–571*f*
  of ovary, 531, 540
  of pineal gland, 368–369
  of renal interstitium, 515–516
Interstitial glands, 535, 540
Interstitial growth, of hyaline cartilage, 149
Interstitial lamellae, 164
Interterritorial matrix, of hyaline cartilage, 152
Intestinal cell kinase (ICK), 106–108
Intestinal phase, of HCl secretion, 451
Intestines. *see* Large intestine; Small intestine
Intracellular plaque, 117
Intracellular receptor family, 24
Intraciliary retrograde transport, 108*b*
Intraciliary transport, 106–108
Intracytoplasmic plaque, 117
Intraepithelial capillaries, 608
Intraflagellar transport, 106–108
Intrafusal fibers, 195
Intralobar ducts
  of pancreas, 476–477
  salivary, 472, 472*f*
Intralobular ducts, salivary, 472, 472*f*
Intramembranous bone formation, 149, 164–165, 165*f*–166*f*
Intramural region, of oviducts, 530*f*, 541
Intraperiod gaps, of Schwann cells, 223
Intraperiod line, of Schwann cells, 223
Intrapulmonary bronchi, 396*t*, 404
Intrapulmonary conducting division, 396*t*
Intratesticular genital ducts, 560*f*, 571–573, 572*t*
Intrinsic muscles
  of larynx, 400
  of tongue, 429, 430*f*
Intrinsic pathway, caspases and, 79
Introns, 65–66
Inverted formin 2 (INF2), 45
Investments
  connective tissue, of peripheral nerves, 233, 233*f*
  of skeletal muscle, 179–191
Involucrin, 377, 382
Involuntary muscle, 203
Iodide, and thyroid hormones, 356, 358*f*
Iodotyrosine dehalogenase, 358
Ion channel-linked receptors, 21
Ion channel-mediated diffusion, 20*f*
Ion channels, 20
Iris, 590–591
Iron-containing pigments, 213–215
Iron deficiency anemia, 280*b*
Iron hematoxylin stain, 2*t*, 12*f*
Islands, blood, 272
Islets of Langerhans, 476, 477*f*, 478, 479*f*, 482*t*
  cells composing, 478–479, 479*f*, 480*t*
Isogenous groups, of chondrocytes, 149
Isotype switching, 317
Isotypes, of immunoglobulins, 317
Isthmus
  of oviduct, 530*f*, 541
  of thyroid, 354–355
Itching (pruriception), 381*b*, 586–587
Ito cells, 486, 493

J

J protein, 318*t*
Jaundice, 492*b*
  hemolytic, 492*b*
  obstructive, 492*b*
Jaw-jerk reflex, 428*b*
Jejunum, 449*t*–450*t*, 458. *see also* Small intestine
Joints, 174–175, 175*f*
Junctional adhesive molecules (JAMs), 112–113
Junctional complexes, 109, 111*f*–112*f*
Junctional epithelium, of gingiva, 429
Junctional folds, 192, 193*f*
Juxtaglomerular apparatus, 503*f*, 512–513, 513*f*–514*f*
  monitoring the filtrate in, 520–521, 521*t*
Juxtaglomerular (JG) cells, 503*f*, 512–513, 513*f*–514*f*, 523*t*
Juxtamedullary nephrons, 500*f*–501*f*, 502, 519

K

Kallikrein, 474
Kallmann syndrome, 353*b*
Kartagener syndrome, 106*b*

Kartogenin, 149
Karyokinesis, 72, 565
Karyoplasm, 13
Karyotyping, 62*f*, 63
KDEL sequence, 34
Keloid, 90*b*, 97*f*
Keratan sulfate
  in bone, 154
  characteristics of, 81–82, 83*t*
Keratin, 51*t*, 382
  of hairs, 389
Keratin filaments, 374, 388
Keratinocytes, 374
Keratohyalin granules, 376
Kerckring, valves of, 453
Ketone bodies, 491
Ketosis, 492*b*
Kidney(s), 499–522, 500*f*–501*f*. *see also* Renal *entries*
  Bowman capsule of, 500*f*–503*f*, 502, 508
  Bowman space of, 502, 503*f*, 508, 513*f*
  calyces of, 499, 500*f*–501*f*, 523
  capsule of, 499, 500*f*–501*f*
  circulation of, 500*f*–501*f*
    arterial supply, 516
    venous drainage, 516–517, 517*f*
  collecting tubules of, 501, 513–515, 514*f*–515*f*, 523*t*
    cortical, 509*f*, 513
    loss of water and urea from filtrate in, 521–522
    medullary, 513–515, 515*f*
    papillary, 499, 515
    structure of, 500*f*–501*f*, 523*t*
  cortex of, 499, 500*f*–502*f*
  disorders of, 500*b*, 502*b*, 504*b*, 522*b*
  distal tubule, 502, 510–512, 513*f*
    convoluted, 500*f*–501*f*, 512, 512*f*, 523*t*
  general functions of, 518–522
  glomerulus of, 502–504, 504*f*–507*f*
  Henle loop of, 500*f*–501*f*
    countercurrent multiplier system and, 519–520, 520*f*
    thick limb of
      ascending, 509*f*, 511, 519, 523*t*
      descending, 508
    thin limbs of, 510–511, 511*t*, 514*f*, 523*t*
      ascending, 509*f*, 510, 519, 523*t*
      descending, 510–511, 519, 520*f*, 523*t*
  hilum of, 499
  innervation of, 517–518
  interstitium of, 515–516
  juxtaglomerular apparatus of, 503*f*, 512–513, 513*f*–514*f*
    monitoring the filtrate in, 520–521, 521*t*
Kidney(s) *(Continued)*
  juxtaglomerular cells of, 503*f*, 512–513, 513*f*–514*f*, 523*t*
  lobated, 500*b*
  lobes and lobules of, 500
  lymphatic supply of, 517
  macula densa of, 502*f*–503*f*, 511–512, 513*f*–514*f*, 523*t*
  medulla of, 499, 500*f*–501*f*, 502, 514*f*
  medullary rays of, 500, 500*f*–502*f*
  mesangial cells of, 503, 504*f*–505*f*
  nephrons of, 500*f*–501*f*, 502–513
  pedicels of, 504, 506*f*
  pelvis of, 499, 500*f*–501*f*, 523
  podocytes of, 502, 503*f*–506*f*, 513*f*
  proximal tubule of, 502, 502*f*–503*f*, 508–511, 523*t*
    convoluted, 503*f*, 508, 509*f*
    regions of, 508, 510*f*
    resorption in, 510, 518–519
  pyramids of, 499, 500*f*–501*f*
  renal corpuscles of, 500, 502–508, 502*f*–503*f*, 523*t*
    filtration in, 518
  sinus of, 499, 500*f*–501*f*
  structure of, 499–500, 500*f*–501*f*
  urinary pole of, 503, 503*f*, 507*f*, 508
  urine formation by, 518–522, 521*t*
  uriniferous tubules of, 500*f*–501*f*, 501–517, 509*f*, 520*f*
    aquaporins in, 522*t*
    structure and function of, 523*t*
  vasa recta of, 516
    and countercurrent exchange system, 522, 522*f*, 523*t*
  vascular pole of, 503, 503*f*
  water loss through, 499
Kiesselbach area, 397*b*
Killer activating receptors, 312–313
Killer inhibitory receptors, 312–313
Kinesin
  in axonal transport, 218
  as microtubule motor protein, 51
  and vesicles, 34
Kinesin-2, 106–108
Kinetochore microtubules, 74
Kinocilium, 605, 605*f*–606*f*
Kit ligand, 534
Klinefelter syndrome, 63*b*, 565*b*
Kohn pore, alveolar, 408, 410*f*–411*f*
Krause end bulbs, 381, 584*f*, 586
Krebs cycle, 44
Kupffer cells, 138, 138*f*, 485, 486*f*–487*f*, 493
Kynurenic acid, 191*b*
Kynurenine, 191*b*
Kynurenine aminotransferase, 191*b*

L

Labia majora, 552
Labia minora, 553
Lacrimal apparatus, 600–601
Lacrimal canaliculi, 601
Lacrimal fluid, 587, 601
Lacrimal gland, 587, 601
Lacrimal punctum, 601
Lacrimal sac, 601
Lactation, 553–554, 554*f*–555*f*
Lacteal, 453, 458
Lactic acid, in vagina, 552
Lactiferous duct, 553, 554*f*
Lactiferous sinus, 553
Lactoferrin, 419, 471, 474
Lacunae
  of bone, 154, 154*f*, 162*f*
  of cartilage, 149, 151*f*
  in implantation, 549
  in placental development, 551
Lamellae, of bone, 161, 162*f*
  interstitial, 164
Lamellar bodies, 375
  of pneumocytes, 411
Lamin A, 51*t*, 55
Lamin B, 51*t*
Lamin B_1, 55
Lamin B_2, 55
Lamin C, 51*t*, 55
Lamina(e)
  basal. *see* Basal lamina
  dental, 424, 425*f*
  elastic. *see* Elastic lamina
  external, 93
  myocardial, 198
  succedaneous, 426
Lamina densa, 95
  glomerular, 504
Lamina lucida, 93–95
Lamina propria
  of alimentary canal, 435, 449*t*–450*t*
  of anal canal, 467
  of bronchioles, 406, 407*f*
  of colon, 463
  defined, 141
  of endometrium, 542
  of esophagus, 437–438, 449*t*–450*t*
  of female urethra, 526
  of gallbladder, 494, 497*f*
  immunological activity of, 459–460, 460*f*–461*f*
  of large intestine, 449*t*–450*t*
  of nasal cavity, 395–397, 399
  of oviduct, 541
  of small intestine, 449*t*–450*t*, 455–456, 455*f*–456*f*, 458*f*
  of stomach, 440, 449*t*–450*t*
  of trachea, 403
  of ureter, 524, 526
  of urinary bladder, 525
  of vagina, 552
Lamina rara, of glomerulus, 504
Lamina reticularis, 93, 93*f*–94*f*, 95

Laminin, 93–95, 504
  in cytoskeleton, 50*f*
  in extracellular matrix, 84
  release of, by capillaries, 300
Langerhans, islets of, 476, 477*f*, 478, 479*f*, 482*t*
  cells composing, 478–479, 479*f*, 480*t*
Langerhans cells
  of epidermis, 374*f*, 378
  of esophagus, 437
  macrophage function of, 138
  of vagina, 552
Lanugo, 386
Large intestine, 463–468
  appendix of, 449*t*–450*t*, 467–468
  colon of, 449*t*–450*t*, 463–467, 464*b*–466*b*, 464*f*–466*f*
  DNES cells and hormones in, 447*t*
  histology of, 449*t*–450*t*
  rectum and anal canal of, 449*t*–450*t*, 467
Laryngeal aditus, 400–401
Laryngitis, 401*b*
Larynx, 396*t*, 400–401
Lateral domains, of hepatocytes, 487–488, 488*f*
Lateral inhibition, 599
Lateral membrane specializations, of epithelium, 109–116
Leaflet, of cell membrane, 13–15, 18*f*
Lens
  of eye, 588*f*, 591, 591*f*–592*f*
    suspensory ligaments of, 590
  of microscope
    condenser, 3, 3*f*
    objective, 3, 3*f*
    ocular, 3, 3*f*
    projection, 3*f*
Lens capsule, 591
Lens fibers, 591, 592*f*
Leptin, 144–145, 147*t*, 538*t*
  mutation of, 145*b*
Leptotene, 76, 565
Leukemia
  acute myeloblastic, 281*b*
  acute myelogenous, 76*b*
Leukocyte(s), 257–265. *see also specific type of leukocytes*
  in connective tissue, 139
Leukocyte adhesion deficiency, 96*b*
Leukotrienes
  basophils and, 263
  eosinophils and, 261
  in inflammatory response, 136
  mast cell release of, 132–133, 136*t*
  neutrophil release of, 260–261
Leydig cells, 559, 569–570, 570*f*–571*f*
Ligand, 21
  in receptor-mediated endocytosis, 36
Ligand-gated channels, 21
Light cells, 513
  of saccule, 604–605
  of taste buds, 431
Light chains
  of immunoglobulin, 316
  of myosin, 189, 206
Light conducting support, for retina, 599
Light meromyosin, 186*f*, 188
Light microscopy, 1–11
  advanced visualization procedures in, 5–7
  digital imaging techniques in, 4
  interpretation of microscopic sections in, 4–5, 4*f*
  microscope for, 3–5, 3*f*
  tissue preparation for, 1–3, 2*t*
Light zones, of lymphoid nodule, 333
Limbus
  of cornea, 589
  of spiral lamina, 608
Limiting membrane
  external
    of ependymal cells, 223
    of retina, 593*f*, 598
  inner, of retina, 593*f*, 600
  internal, of ependymal cells, 223
Limiting plates, of liver, 483*f*–484*f*, 484–486
lincRNA, 68
  H19, 68
  XIST, 68
Linear movements, 610
Lines of Owen, 423
Lingual papillae, 429–433, 430*f*–431*f*
Lingual tonsil, 343, 430*f*
Lining mucosa, 419, 429
Link proteins, 82–83
Linker DNA, 58
Lipase
  adipose triglyceride, 481
  hormone sensitive, 481
  pancreatic, 477
Lipid(s)
  in cytoplasm, 46
  droplets, of neuron, 215
  emulsification of, 461, 462*f*
  in hepatocytes, 490, 491*f*
  liver metabolism of, 491
  in mammary glands, 554
Lipid antigens, 323
Lipid bilayer, of cell membrane, 15
Lipid rafts, 16
Lipochrome pigment, 575–576
Lipofuscin, 46
  in neurons, 213–215
Lipoid nephrosis, 508*b*
Lipolysis, 365
Lipomas, 147*b*
Lipoprotein(s)
  low-density, 252*t*, 364
  plasma, 252*t*
  very-low-density, 252*t*, 490–491, 491*f*
Lipoprotein lipase, 144
Liposarcomas, 147*b*
Lipotropic hormone (LPH), 352
Lips, 419–420
Liquor folliculi, 533*t*, 535
Liver, 481–495, 483*f*. *see also* Hepatic *entries*
  acinus of, 485, 485*f*
  bile manufacture by, 490, 492*f*
  capsule of, 481
  carbohydrate synthesis in, 491–493, 492*f*
  detoxification of drugs and toxins by, 493, 493*b*
  disorders of, 486*b*, 490*b*, 492*b*
  ducts of, 486, 494
  hepatocyte (limiting) plates of, 483*f*–484*f*, 484–486
  hepatocytes of. *see* Hepatocytes
  histophysiology of, 490–493, 491*f*–492*f*
  hormone degradation by, 493
  immune function of, 493
  Kupffer cells of, 485, 486*f*–487*f*, 493
  lipid metabolism in, 491
  lobes of, 481, 483*f*
  lobules of, 485, 485*f*
    classical, 484–485, 485*f*
  lymph flow in, 484–485
  perisinusoidal space of Disse, 485–486, 487*f*–488*f*
  portal areas (triads) of, 483*f*, 484, 497*f*
  protein synthesis in, 489, 489*f*
  regeneration of, 493
  sinusoids of, 483*f*–484*f*, 484–486, 489*f*
  structure of, 481–490, 483*f*
  vascular supply to, 481–490, 483*f*–484*f*
  vitamin storage in, 493
lncRNA, 68
Lobar arteries, of kidney, 516
Lobar bronchi, 404
Lobated kidney, 500*b*
Lobe(s)
  of kidney, 500
  of thymus, 328
  of thyroid gland, 354–355
Lobular carcinoma, 556*b*
Lobule(s)
  of bronchopulmonary segment, 415
  of kidney, 500
  of liver, 485, 485*f*
    classical, 484–485, 485*f*
  of mammary glands, 553
  of thymus, 328
Lobuli testis, 559
Local reaction, to nerve injury, 245

Longitudinal muscle
of myometrium, 543
of ureter, 524
Longitudinal section, 4*f*
Loop of Henle. *see* Henle loop
Loose connective tissue, 141
Low-density lipoprotein (LDL), 252*t*, 364
Low-density lipoprotein cholesterol receptors (LDL-cholesterol receptors), and progesterone, 539
Low-frequency sounds, 611–612
Lubricin, in synovial fluid, 175
Luminal spoke (middle) ring, 56, 60*f*
Luminal surface, of small intestine, 453, 454*f*–456*f*
Lung(s). *see also* Pulmonary *entries*; Respiratory system
gas exchange in, 414–415
gross structure of, 415–416
Lunula, 391
Luteal phase, in menstrual cycle, 546–547, 546*f*
Luteinizing hormone (LH)
interstitial cell-stimulating hormone as, 352
in male reproductive system, 570–571, 571*f*–572*f*
in ovulation, 529–530, 536, 537*f*, 538*t*
physiological effects of, 349*t*–350*t*
Luteinizing hormone receptors, 534
Luteinizing hormone-releasing hormone (LHRH), 347, 349*t*–350*t*, 531, 532*t*, 537*f*, 538*t*
Luteolysis, 540
Lymph, 287, 305
flow of, in liver, 484–485
Lymph nodes, 305–306, 331–335, 331*b*, 335*b*
cortex of, 332–333, 333*f*
diagram of, 332*f*
histophysiology of, 334–335
medulla of, 334, 334*f*
paracortex of, 333
vascularization of, 334
Lymph vessels, 331
Lymphatic anchoring filaments, 306
Lymphatic ducts, 307
Lymphatic supply
of kidney, 517
of lungs, 415–416
of small intestine, 458
Lymphatic vascular system, 305–307
Lymphocyte(s), 264
B. *see* B lymphocytes (B cells)
features of, 258*t*
functions of, 139, 264–265
structure of, 252*f*
T. *see* T lymphocytes (T cells)
types of, 264–265
Lymphoid cell(s), 324–325
interaction among, 324–327
Lymphoid nodules, 333
of alimentary tract, 436*f*
of pharyngeal tonsil, 342*f*
of small intestine, 454*f*
of spleen, 337, 337*f*
Lymphoid organ(s), 327–343
bronchus-associated lymphoid tissue as, 342
gut-associated lymphoid tissue as, 312*t*, 340–342, 341*f*
lymph nodes as, 331–335, 331*b*, 335*b*
mucosa-associated lymphoid tissue in, 340–343
primary, 327
secondary (peripheral), 328
spleen as, 335–340
thymus as, 328–331, 329*f*–330*f*, 329*t*
tonsils as, 342–343
Lymphoid system. *see* Immune system
Lymphoid tissue
bronchus-associated, 312*t*, 342, 416
gut-associated, 312*t*, 340–342, 341*f*
mucosa-associated, 340–343
Lymphomas, 265*b*
Lymphopoiesis, 284–285
Lysosomal acid maltase deficiency, 46*t*
Lysosomal proteins, transport of, 34–35, 35*f*
Lysosomal storage disorders, 40*b*, 41*t*
Lysosomes, 16*f*, 33*f*, 36–39, 40*f*
formation of, 39
in leukocytes, 257
transport of substances into, 39
Lysozyme(s)
in esophagus, 438
in oral cavity, 419
in saliva, 471, 474
in small intestine, 456
in stomach, 446
in tears, 601
Lysyl hydroxylase deficiency, 90*b*

M

M (microfold) cells, 340, 455, 459, 460*f*–461*f*
M line, in skeletal muscle, 180
M phase, 71
Macrophage(s), 136–139, 137*f*
activated, 138
alveolar, 411*f*, 412, 412*b*, 413*f*
in bone marrow, 272
crystals in, 46, 47*f*
development and distribution of, 138, 138*f*
diagram of, 328*f*
elicited, 138
fixed, 138
free, 138
function of, 138–139, 263
Macrophage(s) *(Continued)*
in innate immune system, 312
in killing bacteria, 327
in lymph nodes, 332
in phagocytosis, 36
in renal interstitium, 515
resident, 138
in spleen, 339
tingible body, 330
Macrophage colony-stimulating factor (M-CSF), 159
Macula
of saccule, 604, 604*f*
of utricle, 604, 604*f*
Macula densa, 502*f*–503*f*, 511–513, 513*f*–514*f*, 523*t*
Macula lutea, 588*f*, 592–593
Maculae adherentes, 111*f*–112*f*, 114, 115*f*
Macular degeneration, 613*f*
Main pancreatic duct, 476–477
Majewski polydactyly syndrome, 108*b*
Major basic proteins, of eosinophils, 261
Major dense line, 223
Major histocompatibility complex (MHC) antigens, 263
Major histocompatibility complex (MHC) molecules, 313*b*, 323–324
classes of, 323
loading of epitopes on, 324
synthesis of, by Langerhans cells, 437
and T lymphocytes, 323–324
Malabsorption, 463*b*
Male infertility, 560*b*
Male pronucleus, 548
Male reproductive system, 559–581, 560*f*
accessory genital glands in, 560*f*, 575–578
bulbourethral glands in, 559, 560*f*, 577
ductuli efferentes in, 559–560, 560*f*, 572*t*, 574
ductus (vas) deferens, 559–560, 560*f*, 572*t*
ejaculatory duct in, 560*f*, 572*t*, 575
epididymis in, 559–560, 560*f*, 572*t*, 574, 574*f*
genital ducts in, 571–575, 572*t*
interstitial cells of Leydig in, 559, 569–570, 570*f*–571*f*
pathological considerations for, 581, 581*f*–582*f*
penis in, 559, 560*f*, 577–578, 578*f*, 580*f*
prostate gland in, 560*f*, 576–577, 576*f*–578*f*
rete testis in, 559, 560*f*, 572*t*, 573, 573*f*
seminal vesicles in, 559, 560*f*, 575–576, 576*f*
seminiferous tubules in, 559–568, 560*f*, 562*f*–563*f*

Male reproductive system *(Continued)*
Sertoli cells in, 561–562, 562*f*–563*f*
spermatogenic cells in, 562–568, 562*f*, 564*f*, 566*f*–567*f*
summary of, 559.*e*1
testes in, 559–568, 560*f*
tubuli recti in, 559, 572*t*, 573
Malignant melanoma, 383*b*
Malignant tumor cells, 307*b*
Malleus, 602*f*, 603
Malpighian layer, 374*f*, 375
MALT (mucosa-associated lymphoid tissue), 340–343
Mammary glands, 553–555, 554*f*–556*f*
inactive, 553, 554*f*
lactating (active), 553–554, 554*f*
secretions of, 555
Mammotrophs, 351*f*, 352
Manchette, 566
Mannitol, 243*b*
Mannose, phosphorylation and removal of, 32, 33*f*
Mannose-6-phosphate (M6P), 34
Mannose-6-phosphate receptors, 34, 39
Mantle, of lymphoid nodule, 333
Marfan syndrome, 92*b*, 96*f*
Marginal cells, 607–608
Marginal fold, of capillary, 296
Marginal sinuses, of spleen, 338
Marginal zone, of spleen, 337–338, 337*f*–338*f*
Margination, of neutrophils, 281
Marrow. *see* Bone marrow
Marrow cavity, 154
Masson trichrome stain, 2*t*, 11*f*
Mast cells, 132–136, 134*f*, 281
activation and degranulation of, 134–136, 135*f*
development and distribution of, 133–134
mucosal, 133–134
primary and secondary mediators released by, 136*t*
Masticatory mucosa, 419, 429
Mastocytosis, 137*b*
Matrix granules, 44
Matrix space, mitochondrial, 43*f*, 44
Maturation, of lens fibers, 591
Maturation phase, of spermiogenesis, 566–567, 567*f*
Maturation promoting factor, 536
Maturation zone, 169
Mature (graafian) follicles, 531*f*, 533*t*, 535–536
McArdle syndrome, 46*t*
Mechanically gated channels, 21
Mechanoreceptors, 583–586
encapsulated, 381, 584–586, 584*f*–585*f*
Merkel cell-neurite complexes as, 378
nonencapsulated, 583–584, 584*f*
Mechanotransduction, 158
Meckel diverticulum, 459*b*
Meckel's syndrome, 108*b*
type 1, 108*b*
Median eminence, 348*f*–349*f*
Mediastinum testis, 559
Medulla
of hair shaft, 387–388, 388*f*
of lymph nodes, 334, 334*f*
ovarian, 540
renal, 499, 500*f*–501*f*, 502, 514*f*
of suprarenal glands, 356*t*, 361, 366–368, 366*f*
thymic, 330
Medullary collecting tubules, 513–515, 515*f*
Medullary rays, 500, 500*f*–502*f*
Medullary sinuses, of lymph nodes, 332
Medullipin, 515–516
Megacolon, congenital, 212*b*
Megakaryocyte, 281–284
Meibomian glands, 600
Meiosis, 76–78
chromosome abnormalities during, 78*b*
equatorial division (meiosis II), 76*f*–77*f*, 77–78
of oocytes, 529–530, 536
reductional division (meiosis I), 76–77, 76*f*–77*f*
of spermatocytes, 562*f*, 563, 564*f*, 565
Meiosis-inducing substance, 529–530
Meissner corpuscle, 374*f*, 381, 584, 584*f*–585*f*
Meissner plexus, of digestive tract, 435–436, 456
Meissner submucosal plexus, 238
Melanin, 46
epidermal, 378, 379*b*
in neurons, 213
α–Melanocyte-stimulating hormone, 144–145
Melanocytes
in epidermis, 378–380, 380*f*, 388
in iris, 590–591
Melanoma, malignant, 383*b*
Melanopsin, 599–600
Melanosomes, 378–379, 380*f*, 388
Melatonin, 356*t*, 368, 369*b*
Melena, 448*b*
Membrana granulosa, 531*f*, 535, 536*t*
Membrane, cell. *see* Cell membrane
Membrane attack complex (MAC), 311, 316*b*
Membrane-bound antibodies, 315
Membrane-coating granules, 375, 382
Membrane depolarization, 224
Membrane trafficking, 36
Membrane transport proteins, 19–23, 20*f*
Membranous labyrinth, of ear, 603–610, 604*f*
Membranous urethra, 526
Memory, immunological, 315
Memory cells
of B lymphocytes, 265
functions of, 315
of T lymphocytes, 265
Memory T cells, 321
Menarche, 529
Ménière disease, 611*b*
Meningeal dura mater, 241
Meninges, 240–242, 241*f*
Meningiomas, 242*b*
Meningitis, 242*b*
Menopause, 529
Menses (hemorrhagic discharge), 545
Menstrual cycle, 544–547, 545*f*
Menstrual phase, of menstrual cycle, 545–546, 545*f*
Menstruation, corpus luteum of, 540
Mercaptans, 463
Merkel cell-neurite complexes, 378
Merkel cells, 374*f*, 378, 379*f*, 584, 584*f*
Merkel disk, 584, 584*f*
Merocrine glands, 120, 120*f*
Merocrine mode of exocytosis, 554
Mesangial cells, 503, 504*f*–505*f*
extraglomerular, 503, 512–513, 513*f*–514*f*
intraglomerular, 503–504, 504*f*–505*f*, 508
Mesaxons, 225*f*
Mesenchymal cells
in cartilage, 150–151, 150*b*
in embryonic connective tissue, 141
Mesenchyme, 127
Mesenteric ganglia, 236*f*
Mesentery, 436*f*
Mesoblastic phase, of prenatal hemopoiesis, 272
Mesoderm, 127, 373
Mesonephros, 532
Mesothelial epithelium, 530, 530*f*, 532
Mesothelium, 304
Mesovarium, 529, 530*f*
Messenger ribonucleic acid (mRNA), 24, 65–66, 66*f*
Messenger ribonucleoprotein, 65–66
Metachromasia, 2–3
Metachromatic stain, 2–3
Metaphase
meiotic, 76*f*–77*f*, 77
in oocytes, 536
mitotic, 73*f*, 74
Metaphase plate, 74
Metaphysis, 161–162
Metaplasia, 119*b*, 404*b*
Metaplastic theory, of endometriosis, 544*b*
Metarterioles, 293
Metastasis, of breast cancer, 556*b*
Methionine, 27

Methotrexate, 76*b*
Methyl groups, 64–69
Methylation, of chromosomes, 64*b*
Methyldopa, 213
MHC molecules. *see* Major histocompatibility complex (MHC) molecules
Micelles, 461, 462*f*
Micro RNAs (miRNAs), 67–68, 69*b*
Microbiota, of colon, 466–467, 466*b*
Microbodies, 40
Microfibrils, of elastic fiber, 92, 92*f*
Microfilaments
  of cytoskeleton, 16*f*, 46
  of neuron, 215
Microfold (M) cells, 340, 455, 459, 460*f*–461*f*
Microglia, 587*b*
Microglial cells (microglia), 138, 221, 222*b*
Microphages. *see* Neutrophil(s)
Microprocessor complex, 67–68
Microscope
  compound, 3
  light, 3–5, 3*f*
  scanning electron, 3*f*, 9
  transmission electron, 3*f*, 9
Microscopic sections, interpretation of, 4–5, 4*f*
Microscopy
  confocal, 7, 8*f*
  electron, 7–9
  light, 1–11
Microsomal mixed-function oxidases, 493
Microspikes, 48
Microtubule(s)
  in axons, 217
  in cilia, 106, 109*f*–110*f*
    singlets and doublets of, 105, 397
    subunits of, 105
  in cytoplasm, 50–53, 50*b*, 52*f*
  of neuron, 215
  spindle, 73*f*
Microtubule-associated proteins (MAPs), 50–51, 52*f*
Microtubule-associated protein 2 (MAP-2), 215
Microtubule C, 106–108
Microtubule organizing center (MTOC), 34, 51, 72
Microvilli, 16*f*, 39*f*
  of epithelium, 104–105, 105*f*–108*f*
  of hepatocyte, 485, 488
  of parietal cells, 443, 445*f*
  of renal corpuscle, 503*f*
  of small intestine, 453–454
Micturition, 525–526
Midbody, 74, 75*f*
Middle ear, 601–603, 602*f*
Middle piece, of spermatozoon, 566, 567*f*–568*f*, 568
Middle ring, of nuclear pore complex, 56
Milk, 555
  production of, 553
Milk ejection reflex, 555
Milk teeth, 421, 426
Mineralized components, of teeth, 421–423
Mineralocorticoids, 356*t*, 364–365
Minus end, 189
  of microtubules, 34, 50
  of thin filaments, 47
MIR168a, 69*b*
Mitochondria, 16*f*, 39*f*, 42–45, 43*f*
  in cardiac muscle, 200*f*
  of hepatocytes, 489, 489*f*
  inner membrane of, 44
  intermembrane space of, 43–44
  matrix of, 44
  of neuron, 213
  origin and replication of, 45
  outer membrane of, 43–44
Mitosis, 72–74, 72*f*–73*f*, 207*t*
Mitotic figures, in skin, 375
Mitotic spindle apparatus, 74
Mitral valve, 303, 303*b*
Mixed glands, 120
Mixed peripheral nerves, 233
Mixing contractions, of small intestine, 460
Modiolus, 603
Molars, 421
Molecular layer
  of cerebellar cortex, 244
Moll
  glands of, 384, 600
  space of, 484
Monocyte(s), 138, 263
  features of, 258*t*
  in phagocytosis, 36
  structure of, 252*f*
Monocyte colony-stimulating factor (M-CSF), 274–277
Monocytopoiesis, 281
Monoglycerides, absorption and processing of, 462*f*
Monoiodinated tyrosine (MIT), 358
Monomer, 49–50
Mononuclear phagocyte system, 138, 221
Monosomy, 63*b*, 78*b*
Morgagni, rectal columns of, 467
Morphodifferentiation, in odontogenesis, 426
Morula, 547*f*, 548, 555–556
Motilin, 447*t*
Motor component
  of autonomic nervous system, 234
  of peripheral nervous system, 211
  of somatic nervous system, 234
Motor end plate, 192
Motor endings, 583
Motor neurons, 15*f*, 214*f*, 219
  α-efferent, 195, 197*f*, 198*b*
  γ-efferent, 197*f*
Motor unit, 192
Mounting, 2–3
Mucin, 119, 401–402, 402*f*, 454, 471–472
Mucinogen(s), 119, 121, 454, 471–472
Mucoid cells, of eccrine sweat gland, 383, 385*f*
Mucopolysaccharidosis, 41*t*
Mucosa
  of alimentary canal, 435, 436*f*
  anal, 467
  of esophagus, 437–438, 438*f*
  of gallbladder, 494, 494*f*
  masticatory, 419, 429
  of nasal cavity, 399, 399*b*
  olfactory, 397*f*
  oral, 419–433, 420*t*
  of oviducts, 541, 541*f*
  of small intestine, 453–456
  specialized, in taste perception, 419
  of stomach, 440, 441*f*, 446–451
  of trachea, 401–403, 402*f*
  of ureter, 524
  of vagina, 552
Mucosa-associated lymphoid tissue (MALT), 340–343
Mucosal glands, of prostate, 576
Mucous acini, of salivary glands, 120, 120*f*, 472, 472*f*
Mucous cells, 471–472, 472*f*–473*f*
  esophageal, 438
Mucous glands
  function of, 119
  of urinary bladder, 525
Mucous (internal) aspect, of lip, 420
Mucous neck cells, 441–442, 444*f*
Mucous tissue, 141
Mucus, 471–472
  of colon, 464
  mucin in, 119
  of small intestine, 454
  soluble, 451
  of stomach, 440, 451
  of trachea, 401–402, 402*f*
  visible, 440, 442*f*, 451
Müller cells, of retina, 594*t*, 599
Multidrug resistance proteins (MDR proteins), 23
Multilocular adipose tissue, 133*f*, 146–147
Multilocular fat cells, 131
Multipass proteins, 16–17, 22
Multiple myeloma, bone marrow aspirate of, 285*f*
Multiple sclerosis, 225*b*
Multipolar neurons, 219
Multipotent progenitors, 273

Multipotential hemopoietic stem cells (MHSCs), 273
Multivesicular bodies, 37*f*, 38–39
Mumps, 476*b*
Muscle(s), 179–208
  cardiac, 198–202
    cells of, 198–202, 199*f*
    characteristics of, 181*f*, 207*t*
    contraction of, 198
    intercalated disks of, 198–200, 200*f*–201*f*
    organelles of, 200–202, 202*f*
    regeneration of, 207
  ciliary, 590
  extrinsic
    of eyes, 587, 588*f*
    of larynx, 400
    of tongue, 429
  intrinsic
    of larynx, 400
    of tongue, 429, 430*f*
  involuntary, 203
  longitudinal, of myometrium, 543
  muscle spindles of, 584*f*, 586
  pathological considerations for, 208, 208*f*–209*f*
  regeneration of, 206–208, 207*t*
  skeletal, 179–198
    characteristics of, 180*t*, 181*f*, 207*t*
    connective tissue investments of, 179–191
    contraction and relaxation of, 189–191, 190*f*
    development of, 179
    fine structure of, 180–189
    Golgi tendon organs of, 193–198, 198*b*
    innervation of, 192–193
    light microscopy of, 180, 182–185, 182*f*–184*f*
    muscle spindles of, 193–198, 197*f*
    myofibrils in, 183*f*–184*f*, 185–189, 186*f*–187*f*, 188*t*
    myotendinous junctions of, 191–192
    neuromuscular junctions of, impulse transmission at, 192–193, 193*f*–196*f*
    proteins associated with, 188*t*
    sarcomeres in, 180, 183*f*–184*f*
    sarcoplasmic reticulum in, 182–185, 184*f*–185*f*
    T tubules in, 182–185, 184*f*–185*f*
    triads in, 182, 184*f*–185*f*
    types of muscle fibers in, 180*t*
  smooth, 203–206
    arrector pili muscles as, 374, 374*f*, 381, 388
    of bronchioles, 407*b*
    characteristics of, 181*f*, 207*t*
    contraction of, 205–206, 206*f*
    fine structure of, 204–206
Muscle(s) *(Continued)*
    of gallbladder, 494
    innervation of, 206
    intermediate, 206
    light microscopy of, 203, 203*f*–205*f*
    multiunit, 206
    of prostate gland, 576
    regeneration of, 207–208
    in reticular layer, of dermis, 381
    of seminal vesicle, 575–576
    unitary (single-unit, vascular, visceral), 206
  sphincteral
    of anus, 467
    of gastrointestinal system, 437
    of pupil, 588*f*
    of urethra, 526
    of urinary bladder, 525
  of spleen, 335
  striated, 179, 180*f*
    of facial expression, 381
Muscle contraction
  cardiac, 198, 207*t*
  skeletal, 189–191, 190*f*, 207*t*
    energy sources for, 191
  smooth, 205–206, 206*f*, 207*t*
  uterine, 544
Muscle cramps, 361*b*
Muscle fibers, 179
  red, 180*t*
  skeletal, 180*t*
  white, 180*t*
Muscle phosphorylase deficiency, 46*t*
Muscle spindles, 193–198, 197*f*, 584*f*, 586
Muscle tetany, 361*b*
Muscular arteries, 290–291
Muscularis
  of oviduct, 541
  of ureter, 524
  of vagina, 552
Muscularis externa
  of alimentary canal, 435–436, 436*f*, 449*t*–450*t*
  of anus, 449*t*–450*t*, 467
  of colon, 449*t*–450*t*, 463
  of esophagus, 438, 449*t*–450*t*
  of large intestine, 449*t*–450*t*
  of small intestine, 449*t*–450*t*, 458
  of stomach, 448–451, 449*t*–450*t*
Muscularis mucosae
  of alimentary canal, 435, 449*t*–450*t*
  of anus, 449*t*–450*t*, 467
  of esophagus, 437–438, 449*t*–450*t*
  of large intestine, 449*t*–450*t*
  of small intestine, 449*t*–450*t*, 456, 458*f*
  of stomach, 441*f*, 446–451, 449*t*–450*t*
Myasthenia gravis, 194*b*, 415*b*
Myelin
  oligodendrocytes and, 221
  tubular, 411–412
Myelin sheath, 217
Myelinated axons, 217
  in neuromuscular junction, 193*f*
Myelination, 223
Myeloblast(s), 275*f*, 280, 281*f*, 282*t*, 283*f*
Myeloblastic leukemia, acute, 281*b*
Myelocytes, 280–281
Myelodysplasia, 286*f*
Myelogenous leukemia, acute, 76*b*
Myeloid cell lines, 273
Myeloid phase, of prenatal hemopoiesis, 272
Myeloid progenitors, common, 273
Myenteric plexus, 460–461. *see also* Auerbach plexus
MYO18A, 30
Myoblasts, 179, 206–208
Myocardial infarct, 269*b*, 309*f*
Myocardium, 303–304
Myoepicardial mantle, 198
Myoepithelial cells, 208
  characteristics of, 122
  of eccrine sweat gland, 383–384
  of salivary glands, 472, 472*f*
Myofibrils, 179–180, 207*t*
  structural organization of, 183*f*–184*f*, 185–189, 186*f*–187*f*, 188*t*
Myofibroblasts, 130, 208
Myofilaments, 179
  of smooth muscle, 203
  thin, 198–200
Myoglobin pigments, 179
Myoid cells, 561
Myomesin, 188*t*
Myometrium, 530*f*, 543–544
Myosin
  in contractile bundles, 47–48
  in cytokinesis, 74
  in cytoplasm, 47, 49*t*
  in eccrine sweat glands, 383
Myosin-I, 47–48
Myosin II
  in cytoplasm, 49*t*
  molecular structure of, 186*f*, 189
  in smooth muscle, 204
  in thick filaments, 47–48
    of skeletal muscle, 185, 188*t*
  in vesicle formation, 35
Myosin V, in cytoplasm, 47–48, 49*t*
Myosin light chain kinase, 205
Myosin-IX, 47–48
Myostatin, 207
Myotubes, 179
Myxedema, 359*b*

N

Na^+-K^+ ATPase, 22, 488
Na^+-K^+ ion pumps, 226, 226*f*
Na^+-K^+ pump, 22
NADH dehydrogenase complex, 44

NADPH oxidase, 260–261
  hereditary deficiency, 261*b*
Nail(s), 390–391, 390*f*–391*f*
Nail bed, 390, 390*f*–391*f*
Nail folds, 390
Nail matrix, 390
Nail plate, 390
Nail root, 390, 390*f*–391*f*
Naïve cells, 315, 321
Naris, 395
Nasal cavity, 395–400, 396*t*
  anterior portion (vestibule) of, 395
  histophysiology of, 399–400
  mucosa of, 399, 399*b*
  olfactory region of, 396*t*, 397–399, 397*f*
  posterior aspect of, 395–399
Nasal conchae, 395
Nasal vestibule, 395, 396*t*
Nasolacrimal duct, 601
Nasopharynx, 396*t*, 400
Natriuretic peptides, 144, 503–504
Natural killer (NK) cells, 265, 311, 323
  in innate immune system, 312–313
Natural T reg cells, 323
Navicular fossa, 526
Naxos syndrome, 114*b*
Nebulin, 186*f*, 188, 188*t*
Neck, of spermatozoon, 568, 568*f*
Necrosis, 78–79, 78*t*, 79*f*
  of functionalis layer of endometrium, 545
Negative feedback mechanism, for glucocorticoids, 366
Nephrin, 506*f*
Nephrons, 500*f*–501*f*, 502–513
  cortical, 500*f*–501*f*, 502, 516
  distal tubule of, 502, 510–512, 513*f*
    convoluted, 500*f*–501*f*, 512, 512*f*, 523*t*
  juxtamedullary, 500*f*–501*f*, 502, 519
  proximal tubule of, 502, 502*f*–503*f*, 508–511, 523*t*
    convoluted, 503*f*, 508, 509*f*
    regions of, 508, 510*f*
    resorption in, 510, 518–519
  reduction of number of, 502*b*
  renal corpuscles of, 500, 502–508, 502*f*–503*f*, 523*t*
    filtration in, 518
  thin limbs of Henle loop of, 509*f*, 510–511, 511*t*, 514*f*, 523*t*
Nephrosis, lipoid, 508*b*
Nerve(s)
  functional classification of, 233–234
  regeneration, 245–247, 246*f*
Nerve bundle, 233, 233*f*
Nerve deafness, 611*b*
Nerve fibers, of muscles, 207*t*
Nerve impulses, generation and conduction of, 224–232
Nerve supply
  to blood vessels, 288–289
  of kidney, 517–518
  of lungs, 416
Nervous system
  autonomic, 211, 234–238
  cells of, 211–224
    neurons as, 211–219
  central. *see* Central nervous system (CNS)
  enteric, 436–437
  ganglia of, 211, 238–239
  generation and conduction of nerve impulses in, 224–232
  nerve regeneration in, 245–247, 246*f*
  neurotransmitters in, 227–228, 231–232, 232*t*
  parasympathetic, 235–237, 579
  pathological considerations of, 247
  peripheral, 211, 232–234
  plasticity of, 247
  somatic, 211, 234–238
  sympathetic, 235, 579
  synapses in, 227–232, 227*b*, 228*f*, 598–599
Nervous tissue, 211–247
  autonomic system, 211
  cells of, neuroglial, 211
  development of, 211
Net filtration pressure, 505–508
Network-forming collagens, 88
Neural crest, in odontogenesis, 424
Neural crest cells, 211
Neural groove, 211
Neural plate, 211
Neural tube, 211
Neural tunic. *see* Retina
"Neuro-reticular complex," 292*f*
Neuroblastoma, 218*b*
Neurocrine substance, of DNES cells, 445–446
Neuroepithelial bodies, pulmonary, 403
Neuroepithelial cells
  in saccule and utricle, 605, 605*f*–606*f*
  in semicircular duct, 607, 607*f*
Neuroepithelium, 211
Neurofibrils, 215
Neurofilaments, 51*t*, 215
Neuroglial cells, 219–224, 219*f*
  astrocytes as, 219–221, 220*f*–221*f*
  defined, 211
  ependymal, 223
  microglial, 221–222
  oligodendrocytes as, 221
  Schwann cells as. *see* Schwann cells
Neurohormones, 231
Neurohypophysis, 348*f*, 353–354
Neuromodulators, 231–232
Neuromuscular junctions, impulse transmission at, 189, 192
Neuron(s), 211–219
  axons of, 212, 216–218
    defined, 216–218
    hypothalamic, 347
    myelinated, 217, 217*f*–218*f*
    olfactory cells, 397
    unmyelinated, 218*f*
  bipolar, 215*f*, 218
    in retina, 593*f*, 594*t*, 598–599
  cell body of, 212–215
  classification of, 218–219
  dendrites of, 214*f*, 215–216
  inclusions in, 213–215
  interneurons, 219
  motoneurons (efferent), 219
  multipolar, 219
  postganglionic, 235, 369
  preganglionic, 235
  receptors for, 211
  sensory (afferent), 219
  structure and function of, 212–218, 212*f*, 214*f*
  types of, 215*f*
  unipolar (pseudounipolar), 219
Neuropathic pain, chronic, 587*b*
Neuropeptides, 231
Neurophysin, 353–354
Neuropil, 239, 240*f*
Neuroplasticity, 247
Neurosecretory cells, hypothalamic, 348*f*–349*f*
Neurotendinous spindles (Golgi tendon organs), 193–198, 198*b*
Neurotensin, 447*t*
Neurotoxin(s), 194*b*
  eosinophil-derived, 261
Neurotransmitter(s), 231–232
  excitatory and inhibitory, 21
  receptors, 230
Neurotransmitter-gated channels, 21
Neurotrophins, 247
Neutral proteases
  in inflammatory response, 136
  in mast cells, 132–133, 136*t*
Neutrophil(s), 257–261, 259*f*, 275*f*, 282*t*
  features of, 258*t*
  functions of, 139, 259–261
  granules, 259
  in innate immune system, 312
  structure of, 252*f*
Neutrophil chemotactic factor (NCF)
  in inflammatory response, 136
  mast cell release of, 132–133, 136*t*
Neutrophilic band, 282*t*
Neutrophilic metamyelocyte, 275*f*, 282*t*, 283*f*
Neutrophilic myelocyte, 275*f*, 282*t*, 283*f*
Neutrophilic series, cells of, 282*t*
Neutrophilic stab cell, 275*f*, 283*f*
Newborn, respiratory distress of, 412*b*
Newborn oogenesis homeobox, 533

Newly synthesized mediators, 132–133
Nexin, 105
Nexus. *see* Gap junctions
Nicotinamide adenine dinucleotide (NADH), 44
Nicotinic acetylcholine receptors (nAChRs), 611*b*
Nidi of crystallization, 170
Nidogen, 84
Niemann-Pick disease, 41*t*
Night blindness, 597*b*
Nipple, 553–555
Nissl bodies, 15*f*, 213
Nitric oxide (NO)
  in gas exchange, 414–415
  in hemoglobin, 254
  as neurotransmitter, 231
  in penile erection, 579, 581*b*
  in signaling, 23
  in vasodilation, 293
Nitric oxide synthase, 608
Nociception, 586
Nociceptors, 586–587
Nodes of Ranvier, 223, 224*f*
Non-coding-segments, of DNA, 63–64
Non-growing follicles, 532
Non-Hodgkin disease, 265*b*
Non-snRNP splicing factors, 65–66
Noncoding RNA, 64
Nondisjunction, 78*b*
Nonpolar fatty acyl tails, in cell membrane, 15–16
Nonsteroidal anti-inflammatory drugs (NSAIDs), and ulcers, 453*b*
Nonsyndromic deafness, 117*b*
Nontropical sprue, 463*b*
Norepinephrine
  in autonomic nervous system, 237
  in blood pressure regulation, 356*t*
  in fat release, 144
  as neurotransmitter, 231, 232*t*
  production of, 361, 367–368
  release of
    by blood vessels, 288
    by suprarenal medulla, 368
  in smooth muscle synapses, 206
Norepinephrine receptors, on adipose cells, 143–144
Nuclear bag fibers, 195, 197*f*
Nuclear basket, 56, 60*f*
Nuclear chain fibers, 195, 197*f*
Nuclear envelope, 16*f*, 55–58, 57*f*–58*f*
Nuclear export signals, 57–58
Nuclear lamins, 51*t*, 55
Nuclear layer
  inner, of retina, 593*f*, 598–599
  outer, of retina, 593*f*, 598
Nuclear localization signals (NLSs), 57–58
Nuclear matrix, 69
Nuclear membrane, 55, 57*f*–58*f*
Nuclear pore complex, 56, 60*f*–61*f*
Nuclear pores, 55–58, 59*f*–60*f*
Nuclear region, of rods, 597
Nuclear ring, 56, 60*f*
Nuclear thyroid hormone receptor proteins, 359
Nucleocytoplasmic shuttling signals, 57–58
Nucleolar matrix, 70
Nucleolar-organizing regions (NORs), 70
Nucleolus, 16*f*, 55, 56*f*, 69–70, 70*b*
Nucleolus-associated chromatin, 69
Nucleoplasm, 55, 69
Nucleoplasmic ring, 56
Nucleoporins, 56
Nucleosomes, 58
Nucleotide-gated channels, 21
Nucleotides, 63
Nucleus
  of cell, 55–79, 56*f*
    chromatin in, 58–70, 61*f*
  of muscles, 207*t*
  of neurons, 213
Nucleus pulposus, 153–154
Nuel, space of, 610
Null cells, 265
Nurse cells, 277–280

O

Objective lens, 3, 3*f*
Oblique section, 4*f*
Obstructive jaundice, 492*b*
Occluding junctions, 109
Ocular lens, 3, 3*f*
Oddi, sphincter of, 494, 496*t*
Odontoblastic processes, 423, 426
Odontoblastic zone, 423
Odontoblasts, 423, 426, 426*f*–427*f*
Odontoclasts, 423
Odontogenesis, 424–427, 425*f*
Odor receptor molecule, 399–400
Olfactory bulb, 399–400
Olfactory cells, 397, 398*f*
Olfactory cilia, 397, 397*f*
Olfactory epithelium, 397, 398*f*
Olfactory mucosa, 397*f*
Olfactory receptor cell, 398*f*
Olfactory region, 396*t*, 397–399, 397*f*
Olfactory vesicle, 397, 398*f*
Oligodendrocytes, 221, 222*f*
Oligodendrogliomas, benign, 218*b*
Oncogenes, 71, 76
Oncotic pressure, 505–508
Oocyte maturation inhibitor, 529–530, 532
Oocytes, 529–530
  primary, 529–530, 532, 533*f*, 533*t*
  secondary, 536
Oogonium, 529
Open circulation theory, of spleen, 336, 337*f*
Opioid peptides, 231
Opsins, 595, 598*t*
Opsonins, 318*t*
Optic disk, 592–593
Optic nerve, 588*f*
Optic nerve fiber layer, of retina, 593*f*, 600
Ora serrata, 588*f*
Oral cavity, 419–433
  gingiva (gums) in, 421*f*, 428–429
  lips in, 419–420
  oral mucosa in, 419–433, 420*t*
  palate in, 429
  pathological considerations of, 433*f*
  teeth in, 421–433, 421*f*
  tongue in, 429–433, 430*f*
Oral epithelium, in odontogenesis, 424, 425*f*
Oral mucosa, 419–433, 420*t*
Organ of Corti, 607–611, 608*f*–609*f*, 609*t*
  neuroepithelial cells of, 610
  supporting cells of, 609–610, 609*t*
Organ system, 13
Organelle(s), 13–45, 16*f*–17*f*
  annulate lamella as, 45
  cell membrane as, 13–15
  defined, 13
  endoplasmic reticulum as. *see* Endoplasmic reticulum (ER)
  endosomes as, 36–39, 37*f*, 39*f*
  Golgi apparatus as, 16*f*, 30–32, 31*f*–33*f*
  mitochondria as. *see* Mitochondria
Organs, 13
Orthochromatophilic erythroblast, 275*f*, 277*f*, 279*f*, 280*t*
Orthodox mitochondria, 45
Orthogonal arrays of particles (OAPs), 116*f*
Orthograde degeneration, 246
Orthokeratinization, of gingiva, 428
Osmium tetroxide, as fixative, 9
Osseous spiral lamina, 603
Ossification
  primary center of, 166–167, 168*f*–169*f*
  secondary centers of, 167–168
  zone of, 169–170
Osteoblasts, 154–156, 156*f*–157*f*
  alkaline phosphatase in, 158*b*
Osteocalcin, 155–156
Osteoclast(s), 138, 156*f*, 158–161
  morphology of, 159, 160*f*
Osteoclast-stimulating factor, 156, 361
Osteocytes, 154, 154*f*, 156–158, 158*b*
Osteogenesis. *see* Bone(s), formation of
Osteoid, 156
Osteomalacia, 174*b*
Osteonectin, 85, 156
Osteons, 162*f*
Osteopetrosis, 160*b*

Osteopontin, 155–156, 159
Osteoporosis, 161*b*, 170*b*
Osteoprogenitor cells, 154–155, 154*f*, 164
Osteoprotegerin (OPG), 156, 159
Osterix-null, 165*b*
Otic ganglion, 236*f*
Otitis media, 611*b*
Otoconia, 605*f*–606*f*, 606
Otolithic membrane, 605*f*–606*f*, 606
Otoliths, 606
Otosclerosis, 611*b*
Ouabain, inhibition of Na^+-K^+ pump by, 22
Outer dense fibers, of spermatozoon, 566, 568
Outer dense plaque, 114
Outer leaflet, of cell membrane, 13–15, 18*f*
Outer membrane, mitochondrial, 43–44, 43*f*
Outer nuclear layer, of retina, 593*f*, 598
Outer nuclear membrane, 55, 57*f*–58*f*, 60*f*
Outer plexiform layer, of retina, 593*f*, 598
Outer segments, of rods and cones, 595
Outer table, of calvaria, 163
Oval window, of ears, 603, 612*f*
Ovarian follicles, 529–530, 531*f*, 532–536, 533*t*
  atretic, 540
  dominant, 537, 546
  graafian (mature), 531*f*, 533*t*, 535–536
  primary, 531*f*, 532–535, 533*f*
    multilaminar, 531*f*, 533*t*, 534
    unilaminar, 533, 533*t*
  primordial, 529–532, 531*f*, 533*f*–534*f*, 533*t*
  secondary (antral), 531*f*, 533*t*, 536*t*
Ovarian ligament, 530*f*
Ovaries, 529–555, 530*f*
  cortex of, 531–540, 531*f*, 533*f*, 558*f*
    at onset of puberty, 531–532
  description of, 530
  embryonic development of, 529–530
  medulla of, 530, 540
Ovastacin, 548
Overlap regions, in tropocollagen, 87*f*
Oviducts, 530*f*, 537, 540–541, 541*f*–542*f*
Ovoid cells, in hepatic ducts, 486
Ovulation, 536–537, 537*f*
Ovum, 548
Owen, lines of, 423
Oxidative phosphorylation, 42, 44–45
Oxygen exchange, 414
Oxyhemoglobin, 254, 414
Oxyntic (parietal) cell, 441*f*–442*f*, 442–443, 445*f*
Oxyphil cell, of parathyroid, 360–361
Oxytocin, 208
  functions of, 349*t*–350*t*, 354
  and milk ejection reflex, 538*t*, 555
  neurosecretion of, 353
  as neurotransmitter, 231
  and uterine contractions, 538*t*, 544

P

P-face
  of cell membrane, 17–19, 19*f*
  of microvillar membrane, 112–113, 113*f*
P-site, 26
Pacemaker (sinoatrial node), 303*f*, 304
Pachytene, 77, 565
Pacinian corpuscles, 381, 584*f*–585*f*, 586
Pain, neuropathic, chronic, 587*b*
Pain fibers
  of periodontal ligament, 428
  of pulp, 424
Palate, 429
Palatine tonsils, 342, 430*f*
Palatoglossal fold, 430*f*
Pale-staining fibrillar center, of nucleolus, 70
Palpebral conjunctiva, 600
Palpebral fissure, 600
Pampiniform plexus of veins, 559–560
Pancreas, 471, 476–481, 477*f*–478*f*
  blood supply to, 481
  disorders of, 478*b*, 482*b*, 482*t*
  endocrine, 479*f*, 480*t*, 482*b*
    histophysiology of, 479–481
  exocrine, 476–478, 477*f*–479*f*, 478*b*, 480*t*
    histophysiology of, 477–478
    secretory and duct portions of, 476–477
Pancreatic amylase, 477
Pancreatic cancer, 478*b*
Pancreatic cholera, 482*b*
Pancreatic cholesterol esterase, 477
Pancreatic ducts, 476–478, 477*f*
Pancreatic lipase, 144, 477
Pancreatic polypeptide, 447*t*, 481
Pancreatic polypeptide (PP) cells, 478–479, 480*t*, 481
Pancreatitis, acute, 478*b*, 496*f*
Paneth cells, 456–458, 459*f*
Panniculus adiposus, 373
Papanicolaou (Pap) smear technique, 544*b*
Papilla(e)
  dental, 424–426
  dermal, 373, 376*f*, 381–382, 386
  duodenal (of Vater), 458, 476–477
  lingual, 429–433, 430*f*–431*f*
  renal, 499
Papillary collecting tubules, 499, 515
Papillary layer, of dermis, 375*t*, 376*f*, 381
Papillomaviruses, 383*b*
Paracellular route, 510
Paracortex, of lymph nodes, 333
Paracrine signaling, 23, 119
Paracrine substance, of DNES cells, 445–446
Parafollicular cells, of thyroid, 160–161, 355, 355*f*, 357*f*, 359–360
Paraformaldehyde, as fixative, 9
Parakeratinization, of gingiva, 428
Parallel bundles, 48
Paranasal sinuses, 400
Parasympathetic fibers, of lungs, 416
Parasympathetic innervation
  of gut, 437
  of salivary gland, 474
Parasympathetic nerves, postganglionic, 238
Parasympathetic nervous system, 237–238
  in penile erection, 579
Parasympathetic supply, of gut, 437
Parathyroid glands, 360–361
  cellular organization of, 357*f*, 360–361
  physiological effects of, 356*t*, 361
Parathyroid hormone (PTH)
  in blood calcium, 360
  and bones, 160–161, 361
  physiological effects of, 356*t*, 361
  synthesis of, 360
Parathyroid hormone (PTH) receptors, on osteoblasts, 156
Paratrabecular sinuses, of lymph nodes, 332
Paraventricular nuclei, 348*f*, 353
Parenchyma, of gland, 119
Parietal cell, 441*f*–442*f*, 442–443, 445*f*, 451
Parietal layer, of Bowman capsule, 502, 503*f*
Parietal pleura, 415
Parkinson disease, 231*b*
Parotid gland, 474, 476*b*
Pars ciliaris, of retina, 589
Pars convoluta
  of distal tubule, 511, 523*t*
  of proximal tubule, 508, 509*f*
Pars distalis, of pituitary gland, 347–353, 348*f*, 349*t*–350*t*, 350*f*
Pars fibrosa, of nucleolus, 70
Pars granulosa, of nucleolus, 70
Pars intermedia, of pituitary gland, 353
Pars nervosa, of pituitary gland, 348*f*, 349*t*–350*t*, 354, 354*f*
Pars recta
  of distal tubule, 511, 523*t*
  of proximal tubule, 508, 523*t*
Pars tuberalis, of pituitary gland, 353
β Particles, of glycogen, 490
Passive transport, 20, 20*f*, 22
Pathogen-associated molecular patterns (PAMPs), 313

Pax5 transcription factor, 276*t*
Pectinate line, 467
Pedicels
  of astrocytes, 220–221
  of podocytes, 504, 506*f*
Peg cells, of oviduct, 541, 542*f*
Pelvis, renal, 499, 500*f*–501*f*, 523
Pemphigus vulgaris, 114*b*
Penicillar arteries, 335
Penile urethra, 526
Penis, 559, 560*f*, 578–581, 578*f*, 580*f*
  structure of erectile tissue in, 579
Pentameric form, 318*t*
Pepsin, 439, 451
Pepsinogen, 438, 443, 451
Peptidyl transferase, 28
Periarterial lymphatic sheath (PALS), 335
Peribiliary capillary plexus, 484
Pericarditis, 305*b*
Pericellular capsule, of hyaline cartilage matrix, 152
Pericentrin, 51
Pericentriolar material, 51
Perichondrium, in cartilage, 149
Perichromatin granules, 69
Pericranium, 163
Pericytes
  in capillaries, 288, 296–297, 297*f*
  in connective tissue, 130–131
  in postcapillary venules, 301, 302*f*
Perikaryon, 212–215, 212*f*
Perilymphatic space, 603
Perimysium, 179
Perineurium, 233, 233*f*
Perinuclear cisterna, 55
Periodic acid-Schiff (PAS) reagent, 2*t*, 5, 12*f*
Periodontal ligament (PDL), 427–428, 428*f*
  pulp and, 423, 424*f*
  structure and function of, 421*f*
Periosteal dura mater, 240
Periosteocytic space, 158
Periosteum, 154, 162–163
Peripheral blood stream, 285*f*
Peripheral nerve fibers, classification of, 234*t*
Peripheral nerves, 232–234, 233*f*
Peripheral nervous system, 211, 232–234
  peripheral nerves as, 232–234, 233*f*
Peripheral proteins, in cell membrane, 16, 18*f*
Peripheral receptors, specialized, 583–587
Perisinusoidal space of Disse, 485–486, 487*f*–488*f*
Perisinusoidal stellate cells, 486
Peristalsis, 437, 451
Peristaltic activity, 435
Peristaltic contractions, 575
Peristaltic rush, 461*b*
Peristaltic waves, 460–461
Peritrichial (naked) nerve endings, 583, 584*f*
Peritubular capillary network, 516
Perivascular glia limitans, 242
Perivitelline space, 548
Perlacan, 95
Pernicious anemia, 442*b*
Peroxisome proliferator-activated receptor γ coactivator 1α (PCG-1α), 191*b*
Peroxisomes, 40–41, 42*f*
Peyer patches, 340, 458
Phagocyte system, mononuclear, 221
Phagocytes, 36
Phagocytosis, 36
  by Kupffer cells, 493
  neutrophils in, 260*f*
Phagosomes, 36, 39, 260
Phakinin, 51*t*
Phalangeal cells, 609, 609*t*
  inner, 610
  outer, 610
Phalangeal process, 610
Pharyngeal tonsil, 342–343, 342*f*, 400
Pharyngoesophageal sphincter, 439
Phosphate group, in cell membrane, 16
Phosphatidylglycerol, 411
Phosphatidylinositol, metabolites of, 345–346
Phosphatidylinositol-4-phosphate (PtdIns4P), 30
Phosphocreatine, in muscle contraction, 191
Phosphocreatine kinase, 191
Phosphodiesterase (PDE), 581*b*
Phospholipase A, in basophil function, 263
Phospholipase A_2, mast cell release of, 135
Phospholipase C, 25
Phospholipid molecule, in cell membrane, 15–16
Phospholipid phosphatidylinositol bisphosphate (PIP_2), 25
Phospholipids, in cell membrane, 15
Phosphorylase, 481
Photopsin, 597
Photosensitive retinal glial cells, 599–600
Photosensory organs, 587
Phrenic arteries, inferior, 363
Physical support, for retina, 599
Physiological support, for retina, 599
Pia-arachnoid, 242
Pia-glial membrane, 220–221
Pia mater, 241*f*, 242
Pigment cells, 593*f*, 594*t*
Pigment epithelium
  of ciliary body, 589–590
  of retina, 589, 592, 593*f*, 594–595
Pigment stones, 496*b*
Pigments, in cytoplasm, 46
Pillar cells, 609, 609*t*
Pineal gland, 356*t*, 368–369, 369*f*
Pinealocytes, 368, 369*f*
Pinna, 601, 602*f*
Pinocytosis, 36, 37*f*
Pinocytotic vesicles, 38*f*
  in capillaries, 300*f*
piRNA, 68–69
Pit cells, 486
Pitch, of sound, 401
Pituicytes, 354, 354*f*
Pituitary adenomas, 355*b*
Pituitary gland, 346–354
  adenohypophysis of, 346–353
  blood supply to, 347, 349*f*
  control of secretion of, 347
  neurohypophysis of, 346, 348*f*, 353–354
  pars distalis of, 347–353, 348*f*, 349*t*–350*t*, 350*f*
  pars intermedia of, 353
  pars nervosa of, 348*f*, 349*t*–350*t*, 354, 354*f*
  pars tuberalis of, 353
Pituitary hormones, physiological effects of, 347, 349*t*–350*t*
Piwi protein, 68
Placenta, 549–551, 550*f*–551*f*, 552*b*
  hemochorioendothelial type of, 543
Placenta previa, 552*b*
Placental barrier, 551
Placental septa, 551
Plakin proteins, 117
Plakins, 50
Plakoglobins, 114, 114*b*
Plakophilins, 114
Plaques, of urinary bladder, 524
Plasma, 251–252, 252*t*
Plasma cells, 416
  in immune response, 265
  in lymphoid nodule, 319, 333
  structure and function of, 139, 139*f*–140*f*
Plasma fibronectin, 84
Plasma lipoproteins, 252*t*
Plasma membrane. *see* Cell membrane
Plasma membrane infoldings, in epithelium, 117
Plasmalemma. *see* Cell membrane
Plasmin, 269
Plasminogen activators, 269
Plasticity, neuronal, 247
Platelet(s), 265–271
  activation, 269
  adhesion of, 267
  aggregation of, 267
  formation of, 281–284, 284*f*
  function of, 267–269

Platelet(s) *(Continued)*
structure of, 252*f*
tubules and granules of, 266
Platelet-activating factor (PAF)
in inflammatory response, 136
mast cell release of, 132–133, 136*t*
Platelet-derived growth factor, 294*b*
Platelet factor 3, 269
Plates, tarsal, 600
Pleats, of pharyngeal tonsil, 342
Plectin, 50, 117
of skeletal muscle, 182
Pleura, 415
Pleural cavities, 415
Plexiform layer
inner, of retina, 593*f*, 599
outer, of retina, 593*f*, 598
Plexus(es). *see specific plexus*, e.g., *Auerbach plexus*
Plicae circulares, 453
Ploidy, 62*f*, 63
Pluripotent stem cells, 555–556
induced, 556–557
Pluripotential hemopoietic stem cells (PHSCs), 273
Plus end
of microtubules, 34, 50
of thin filaments, 47, 189
Pneumocytes
type I, 408–410, 411*f*
type II, 410–412, 412*f*–413*f*
Podocalyxin, 504–505
Podocin, 506*f*
Podocytes, 502, 503*f*–506*f*, 504, 513*f*
Podoendin, 504–505
Podoplanin, 504–505
Polar body
first, 536
second, 548
Polar head, in cell membrane, 15–16, 18*f*
Polar microtubules, 74
Polar molecules, 23
Poliomyelitis, 415*b*
Polychromatophilic erythroblast, 275*f*, 277*f*, 280*t*
Polycomb repressive complex 2, 68
Polycystic kidney disease, 500*b*
Polycystic ovarian disease, 558*f*
Polycythemia, secondary, 277*b*
Polydipsia, 482*b*
Polymerization, in microtubules, 50
disruption of, 50*b*
Polymorphonuclear leukocytes. *see* Neutrophil(s)
Polypeptides
in hormones, 345
lamina-associated, 55
Polyphagia, 482*b*
Polyphosphoinositide, 47
Polyribosomes, 26, 28*f*
Polysome, 26–27, 28*f*
Polyspermy, 548
Polyubiquitinated protein, 41
Polyuria, 482*b*
Pompe's disease, 41*t*, 46*t*
Pore(s)
alveolar, 408, 410*f*–411*f*
in capillaries, 298
nuclear, 55–58, 59*f*–60*f*
sweat, 374*f*, 382
taste, 430–431, 431*f*–432*f*
Pore protein, 26, 29
Porins, 43–44
Porta hepatis, 483–484
Portal areas (triads), 483*f*, 484, 497*f*
Portal lobule, 485, 485*f*
Portal veins, 336, 483–484, 483*f*
hypophyseal, 347, 349*f*
Postcapillary venules, 301
of lymph nodes, 333
Posterior chamber, of eye, 588*f*
Postganglionic fibers, autonomic, 235
Postganglionic nerves, parasympathetic, 238–239
Postganglionic sympathetic neurons, 369
Postsynaptic membrane, 192, 227–228, 228*f*, 230
Postsynaptic potential, excitatory and inhibitory, 228
Posttranslational modification, 88
Potassium/chloride cotransporter 2 (KCC2), 587*b*
Potassium ions (K^+), in HCl production, 452
Potassium ion channels, 605*b*
Potassium (K^+) leak channels, 21, 226, 226*f*
Potassium permanganate, as fixative, 9
Power stroke, 190*f*
Preacrosomal granules, 566
Preadipocytes, 147
Precapillary sphincter, 293
Precursor cells, 273–277, 275*f*
Precursor messenger RNA (pre-mRNA), 65–66
Pre-Descemet layer, of cornea, 589
Preformed mediators, 132–133
Preganglionic fibers, 235
Pregnancy
corpus luteum of, 540
mammary glands during, 553–554
maternal changer during, 551–552, 552*t*
Pregnenolone, 539
Pre-miRNA, 67–68
Preprocollagen, 88
Preproinsulin, 479–480
Preproparathyroid hormone, 360
Prepuce, of penis, 578
Presbyopia, 591*b*
Pressure
Pacinian corpuscles and, 586
Ruffini corpuscles and, 586
Presynaptic membrane, 192, 228
Presynaptic vesicles, 229*f*
Primary cilia, 108
Primary mediators, 132–133
Primary miRNA (pri-miRNA), 67–68
Primary ossification center, 164
Primary response, 24
Primary saliva, 474
Primary transcript, 65
Primitive germ cells, 529
Primitive sex cords, 529
Primitive streak, 547*f*
Primordial follicle, 529–532, 531*f*, 533*f*–534*f*, 533*t*
Principal cells
of cortical collecting tubules, 513, 521–522
of ductus epididymis, 574*f*, 575
of thyroid, 355–357, 355*f*, 357*f*
Principal duct, salivary, 472
Principal piece, of spermatozoon, 566, 567*f*–568*f*, 568
Prisms, in enamel, 422
Procentriole, 52–53
Procentriole organizers, 106–108
Procollagen molecules, 88–89
Procollagen peptidase, 88–90
Proerythroblast, 275*f*, 277–280, 277*f*–278*f*, 280*t*
Profilin, 46, 49*t*
Progenitor cells, 273–277, 275*f*
Progesterone
in female reproductive system, 537*f*, 538*t*
in follicular development, 535
in lactation, 553
in mammary gland development, 553
in menstrual cycle, 545, 545*f*
in placental development, 551
Progesterone-progesterone receptor complexes, 546
Programmed cell death. *see* Apoptosis
Progressive multifocal leukoencephalopathy, 221*b*
Proinsulin, 479–480
Projection lens, 3*f*
Prolactin
in lactation, 553, 555
in mammary gland development, 553
in pars distalis, 349*t*–350*t*, 352
synthesis of, 551
Prolactin inhibitory factor (PIF), 347, 349*t*–350*t*
Prolactin-releasing hormone (PRH), 347, 349*t*–350*t*
Proliferation, zone of, 168–169
Proliferative (follicular) phase, of menstruation, 545*f*, 546

Proline, 87
tritiated, 7, 7*f*
Prometaphase, of cell cycle, 73*f*, 74
Promyelocyte, 275*f*, 282*t*, 283*f*
Pronucleus
female, 548
male, 548
Proopiomelanocortin (POMC), 353
Proparathyroid hormone, 360
Propeptides, 88
Prophase
meiotic, 76–77, 76*f*–77*f*
in oocytes, 532
in spermatocytes, 565
mitotic, 72–74, 73*f*
Proplatelets, 284
Proprioception, 198*b*
Proprioceptive fibers, of periodontal ligament, 428, 428*b*
Proprioceptors, 583
Propulsive contractions, of small intestine, 460–461
Prostacyclins
capillary release of, 300
and clot formation, 267
Prostaglandin(s), 499, 544
inhibition of HCl release by, 452
mast cell release of, 132–133, 136*t*
placental manufacture of, 551
Prostaglandin D_2, in inflammatory response, 136
Prostate gland, 560*f*, 576–577, 576*f*–578*f*
Prostatic concretions, 576–577
Prostatic hyperplasia, benign, 582*f*
Prostatic hypertrophy, benign, 577*b*
Prostatic secretions, 577
Prostatic-specific antigen, 577
Prostatic urethra, 526
Proteases, neutral
in inflammatory response, 136
in mast cells, 132–133, 136*t*
Proteasomes, 41–42, 324
Protein(s)
actin-binding, 49*t*
androgen-binding, 562, 571, 572*f*
associated with skeletal muscle, 188*t*
band 3, 254, 256*f*
band 4.1, 255, 256*f*
in blood, 252*t*
bone morphogenic, 425
capping, 47, 49*t*
carrier, 22–23
in cell membrane, 15
cell-surface, 315
channel, 20–22
class II-associated invariant, 324
clotting, 252*t*
complement. *see* Complement proteins
CRE-binding, 24–25
docking, 26, 29–30
Protein(s) *(Continued)*
endogenous, 324
eosinophilic cationic, 261
exogenous, 324
G, hormone receptor complexes and, 346
glial fibrillary acidic, 51*t*
glycoproteins as. *see* Glycoprotein(s)
in hormones, 345
integral, 16, 18*f*
J, 318*t*
lipoproteins as. *see* Lipoprotein(s)
lysosomal, transport of, 34–35, 35*f*
major basic, 261
in mammary glands, 554
membrane transport, 19–23, 20*f*
microtubule-associated, 50–51, 52*f*, 215
multipass, 16–17, 22
nuclear thyroid hormone receptor, 359
peripheral, 16, 18*f*
polyubiquitinated, 41
pore, 26, 29
Ran-binding, 56
regulated secretory, transport of, 35
ribosome receptor, 26
secretory, 33*f*
signal recognition particle receptor, 26, 29–30
sorting of, 33*f*
Tamm-Horsfall, 511
transmembrane, 16
transmembrane linker, 96
vesicle coat, 230
Protein C3, 316*b*
Protein kinase regulator protein, 481
Protein kinases
A-kinase, 24–25
adenosine monophosphate (AMP)-activated, 145
C-kinase, 25
calcium-calmodulin-dependent (CaM-kinases), 25
in cell cycle, 71
cycle adenosine monophosphate (cAMP)-dependent, 201
cyclin-dependent (CDKs), 71
Protein synthesis, 26–35
cytosolic, 27–29, 29*f*
in liver, 489, 489*f*, 491–493, 492*f*
on rough endoplasmic reticulum, 29–30, 30*f*
by smooth muscle, 203
Protein translocators, 29
Proteinuria, 508*b*
Proteoglycans
in extracellular matrix, 82–83, 84*f*
of cartilage, 149
functions of, 83
Proteolysis, 41, 365
Prothrombin, 269, 269*b*
Protofilaments, 50
Proton motive force, 45
Proto-oncogenes, 71, 76
Protoplasm, 13
Protoplasmic astrocytes, 220–221, 220*f*
Proximal tubule, 502, 502*f*–503*f*, 508–511, 523*t*
convoluted, 503*f*, 508
regions of, 508, 510*f*
resorption in, 510, 518–519
Pruriceptors, 586–587
Pseudomembranous colitis, 464*b*–465*b*
Pseudostratified columnar epithelium, 100*t*, 101*f*, 102–103, 104*f*
Psoriasis, 383*b*
Pterygopalatine ganglion, 236*f*
Ptyalin, 471, 474
PU.1 transcription factor, 276*t*
Puberty, ovarian cortex at onset of, 531–532
Pulmonary arteries, 405*f*, 415–416
Pulmonary circuit, 287
Pulmonary neuroepithelial bodies, 403
Pulmonary surfactant, 411, 412*b*
Pulmonary trunk, 289
Pulmonary veins, 303, 405*f*, 416
Pulp
of spleen
red, 335
white, 335
of tooth, 421*f*, 423–424, 424*b*, 424*f*
Pulp arteriole, 335
Pulp chamber, 421
Pulp core, 423
Pulp stones (denticles), 424
Pulp veins, 336
Pupil
dilator muscle of, 590
sphincter muscle of, 588*f*
Pupillary aperture, 590
Pupillary zone, 590
Purines, 63
Purkinje cells, 14*f*, 244
Purkinje fibers, 303*f*–304*f*, 304
Pus, 261
Pyelonephritis, 526*b*–527*b*
Pyloric sphincter, 451
Pyloric valve, 439
Pylorus, 440, 446–451, 448*f*, 449*t*–450*t*
Pyramidal lobe, of thyroid, 354–355
Pyramids, renal, 499, 500*f*–501*f*
Pyrimidines, 63

Q

Quanta, 192

R

rab3a protein, 230
Radial spokes, of cilia, 105
Radiation therapy, demyelination due to, 225*b*

Radioautography, 6
Raft proteins, 106–108
Ran-binding protein, 56
Ranvier, nodes of, 223, 224*f*
Rappaport, acinus of, 485
Raschkow plexus, 424
Rathke cysts, 353
Rathke pouch, 346
Reaginic antibody, 318*t*
Receptor(s)
  for acetylcholine, pancreatic, 476
  B-cell, 317
  cargo, 36
  catalytic, 346
  cell-surface, 24–25, 345
  enzyme-linked, 24
  Fc
    of antibodies, 316
    FcεRI, on basophils, 262
    on macrophages and neutrophils, 36
    on mast cells, 134–135
  G-protein-linked, 24–25, 25*f*
  gated ion-channel, 227–228
  for hormones, 345–346
  immunoglobulin E (IgE), 262
  insulin, 143–144
  ion channel-linked, 21
  killer activating, 312–313
  killer inhibitory, 312–313
  luteinizing hormone, 534
  mannose-6-phosphate, 34, 39
  mechanoreceptors as, 378, 381, 583–586, 584*f*–585*f*
  for neurons, 211
  neurotransmitter, 230
  nociceptors as, 586–587
  nuclear thyroid hormone, 359
  olfactory, 398*f*
  parathyroid hormone (PTH), 156
  ribosome, 26
  selectin, 259
  sensory, 583
  sensory nerve action potential (SNARE), 230
  signal recognition particle, 26, 29
  specialized peripheral, 583–587
  steroid hormone, 24
  T-cell, 315, 319
  thermoreceptors as, 586
  toll-like, 312*t*, 313*b*, 314*t*
  transferrin, 243*b*
Receptor coupling factors, 134
Receptor for activation of nuclear factor kappa B ligand (RANKL), 156, 159
Receptor-mediated endocytosis, 36, 37*f*–38*f*
Receptor-mediated transport
  in blood-brain barrier, 242
  in nuclear pore, 56
Rectal columns of Morgagni, 467
Rectal examination, 468*b*
Rectum, 449*t*–450*t*, 467
Recycling endosome, 37–38
Red blood cells (RBCs). *see* Erythrocyte(s)
Red bone marrow, 161
Red marrow, 271–272
Red pulp, of spleen, 335
5α-Reductase, 571
Reductional division (meiosis I), 76–77, 76*f*–77*f*
Reflex arc, simple, 198*b*
Reflex(es), somatic *vs*. visceral, 235*f*
Refractory period, of voltage-gated channels, 21, 226
Regeneration
  of liver, 493
  of muscles, 206–208, 207*t*
  of nerve, 245–247, 246*f*
Regenerative cells
  of colon, 463, 464*f*
  of small intestine, 454*f*, 456
  of stomach, 441–442, 441*f*
Regulated secretory pathway
  of glands, 119
  of Golgi apparatus, 34
Regulated secretory proteins, transport of, 35
Regulatory chains, of myosin, 206
Regulatory component, of A-kinase, 24–25
Regulatory particle, 41
Regulatory RNAs, 67–69
Regulatory T cells (T reg cells), 265, 323
Regurgitation theory, of endometriosis, 544*b*
Reinke, crystals of, 46, 570
Relaxin, 537*f*, 538*t*, 544
Release factor, in protein synthesis, 28
Releasing hormones (factors), 347
Renal artery, 363, 500*f*–501*f*, 516
Renal cell carcinoma, 527*f*
Renal corpuscles, 500, 502–508, 502*f*–503*f*, 523*t*
  filtration in, 518
Renal cortex, 499, 500*f*–502*f*
Renal glomerulus, 298
Renal innervation, 517–518
Renal interstitium, 515–516
Renal medulla, 499, 500*f*–501*f*, 502, 514*f*
Renal papilla, 499
Renal pelvis, 499, 500*f*–501*f*, 523
Renal pyramids, 499, 500*f*–501*f*
Renal sinus, 499, 500*f*–501*f*
Renal vein, 500*f*–501*f*, 516
Renin, in blood pressure regulation, 294
Rennin, 439, 451, 499, 513, 514*f*, 521
Reparative dentin, 423
Reproductive system. *see* Female reproductive system; Male reproductive system
Reserve cartilage, zone of, 168
Reserve cells, 563
Residual bodies, 39, 46
Resistin, 146, 147*t*
Resolution, 3
Resorption
  of bone, 159–160, 161*f*
  of periodontal ligament collagen, 427
  in proximal tubule, 510, 518–519
Respiration
  external, 395
  internal, 395
Respiratory bronchioles, 396*t*, 407, 408*f*–409*f*
Respiratory burst, 260–261
Respiratory chains, 44
Respiratory distress of newborn, 412*b*
Respiratory epithelium, 395–397
  tracheal, 401–403, 402*f*–403*f*
Respiratory regulators, 403
Respiratory system, 395–416
  alveolar ducts in, 396*t*, 407–408, 408*f*–410*f*
  alveolar macrophages in, 411*f*, 412, 413*f*
  alveoli in, 396*t*, 408–415, 409*f*–411*f*
  blood-gas barrier in, 411*f*, 414
  bronchi in, 396*t*, 403–404
  bronchial tree in, 403–407, 405*f*
  bronchioles in, 396*t*, 404–406, 405*f*–408*f*
  characteristic features of, 395, 396*t*
  conducting portion of, 395–407, 396*t*
  gas exchange in, 414–415
  gross structure of lungs in, 415–416
  interalveolar septa in, 408, 409*f*, 411*f*, 414
  larynx in, 396*t*, 400–401
  mechanism of ventilation in, 415
  mucosal immune defense of, 416
  nasal cavity in, 395–400, 396*t*
  nasopharynx in, 396*t*, 400
  paranasal sinuses in, 400
  pathological considerations of, 417*f*
  pleural cavities in, 415
  pneumocytes in, 408–412, 411*f*–413*f*
  respiratory portion of, 396*t*, 407–416
  trachea in, 396*t*, 401–403, 402*f*
  vascular and lymphatic supply of, 415–416
Resting membrane potential, 182
Resting potential, 192, 224–226
Rete apparatus
  of lips, 420
  of skin, 373
Rete testis, 559, 560*f*, 572*t*, 573, 573*f*
Reticular cells
  adventitial, of bone marrow, 272, 272*b*
  epithelial, 320
  stellate, of spleen, 339

Reticular fibers, 85
in bone marrow, 272
in smooth muscle, 203
in spleen, 335
Reticular layer, of dermis, 374*f*, 375*t*, 381
Reticular tissue, 143, 143*f*
Reticulocyte, 275*f*, 280*t*
Reticuloendothelial system, 138
Reticulum
of clot, 269
stellate, in odontogenesis, 424
Retina, 587, 588*f*, 592–600, 592*f*–594*f*, 594*t*
blind spot of, 592–593
cones of, 587, 592, 593*f*–594*f*, 594*t*, 597–598, 598*b*, 598*f*
detachment of, 595*b*
external (outer) limiting membrane of, 593*f*, 598
ganglion cell layer of, 593*f*, 594*t*, 599–600
image processing in, 600
inner limiting membrane of, 593*f*, 600
inner plexiform layer of, 593*f*, 599
optic nerve fiber layer of, 593*f*, 600
outer nuclear layer of, 593*f*, 598
outer plexiform layer of, 593*f*, 598
pars ciliaris of, 589
pigment epithelium of, 589, 592, 593*f*, 594–595
rods of, 587, 592, 593*f*–594*f*, 594*t*, 595–597
Retina proper, 592
11-*cis* Retinal, 597
Retinal artery, central, 593
Retinal isomerase, 597
activated, 597
Retinol-binding protein-4 (RBP-4), 144, 146, 147*t*
Retrograde intraciliary transport, 106–108
Retrograde reaction, to nerve injury, 247
Retrograde transport, 34
axonal, 217
Retromer, 33
Retroperitoneal area, 544
Retzius, striae of, 421*f*, 422
RGD sequence, 84
Rheumatic fever, 303*b*
Rheumatic heart valve disease, 303*b*, 308*f*
Rhodopsin, 595
Rhodopsin kinase, 597
Ribonucleic acid (RNA), 55, 64–69
messenger, 24, 65–66, 66*f*
precursor messenger, 65–66
regulatory, 67–69
ribosomal, 25–26, 67, 67*f*
splicing of, 65–66
Ribonucleic acid (RNA) *(Continued)*
transfer, 26, 66–67
types of, 65*t*
Ribonucleoprotein particles (RNPs), 67
heterogeneous nuclear, 65–66
messenger, 65–66
small nuclear, 65–66
Ribosomal RNA (rRNA), 25–26, 67, 67*f*
45S (pre-rRNA), 67
Ribosome(s), 25–26
protein synthesis on, 26
Ribosome receptor protein, 26
Ribozymes, 26
Rickets, 174*b*, 361*b*
Rigor mortis, 191*b*
Rima glottidis, 401
RNA. *see* Ribonucleic acid (RNA)
RNA-induced silencing complex-miRNA (RISC-miRNA), 67–68
RNA polymerases, 64
RNase, pancreatic, 477
RNAse III enzyme, 67–68
Rod segments, in enamel, 422
Rods, of retina, 587, 592, 593*f*–594*f*, 594*t*, 595–597
hyperpolarized, 595–597
inner segment of, 595–597
layers of, 595–598
nuclear region of, 597
outer segment of, 595, 596*f*, 598*f*
synaptic region of, 597
Root
of lung, 415
of tongue, 429
of tooth, 421, 421*f*, 427
Root canal, 421, 421*f*
Root sheath, of hair, 387–388, 388*f*
Roseola, 397*b*
Rosettes, 45
Rough endoplasmic reticulum. *see* Endoplasmic reticulum (ER), rough
Round ligament, 530*f*
Round window, of ears, 603
Ruffini corpuscles, 381, 584*f*, 586
Ruffled border, of osteoclast, 159
Rugae, 440

S

S phase, of cell cycle, 70*f*, 71
Saccule, 603–606, 604*f*–605*f*
Sacral spinal nerves (spinal outflow), 437
Saddle embolus, 269*b*
Saliva, 474
primary, 474
secondary, 474
Salivary amylase, 419, 471, 474
Salivary glands, 474–476
anatomy of, 471–474, 472*f*
autonomic innervation of, 474
disorders of, 476*b*
duct portions of, 472–473, 472*f*
Salivary glands *(Continued)*
histophysiology of, 473–474
major, 419, 471–476
minor, 419, 473
parotid, 474
salivon, 473
secretory portions of, 471–472, 472*f*
sublingual, 472*f*–473*f*, 475
submandibular, 475–476, 475*f*, 476*b*
vascular supply, 473
Saltatory conduction, 234
Sarcoglycan complex, 183–185
Sarcolemma, 179, 192
Sarcoma
liposarcoma as, 147*b*
malignant, 218*b*
Sarcomeres
in cardiac muscle, 207*t*
in skeletal muscle, 180, 183*f*–184*f*, 186*f*, 207*t*
in smooth muscle, 207*t*
Sarcoplasm, 179
Sarcoplasmic reticulum, 26
of cardiac muscle, 207*t*
corbular, 201
of skeletal muscle, 179, 180*t*, 182–185, 184*f*–185*f*, 207*t*
of smooth muscle, 207*t*
terminal cisternae of, 182, 184*f*
Satellite cells, 180, 238
Satellite oligodendrocytes, 221
Scaffold, of nuclear pore, 60*f*
Scala media, 607–610, 608*f*–609*f*
Scala tympani, 607
Scanning electron microscopy, 3*f*, 9
Schlemm, canal of, 588*f*, 589
Schmidt-Lanterman, clefts of, 223
Schwann cells, 223–224
in anterograde reaction, 246
in endoneurium, 233
in myelinated nerve fiber, 218*f*, 223
in unmyelinated nerve fiber, 218*f*, 223
Schwann tubes, 247
Sclera, 587–588, 588*f*
Sclerocorneal junction, 589
Scotopsin, 597
Scrotum, 560*f*
Scurvy, 90*b*, 174*b*
Sebaceous glands, 374, 374*f*, 381, 385–386, 386*b*, 386*f*
Sebum, 385
Second messenger system, 23, 231, 345–346
Second polar body, 548
Secondary (antral) follicles, 531*f*, 533*t*, 536*t*
Secondary bronchi, 396*t*, 404
Secondary mediators, 132–133
Secondary response, 24
Secondary saliva, 474

Secretin, 444, 447*t*, 476–478
Secretomotor in function, 237
Secretory activity, of small intestine, 437, 460
Secretory antibody, 318*t*
Secretory component, of immunoglobulins, 318*t*, 459–460, 471
Secretory endings, 583
Secretory granules
  of cytoplasm, 16*f*, 34
  in glands, 119
  in neuron, 215
  pancreatic, 476, 478*f*
  in stomach, 440, 443*f*
Secretory immunoglobulin A (IgA), 419, 459–460, 474
Secretory (luteal) phase, in menstrual cycle, 546–547, 546*f*
Secretory portions
  of pancreas, 476–477, 477*f*
  of salivary glands, 471–472, 472*f*
Secretory proteins, 33*f*
Secretory unit, of eccrine sweat glands, 382–384, 384*f*
Sectioning, in tissue preparation, 2
Segmental arteries, of kidney, 516
Selectin molecules, 259
Selectin receptors, 259
Self-antigens, 330
Self-epitopes, 331
Self-MHC molecules, 331
Self/nonself recognition, 314
Semen, 559, 577–579
Semicircular canals, 603
Semicircular ducts, 603–604, 607, 607*f*
Semilunar valves, 303
Seminal fluid, fructose-rich, 576, 579
Seminal vesicles, 559, 560*f*, 575–576, 576*f*
Seminiferous epithelium, 559, 561, 561*f*–563*f*
  cycle of, 568–571, 569*f*
  wave of, 568–569
Seminiferous tubules, 559–568, 560*f*
  differentiation of spermatogonia in, 562*f*, 563–565, 564*f*, 567*f*–568*f*
  epithelium of, 559, 561, 561*f*–563*f*
    cycle of, 568–571, 569*f*
    wave of, 568–569
  interstitial cells of Leydig in, 559, 569–570, 570*f*–571*f*
  meiotic division of spermatocytes in, 562*f*, 563, 564*f*, 565
  Sertoli cells of, 561–562, 562*f*–563*f*
  spermatogenic cells of, 562–568, 562*f*, 564*f*, 567*f*
  structure of spermatozoa in, 567–568, 568*f*
  transformation of spermatids in, 565–567, 566*f*–568*f*
Seminoma, 581*f*
Senses, special, 583–612
  pathological considerations for, 612, 612*f*–613*f*
Sensory component, of peripheral nervous system, 211
Sensory components, of alimentary canal, 437
Sensory endings, 583
Sensory fibers, of pulp, 424
Sensory ganglion, 212, 212*f*
Sensory nerve fibers
  group Ia, 195, 197*f*
  group II, 195, 197*f*
Sensory neurons, 219
Sensory receptors, 583
Septa, of glands, 121–122
Septal cells, 410, 412*f*–413*f*
Septum membranaceum, 305
Serine/threonine phosphoprotein phosphatases, 25
Serosa
  of alimentary canal, 436, 436*f*, 449*t*–450*t*
  of colon, 463
  of esophagus, 438, 449*t*–450*t*
  of large intestine, 449*t*–450*t*
  of oviducts, 541
  of small intestine, 449*t*–450*t*, 458
  of stomach, 449*t*–450*t*, 451
  of uterus, 544
Serotonin, 232*t*, 447*t*
Serous cells
  of esophagus, 438
  of respiratory epithelium, 403
  of salivary gland, 471, 472*f*
Serous demilunes, 120, 120*f*
  of salivary gland, 472*f*, 475
Serous glands, 120, 430*f*–431*f*
Serous secretion, of parotid gland, 474
Sertoli cells, 530*b*, 561–562, 562*f*–563*f*
Serum, 251
  vs. cerebrospinal fluid, 244*t*
Sex chromatin, 62–63
Sex chromosomes, 59–60, 257
Sex cords
  cortical, 529
  primitive, 529
Sex determining region of the Y chromosome (SRY), 530*b*
Sharpey fibers
  in bone, 154
  in cementum, 423
  in periodontal ligament, 427
Sheath cells, 293
Sheathed arteriole, 335
Shock, anaphylactic, 263*b*
Sickle cell anemia, 255*b*
Sieve plates, 483*f*, 485
Signal peptidase, 30, 30*f*
Signal peptide, 29
Signal recognition particle receptor protein, 26, 29–30
Signal-recognition particle (SRP), 29, 30*f*
Signal transduction, 24, 319, 345
Signal transduction pathways, 71
Signaling cell
  in cytoplasm, 23
  in glands, 119
Signaling molecules, 23–24
  neurotransmitter as, 231
  receptors for, 16–17
Sildenafil (Viagra), 581*b*
Silencing RNAs, 68, 69*b*
Silver stain, 2*t*, 11*f*
Simple diffusion, 20
Simple epithelium, 99, 100*t*, 101*f*
  columnar, 100, 101*f*–102*f*
  cuboidal, 100, 101*f*
Simple reflex arc, 198*b*
Singlets, of microtubules, 105, 397
Sinoatrial node, 303*f*, 304
Sinus(es)
  anal, 467
  carotid, 293
  lactiferous, 553, 554*f*
  of lymph nodes, 332
  marginal, of spleen, 338
  paranasal, 400
  renal, 499, 500*f*–501*f*
  splenic, 338–339
Sinusoid(s)
  in bone marrow, 272
  hepatic, 483*f*–484*f*, 484–486, 489*f*
Sinusoidal (discontinuous) capillaries, 298, 298*f*
Sinusoidal domains, of hepatocytes, 488, 488*f*
Sinusoidal lining cells, 485, 486*f*
Sister chromatids, 72, 72*f*
Skeletal muscles. *see* Muscle(s), skeletal
Skin, 373–391. *see also* Integument
  dermis (corium) of, 373, 380–382
  epidermis of. *see* Epidermis
  functions of, 373
  glands of, 373, 382–386
  histophysiology of, 382
  hypodermis of, 373, 374*f*, 380
  structure of, 374*f*
  thick, 374, 374*f*, 375*t*, 376*f*
  thin, 374, 374*f*
Skull cap, 163
Slit diaphragms, 504–505, 506*f*–507*f*, 508*b*
Slow-reacting substance of anaphylaxis (SRS-A), 263
Slow sodium channels, 201
Small-granule mucous cells, 402*f*, 403
Small intestine, 452–463
  absorption by, 462–463, 462*f*
  Auerbach myenteric plexus of, 458
  Brunner glands of, 456*f*, 457–458

Small intestine *(Continued)*
crypts of Lieberkühn of, 453, 454*f*–455*f*, 455–456
digestion by, 461
DNES cells and hormones in, 447*t*, 454–455, 460
epithelium of, 449*t*–450*t*, 453–455
glands of, 449*t*–450*t*, 460
goblet cells of, 454, 454*f*, 456*f*
histology of, 449*t*–450*t*, 453–458
histophysiology of, 458–463
lacteal of, 453, 458
lamina propria of, 449*t*–450*t*, 455–456, 455*f*–456*f*, 458*f*
luminal surface of, 453, 454*f*–456*f*
lymphatic and vascular supply of, 458
lymphoid nodule of, 454*f*
M cells (microfold cells) of, 455, 459, 460*f*–461*f*
microvilli of, 453–454
mucosa of, 453–456
muscularis externa of, 449*t*–450*t*, 458
muscularis mucosae of, 449*t*–450*t*, 456, 458*f*
Paneth cells of, 456–458, 459*f*
Peyer patches in, 458
plicae circulares (valves of Kerckring) of, 453
regenerative cell of, 454*f*, 456
regional differences in, 458
secretory activity of, 460
serosa or adventitia of, 449*t*–450*t*, 458
submucosa of, 449*t*–450*t*, 456–458, 456*f*
surface absorptive cells of, 453–454, 454*f*, 456*f*–457*f*
villi of, 453, 454*f*–456*f*
Small-molecule transmitters, 231
Small nuclear ribonucleoprotein particles (snRNPs), 65–66, 69
Smooth endoplasmic reticulum (SER). *see* Endoplasmic reticulum (ER), smooth
Smooth muscle. *see* Muscle(s), smooth
Smooth muscle cells, in dermis, 381
Smooth muscle coat, of seminal vesicle, 575–576
Sneeze reflex, 399*b*
Sodium, in glomerular ultrafiltrate, 510
1-sodium, 1-potassium, 2-chloride co-transporters, 511, 519
Sodium adenosine triphosphatase (Na^+-ATPase), 472
Sodium ions, as second messenger, 345–346
Sodium-potassium (Na^+-K^+) pump, 22
Sodium pumps, 518–519
Soft palate, 429
Soluble mucus, 451
Soma, neuron, 212*f*, 213–215
Somatic afferent pathways, 583
Somatic nervous system, 211, 234
Somatic reflex, 235*f*
Somatomammotropin, chorionic, 551
Somatostatin, 480*t*, 481
inhibition of HCl release by, 452
as neurotransmitter, 231
in pars distalis, 349*t*–350*t*, 351–352
production of, 447*t*
Somatotrophs, 351–352, 351*f*
Somatotropin
effect on cartilage, 152*t*
in mammary gland development, 553
physiological effects of, 349*t*–350*t*
secretion by acidophils, 351–352
in T-cell development, 331
Somatotropin-releasing hormone (SRH), 347, 349*t*–350*t*, 351–352
Sonic hedgehog, 425
Space of Disse, 485–486, 487*f*–488*f*
Space of Moll, 484
Space of Nuel, 610
Specific granules, of neutrophils, 259
Specificity
of adaptive immune system, 314
of antibody, 316
Spectrin, 48, 49*t*
Spectrin tetramers, in erythrocyte cell, membrane, 255
Sperm, 567
Spermatic cord, 559–560
Spermatids, 562, 564*f*
transformation of, 565–567, 566*f*–568*f*
Spermatocytes, 562, 562*f*, 564*f*
secondary, 565
Spermatocytogenesis, 563
Spermatogenesis, 563, 564*f*, 566*f*–568*f*
cycle of, 568–571, 569*f*
six stages of, 568, 569*f*
Spermatogenesis and oogenesis helix-loop-helix 1, 533
Spermatogenic cells, 562–568, 562*f*–564*f*, 566*f*–567*f*
Spermatogonia, 562, 562*f*, 564*f*
dark type A, 563
differentiation of, 562*f*, 563–565, 564*f*, 567*f*–568*f*
pale type A spermatogonia, 563
type B, 565
Spermatozoa, 562
capacitation of, 541, 548, 567, 573
defined, 559
hyperactivity of, 548
maturation of, 548
structure of, 567–568, 568*f*
Spermiation, 566–567
Spermiogenesis, 565–567, 566*f*–568*f*
Spherocytosis, hereditary, 256*b*
Sphincter ampullae, 494, 496*t*
Sphincter choledochus, 494, 496*t*
Sphincter muscles
anal, 467
of gastrointestinal system, 437
of pupil, 588*f*
of urethra, 526
of urinary bladder, 525
Sphincter of Oddi, 494, 496*t*
Sphincter pancreaticus, 494, 496*t*
Sphingolipidosis, 41*t*
Spicules, 161
Spike trigger zone, 216–217, 224
Spina bifida, 212*b*
Spinal cord, development of, 211
Spinal dura mater, 241
Spinal nerves, 233
Spine(s), of dendrites, 216
Spiral ganglion, 603
Spiral lamina, limbus of, 608
Spiral prominence, 608
Splanchnic nerves, preganglionic sympathetic, 366
Spleen, 335–340
diagram of, 336*f*
histophysiology of, 339–340, 340*f*
marginal zone of, 337–338, 338*f*
red pulp of, 338–339
rupture of, 340*b*
vascular supply of, 335–336, 337*f*
white pulp of, 337–338
Splenic artery, 335
Splenic cords, 339, 339*f*
Splenic phase, of prenatal hemopoiesis, 272
Splenic sinuses, 336
Splenic vein, 336
Spliceosomes, 65–66
Spongiocytes, 364
Spongiosa, of dental alveolus, 428
Spongy bone, 162*f*
Spongy urethra, 526
Sprue, 463*b*
Squames, 377
Squamous alveolar cells, 408–410, 411*f*
Squamous cell carcinoma, 383*b*
Squamous epithelium
simple, 99–100, 100*t*, 101*f*–102*f*
stratified
keratinized, 100*t*, 101, 101*f*, 103*f*
ectodermally derived, 373
nonkeratinized, 100–101, 100*t*, 101*f*, 103*f*
of oral cavity, 419
of thin limbs of Henle loop, 514*f*
Squamous metaplasia, 119*b*
Stable phase, of cell cycle, 70–71
Staining, 2–3, 2*t*
Stapedius, 602*f*, 603
Stapes, 602*f*, 603
Start codon, 27, 65
Steatosis, 54*f*

Steel factor, 274–277, 276*t*
Stellate cells
of cerebellar cortex, 244
perisinusoidal, 486
Stellate reticular cells
of lymph nodes, 332
of spleen, 339
Stellate reticulum (SR), in odontogenesis, 424
Stellate veins, of kidney, 516
Stem, of goblet cell, 401–402
Stem cell(s), 273–277, 275*f*, 555–557
of colon, 463, 464*f*
of small intestine, 454*f*, 456
of stomach, 441–442, 441*f*
Stem cell factor, 281, 534
Stem cell therapy, 556
Stereocilia
of ductus epididymis, 574–575
function of, 104–105
of mechanically gated channels, 21
of neuroepithelial hair cells, 605, 605*f*–606*f*
Sterility, 580*b*
Steroid hormone(s)
production of, by suprarenal glands, 363
in signaling, 23
Steroid hormone receptors, 24
Steroidogenic acute regulatory (StAR) proteins, and progesterone, 539
Stickler syndrome, 90*b*
Stigma, of oocyte, 536
Stimulus transmission, at neuromuscular junctions, 192
Stomach, 439–452. *see also* Gastric *entries*
body (corpus) of, 440
carcinoma of, 453*b*
cardiac, 446–451, 449*t*–450*t*
diffuse neuroendocrine system cell of, 441*f*, 444–446, 447*t*, 448*f*
emptying contents of, 451
epithelium of, 440, 442*f*–443*f*, 449*t*–450*t*
fundic, 440, 441*f*, 449*t*–450*t*
glands of, 440, 440*t*, 441*f*, 449*t*–450*t*
histology of, 440–446, 441*f*, 449*t*–450*t*
histophysiology of, 451–452
hydrochloric acid production by, 451–452, 452*f*
lamina propria of, 440, 449*t*–450*t*
mucosa of, 440, 441*f*, 446–451
mucous neck cells of, 441–442, 444*f*
muscularis externa of, 448–451, 449*t*–450*t*
muscularis mucosae of, 446–451, 449*t*–450*t*
parietal (oxyntic) cell of, 441*f*–442*f*, 442–443, 445*f*
Stomach *(Continued)*
pyloric, 440, 446–451, 448*f*, 449*t*–450*t*
regenerative (stem) cell of, 441–442, 441*f*
serosa or adventitia, 449*t*–450*t*, 451
submucosa of, 446–448, 449*t*–450*t*
surface-lining cells of, 440, 441*f*–443*f*
zymogenic (chief) cell of, 441*f*–442*f*, 443–444, 446*f*
Stones
cholesterol, 496*b*, 497*f*
in gallbladder, 496*b*
pigment, 496*b*
pulp (denticles), 424
Stool, fatty, 490*b*
Stop codons, 28, 65
Straight arteries, of endometrium, 543, 543*f*
Stratified epithelium, 99, 100*t*, 101*f*
Stratum basale, 374–375, 374*f*, 375*t*, 376*f*, 383*b*
Stratum corneum, 374, 374*f*, 375*t*, 377–380
Stratum germinativum, 374–375, 374*f*, 375*t*, 376*f*
Stratum granulosum, 374, 374*f*, 375*t*, 376–377
Stratum intermedium, in odontogenesis, 426
Stratum lucidum, 374, 374*f*, 375*t*, 377
Stratum spinosum, 374–376, 374*f*, 375*t*, 376*f*–377*f*
Stratum vasculare, of myometrium, 543
Stress fibers, 48
Stretch reflex, 195
Stretching, and Ruffini corpuscles, 586
Stria vascularis, 607
Striae of Retzius, 421*f*, 422
Striated anchoring fibers (SAFs), 118*f*
Striated border, 104
Striated ducts
defined, 117
of salivary gland, 472, 472*f*
Striated muscle, 179, 180*f*
Stroma
of cornea, 589
of gland, 119
of kidney, 501
of ovarian cortex, 531
of prostate gland, 576
Stromal cells
ovarian, 531, 534
uterine, 548
Subarachnoid space, 241, 241*f*
Subcapsular epithelium, of lens, 591, 591*f*
Subcapsular plexus, 363
Subcapsular sinus, of lymph nodes, 332, 333*f*
Subcapsular space, 197*f*
Subdural space, 241
Subendocardial layer, 303
Subendothelial layer, of blood vessels, 288
Sublingual gland, 472*f*–473*f*, 475
Sublobular vein, of liver, 484
Submandibular ganglion, 236*f*
Submandibular gland, 475–476, 475*f*, 476*b*
Submucosa
of alimentary canal, 435, 436*f*, 449*t*–450*t*
of anal canal, 467
of colon, 464*f*
of esophagus, 438, 449*t*–450*t*
of large intestine, 449*t*–450*t*
of small intestine, 449*t*–450*t*, 456–458, 456*f*
of stomach, 441*f*, 446–448, 449*t*–450*t*
of trachea, 402*f*, 403
Submucosal glands, of prostate, 576
Submucosal lymphatic plexus, 458
Submucosal (Meissner) plexus, of digestive tract, 435–436, 456
Submucosal vascular plexus, 458
Subosteoclastic compartment, 159
Substance P, 231, 447*t*
Subunits, ribosomal, 25–26
Succedaneous lamina, 426
Succedaneous teeth, 421, 426
Sulci, 244
Sulcus terminalis, of tongue, 429
Superficial fascia, 373
Superoxides, 261
Supporting cells
of maculae, 605*f*, 606
of organ of Corti, 609–610, 609*t*
of semicircular ducts, 607, 607*f*
Supraoptic nuclei, 348*f*, 353
Suprarenal (adrenal) glands, 356*t*, 361–368, 362*f*
blood supply to, 362–363, 363*f*
cortex of, 356*t*, 361, 362*f*, 363–366, 364*f*
medulla of, 356*t*, 361, 366–368, 366*f*
zona fasciculata of, 364–365
zona glomerulosa of, 364, 364*f*
zona reticularis of, 365
Suprarenal arteries, 363
Surface absorptive cells
of colon, 463, 464*f*
of small intestine, 453–454, 454*f*, 456*f*–457*f*
Surface immunoglobulins, 317
Surface-lining cells, of stomach, 440, 441*f*–443*f*
Surface-opening tubule system, 266*t*
Surface remodeling, 171
Surfactant, 411, 412*b*
Surfactant apoproteins, 411
Suspensory ligaments, of lens, 588*f*, 590

Sustentacular cells, 397–399, 398*f*
Sweat glands
  apocrine, 384–385
  eccrine, 374*f*, 382–384, 384*f*–385*f*
Sweat pore, 374*f*, 382
Sweating, 499
Swell bodies, 399*b*
Symbiotic relationship, 45
Symmetric synapses, 231
Symmetrical division, 555–556
Sympathetic chain ganglia, 238
Sympathetic fibers
  of dental pulp, 424
  of lungs, 416
  preganglionic, 237
Sympathetic ganglion cells, of suprarenal gland, 366
Sympathetic innervation
  of gut, 437
  of salivary gland, 474
Sympathetic nervous system, 236*f*, 237
  in ejaculation, 579
Symport transport, 20*f*, 22
Synapses, 227–232, 227*b*
  asymmetric, 231
  axoaxonic, 228
  axodendritic, 228, 240*f*
    retinal, 598–599
  axosomatic, 228
  chemical, 227–228
  dendrodendritic, 228
  electrical, 227
  morphology of, 228–231
  symmetric, 231
Synapsin-I, 228
Synapsin-II, 230
Synaptic clefts, 230*f*
  primary, 192
  secondary, 192
Synaptic region, of rods, 597
Synaptic ribbons
  in hair cells, 606
  in pinealocytes, 368
  in retina, 598–599
Synaptic signaling, 23
Synaptic vesicles, 206
Synaptonemal complex, 77
Synarthroses, 174
Synchondrosis, 174
Syncoilin, 51*t*
Syncytiotrophoblasts, 549
Syncytium, of spermatids, 566–567
Syndecans, 83
Syndesmosis, 175
Synemin, 50
Synostosis, 174
Synovial fluid, 175
Synovial layer, 175
Synovial membrane, 175
Synthetic phase, of cell cycle, 71
Syntrophin, 185
Systemic circuit, 287
Systole, 294

T

T-cell receptor complex, 319
T-cell receptors (TCRs), 315, 319
α/β T cells, maturation of, 320–321
T helper cells, 320*t*, 322–323, 343*f*
  diagram of, 326*f*
  functions of, 265
  in humoral immune response, 325, 326*f*
  in killing of virally transformed cells, 325–327, 326*f*
T killer cells, 265
T lymphocytes (T cells), 540
  cytotoxic, 265
  effector, 265
  functions of, 264–265
  in thymus, 319–323
T memory cells, 321
T tubules
  of cardiac muscle, 207*t*
  of skeletal muscle, 182, 207*t*
Tactile discrimination, 584
Taeniae coli, 463
Tail, of spermatozoon, 568
Talin, in cytoskeleton, 48, 50*f*
Tamm-Horsfall protein (uromodulin), 511
Tanycytes, 223
Target cells
  in cytoplasm, 23
  of glands, 119
  of hormones, 345
Tarsal plates, 600
Tastants, 431
Taste buds, 419, 430–433, 431*f*–432*f*, 432*t*
Taste hairs, 431
Taste pore, 430–431, 431*f*–432*f*
Tay-Sachs disease, 40*b*, 41*t*
Tear film, 600
Tears, 601
Tectorial membrane, 21, 608
Teeth (tooth), 421–433, 421*f*
  accessional, 426
  alveolus of, 421, 421*f*, 428
  cementum of, 421*f*–422*f*, 423, 427
  cervix of, 421, 421*f*
  crown of, 421, 421*f*
  deciduous (milk), 421, 426
  dentin of, 421*f*–422*f*, 422–423
  enamel of, 421*f*, 422, 426–427
  gingiva (gums) of, 421, 421*f*, 428–429
  mineralized components of, 421–423
  molars, 421
  odontogenesis of, 424–427, 425*f*
  periodontal ligament of, 421, 421*f*, 423, 424*f*, 427–428, 428*f*
Teeth (tooth) *(Continued)*
  pulp of, 421*f*, 423–424, 424*b*, 424*f*
  root canal of, 421, 421*f*
  root of, 421, 421*f*, 427
  structures associated with, 427–429
  succedaneous, 421, 426
Tektin, 105–106
Tektin rod, 105–106
Telogen phase, of hair growth, 388–389
Telomerase, 62, 556
Telomeres, 62, 63*b*
Telophase
  meiotic, 76*f*–77*f*, 77
    in spermatocytes, 565
  mitotic, 73*f*, 74
Tenascin, 84
Tensor tympani, 602*f*, 603
Terminal arbor, 216
Terminal arterial capillaries, of spleen, 335
Terminal arterioles, 298
Terminal bars, 109
Terminal boutons, 213
Terminal bronchioles, 396*t*, 407
Terminal cisternae, of sarcoplasmic reticulum, 184*f*
Terminal ductules, of mammary gland, 553
Terminal ganglia, 237
Terminal glycosylation, 33*f*
Terminal hairs, 386
Terminal (principal) duct, salivary, 472
Terminal signal complex, 29*f*
Terminal web, 48, 104, 107*f*
Territorial matrix, of hyaline cartilage, 152
Tertiary bronchi, 396*t*, 404
Tertiary granules, of neutrophils, 259
Testicular artery, 559–560
Testicular lobules, 560*f*
Testicular transferrin, 562
Testis determining factor, 530*b*
Testis(es), 559–568, 560*f*
  cycle of seminiferous epithelium in, 568–571, 569*f*
  differentiation of spermatogonia in, 562*f*, 563–565, 564*f*, 567*f*–568*f*
  general structure of, 559–560, 560*f*
  histophysiology of, 570–571, 571*f*–572*f*
  interstitial cells of Leydig in, 559, 569–570, 570*f*–571*f*
  meiotic division of spermatocytes in, 562*f*, 563, 564*f*, 565
  seminiferous tubules of, 559–568, 560*f*, 562*f*–563*f*
  Sertoli cells of, 561–562, 562*f*–563*f*
  spermatogenic cells of, 562–568, 562*f*, 564*f*, 566*f*–567*f*
  structure of spermatozoa in, 567–568, 568*f*

Testis(es) *(Continued)*
transformation of spermatids in, 565–567, 566*f*–568*f*
vascular supply to, 559–560
Testosterone
effect on cartilage, 152*t*
in male reproductive system, 559, 570–571, 571*f*–572*f*
Tetany, 331*b*, 361*b*
Tetrads, of spermatocytes, 565
T_H0 cells, 322
T_H1 cells, 322, 326*f*
macrophages in killing bacteria, 327
T_H2 cells, 322
T_H3 cells, 323
Thalassemia, 255*b*
Theca, of goblet cell, 121, 401–402
Theca externa, 531*f*, 533*t*, 534, 536
Theca folliculi, 531*f*
Theca interna, 531*f*, 533*t*, 534, 540
Theca-lutein cells, 531*f*, 540
Thermogenins, 45, 147
Thermoreceptors, 586
Thick filaments
in cytoskeleton, 47–48
in skeletal muscle, 185, 187*f*
in smooth muscle, 204
Thick skin, 374, 374*f*, 375*t*, 376*f*
Thin filaments
of cytoskeleton, 46–48, 48*f*–50*f*
in skeletal muscle, 185, 187*f*
in smooth muscle, 204
Thin skin, 374, 374*f*
Thiocyanate ions, 471, 474
Thoracic duct, 307
Thoracolumbar outflow, 237
Thoroughfare channel, 299
Thrombin, 269
Thrombocytopenia, 269*b*
Thromboembolism, 269*b*
Thrombomodulin, and clot formation, 267
Thromboplastin, in clot formation, 267–269
Thrombopoietin, 276*t*
Thrombospondin, 269
Thromboxane A_2, in clot formation, 269
Thromboxanes
in inflammatory response, 136
mast cell release of, 132–133, 136*t*
Thrombus, 269
Thymic corpuscles, 330–331, 330*f*
Thymic-independent antigens, 319
Thymine, 63
Thymocytes, 320
Thymosin, 46, 49*t*
Thymus, 328–331, 329*f*–330*f*, 329*t*
cortex of, 328–330, 329*t*
histophysiology of, 331
medulla of, 330–331
Thymus *(Continued)*
T lymphocytes in, 264
vascular supply to, 331
Thyrocalcitonin, 356*t*, 359
Thyroglobulin, 355, 358, 358*f*
Thyroid follicle, 355, 357*f*
Thyroid gland, 354–360
cellular organization of, 355–357, 357*f*
hormones of. *see* Thyroid hormone(s)
schematic diagram of, 355*f*
Thyroid hormone(s)
functions of, 356*t*
physiological effects of, 359
release of, 358–359
synthesis of, 357–358, 358*f*
and thyrotrophs, 352
Thyroid hormone receptor proteins, nuclear, 359
Thyroid peroxidase, 358
Thyroid-stimulating hormone-releasing hormone, 347, 349*t*–350*t*, 352
Thyroid-stimulating hormone (TSH), 349*t*–350*t*, 352, 355, 356*t*, 358
Thyroidectomy, 359*b*
Thyrotrophs, 352
Thyrotropin, 352
chorionic, 538*t*, 551
Thyroxine (T_4)
in colloid, 355
effect on cartilage, 152*t*
functions of, 355, 356*t*
physiological effects of, 359–360
release of, 358–359
synthesis of, 357–358, 358*f*
and thyrotrophs, 352
Tight junction(s), of capillaries, 296
Tight junction strands, 111*f*, 112–113, 113*f*
Tingible body macrophages, 330
Tissue(s)
defined, 81
types of, 13
Tissue preparation, for light microscopy, 1–3, 2*t*
Tissue thromboplastin, in clot formation, 271
Titin, 188*t*
Tolerance
drug, 493*b*
immunological, 315
Toluidine blue, 2–3
Tomes process, 426–427
Tongue, 429–433, 430*f*
papillae in, 429–433, 430*f*–431*f*
taste buds in, 430–433, 431*f*–432*f*, 432*t*
Tonofibrils, 375, 377*f*
Tonofilaments, 117, 375
Tonsils, 342–343, 400, 430*f*
Tooth. *see* Teeth (tooth)
Tooth germ, 424
Touch, Pacinian corpuscles and, 586
Toxins, detoxification of, in liver, 493
Trabeculae
arachnoid, 241
bony, 161
of lymph nodes, 335, 336*f*
of spleen, 335
Trabecular arteries, 335
Trabecular meshwork, of scleral sulcus, 589
Trabecular veins, 336*f*
Trachea, 396*t*, 401–403, 402*f*
adventitia of, 402*f*, 403
mucosa of, 401–403, 402*f*
submucosa of, 402*f*, 403
trans Golgi network, 32, 33*f*
collagen synthesis in, 88, 89*f*
sorting in, 34–35
in spermiogenesis, 566
Transcription, 65–66, 66*f*
Transcription factors, 71
Transcription factory, 60–62
Transcytosis, 37–38, 299, 300*f*, 459
Transducin, in photoreception, 597
Transduction, 24, 345
Transfer ribonucleic acid (tRNA), 26, 66–67
Transfer vesicles, 30, 32
Transferrin, 340
testicular, 562
Transferrin receptors, 243*b*
Transforming growth factor (TGF), 382
Transient cells, of connective tissue, 127–129, 139
Transient receptor V1 channels, 586
Transition zone, 106–108
Transitional endoplasmic reticulum (TER), 30, 33*f*
Transitional epithelium, 100*t*, 101*f*, 102
Translation, 26–35
Translocators, protein, 29
Transmembrane collagens, 88
Transmembrane linker proteins, 96, 113
Transmembrane proteins, 114
in cell membrane, 16
Transmission electron microscope, 3*f*, 9
Transneuronal degeneration, 247
Transport
active, 20, 20*f*, 22
primary, by Na^+-K^+ pump, 22
secondary, by coupled carrier proteins, 22
along constitutive pathway, 35
anterograde, 34
axonal, 217
antiport, 20*f*, 22
coupled, 20*f*, 22
of lysosomal proteins, 34–35, 35*f*
passive, 20, 20*f*, 22
receptor-mediated, 56, 242
of regulated secretory proteins, 35

Transport *(Continued)*
retrograde, 34
axonal, 217
symport, 20*f*, 22
uniport, 20*f*, 22
via membrane transport proteins, 19–23, 20*f*
Transport vesicles, 33
Transporter, in nuclear pore complex, 56
Triad(s)
in retina, 598
in skeletal muscle, 182, 184*f*–185*f*
Tricarboxylic acid cycle, 44
Trichohyalin granules, 388
Trichromatic cone, 598*b*
Tricuspid valve, 303
Triglycerides
absorption and processing of, 462, 462*f*
in cytoplasm, 46
digestion of, 144
Trigone, of urinary bladder, 525
Trigonum fibrosum, 305
Triiodothyronine (T_3)
in colloid, 355
functions of, 355, 356*t*
physiological effects of, 359–360
release of, 358–359
synthesis of, 357–358, 358*f*
and thyrotrophs, 352
Trisomy, 78*b*
Trisomy 21, 63*b*
Tritium (^3H), in autoradiography, 6, 7*f*
Trophic influence, 247
Trophic relationships, axonal, 217
Trophoblast, 547*f*, 549
Tropocollagen molecules, 86–90, 87*f*
Tropoelastin, 91–92
Tropomodulin, in skeletal muscle, 186*f*, 188, 188*t*
Tropomyosin, 204
in skeletal muscle, 186*f*, 188*t*, 189
in smooth muscle, 205
Troponin
in skeletal muscle, 186*f*, 188*t*
in smooth muscle, 204
Trypsin, 477
Trypsin inhibitor, 477
Tubular glands, 121
of endometrium, 542, 543*f*, 546
simple coiled, 382
Tubular myelin, 411–412
Tubules. *see also* Microtubule(s)
dentinal, 423
of platelets, 266, 266*t*
seminiferous, 559–568, 560*f*, 562*f*–563*f*
uriniferous, 500*f*–501*f*, 501–517, 509*f*, 520*f*
aquaporins in, 522*t*
structure and function of, 523*t*
Tubuli recti, 559, 572*t*, 573
α Tubulin, 48*f*
β Tubulin, 48*f*
γ–Tubulin, 51
Tubulin dimers, 48*f*, 218
γ–Tubulin ring complex, 50
γ-Tubulin rings, 72
Tubuloacinar (tubuloalveolar) glands, 121
compound
of mammary gland, 553
prostatic, 576, 576*f*
salivary, 471
Tubulocisternal network (TC), 445*f*
Tubulovesicular system, of parietal cells, 443, 445*f*
Tumor growth factor β, 486
Tumor necrosis factor (TNF), 538*t*
in apoptosis, 78
neutrophils and, 259
Tumor necrosis factor-α (TNF-α), 146, 147*t*, 540
mast cell release of, 132–133
in small intestine, 456
Tumors. *see* Cancer
Tunic(s), vessel, 287–288
Tunica adventitia, 288, 288*f*
of arterioles, 289*t*, 291
of elastic arteries, 289*t*, 290
of large veins, 302
of lymphatic ducts, 307
of metarteriole, 289*t*
of muscular arteries, 289*t*, 291
thickness of, 294
of veins, 301*t*
Tunica albuginea
of ovary, 530
of penis, 578*f*
of testis, 559
Tunica fibrosa, 587–589
cornea in, 588–589, 588*f*
sclera in, 587–588, 588*f*
Tunica intima, 287–288, 288*f*
of arteriole, 289*t*, 291
of elastic arteries, 289*t*, 290
of lymphatic ducts, 307
of metarteriole, 289*t*
of muscular arteries, 289*t*, 291, 291*f*
of veins, 301*t*
Tunica media, 288
of arterioles, 289*t*, 291, 292*f*
of elastic arteries, 289*t*, 290
of lymphatic ducts, 307
of metarteriole, 289*t*
of muscular arteries, 289*t*, 291
thickness of, 294
of veins, 301*t*
Tunica propria, 561
Tunica vaginalis, 559
Tunica vasculosa, 559, 589–591
choroid in, 588*f*, 589
ciliary body of, 589–590, 592*f*
iris in, 590–591
Turner syndrome, 63*b*
Tympanic cavity, 601–602, 602*f*
Tympanic lip, 608
Tympanic membrane, 602, 602*f*
Tyrosinase, 378–379, 388
Tyrosine
deiodinated, 358
monoiodinated, 358

U

Ubiquitin-activating enzyme, 41
Ubiquitin-conjugating enzymes, 41
Ubiquitin ligases, 41
Ubiquitination, 41
Ulcers, 453*b*
Ultrafiltrate, 510, 518
Ultraviolet light, 379*b*
Ultraviolet rays, 380*b*
Ungated channels, 21
Unilocular fat cells, 131, 144
Unipolar neurons, 219
Uniport transport, 20*f*, 22
Unipotential progenitor cells, 274–277, 281
Unit membrane, 13–15, 18*f*
Unmyelinated axons, 217
Urate oxidase, 40
Urea, 492, 492*b*
Ureters, 499, 500*f*–501*f*, 523–524
Urethra, 499
female, 526
male, 526
Urinary bladder, 499, 524–525, 524*f*–525*f*, 525*b*
Urinary incontinence, 526*b*
Urinary pole, of kidney, 503, 503*f*, 507*f*, 508
Urinary space, 502, 502*f*–503*f*, 505–508
Urinary system, 499–527
excretory passages of, 523–526
intake and loss of water and, 499
kidney in, 499–522, 500*f*–501*f*
pathological considerations for, 527, 527*f*
Urinary tract infections, 526*b*–527*b*
Urine, 499
formation of, 501, 518–522, 521*t*
Uriniferous tubules, 500*f*–501*f*, 501–517, 509*f*, 520*f*, 522*t*
structure and function of, 523*t*
Urogastrone, 452, 458
Uronic acid, 81
Urticaria pigmentosa, 137*b*
Uterine contractions, 544
Uterine tube, 530*f*
Uterus, 530*f*, 541–544
broad ligament of, 529, 530*f*

Utricle, 604–606, 604*f*–605*f*
Uvea, 588*f*
Uvula, 429, 430*f*

V

Vagina, 530*f*, 552
Vagus nerve, 437
Valves
  anal, 467
  ileocecal, 463
  of Kerckring, 453
  of lymphatic vessel, 331–332
  pyloric, 439
  of veins, 302
Varices, esophageal, 303*b*
Varicose veins, 303*b*
Vas deferens, 559–560, 560*f*, 572*t*, 575
Vasa recta, 516
  and countercurrent exchange system, 522, 522*f*, 523*t*
  histophysiology of, 522*f*
Vasa vasorum, 288
Vascular compartment, of bone marrow, 272
Vascular (lymphatic) dissemination theory, of endometriosis, 544*b*
Vascular feet, of astrocytes, 220–221
Vascular pole, of kidney, 503, 503*f*
Vascular supply
  to bone marrow, 272
  to kidneys, 500*f*–501*f*, 516–517, 517*f*
  to liver, 481–490, 483*f*–484*f*
  to lungs, 415–416
  to pituitary gland, 346–347, 349*f*
  to placenta, 550*f*
  to salivary glands, 473
  to small intestine, 458
  to spleen, 335–336, 337*f*
  to testes, 559–560
  to thymus, 331
Vascular system. *see* Cardiovascular system; Lymphatic vascular system
Vascular tunic, of eye, 587, 588*f*
Vasectomy, 575*b*
Vasoactive intestinal peptide (VIP), 447*t*, 480*t*, 481
Vasoconstriction, 237
Vasodilation, 293
Vasomotor center, in brain, 293
Vasomotor nerves, 288, 293
Vasomotor tone, 293
Vasopressin
  in blood pressure regulation, 294
  physiological effects of, 349*t*–350*t*, 354
  synthesis of, 353
  in urine formation, 520*f*, 521
Veins, 300–302. *see also specific vein*
  characteristics of, 301*t*
  classification of, 301–302
  defined, 287
  hepatic, 483–484, 483*f*
Veins *(Continued)*
  large, 301–302, 302*f*
  medium, 301
  pulmonary, 303
  small, 301
  valves of, 302
  varicose, 303*b*
Vellus hairs, 386
Velocity-dependent channels, 21
Vena cava, 301, 483*f*
Venae rectae, 516–517
Venous sinusoids, of spleen, 336*f*
Ventilation, 395
  mechanism of, 415
Ventral horns, 239–240
Ventricles, of heart, 303
Venules, 301
  hepatic, 484
  high endothelial, 334
  postcapillary, 333
Vermiform appendix, 467
Vermiform granules, 378
Vermilion zone, 420
Verner-Morrison syndrome, 482*b*
Very-low-density lipoprotein (VLDL), 252*t*, 490–491, 491*f*
Vesicle coat protein AP-2, 230
Vesicular-tubular clusters (VTC), 32
Vesicular zone, of osteoclast, 159, 160*f*
Vessels
  blood. *see* Blood vessels
  lymphatic, 306–307, 306*f*
Vestibular apparatus, 610
Vestibular (balance) mechanism, 583
Vestibular folds, of larynx, 401
Vestibular lip, 608
Vestibular membrane, 607
Vestibule, 553
  of inner ear, 603
  nasal, 395, 396*t*
Vestibulocochlear apparatus. *see* Ear(s)
Viagra (Sildenafil), 581*b*
Vibration, Pacinian corpuscles and, 586
Vibrissae, of nasal cavity, 395
Villin, 48, 108*f*
Villus(i)
  chorionic, 550, 550*f*–551*f*
  free, 551
  of jejunum, 458
  microvilli. *see* Microvilli
  of small intestine, 453, 454*f*–456*f*
Vimentin, 51*t*
  in capillaries, 295
  in nuclear membrane, 55
  of skeletal muscle, 182
  in smooth muscle, 204–205
Vincristine, 76*b*
Vinculin, 48, 50*f*, 113
Virally transformed cells, T-helper cell-mediated killing of, 325–327, 326*f*
Virgin cells, 315
Visceral afferent pathways, 583
Visceral layer
  of Bowman capsule, 502, 503*f*, 504–508, 506*f*–507*f*
  of pericardium, 304
Visceral motor component, 235
Visceral nervous system, 235
Visceral pleura, 415
Visceral reflex, 235*f*
Visceromotor nuclei, 237
Visible mucus, of stomach, 440, 442*f*, 451
Visual purple (rhodopsin), 595
Vitamin(s)
  effect on cartilage, 152, 152*t*
  storage, in liver, 493
Vitamin A, 597
Vitamin A deficiency, bone effects of, 174*t*
Vitamin C deficiency, bone effects of, 174*t*
Vitamin D deficiency
  bone effects of, 174*t*
  cartilage effects of, 152*t*
Vitamin K deficiency, 269*b*
Vitreous body, 588*f*, 591, 592*b*
Vitreous cavity, 591
Vitreous opacities, 592*b*
Vocal fold, 401
Vocal ligament, 401
Volkmann canals, 162*f*, 163, 164*f*
Voltage-gated channels, 21
  calcium ion, 230
  calcium release, 192
  potassium (K^+), 226
  sodium (Na^+), 226
Volume transmission, 231–232
von Ebner glands, 430
von Gierke disease, 46*t*
von Willebrand disease, 290*b*
von Willebrand factor, in clot formation, 267–269

W

W ganglion cells, 599
Wallerian degeneration, 246
Warts, 383*b*
Water
  in cytoplasm, 13
  in gastric juices, 451
Wave of depolarization, 227
Wax glands, 384
Weibel-Palade bodies, 290
Weigert elastic stain, 2*t*, 11*f*
Wharton jelly, 141
White adipose tissue, 131, 143–144
White blood cells (WBCs). *see* Leukocyte(s)
White fibers. *see* Collagen fibers
White matter, 217

White pulp, 336*f*
of spleen, 335
Wilson disease, 490*b*
Wright stains, 2*t*, 12*f*

X

X chromosome, 59–60, 62*f*
X ganglion cells, 599
X-linked recessive disorder, 353*b*
Xeroderma pigmentosum (XP), 380*b*
XO chromosome, 63*b*
XX chromosomes, 59–60
XXY chromosomes, 63*b*, 565*b*
XY chromosomes, 59–60

Y

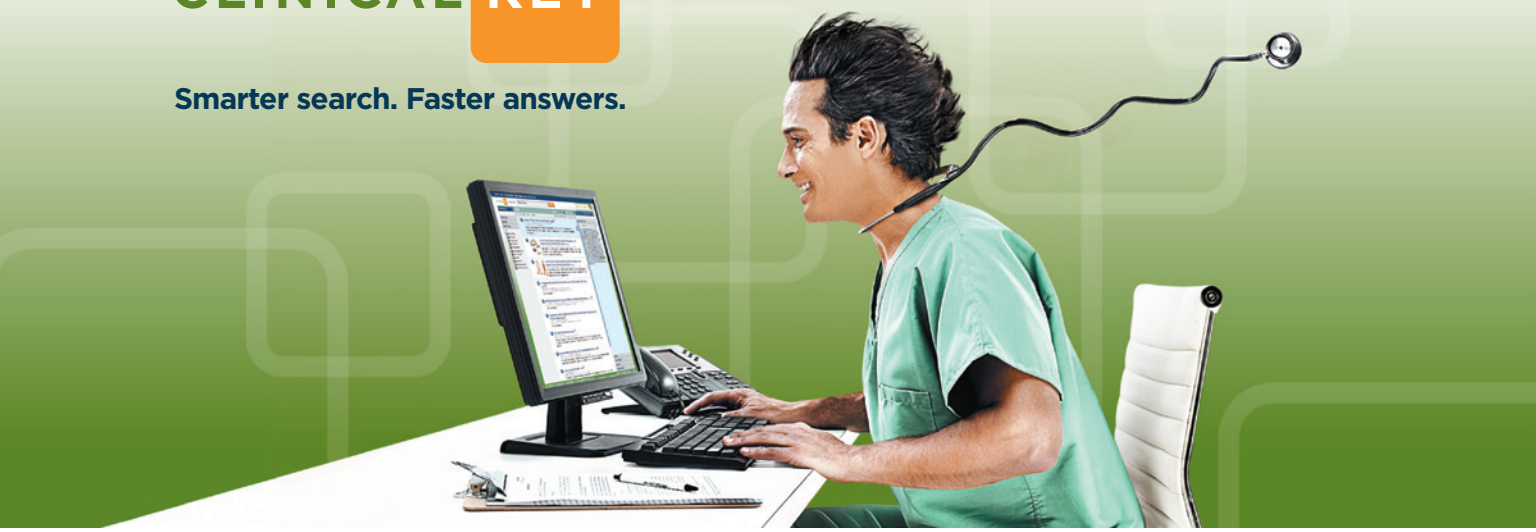
Y chromosomes, 59–60
Y ganglion cells, 599
Yellow bone marrow, 161, 271–272
Yolk sac, 547*f*

Z

Z disk (Z line)
in cardiac muscle, 200*f*
in skeletal muscle, 180, 183*f*–184*f*, 186*f*
Zeis, glands of, 600
Zona arcuata, 607
Zona fasciculata, of suprarenal gland, 364–365
Zona glomerulosa, of suprarenal gland, 364, 364*f*
Zona intermedia, of pituitary gland, 353
Zona pectinata, 607
Zona pellucida, 531*f*, 533*t*, 534, 548
Zona reticularis, of suprarenal gland, 365
Zone(s)
dark, of lymphoid nodule, 333
marginal, of spleen, 337–338, 337*f*
Zonulae adherentes, 111*f*–112*f*, 113–114
Zonulae occludentes, 111–113, 111*f*–113*f*, 233
Zonule fibers, 590
Zonulin, 482*b*
Zygote, 548
Zygotene, 77, 565
Zymogen granules, 476, 477*f*–478*f*
Zymogenic cell, 441*f*–442*f*, 443–444, 446*f*

CLINICAL KEY

Smarter search. Faster answers.



Smarter, Faster Search for Better Patient Care

Unlike a conventional search engine, ClinicalKey is specifically designed to serve doctors by providing three core components:

1 Comprehensive Content

The most current, evidence-based answers available for every medical and surgical specialty.

2 Trusted Answers

Content supplied by Elsevier, the world's leading provider of health and science information.

3 Unrivaled Speed to Answer

Faster, more relevant clinical answers, so you can spend less time searching and more time caring for patients.

Start searching with ClinicalKey today!

Visit ***ClinicalKey.com*** for more information and subscription options.

ELSEVIER