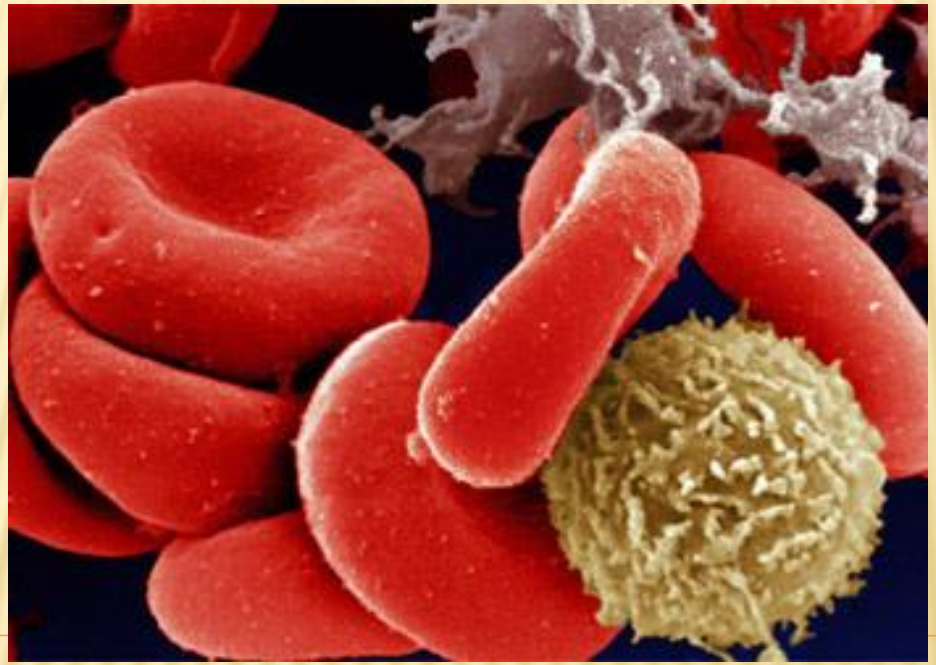
BLOOD PHYSIOLOGY – LECTURE 2

Hematopoiesis. Erythrocytes. Respiratory gases transportation. Blood groups.



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Discipline of Physiology and Fundamental Neurosciences, www.fiziologie.ro

Hematopoiesis: the formation of blood cells

•Hematopoiesis is the process that generates blood cells of all lineages.

•Calculations based on the blood volume and the level and half-life of each type of blood cell in the circulation indicate that each day an adult produces ~ 200 billion erythrocytes, 100 billion leukocytes, and 100 billion platelets.

•These rates can increase by a factor of 10 or more when the demand for blood cells increases.

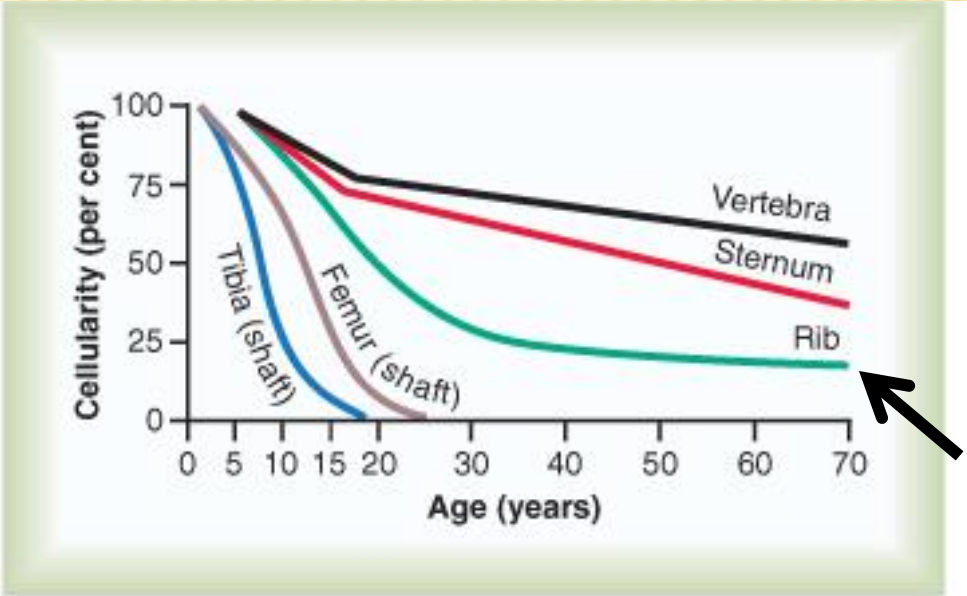
HEMATOPOIESIS: THE FORMATION OF BLOOD CELLS

Stages:

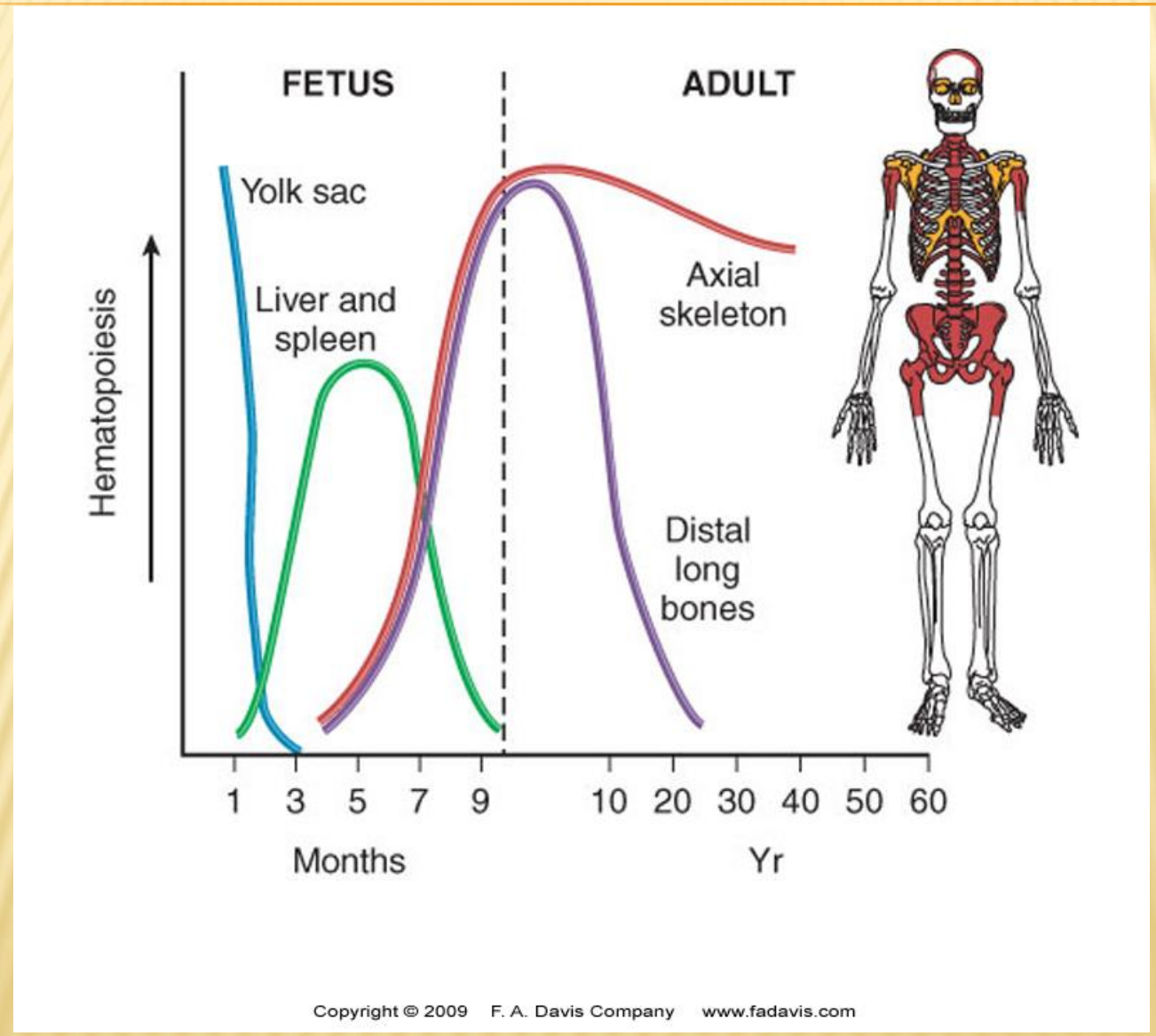
- **embryonic**: up to 2 months – **yolk sac**
- **fetal**: 2-7 months – **liver**, spleen, lymph nodes
  after 3 months – start in the bone marrow
- **after birth**: in the **bone marrow.**

Lymphocytes are also produced in other lymphoid tissues; monocytes also in spleen, mostly when there is an excess demand for bood cell formation (**extramedullary hemopoiesis**).

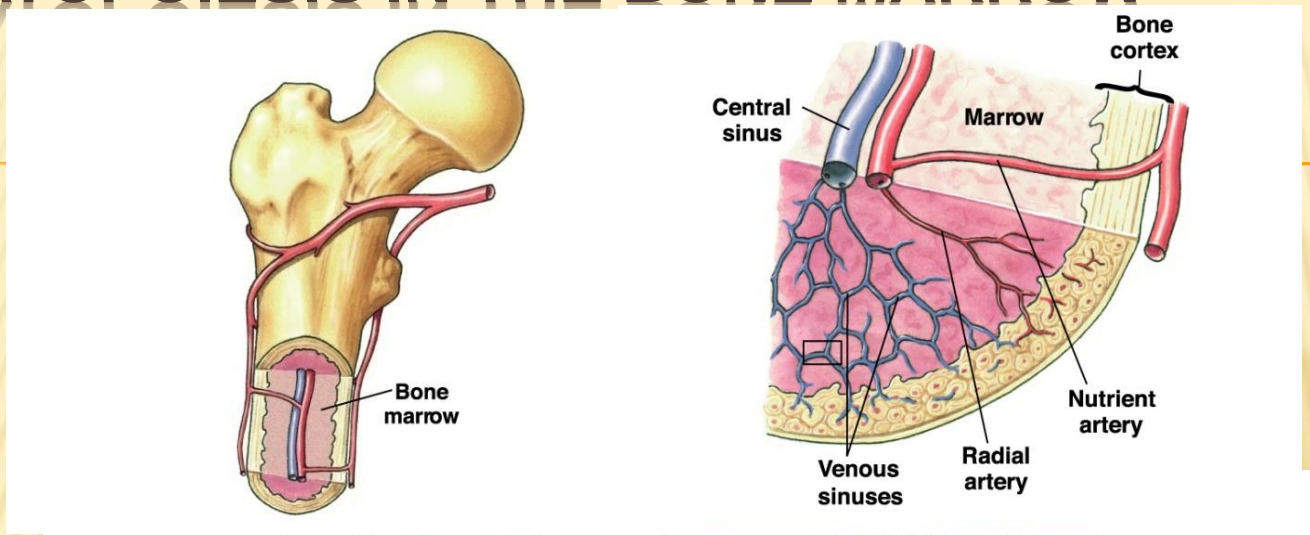
- **up to 5 years**: bone marrows of essentially all bones
- **after 20 years**: bone marrows of the membranous bones (vertebrae, sternum, ribs, iliac); proximal portions of femural bones.

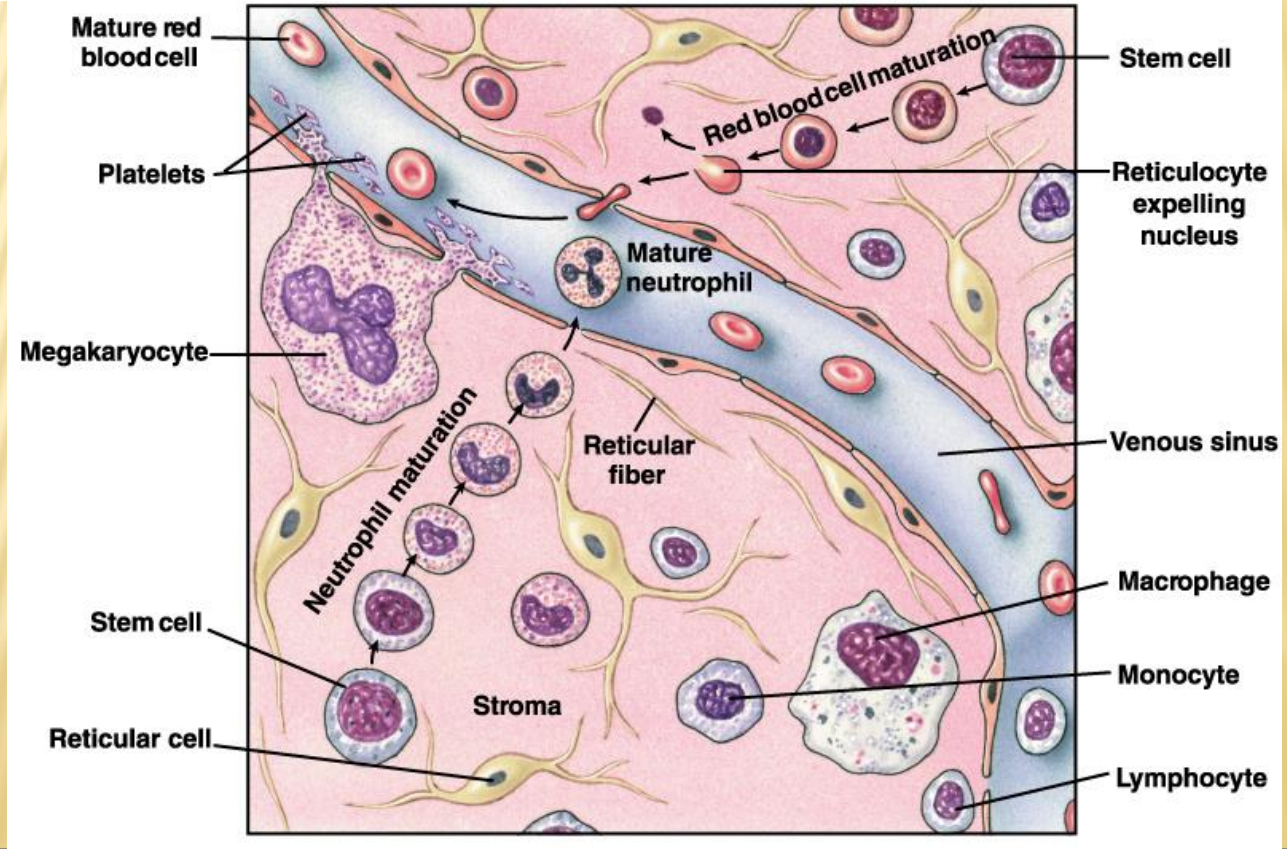


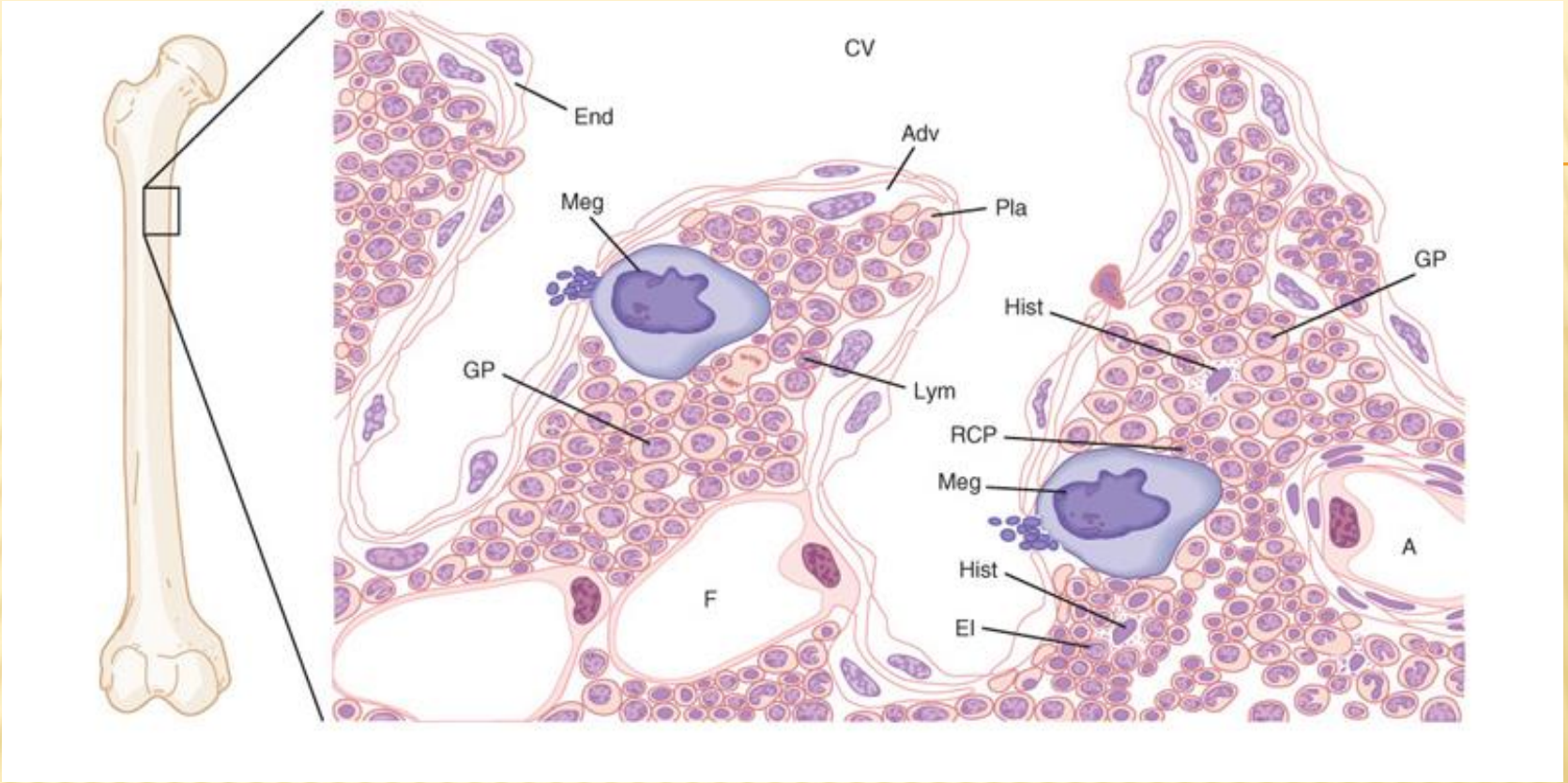
Relative rates of red blood cell production in the bone marrow of different bones at different ages



HEMATOPOIESIS IN THE BONE MARROW







- The blood vessels constitute a barrier, inhibiting immature blood cells from leaving the bone marrow. Only mature blood cells contain the membrane proteins required to attach to and pass the blood vessel endothelium. Hematopoietic stem cells may also cross the bone marrow barrier, and may thus be harvested from blood.
- There is biologic compartmentalization in the bone marrow, in that certain cell types tend to aggregate in specific areas. For instance, erythrocytes, macrophages, and their precursors tend to gather around blood vessels, while granulocytes gather at the borders of the bone marrow.

BONE MARROW

In the adult- bone marrow – is located inside spongy bone

- In a normal adult, ½ of the bone marrow is hematopoietically active (red marrow) and ½ is inactive, fatty marrow (yellow marrow).
- The marrow contains both Erythroid (RBC) and leukocyte (WBC) precursors as well as platlet precursors.
- Early in life most of the marrow is red marrow and it gradually decreases with age to the adult level of 50%.
- In certain pathologic states the bone marrow can increase its activity to 5-10X its normal rate.
  - When this happens, the bone marrow is said to be hyperplastic because it replaces the yellow marrow with red marrow.

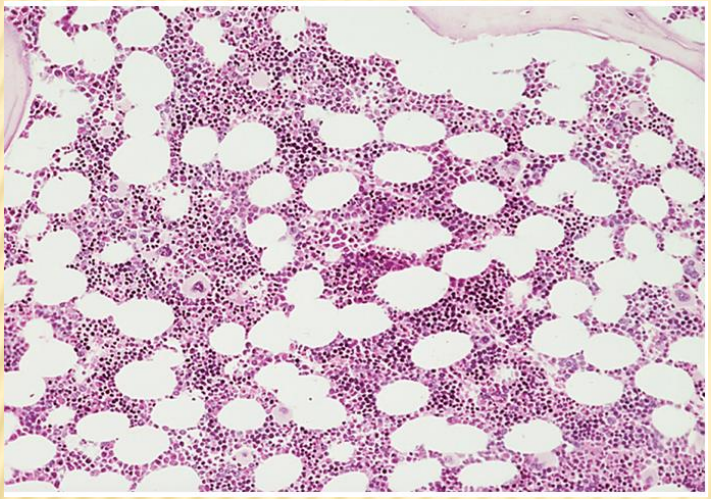
BONE MARROW

- This occurs in conditions where there is increased or ineffective hematopoiesis.
- The degree to which the the bone marrow becomes hyperplastic is related to the severity and duration of the pathologic state.
- Pathologic states that cause this include:
  - Acute blood loss in which there is a temporary replacement of the yellow marrow
  - Severe chronic anemia – erythropoiesis (RBC production) may increase to the extent that the marrow starts to erode the bone itself.
  - Malignant disease – both normal red marrow and fatty marrow may be replaced by proliferating abnormal cells.

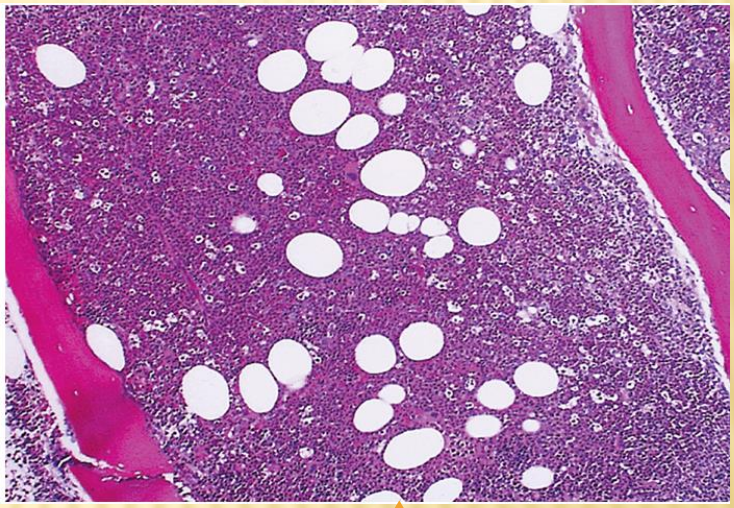
BONE MARROW

- The hematopoietic tissue may also become inactive or hypoplastic. This may be due to:
  - Chemicals
  - Genetics
  - Myeloproliferative disease that replaces hematopoietic tissue with fibrous tissue

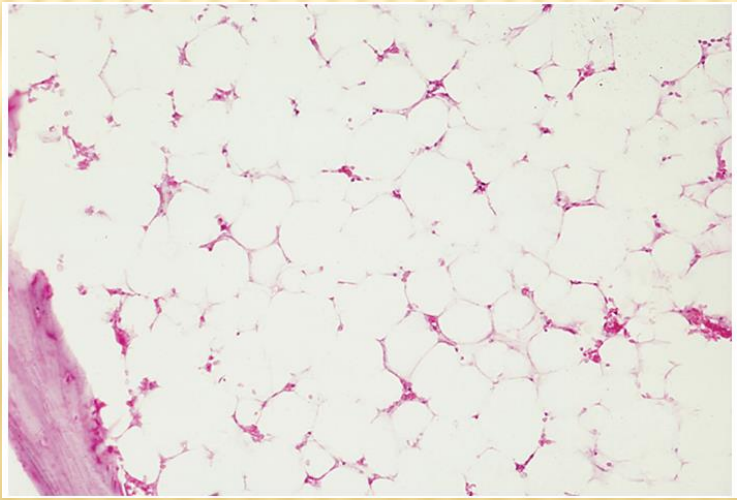
BONE MARROW



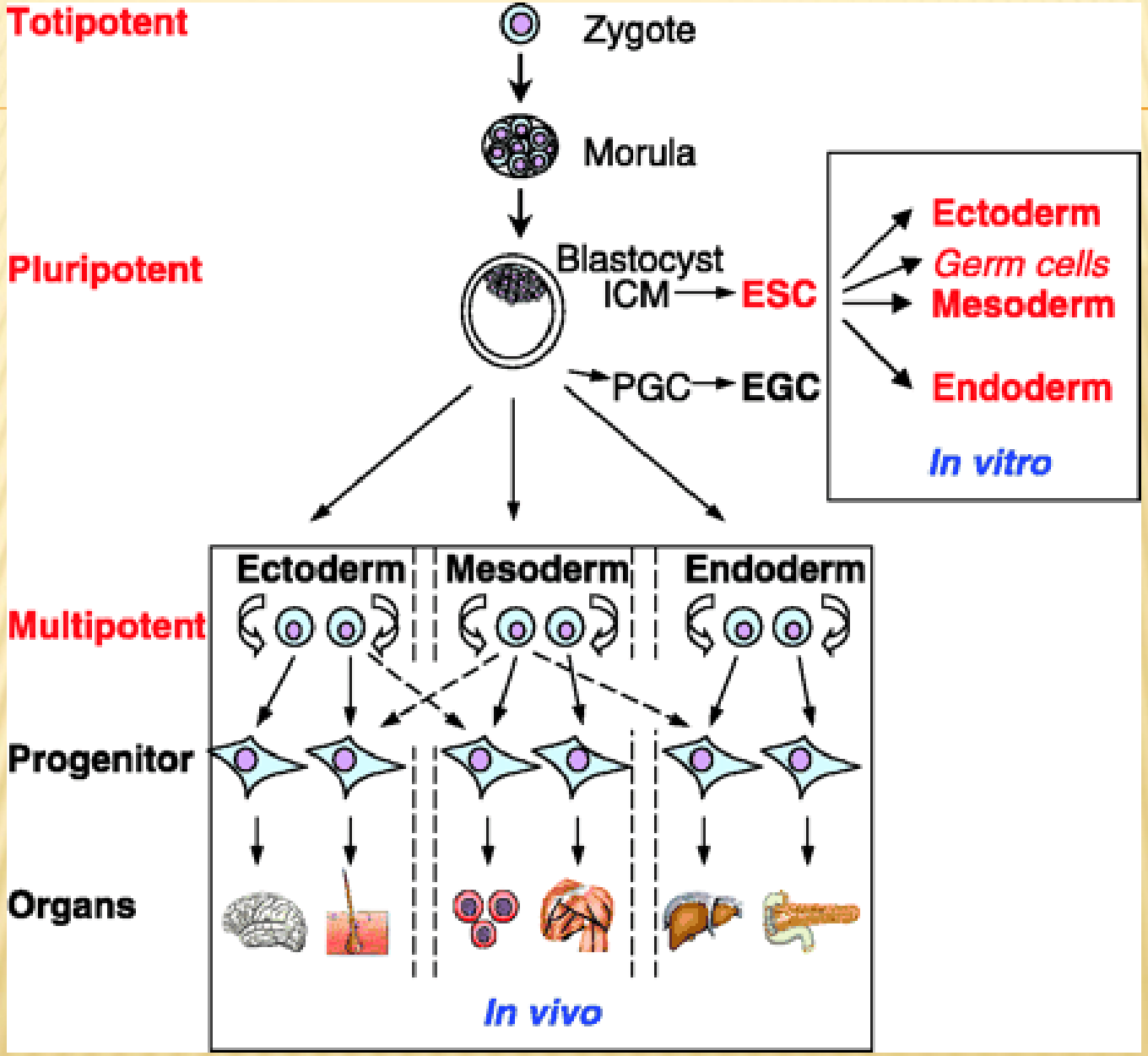
Normal



Hyperplastic



Hypoplastic


Totipotent
Zygote
Morula
Pluripotent
Blastocyst
ICM
ESC
PGC
EGC
Ectoderm
Germ cells
Mesoderm
Endoderm
In vitro
Multipotent
Ectoderm
Mesoderm
Endoderm
Progenitor
Organs
In vivo

Hematopoiesis: stem cells

3 functionally different stem cells:

-pluripotent hematopoietic stem cells (HSC) which can give rise to any blood cell

-multipotent= progenitor

-myeloid stem cells which can give rise to erythrocytes, granulocytes, monocytes and platelets

-**lymphoid stem cells** which gives rise only to lymphocytes

*

STEM CELLS

All stem cells possess 2 fundamental properties:

- **self-renewal**: producing more stem cells through mitosis

  →more stem cells (a mouse that has had all its blood stem cells killed by a lethal dose of radiation can be saved by the injection of a single living stem cell !).

- **differentiation and commitment** into a mature specialized blood cell

Stem cells are attached to osteoblasts lining the inner surface of bone cavities (probably by adherens junctions); their number decreases with age. With time their capability for self-renewal diminishes.

- **All hematopoietic cells arise from a single type of cell – the hematopoietic stem cell (HSC).**

- **HSCs are pluripotent (can give rise to differentiated blood cells of all lineages**

- **HSCs are rare (1 in every 10^5 nucleated cells in adult bone marrow)**

- **Are mainly quiescent, undifferentiated cells that on occasion produce by mitosis 2 kinds of progeny (asymmetric division) :**

  - **more stem cells (HSCs have a limited ability to self-renew)**
  - **progenitor cells that can undergo further divisions and become progressively more differentiated and more restricted in their capacity for self renewal**

Progenitor cells and precursor cells

Cycling stem cells renew and give rise to more mature multipotent progenitor cells, which are more restricted in the offspring which they will generate. This is associated with tremendous amplification in cell number.

Progenitor cells

- **are multipotent**
- **do not self-renew or have only an extremely limited capacity**
- **respond best to multiple cytokines**
- **is a compartment of hematopoiesis that expands the number of cells dramatically**
- **are named by the types of colonies they give rise to:**

The pluripotent HSC gives rise to lymphoid and myeloid stem cells, the latter of which gives rise to CFU-GEMM

CFU-GEMM is a multipotent cell giving rise to granulocyte, erythroid, monocyte, and megakaryocyte colonies

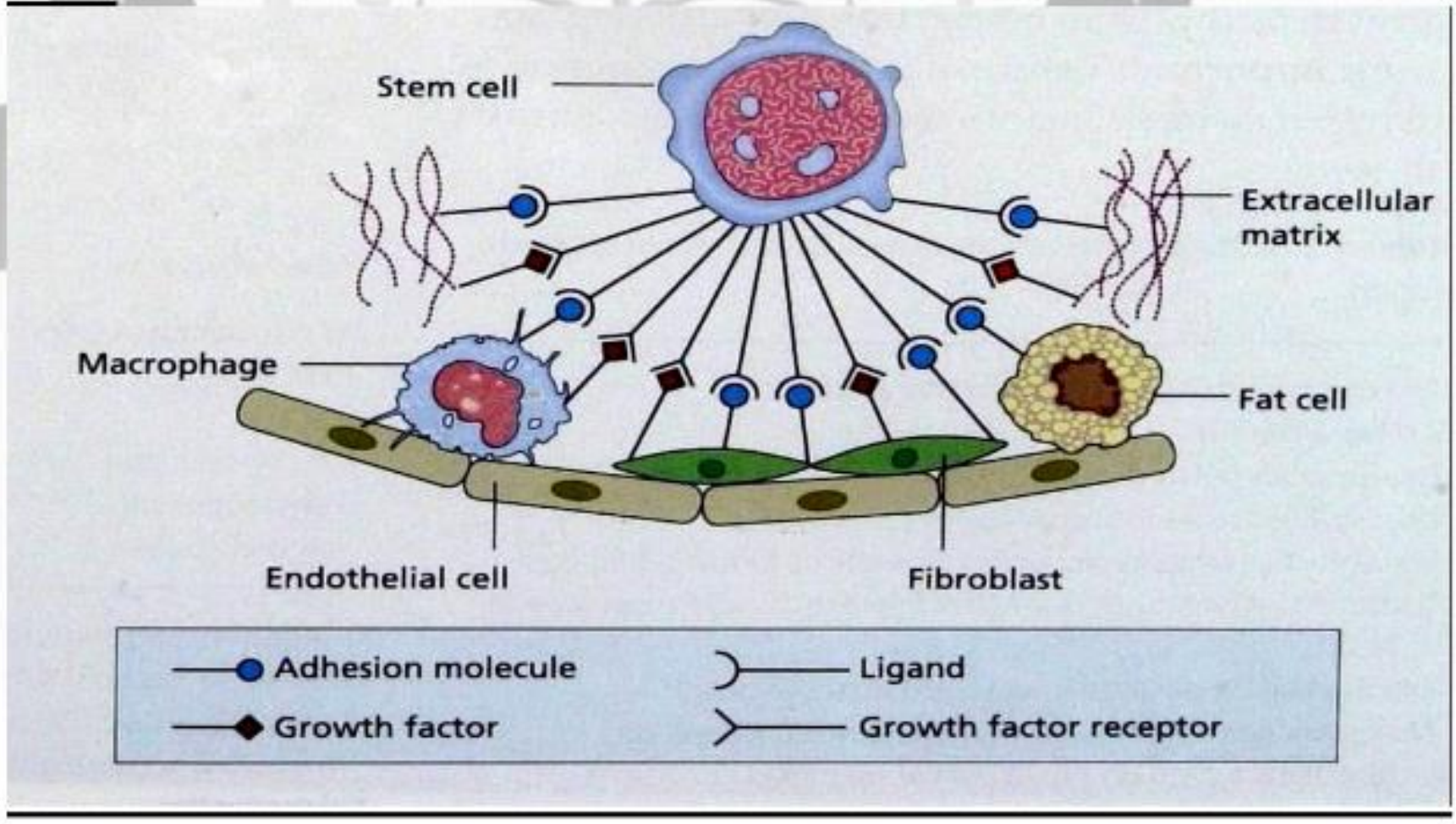
CFU-GM gives rise to both granulocyte and monocyte colonies

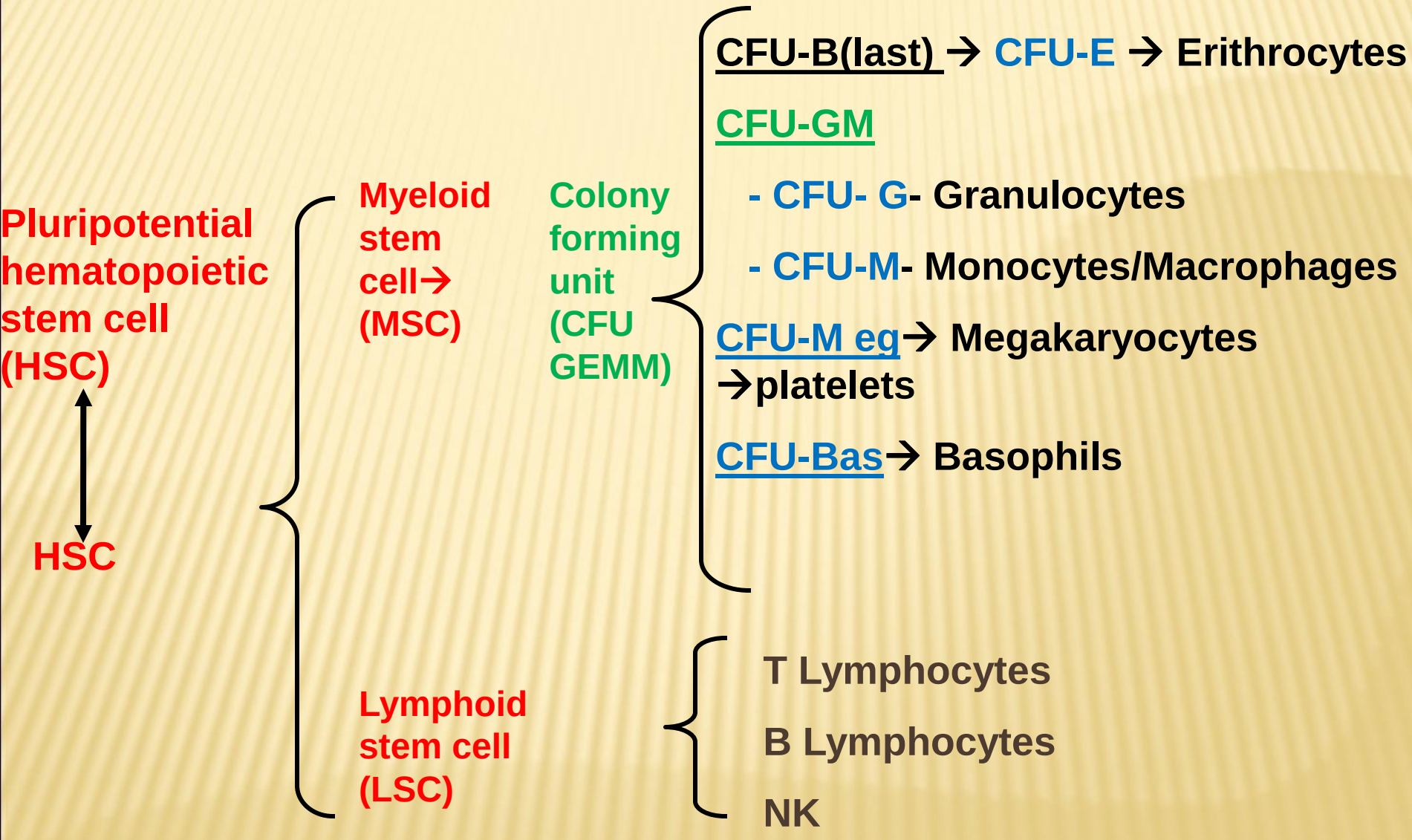
Progenitor cells and precursor cells

Precursor cells (“Committed” precursor cells)

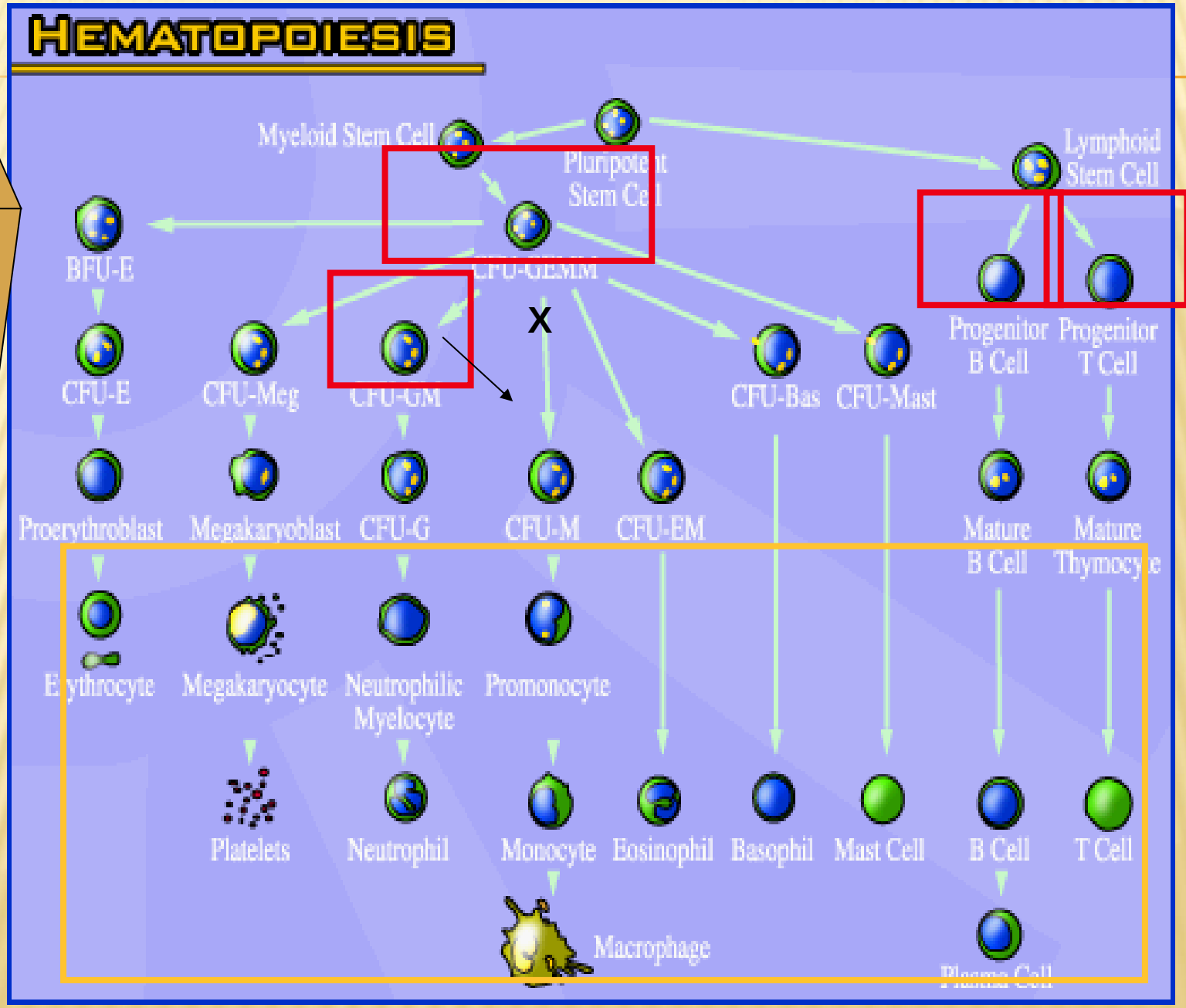
- blast cells committed to unilinear differentiation – much more mature than progenitor cells
- do not self-renew
- respond best to one or 2 cytokines
- still replicate until near terminal differentiation
- progeny increasingly acquire specific differentiation markers and functions
- include CFU-G, CFU-M, CFU-E, and CFU-Baso, giving rise respectively to granulocytes, monocytes, eosinophils, and basophils

- **Interaction of stromal cells, growth factors and haemopoietic cells**



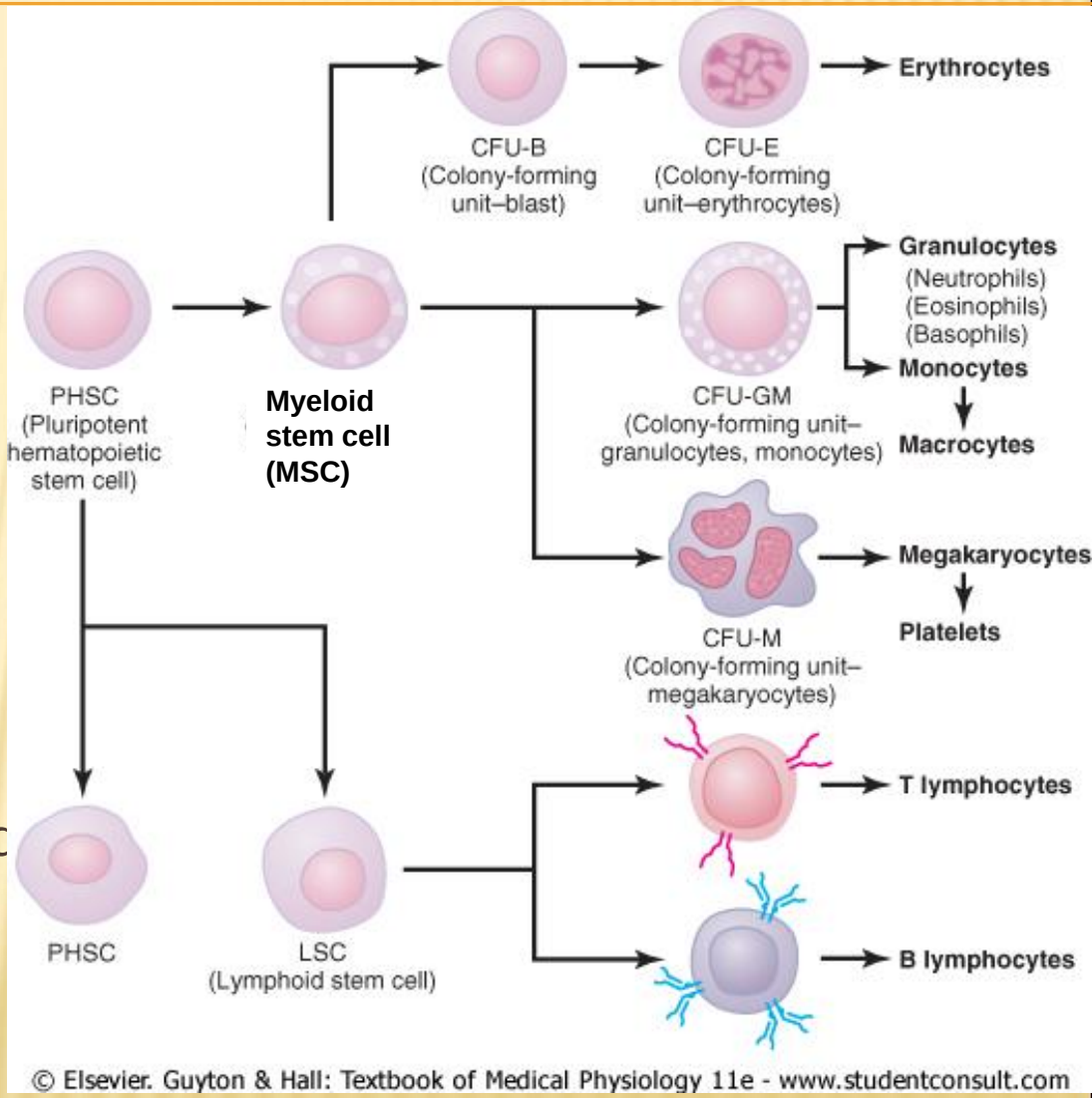

Pluripotential hematopoietic stem cell (HSC)
HSC
Myeloid stem cell→ (MSC)
Lymphoid stem cell (LSC)
Colony forming unit (CFU GEMM)
CFU-B(last) → CFU-E → Erithrocytes
CFU-GM
- CFU- G- Granulocytes
- CFU-M- Monocytes/Macrophages
CFU-M eg→ Megakaryocytes →platelets
CFU-Bas→ Basophils
T Lymphocytes
B Lymphocytes
NK

Hematopoiesis



PROGENITOR CELLS

- the path that is taken, to a ***committed stem cell***, on a particular line of differentiation, is regulated by the need for more of a certain type of blood cell which is, in turn, controlled by grows inducers (cytokines: IL-3, IL-7, IL-11, etc.)
- committed cells → **colony forming units (CFU)**:
  CFU erythrocytes (E)/ granulocytes & monocytes (G/M)/ megakaryocytes (Meg)
- **differentiation inducers** then act on CFUs → final adult blood cells
- hypoxia, infectious diseases: control grows & differentiation inducers



GROWTH AND DIFFERENTIATION INDUCERS (CYTOKINES, HORMONES) FOR THE FORMATION OF BLOOD CELLS …

Interleukin-3 (**IL-3**) promotes **growth** of most of the different types of stem cells

Interleukin-7 (**IL-7**) - major cytokine in stimulating bone marrow stem cells to start down the path leading to the various **lymphocytes** (mostly B cells and T cells).

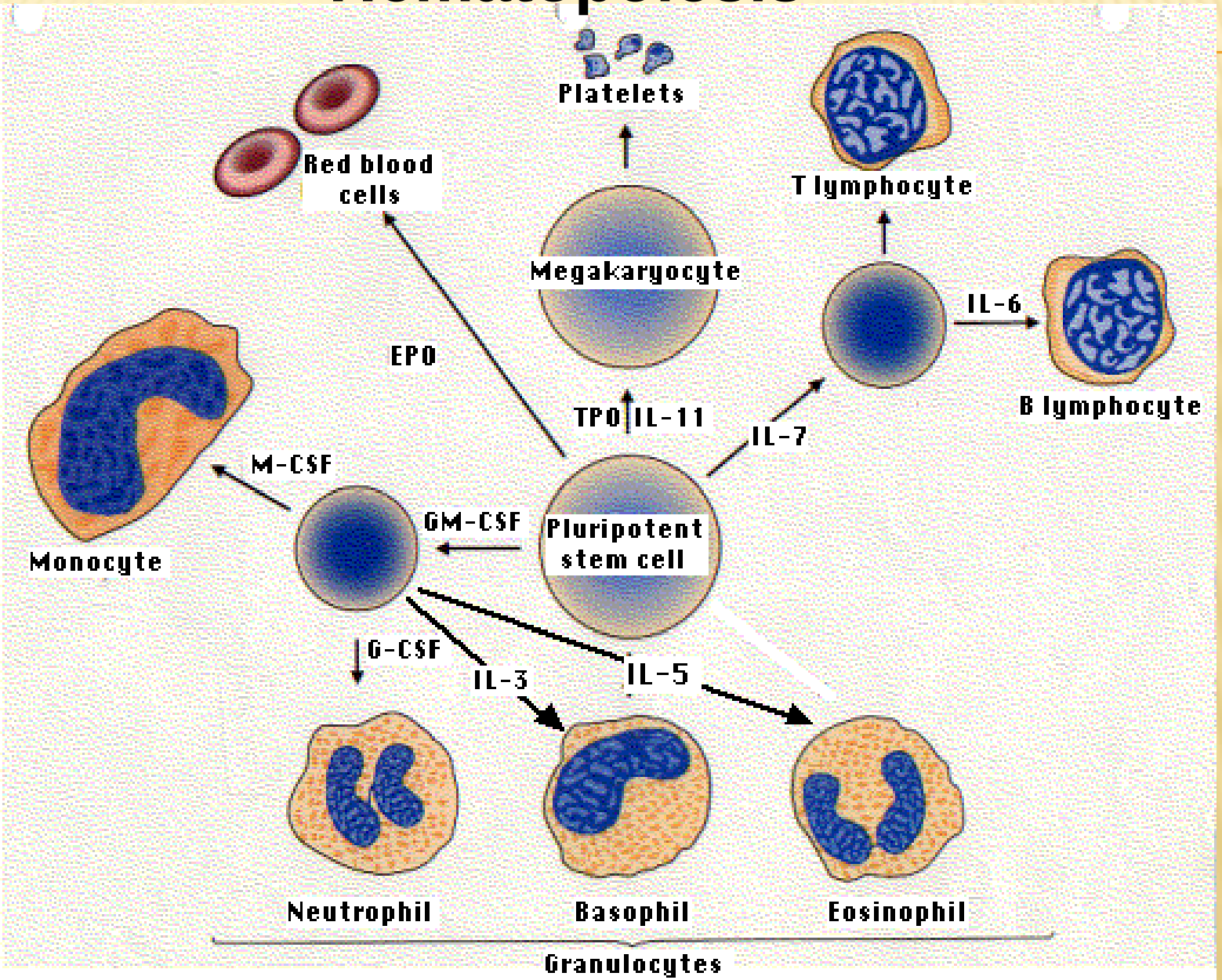
Erythropoietin (**EPO**), produced mostly by the kidneys, enhances the production of **red blood cells**

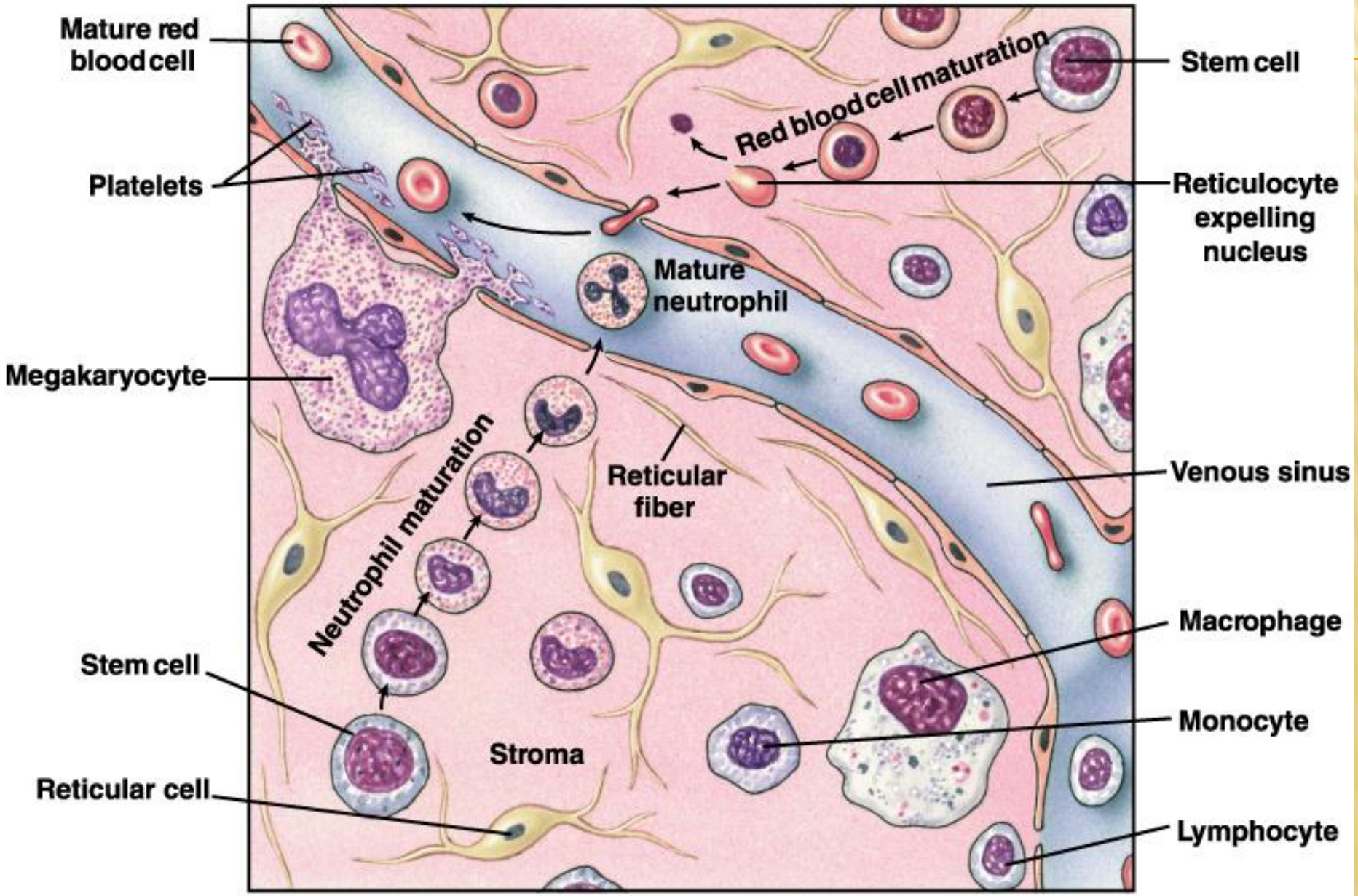
Thrombopoietin (**TPO/** megakaryocyte growth and development factor), assisted by **Interleukin-11** (**IL-11**), stimulates the production of **megakaryocytes**. Their fragmentation produces **platelets**.

Granulocyte-monocyte colony-stimulating factor (**GM-CSF**), as its name suggests, sends cells down the path leading to both those cell types. In due course, one path or the other is taken.

- Under the influence of **granulocyte colony-stimulating factor** (**G-CSF**), they differentiate into **neutrophils**.
- Stimulated by **interleukin-5** (**IL-5**) they develop into **eosinophils**.
- Stimulated by **IL-3** they differentiate into **basophils**
- Stimulated by **macrophage colony-stimulating factor** (**M-CSF**) the granulocyte/macrophage progenitor cells differentiate into **monocytes**, the precursors of **macrophages**.

Hematopoiesis




Mature red blood cell
Platelets
Megakaryocyte
Stem cell
Reticular cell
Stroma
Neutrophil maturation
Reticular fiber
Mature neutrophil
Red blood cell maturation
Stem cell
Reticulocyte expelling nucleus
Venous sinus
Macrophage
Monocyte
Lymphocyte

Blast → Mature

Making lots of protein → Making less protein

Nucleus:	dispersed chromatin	clumped chromatin
Nucleoli:	more	fewer
Cytoplasm:	more ribosomes	fewer ribosomes
	basophilic	acidophilic
Golgi:	acentric nucleus	central nucleus
	(except in RBC precursors)	

Genesis of Red Blood Cells

Bone Marrow

CFU-E

↓

PROERYTHROBLAST

↓

BASOPHIL ERYTHROBLAST (Hb→)

↓

POLYCHROMATOPHIL ERYTHROBLAST

↓

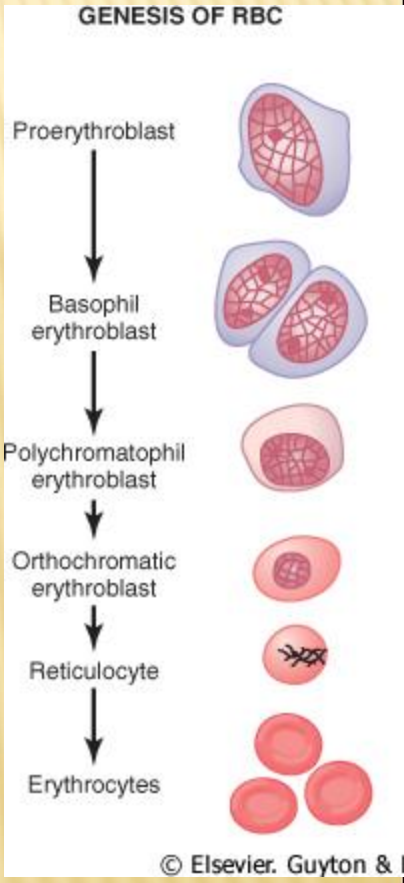
ORTHOCHROMATIC ERYTHROBLAST (34% Hb)

Blood

RETICULOCYTE (remnants of organells)

↓ passes the capillary wall through diapedesis;
mature in 1-2 days;
~1% in the blood

ERYTHROCYTE



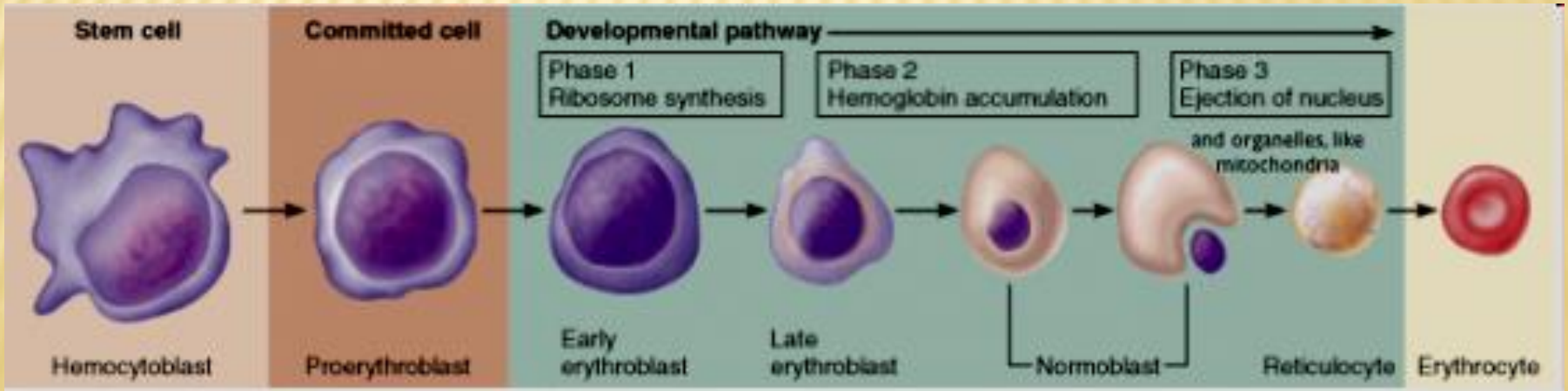
RED BLOOD CELLS (RBC)

- Erythropoiesis: HSC → proerythroblast (1) → progressively smaller normoblasts*→ **reticulocytes** (still RNA…)→ mature erythrocytes (16)

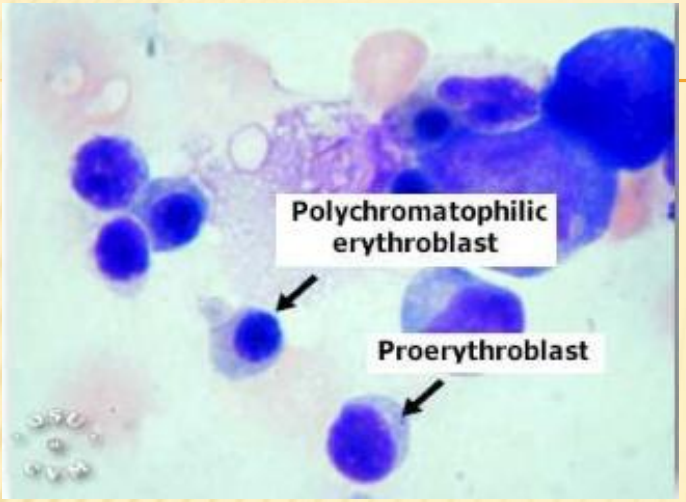
  - nucleus is squeezed out of the cell & ingested by macrophage → RBC are terminally differentiated (never divide).
    - spends 1-2 days in the marrow, circulates 1-2 days in peripheral blood (0.5-1%), mature mainly in the spleen

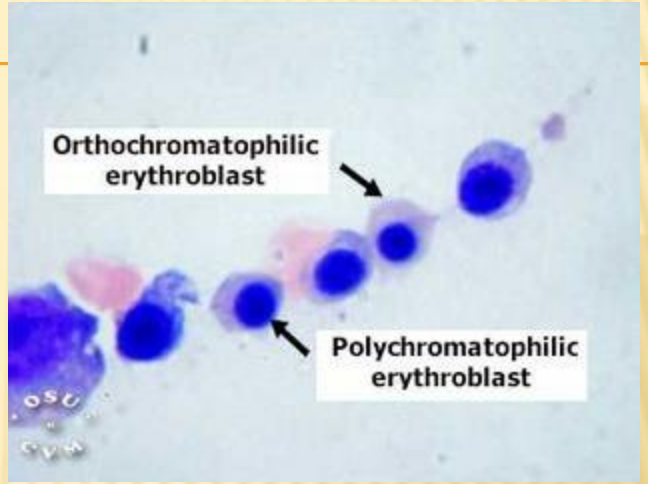
  *Obs: Nucleated cells (normoblasts) appear in the blood in case of extramedullary erythropoiesis or in case of some marrow diseases

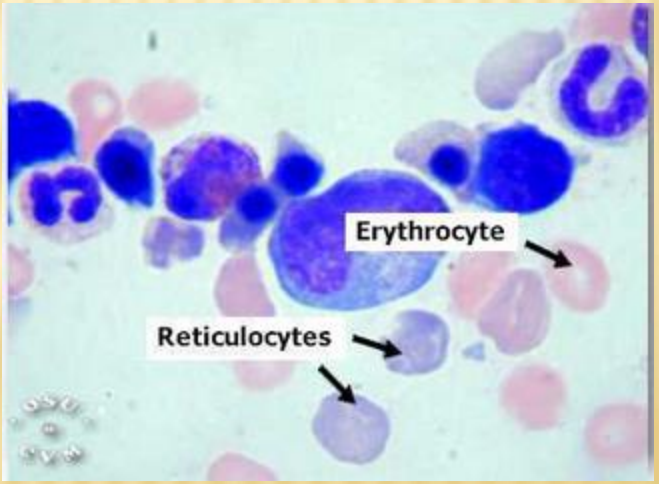
- RBC precursors (up to reticulocytes) manufacture **hemoglobin** until it accounts for some 90% of the dry weight of the cell: **34 g/dl of cell fluid - metabolic limit of cell Hb-forming mechanism**



- **Proerythroblast:** nucleus still rather large, taking up most of the cell; nucleus not condensed; cytoplasm still very blue or basophilic
- **Basophilic erythroblast:** not shown; very difficult to distinguish from the proerythroblast
- **Polychromatophilic erythroblast:** nucleus is more condensed than that of the proerythroblast; cytoplasm less blue, more grayish
- **Orthochromatophilic erythroblast:** nucleus more condensed, smaller than that of previous cells and looks pyknotic by comparison; cytoplasm beginning to take on a more pinkish cast


Polychromatophilic erythroblast
Proerythroblast


Orthochromatophilic erythroblast
Polychromatophilic erythroblast


Erythrocyte
Reticulocytes

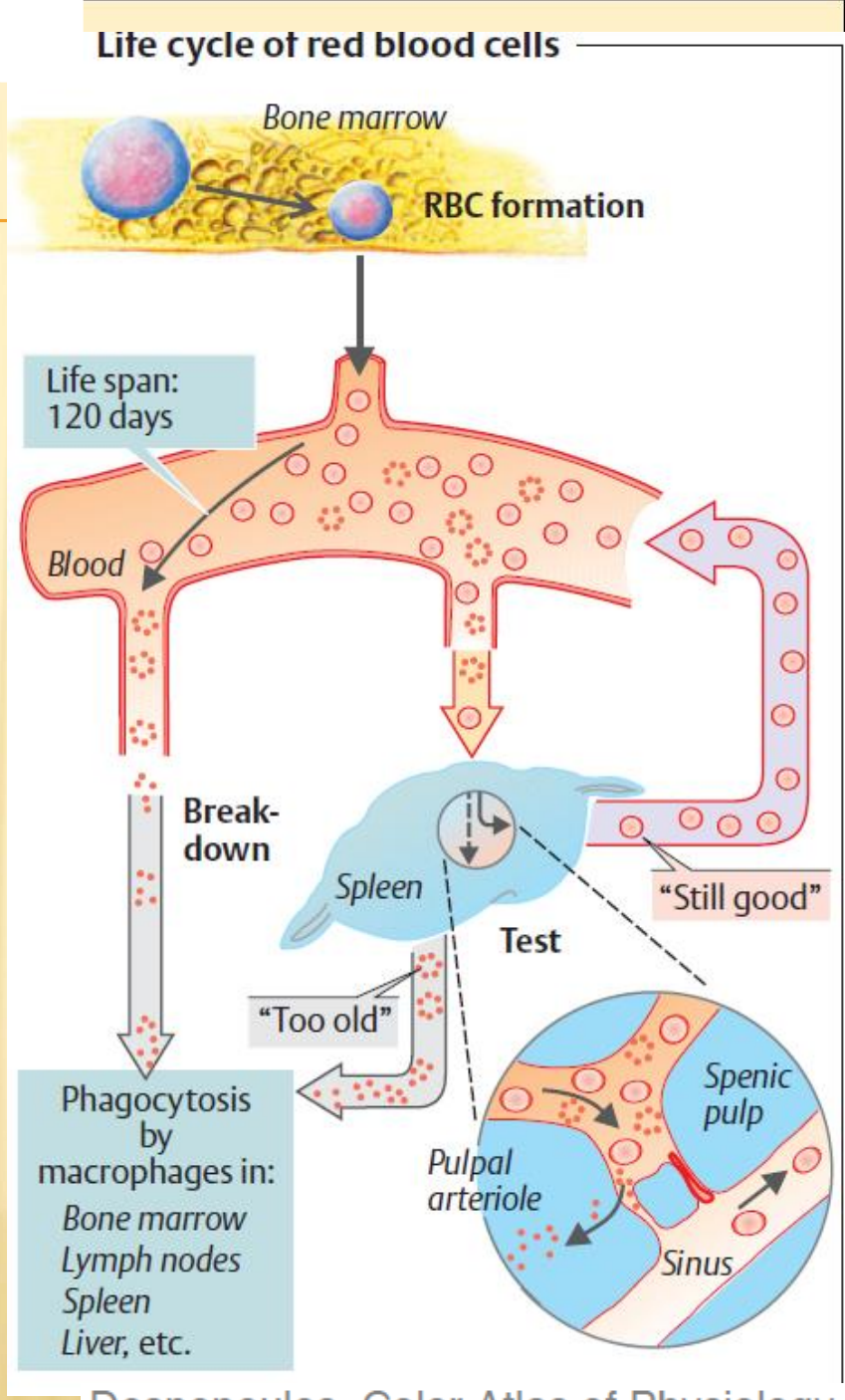
RBCs have a life-span of **120 days** and then are ingested by phagocytic cells in the liver and spleen:

- ~ 3 millions RBCs die & are scavenged/ day

- break up in the bloodstream =*hemolysis*, but the majority are engulfed by macrophages of the reticulo-endothelial system (RES).

- the **iron** from hemoglobin is reused.

- the remainder of the **heme** portion of the molecule is degraded into **bile pigments** and excreted by the liver



Despopoulos, Color Atlas of Physiology

OLD RBC

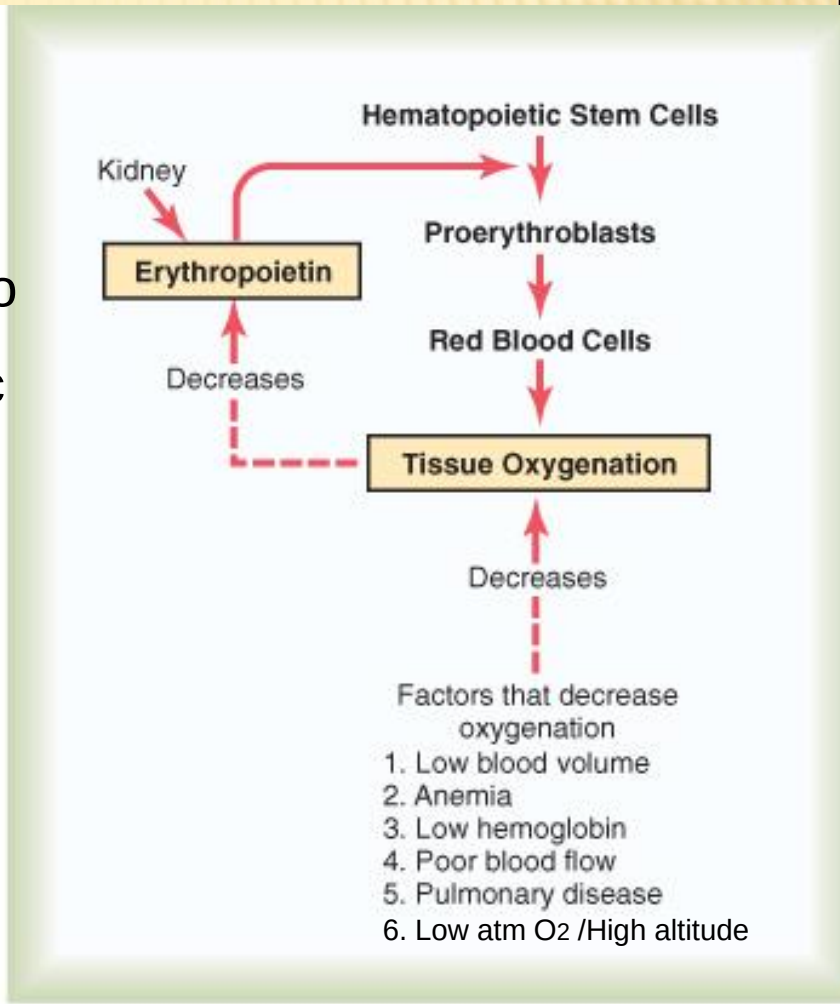
- Spherical
- Low K, Ca, water content, cholesterol, ATP, 2,3 DPG, -SH
- Low glucose use
- Lower enzyme activity
- Low shape changing ability- more fragile
- Higher MetHb (low metHb reductase activity)
- Low sialic acid residues on the membrane
- RBC senescence factor- GP present only on low sialic acid membrane RBC + exposed phosphatidil serine→ signal for macrophages

RBC PHYSIOLOGICAL HEMOLYSIS

- RES- spleen, etc
- Phagocytosis- red spleen pulp
- Results:
  - Globin→AA
  - Hem→ Bilirubin
  - A low amount of HB- bound to haptoglobin→ liver

REGULATION OF RBC GENESIS

- RBC precursors mature in the bone marrow closely attached to a macrophage.
- Time for the transition from proerythroblast to reticulocitocyte: 5 days (just 2 days in anemic stress → macrocytes with >25 % HbF)
- Erythropoiesis rate: 5 x 10^4 RBC / day / µl, stimulated by erythropoietin (EPO)
- Reticulocytes in the blood: 0,5 –1%



ERYTHROPOIETIN

Glycoprotein, MW = 46kDa, $T^{1/2}$ = 6 - 9 hours

Mechanism of action:

↑ the committment of stem cells to proerythroblasts

↑ the differentiation of erythroblastic stages

Synthesized

- 90% kidneys by **peritubular fibroblasts in the renal cortex**
- the rest of 10% formed mainly in the liver- perisinusoidal cells

- stimulus = renal hypoxia

→ ↑ in EPO conc. after minutes to hours, with a maximum level after 24 h

→ after 3 - 5 days: ↑ RBC number. → 10 x

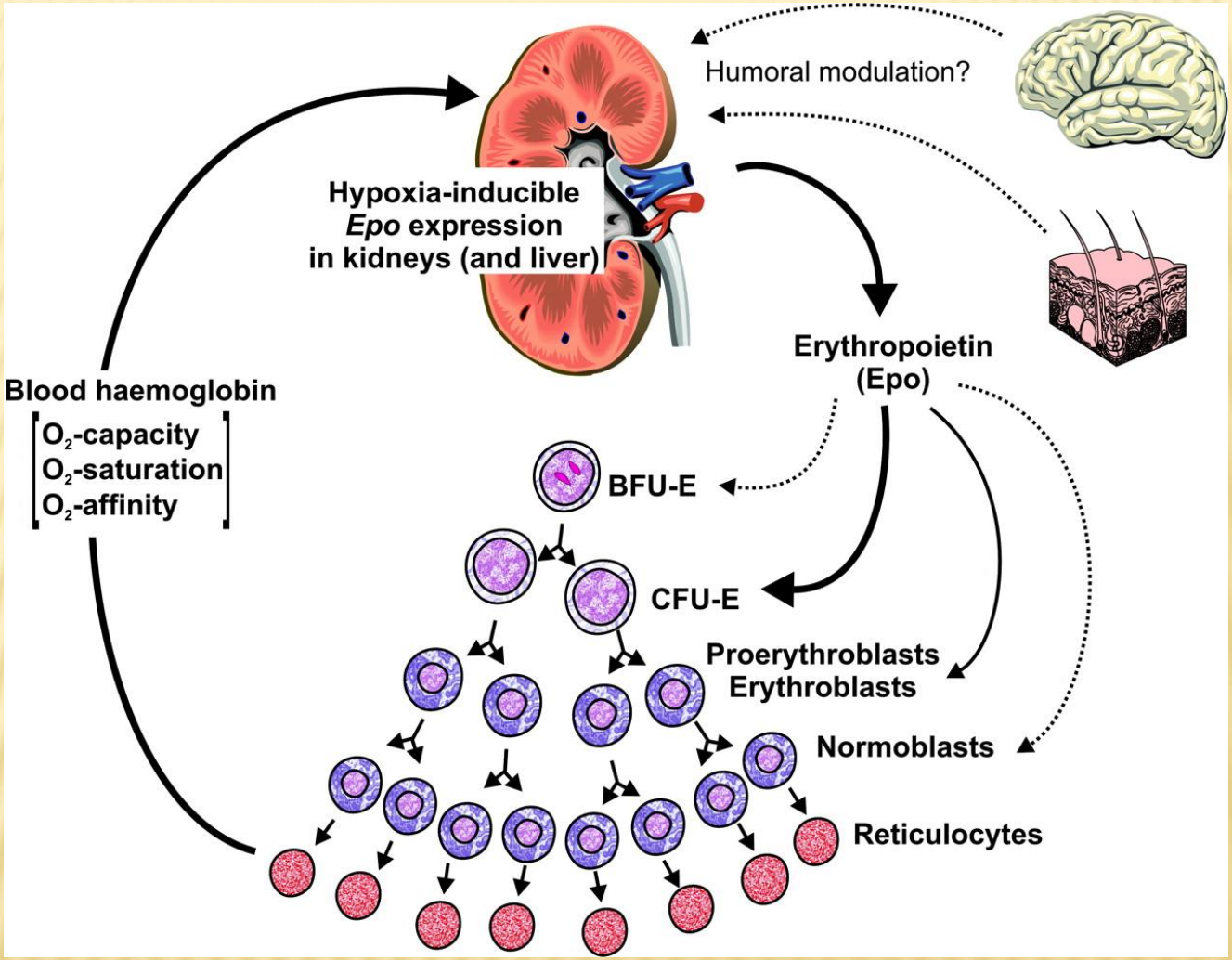
- other non-renal hypoxia sensors act through E, NE, PG, androgens → (+) EPO production

REGULATION OF ERYTHROPOIETIN CONTROL MECHANISM- TRANSCRIPTIONAL LEVEL

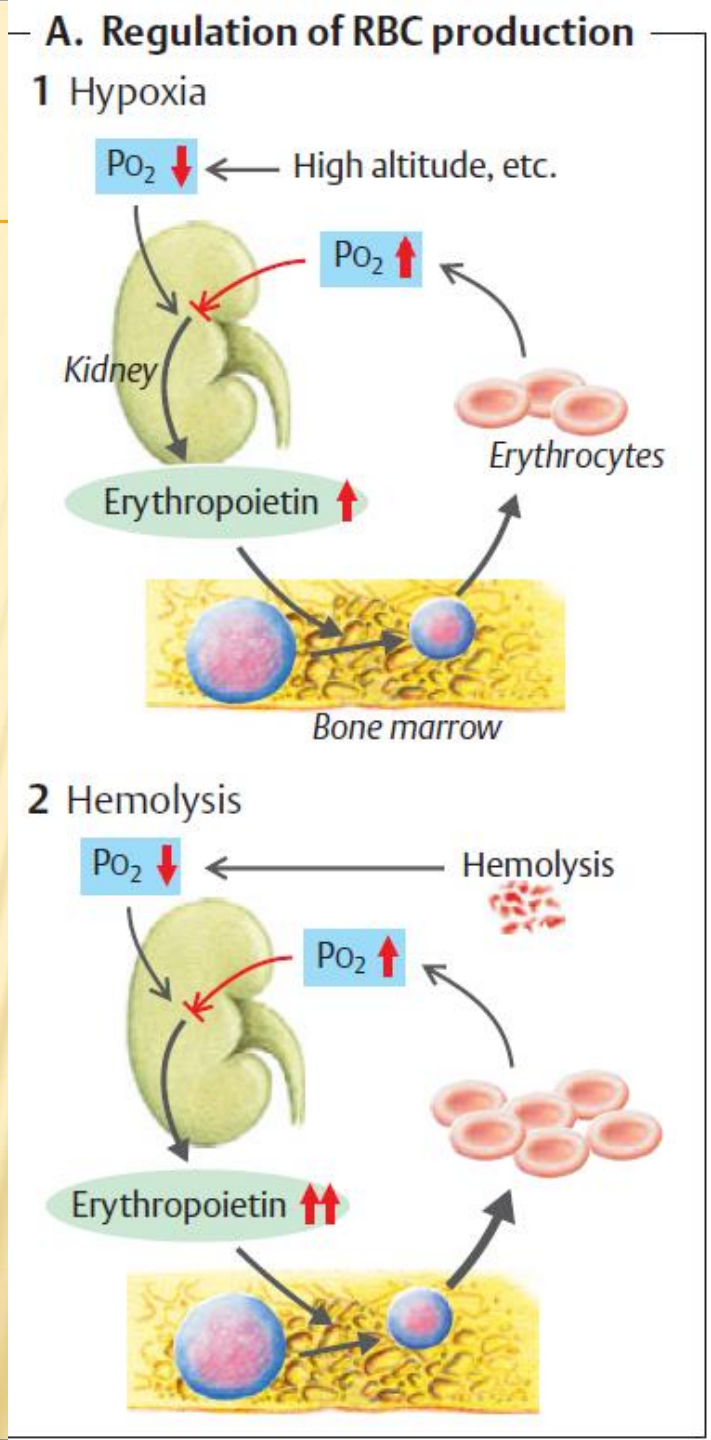
- Hypoxia
  - Attenuates inhibition of Epo promoter GATA 2
  - Promotes availability of hypoxia- inducible transcription factors (HIF-2)- which are inactivated in normoxia by enzymatic hydroxilation

- Therapeutically used: 50 – 300 U / kg, 3 times / week in kidneys diseases, transplant, anemiapulmonary diseases, blood loss…

Figure 1. Diagram of the feedback regulation of erythropoiesis Lack of O2 (hypoxia) is a stimulus for the synthesis of erythropoietin (Epo), primarily in the kidneys.

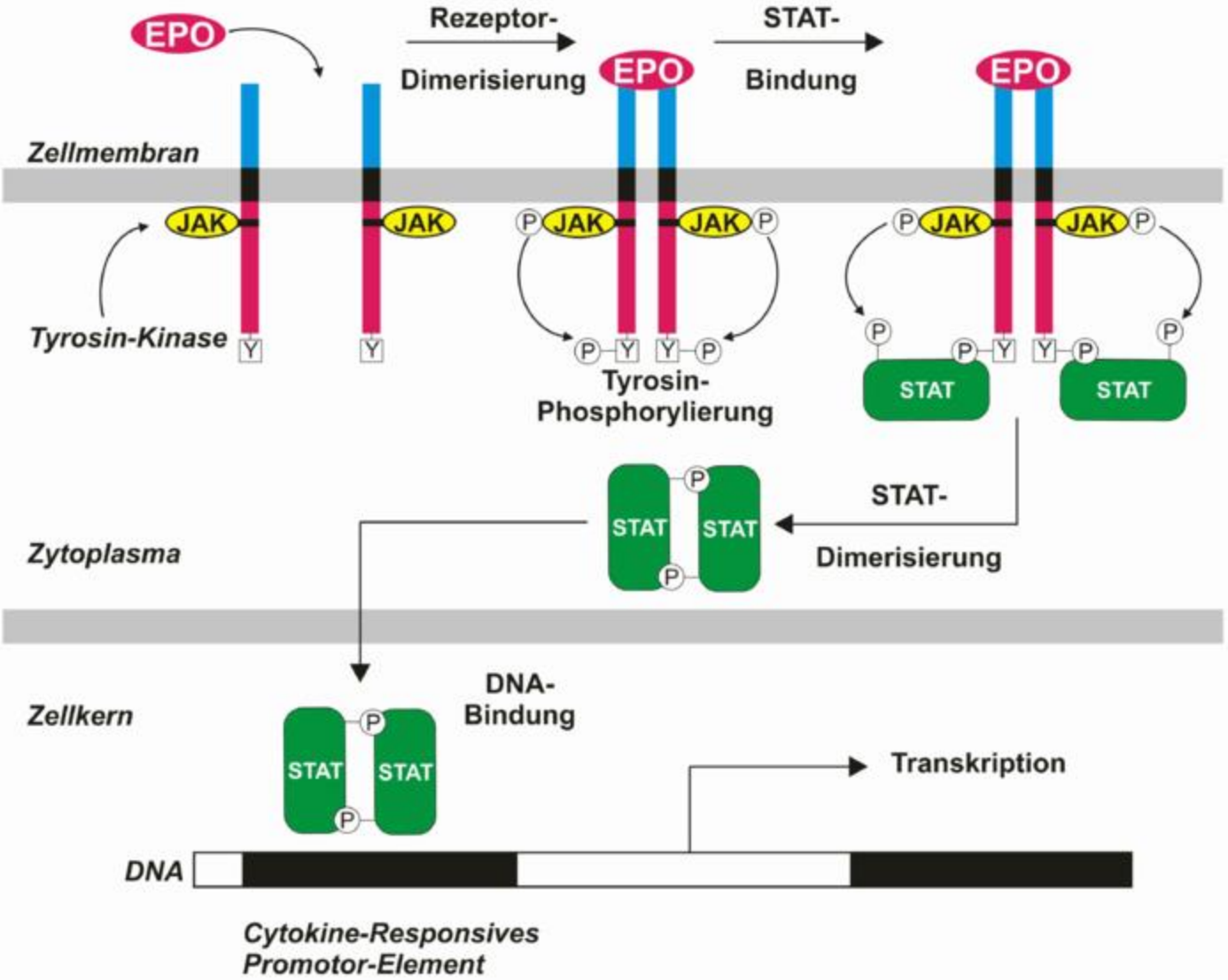


Jelkmann W J Physiol 2011;589:1251-1258


A. Regulation of RBC production
1 Hypoxia
P_{O_2}
High altitude, etc.
P_{O_2}
Kidney
Erythrocytes
Erythropoietin
Bone marrow
2 Hemolysis
P_{O_2}
Hemolysis
P_{O_2}
Erythropoietin

EPO

- EPO-R→ JAK2 tyrosin- kinase activation→ STAT5 transcription factor activation via phosphorilation→ gene activation→RBC precursor differentiation and survival


EPO
Rezeptor-
Dimerisierung
EPO
STAT-
Bindung
EPO
Zellmembran
JAK
JAK
JAK
JAK
JAK
JAK
Tyrosin-Kinase
Y
Y
P
Y
Y
P
Tyrosin-
Phosphorylierung
STAT
STAT
STAT-
Dimerisierung
STAT
STAT
Zytoplasma
DNA-
Bindung
Zellkern
STAT
STAT
Transkription
DNA
Cytokine-Responsives
Promotor-Element

B_{12} VITAMIN & FOLIC ACID

Act on the final maturation of RBC.

Both are essential for DNA synthesis through the formation of an essential DNA building block, thymidine triphosphate

B12 Vitamin:

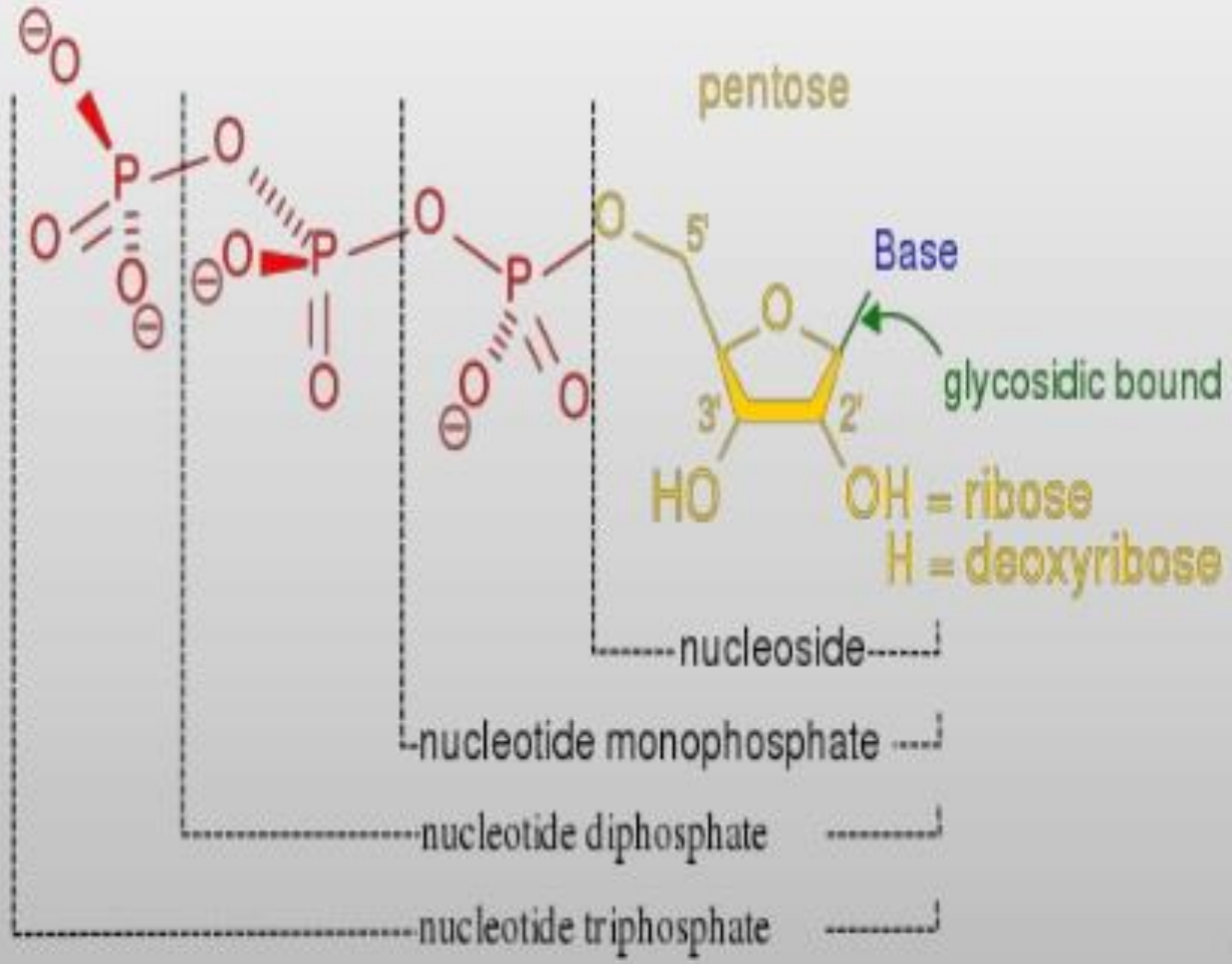
- the body uses 1-3 µg/day of B12 vitamin
- hepatic stores amounts 1000-3000 µg (enough for 3-4 years…)
- intrinsic factor needed for absorption …

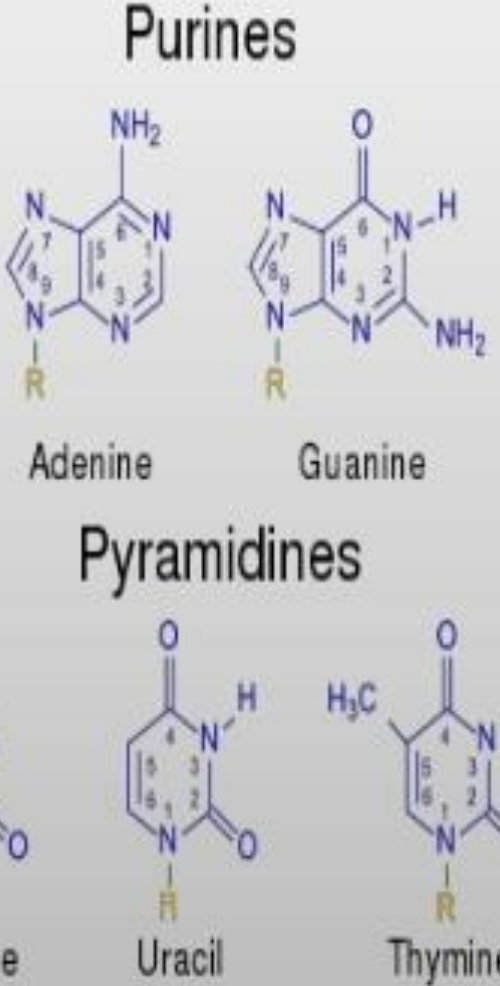
B12 Vit. & folic acid deficiency → proliferation & maturation failure:

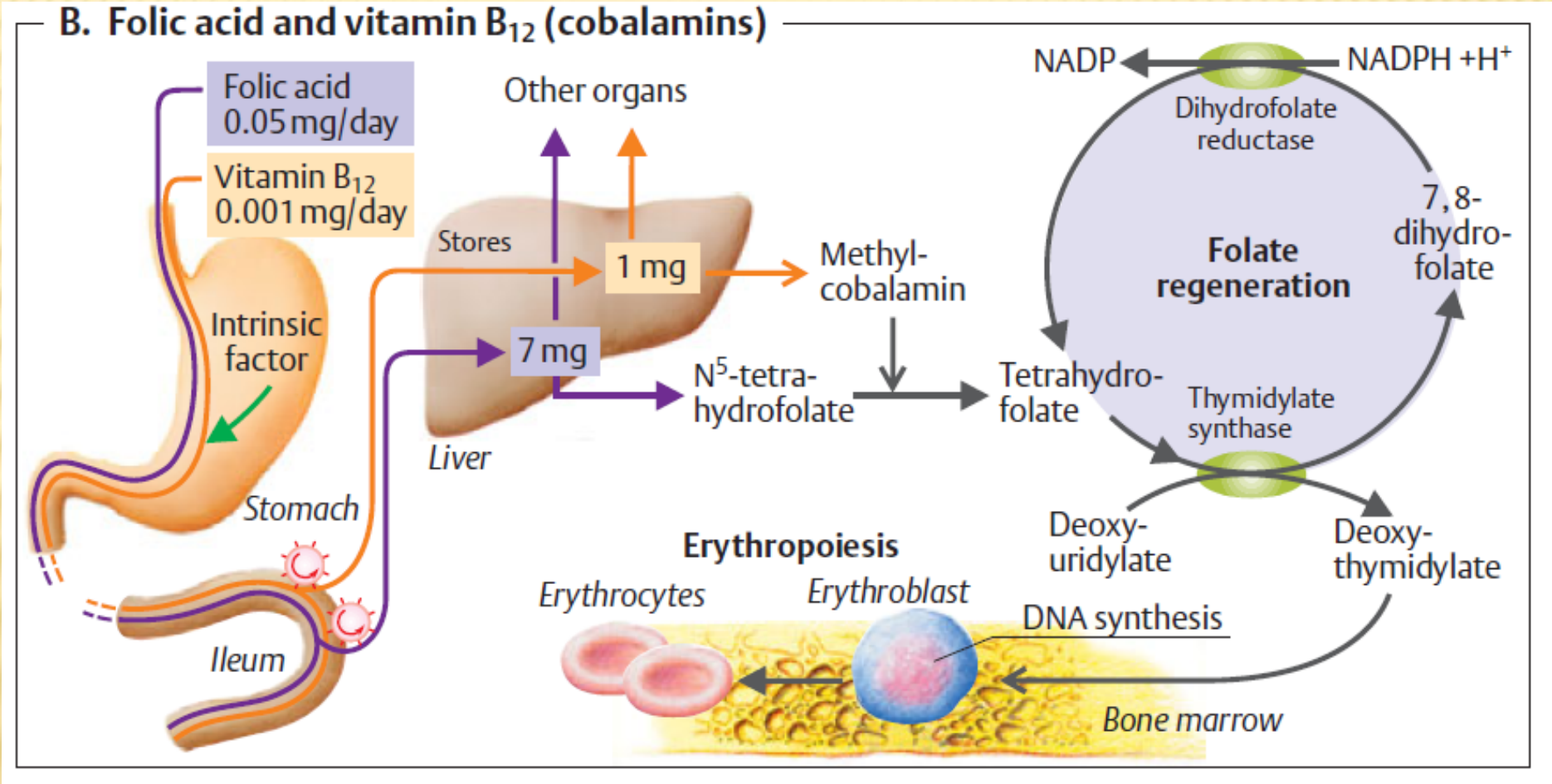
- pernicious anemia → macrocytes (large, oval, fragile)→ short life
- causes:

atrophic gastric mucosa→ intrinsic factor deficiency→ no B12 vit abs./ genetic absence intrinsic factor

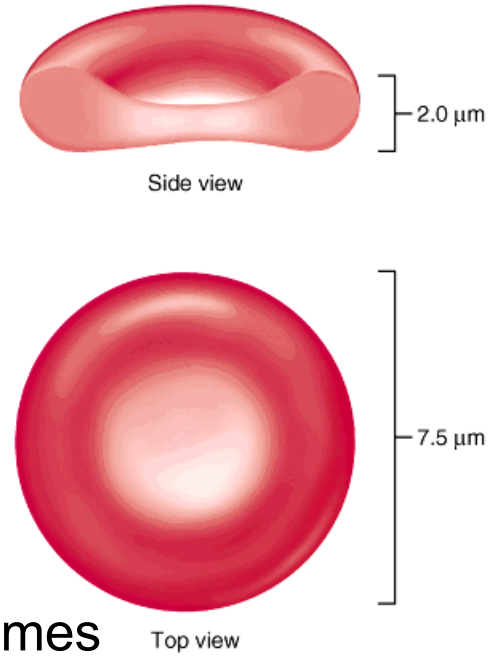
- **Chemically, DNA is a long polymer of simple units called nucleotides, with a backbone made of sugars and phosphate groups joined by ester bonds. Attached to each sugar is one of four types of molecules called bases. It is the sequence of these four bases along the backbone that encodes information.**






B. Folic acid and vitamin B_{12} (cobalamins)
Folic acid
0.05 mg/day
Vitamin B_{12}
0.001 mg/day
Intrinsic
factor
Stomach
Ileum
Other organs
Stores
1 mg
7 mg
Liver
Methyl-
cobalamin
N^5-tetra-
hydrofolate
Tetrahydro-
folate
NADP
NADPH +H^+
Dihydrofolate
reductase
7,8-
dihydro-
folate
Folate
regeneration
Thymidylate
synthase
Deoxy-
uridylate
Deoxy-
thymidylate
Erythropoiesis
Erythrocytes
Erythroblast
DNA synthesis
Bone marrow

RBCs

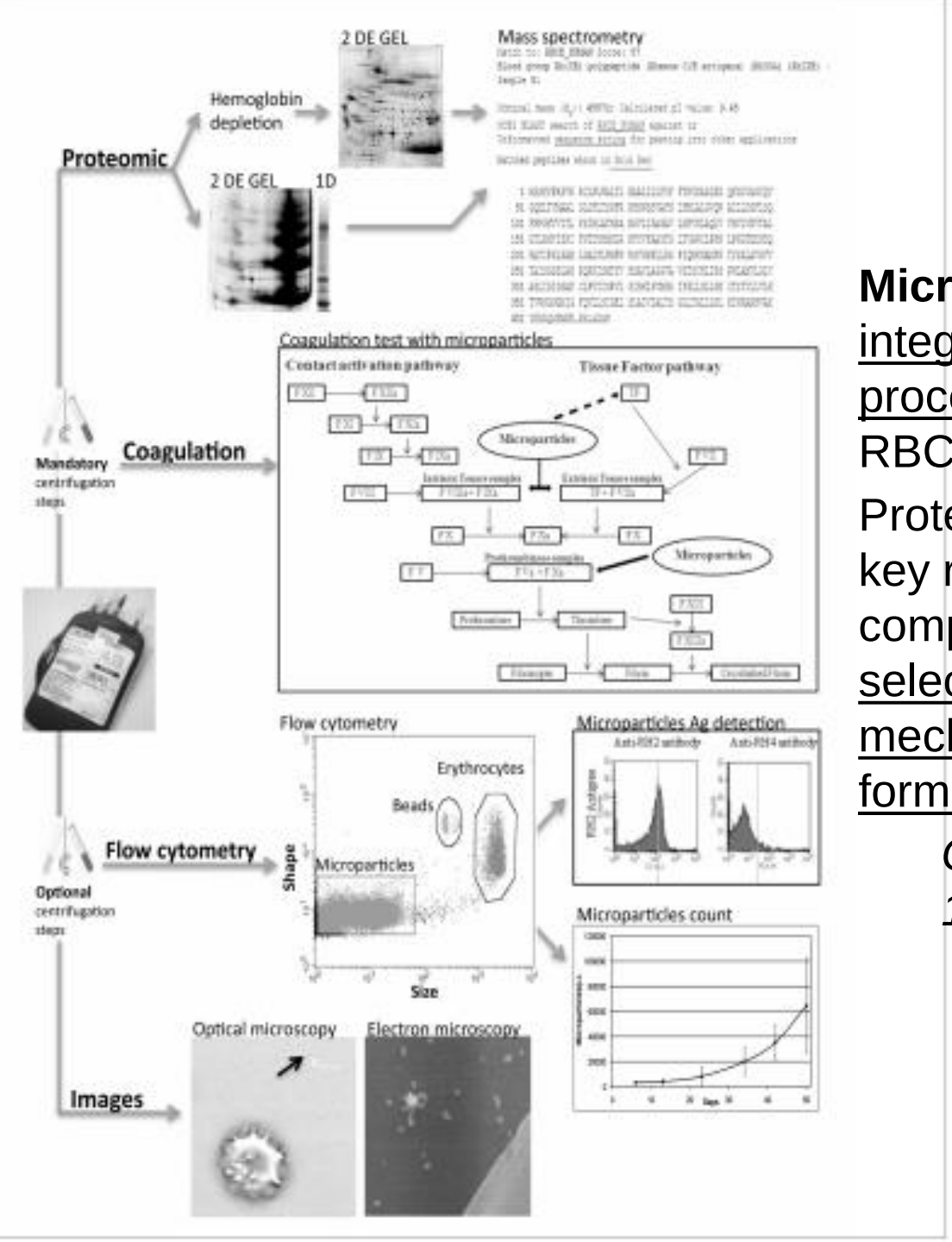


- cells that lack nucleus and membranous organelles
  - no mitochondria, **no aerobic metabolism**;
  - no ribosomes, RNA and protein synthesis,
    no renew of enzymes or membrane components
    → loss of plasma membrane flexibility with age
    – hemolysis in the spleen
- RBCs = simple membranous 'bag' filled with Hb and enzymes
- normal flexible membrane
  → shapes (round, slightly oval, parachute-like in the capillaries)
  → cytoskeleton particularities (attachment proteins, actin, filaments)
- biconcave shape that modify with *osmotic changes*(!), large surface-to-volume ratio to maximize fast gaseous exchange

  Obs: ***poikilocytosis*** refers to an excessive variation in RBC shape
- sizes: 7.8 / 2.5 / 1 µm

  Obs: ***anisocytosis*** refers to excessive variation in RBC size.

  Red cell distribution width (RDW) is a measurement of the size variation of RBCs!
- volume: 80 - 95 μm^3
- surface: 135 μm^2



Microparticles (MP) release is an integral part of the erythrocyte ageing process, preventing early removal of RBCs.

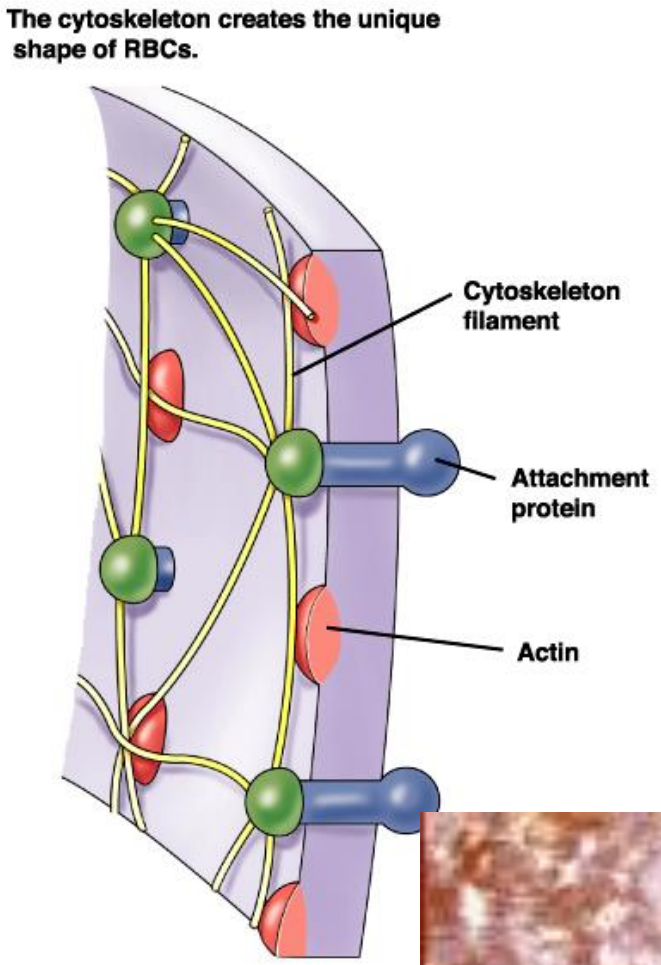
Proteomics analyses have outlined the key role of band 3-ankyrin anchoring complex and the occurrence of selective RBC membrane remodelling mechanisms in microparticles formation.

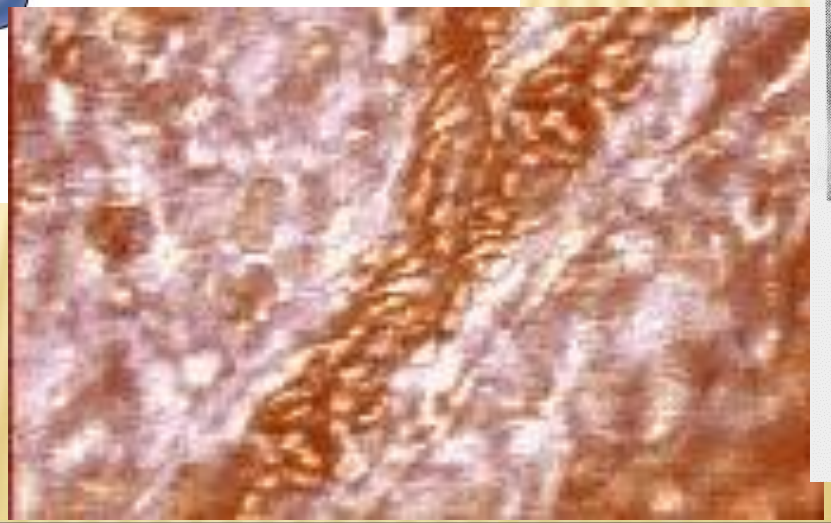
Curr Opin Hematol. 2010 Nov; 17(6):571-7

MICROPARTICLES

- Small phospholipid vesicles, circulating in the blood stream
- They are realeased by cells (ectosomes)
- Contain membrane proteins from their mother cells
- Facilitate cell-cell interactions, induce cell-signaling, promote coagulation, transfer R between 2 cells?

Biconcave shape vs. Membrane flexibility





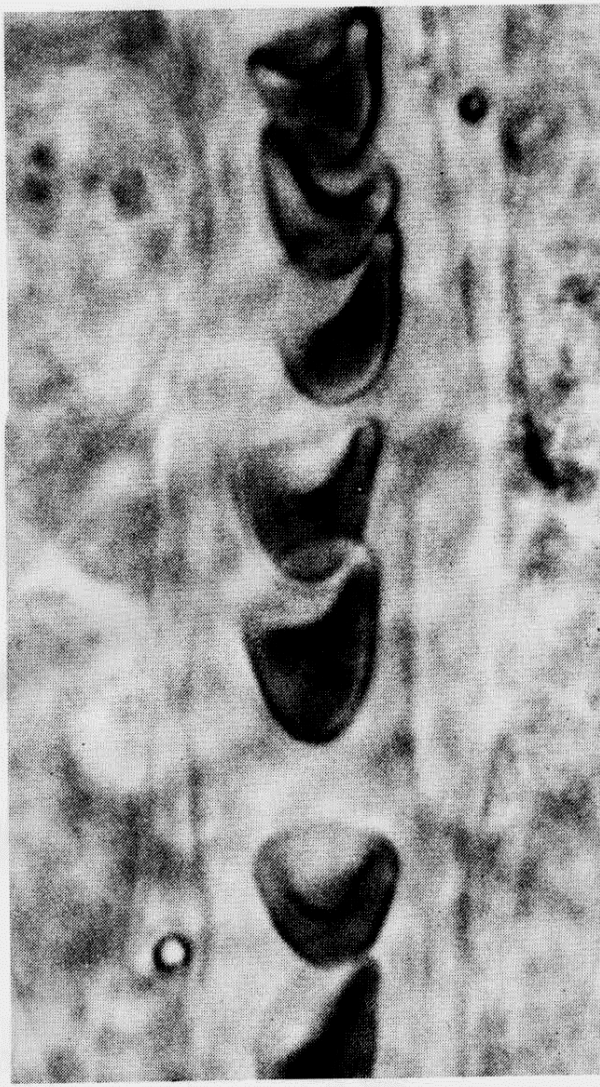


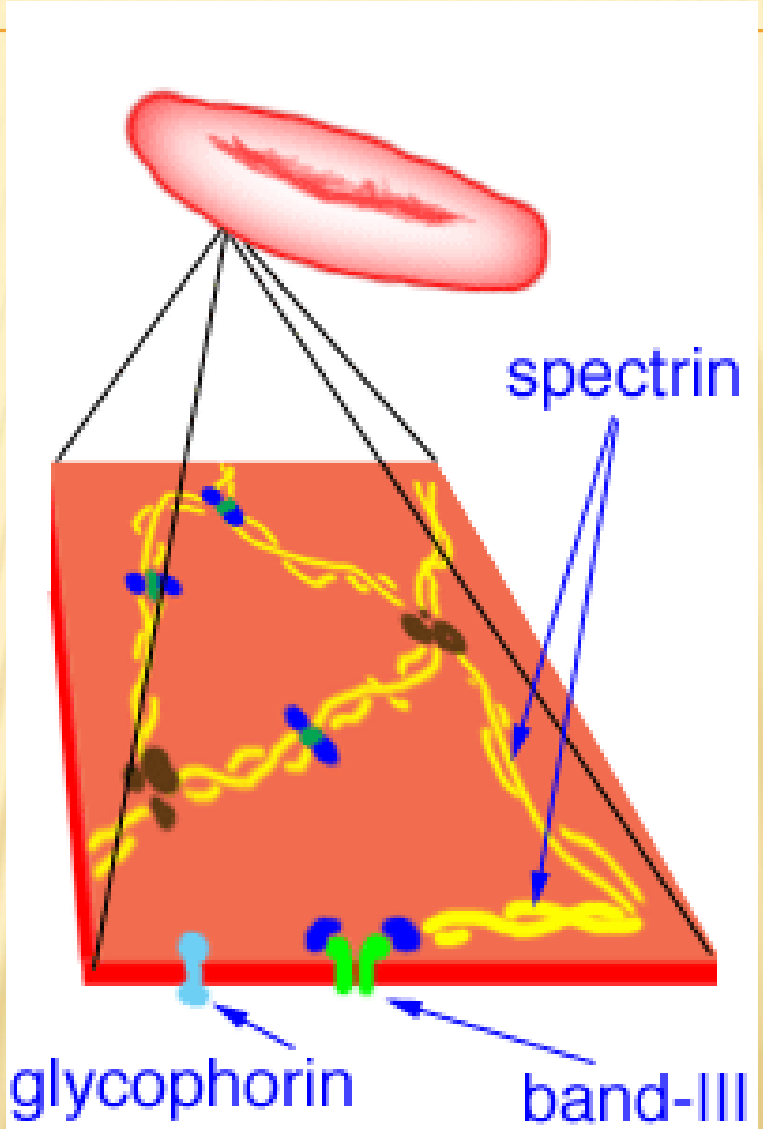
Figure
Erythrocytes flowing through a small blood vessel. [From P. I. Brånemark. *Intravascular Anatomy of Blood Cells in Man* (Basel: S. Karger AG, 1971).]

RBC MEMBRANE

- **Spectrin** is found on the inner, cytoplasmic, side of the cell membrane.
- It is a long, fibrous molecule that makes up about 30% of the total protein.
- It consists of two very large polypeptide chains that wind themselves into a complex that stretches between other protein molecules, such as *actin*, and several other proteins, including the *band-III* type and *ankyrin*.

RBC MEMBRANE

- Together, these proteins appear to form a mesh or network on the inner surface of the red blood cell, which may in turn be responsible for holding the cell in its typical biconcave shape, even as it squeezes through some very, very narrow capillaries in the blood stream

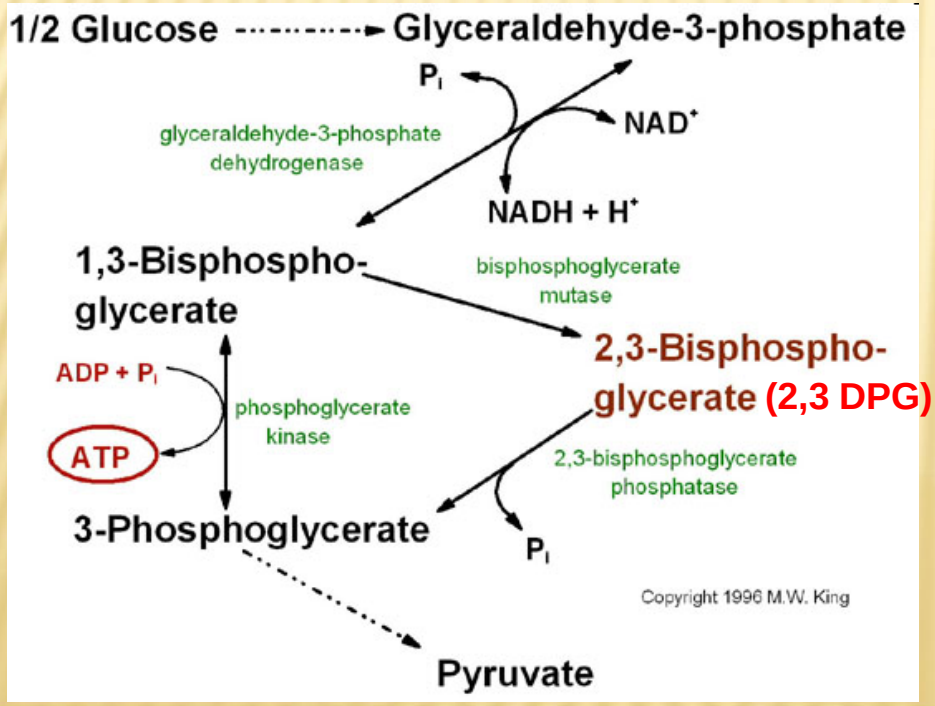

spectrin
glycophorin
band-III

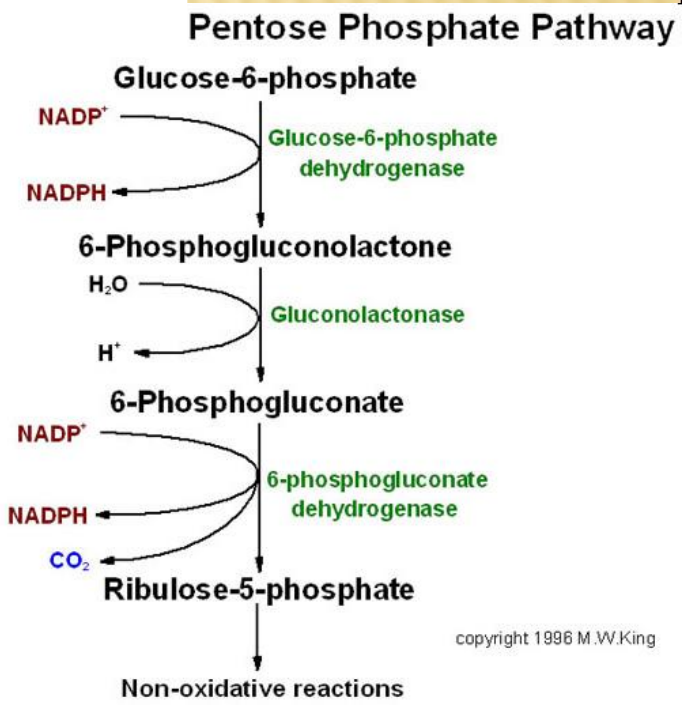
RED BLOOD CELL METABOLISM

Glycolysis is the primary source of ATP:

- 90% of glycolysis occurs through ***Embden Meyerhof pathway***, with the particularity that the phosphoglycerate kinase step is by-passed to produce **2,3 DPG** that influences the O_2 affinity of Hb (Rapaport Shunt)
- 10% of glycolysis occurs through ***pentose phosphate pathway***, that generates **NADPH**
- **NADPH** generated by glycolysis is required for glutathione reduction, important to protect sulphydryl groups of Hb against oxidation; important to reduce methemoglobin (continuously formed by Hb autooxidation) and to keep it <1% of Hb content; also protects plasma membranes.

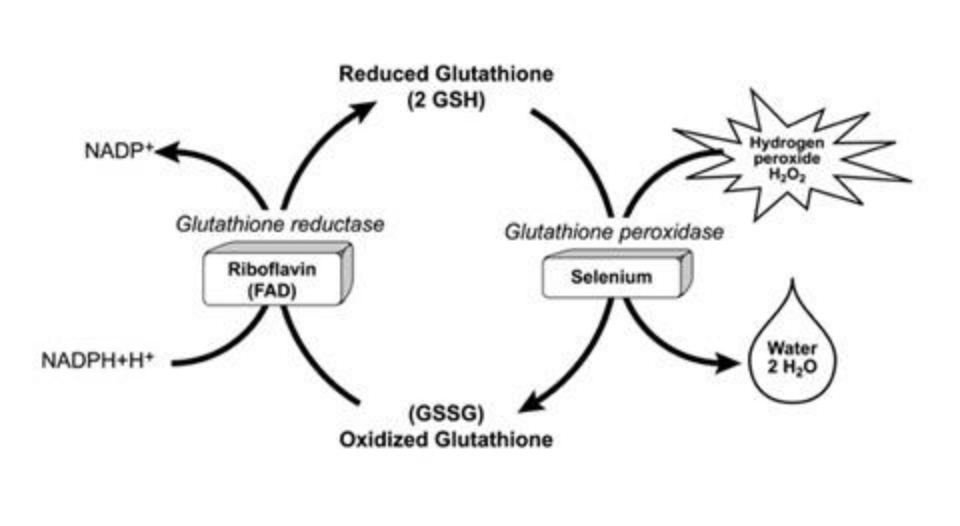
Embden Meyerhof pathway





GLUTATHIONE

- Tripeptid- Gli Cys Glu
- Thiol group (Cys)- reducing agent
- Protects cell from free radicals



Clin Hemorheol Microcirc. 2009;43(3):223-32.

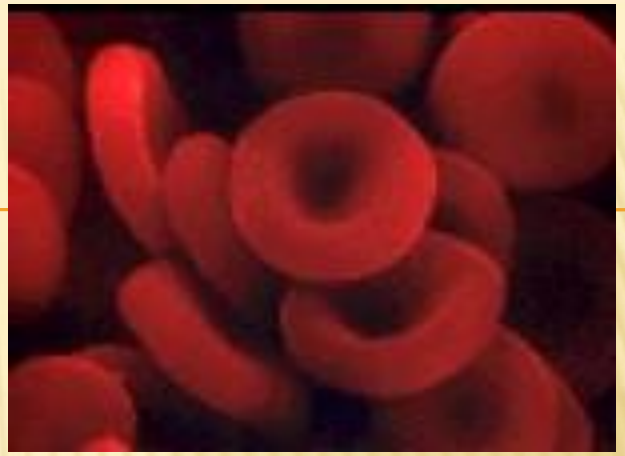
Extra- and intracellular signaling pathways under red blood cell aggregation and deformability changes.

Muravyov AV, Tikhomirova IA, Maimistova AA, Bulaeva SV.

Department of Medicine and Biology, University of Yaroslavl, 150000 Yaroslavl, Russia.

Exposure of red blood cells (RBCs) to catecholamines (epinephrine, phenylephrine, an agonist of alpha1-adrenergic receptors, clonidine, an agonist of alpha2-adrenergic receptors and isoproterenol, an agonist of beta-adrenergic receptors) led to change in the RBC microrheological properties. When forskolin (10 microM), an AC stimulator was added to RBC suspension, the RBC deformability (RBCD) was increased by 17% ($p<0.05$). Somewhat more significant deformability rise appeared after RBC incubation with dB-AMP (by 27%; $p<0.01$). Red blood cell aggregation (RBCA) was significantly decreased under these conditions ($p<0.01$). All drugs having cyclic nucleotide phosphodiesterase (PDE) activity increased red cell deformability similarly. Some more changes of deformability was found after RBC incubation with pentoxifylline--25% ($p<0.05$) and IBMX incubation was accompanied only by 15% rise of RBC deformability. The drugs with PDE inhibitory activity reduced red cell aggregation. The most significant RBCA reduction effect was found under cell incubation with pentoxifylline and inhibitor PDE1-vinpocetine. On the whole RBCA reduction averaged 36% ($p<0.05$) under RBCs incubation with PDE inhibitors. The rise of Ca2+ influx, stimulated by A23187, was accompanied by an increase of RBCA, whereas red cell deformability was changed insignificantly. At the same time Ca2+ entry blocking into the red cells by verapamil or its chelating in medium by EGTA led to significant RBCA decrease and deformability rise ($p<0.05$).On the whole the total data clearly show that the **red cell aggregation and deformation changes were connected with an activation of the different intracellular signaling pathways**. It seems reasonable to suppose that RBCA decrease was mainly associated with an activation of the adenylyl-cyclase-cAMP system, while the red cell deformability was closely associated with Ca2+ control mechanisms.

RED BLOOD CELLS (RBC)



RBC are most abundant of all the cells of the blood

RBC count - 2 years < 4,000,000 / mm^3

- men: 5,200,000 ± 300,000 / mm^3;
- women: 4,700,000 ± 300,000 / mm^3
- changes - physiological: pregnancy, high altitude
- pathological : anemia,

polycytemia: Ht > 52 (M), 47 (F)
hypoxia, high altitude
polycytemia vera (malignant)
erythropoietin independence, JAK2

Increased blood viscosity and resistance to blood flow, stagnant hypoxia, peripheral cyanosis

Hematocrit – 40 – 45 %

Hemoglobin – 14 – 16 g/dl blood
up to 34 g/dl of cell fluid (metabolic limit of cell Hb-forming mechan.)

1 g pure Hb combine with 1.34 ml O_2
14 – 16 g Hb/dl blood combine with 19 – 21 ml O_2

Chromicity – normochromia, hypochromia (decreased MCHC or thinner cells), hyperchromia (increase of cell thickness) (e.g. spherocytosis – ↑ MCHC and thickness)

Pathol Biol (Paris). 2004 Jun;52(5):280-4.

Polycythemia and oxygen sensing.

Maran J, Prchal J.

802E Medicine Divison of Hematology/Oncology, Baylor College of Medicine and Houston VA Medical Center, One Baylor Plaza, MS 525D, Houston, TX 77030, USA.

Polycythemias can be differentiated based on the responsiveness of erythroid progenitors to circulating cytokines. Primary polycythemias are characterized by an augmented response due to acquired somatic or inherited germ-line mutations that are expressed within hematopoietic progenitors causing increased proliferation or decreased apoptosis and resulting in accumulation of red blood cells. In terms of oxygen requirements, primary polycythemias can be viewed as the production of hemoglobin fully dissociated from the tissue oxygen needs and from the oxygen sensing pathway. Polycythemia vera (PV) is the most common primary polycythemia. PV bone marrow progenitors cells can form erythroid colonies in the absence of exogenous erythropoietin in vitro. These endogenous erythroid colonies (EEC) are useful in differentiating PV and secondary polycythemias. They also can differentiate PV where this feature is independent of Epo signalling from primary familial and congenital polycythemia. In this autosomal dominant primary polycythemia, at variance with PV, EEC formation is abolished by anti-Epo and anti-Epo receptor neutralising antibodies. Mutations of the EPOR have been described and resulted in nine cases in truncated EPORs lacking the cytoplasmic carboxy-terminal of the receptor which possesses a negative growth regulatory domain. However, recent data suggest that different mutations may cause PFCP in most cases. Secondary polycythemia can be viewed as either physiological response to satisfy the oxygen needs of the tissues, resulting for instance from high affinity hemoglobins or BPG mutase deficiency, or as the result of germ-line or somatic mutations disturbing the oxygen sensing pathway or its target: Epo. Chuvash polycythemia is a frequently symptomatic disorder with an autosomal recessive inheritance and inappropriately high Epo levels. The erythroid progenitors are hypersensitive to Epo linking this condition to both primary and secondary polycythemia. A germline missense mutation at nucleotide 598 in both alleles of the von Hippel-Lindau gene results in increased hypoxia inducible factor-1 (HIF-1) expression in normoxic conditions. HIF-1 controls the expression of many genes including Epo. Identifying causal defects in other situations like post-renal transplant erythrocytosis and cases of autosomal dominant polycythemia with high Epo levels will help further understanding of the regulation of erythropoiesis.

Intern Emerg Med. 2010 Oct;5(5):375-84. Epub 2010 Mar 16.

Polycythemia vera.

Landolfi R, Nicolazzi MA, Porfidia A, Di Gennaro L.

Institute of Internal Medicine and Geriatrics, Haemostasis Research Center, Catholic University School of Medicine, Largo Agostino Gemelli 8, Rome, Italy. rlandolfi@rm.unicatt.it

The diagnostic approach to a patient with polycythemia has been greatly simplified by the introduction of new genetic testing in addition to traditional tests, such as measurement of red cell mass and serum erythropoietin (Epo) level. Clonal erythrocytosis, which is the diagnostic feature of polycythemia vera (PV), is almost always associated with a JAK2 mutation (JAK2V617F or exon 12). Therefore, in a patient with acquired erythrocytosis, it is reasonable to begin the diagnostic work-up with JAK2 mutation analysis to distinguish PV from secondary erythrocytosis. The clinical course of PV is marked by a high incidence of thrombotic complications that represent the main cause of morbidity and mortality in these patients. Blood hyperviscosity as well as platelet and leukocyte quantitative, and qualitative abnormalities play a major role in the pathogenesis of thrombophilia. Prevention of vascular events and minimizing the risk of disease transition into acute leukaemia are the main targets of the whole PV treatment strategy. This can rely on the use of low-dose aspirin in most patients, while the choice of the optimal cytoreductive strategy is based on the individual vascular risk. Phlebotomy is still the preferred treatment in subjects at low risk, while hydroxyurea or pipobroman is usually administered to most elderly subjects or subjects with a previous vascular history. The use of pegylated interferon, imatinib, and JAK2 inhibitors is currently being evaluated.

The oxygen carrying capacity (OCC)

= maximum amount of oxygen that can be carried in a deciliter (100 mL) of blood, including both oxygen bound to hemoglobin and oxygen dissolved in plasma.

- **1 g Hb** can combine with and transport **1.34 mL oxygen.**
- For each 1 mmHg of PaO_2 there is **0.003 mL of oxygen dissolved/100 mL** of blood

  →**oxygen carrying capacity in 100 mL of blood** can be calculated as follows:

$$OCC = (\text{Blood Hemoglobin} \times 1.34) + (0.003 \times PaO_2)$$

Example: $(15\ \text{g}/100\ \text{mL blood} \times 1.34\ \text{mL } O_2)$

$+(0.003\ \text{mL } O_2/\text{mm Hg}/100\ \text{mL blood} \times 100\ \text{mm Hg})$

$= 20.1 + 0.3 = 20.4\ \text{mL of } O_2/100\ \text{mL of blood}$

FUNCTIONS OF RBC

-RBCs transport **Hb**, which carries O_2 , CO_2

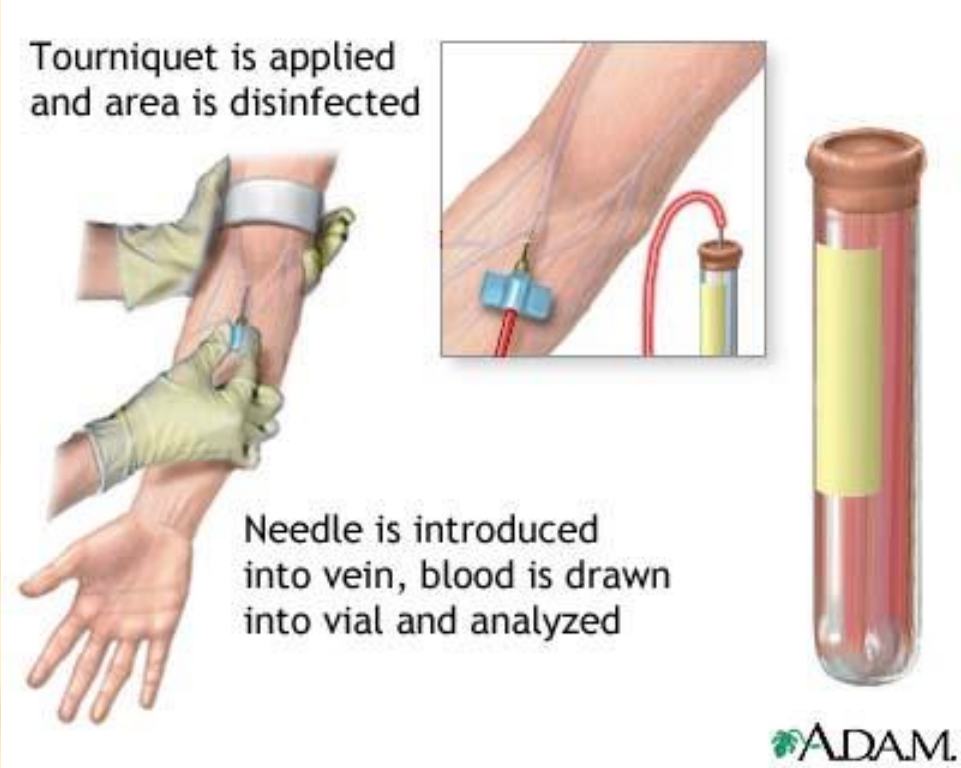
- if free Hb in the plasma:
  1. 3% Hb would leak through the capillary membrane with each pass
  2. plasma viscosity would markedly increase
  3. blood osmotic pressure would increase

-RBCs contain large quantities of carbonic anhydrase (CA): transport of CO2 from the tissues to the lungs in the form of HCO3-

-as a protein, Hb act as an acid-base buffer

The red color of whole blood stems from hemoglobin. Oxygenated iron in hemoglobin gives the blood a bright red color. Deoxygenated blood is darker red, which can be seen when venous blood samples are taken. Veins, when seen through the skin, typically appear blue in color as a result of the deflection of light when it penetrates the skin.

Blood tests



Blood is drawn from a vein (venipuncture), usually from the inside of the elbow or the back of the hand. A needle is inserted into the vein, and the blood is collected in an air-tight vial or a syringe. Preparation may vary depending on the specific test.

Blood tests

Erythrocyte parameters

1.red cell count
2.hematocrit
3.hemoglobin
4.erythrocyte indices :
    a.Mean corpuscular volume (MCV)
    b.Mean corpuscular hemoglobin (MCH)
    c.Mean corpuscular hemoglobin concentration (MCHC)

Leukocyte parameters

1. white cell count
2. leukocyte formula: Neutrophil granulocytes, Lymphocytes, Monocytes, Eosinophil granulocytes, Basophil granulocytes

Platelet parameters

1.platelet count
2.platelet size

Erythrocyte indices

The **mean cell** (or **corpuscular**) **volume (MCV)** is the index most often used. It reflects the average volume of each red blood cell (RBC) and is calculated as follows:

$$\text{MCV} = \frac{\text{Hematocrit}}{\text{RBC Count (cells/L)}} \qquad (1)$$

$$\text{Example: } 0.450 \div \left(5 \times 10^{12}\ \text{cells/L}\right) = 0.090 \times 10^{-12}\ \text{L/cell}$$
$$= 90\ \text{fL}\ \left(1\ \text{fL} = 10^{-15}\ \text{L}\right)$$

Normocytic - normal size cells; microcytic – cells with a low MCV, macrocytic – cells with a high MCV. These size categories are used to classify anemias.

The **mean cell (**or **corpuscular) hemoglobin (MCH)** value is an estimate of the average hemoglobin content of each red blood cell.

$$\text{MCH} = \frac{\text{Blood Hemoglobin (g/L)}}{\text{RBC Count (cells/L)}} \qquad (2)$$

$$\text{Example: } 150\ \text{g/L} \div \left(5 \times 10^{12}\ \text{cells/L}\right)$$
$$= 30 \times 10^{-12}\ \text{g/cell} = 30\ \text{pg/cell}$$

MCH values usually rise or fall as the MCV is increased or decreased. The MCH is often related to the MCHC as RBC count is usually related to the hematocrit.

The **mean cell** (or **corpuscular**) **hemoglobin concentration (MCHC)** provides an index of the average hemoglobin content in the mass of circulating red blood cells. It is calculated as follows:

$$\text{MCHC} = \frac{\text{Blood Hemoglobin (g/L)}}{\text{Hematocrit}} = \frac{\text{MCH}}{\text{MCV}} \qquad (3)$$

$$\text{Example: } 150\ \text{g/L} \div 0.45 = 333\ \text{g/L}$$

Low MCHC indicates deficient Hb synthesis→ hypochromic cells.
High MCHC values do not occur in erythrocyte disorders, because normally the Hb concentration is close to the saturation point in red cells (here discuss hyperchromia)

RBC INDICES

- MCV= mean corpuscular volume= the mean volume of one red blood cell

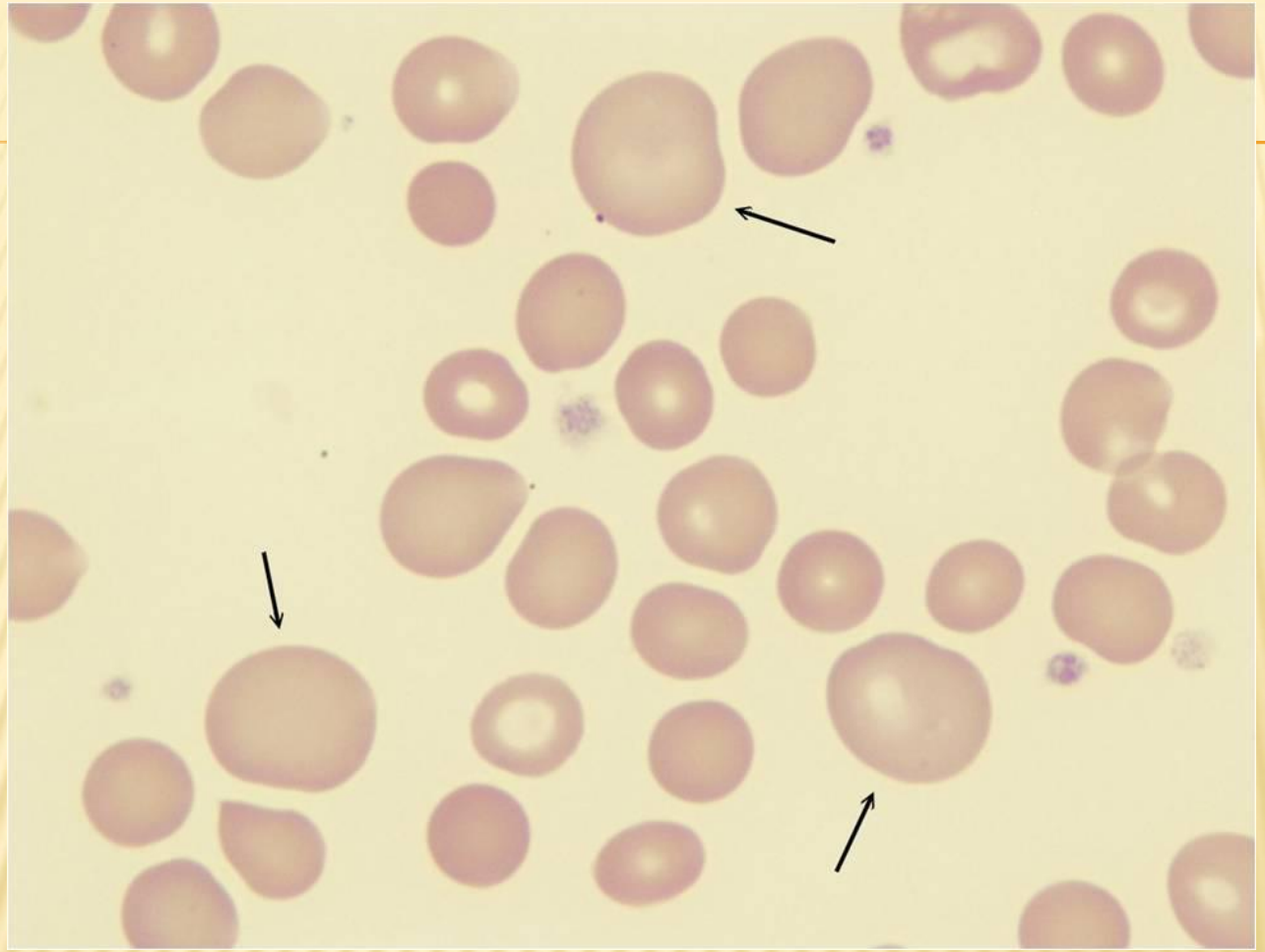
- MCV= HT x 10/ red cell count fL (femtoliter= 10^{-15} l)

- Normal value

80- 100 fL

- indicates SIZE

- 80- 100 fL= normocytes= RBC have normal volume
- < 80 fL= microcytes= RBC have small volume
- > 100 fL= macrocytes= RBC have large volume



Macrocytes and normocytes are seen.

RBC INDICES

- MCHC= mean hemoglobin concentration in all RBCs

Hb conc x 100/ HT

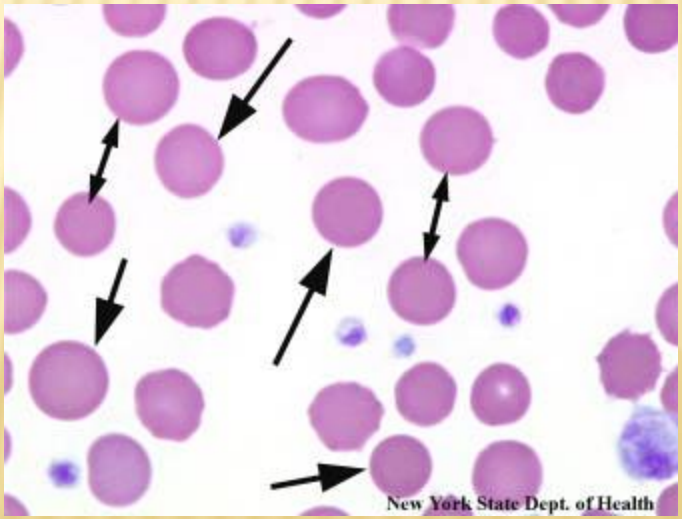
Normal value= 320-360 g/L (32- 36 g/ 100 ml RBC)

Indicates COLOUR:

- 32- 36- normochromic RBC
- < 32- hypochromic RBC
- HYPERCHROMIA DOESN'T EXIST!
- (Hb precipitates and hemolysis occures)

RBC INDICES

- Hb concentration never exceeds 36 %
- Lack of central bleach is usually caused by change in shape (spherocytosis), not by change in Hb content.



RBC INDICES

- MCH= mean cell hemoglobin in one RBC=

Hb concentration x 10/ red cell count

Normal value= 27- 32 pg/ cell

Indicates COLOUR

ANEMIA= LOW RBC COUNT AND/ OR LOW HEMOGLOBIN

With regard to the indices we can classify anemia into the following categories:

I. normochromic, normocytic anemia

- Normal MCV, normal MCHC

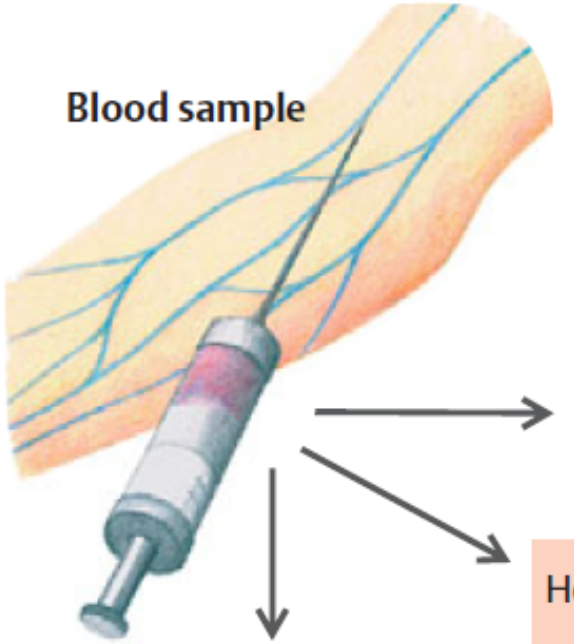
II. hipochromic, microcytic anemia

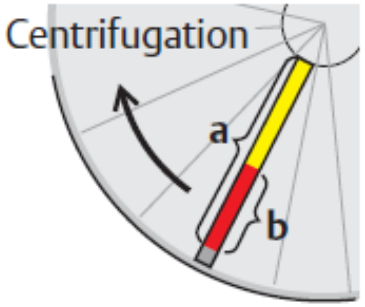
- MCV < 80 fL, MCHC < 32 %

III. normochromic, macrocytic anemia

- MCV > 100 fL, normal MCHC

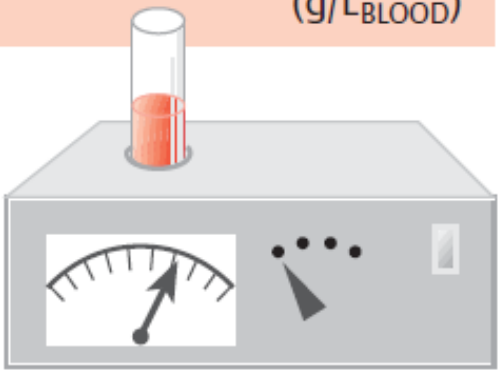
C. Erythrocyte parameters MCH, MCV and MCHC



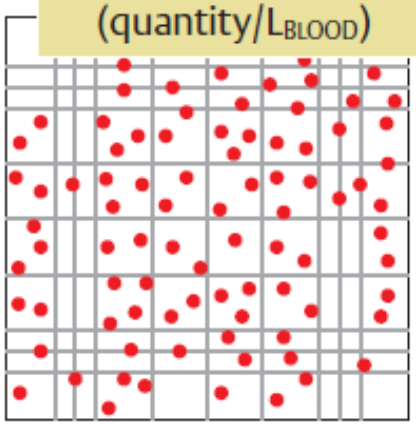


Hematocrit (Hct) = **b/a**
(L_{RBC}/L_{Blood})

Hemoglobin concentration
(g/L_{BLOOD})



Red cell count (RCC)
(quantity/L_{BLOOD})



MCH (mean Hb mass/RBC)

$$= \frac{\text{Hb conc.}}{\text{red cell count}} \text{ (g/RBC)}$$

Normal: 27–32 pg

MCV (mean volume of one RBC)

$$= \frac{\text{Hct}}{\text{red cell count}} \text{ (L/RBC)}$$

Normal: 80–100 fl

MCHC (mean Hb conc. in RBCs)

$$= \frac{\text{Hb conc.}}{\text{Hct}} \text{ (g/L}_{RBC}\text{)}$$

Normal: 320–360 g/L

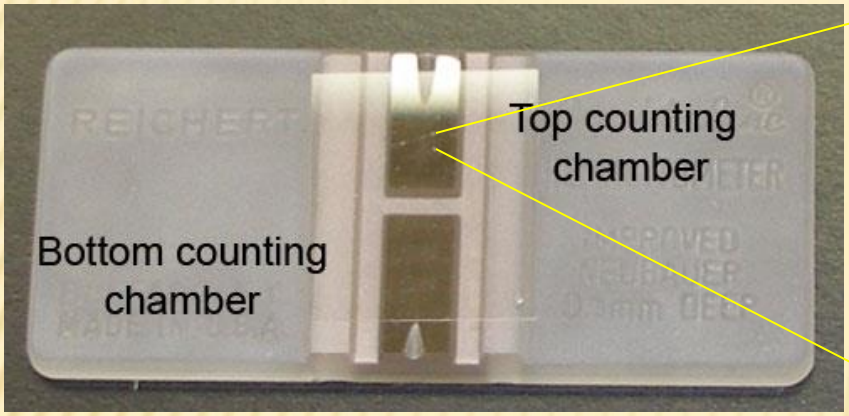
RBC count

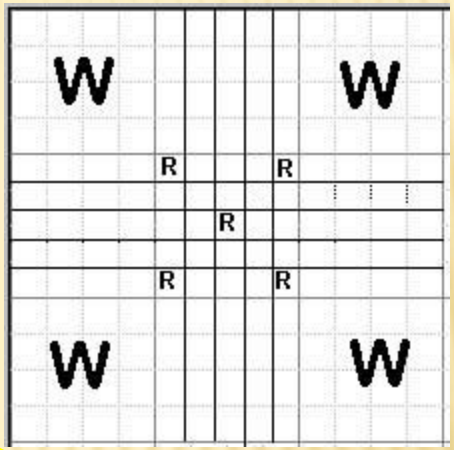
1. Blood is drawn for the Total RBC count using a ***red blood cell diluting pipette*** (note the [red] crystal in the dilated portion of the pipette).

2. Red blood cell diluting fluid is drawn and mixed with the blood sample. This fluid functions to lyse the WBCs in the blood sample.

RBC COUNT

Hemacytometer = blood cell counting slide

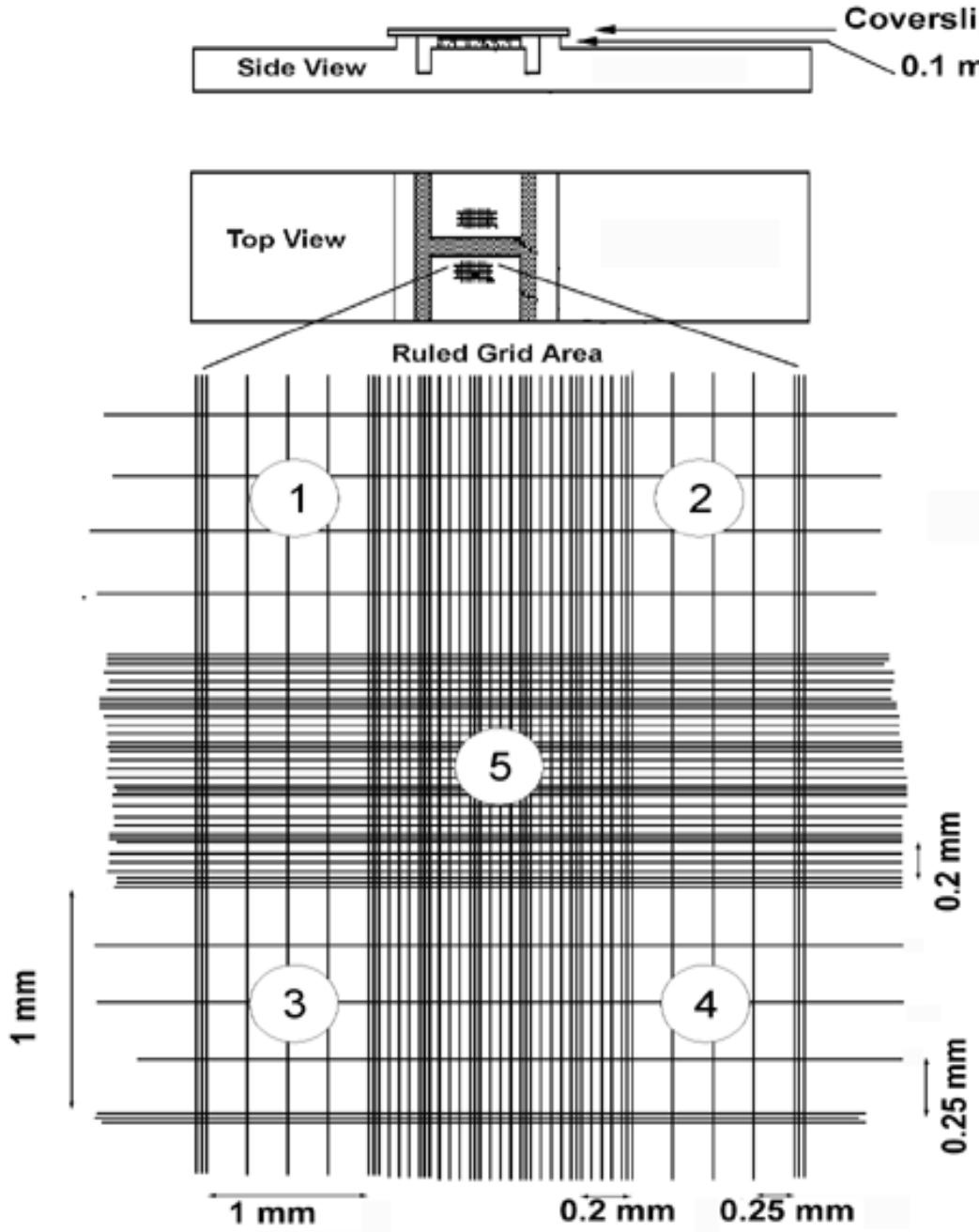


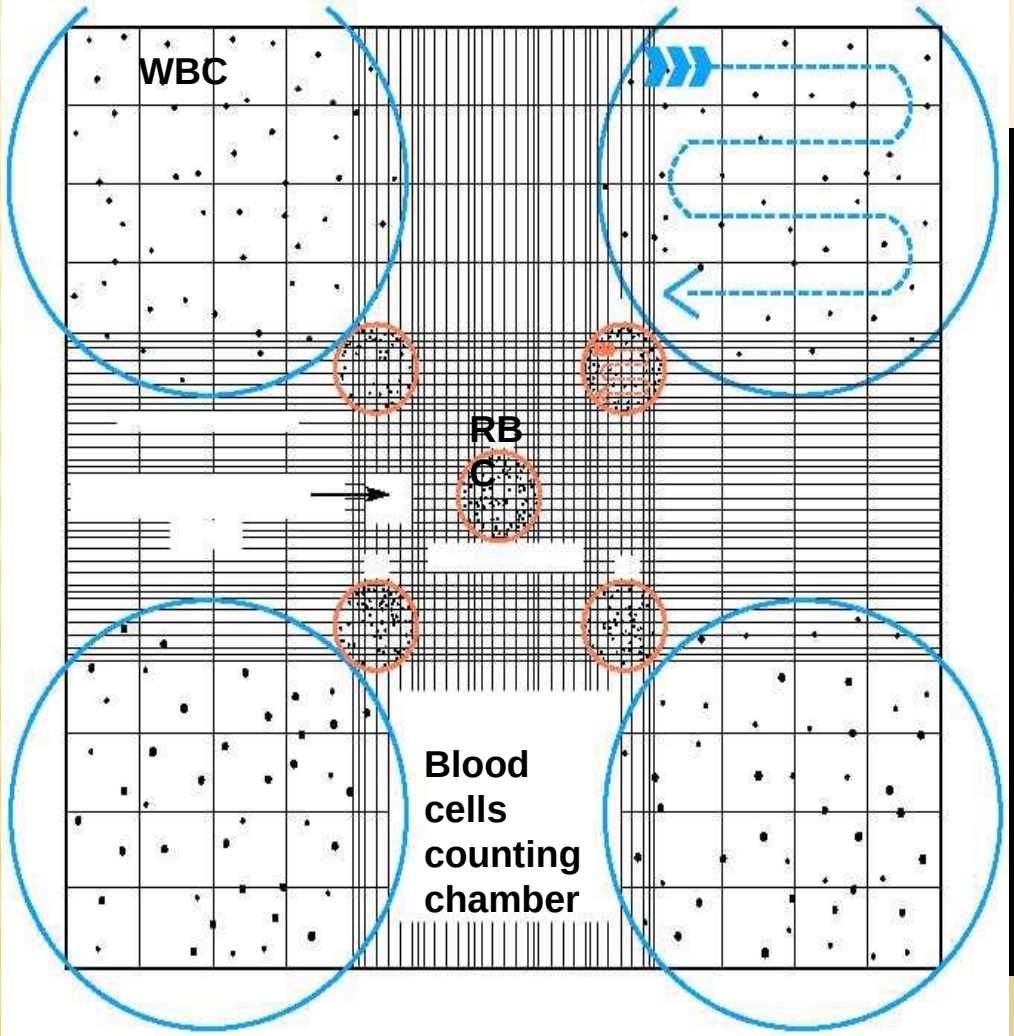


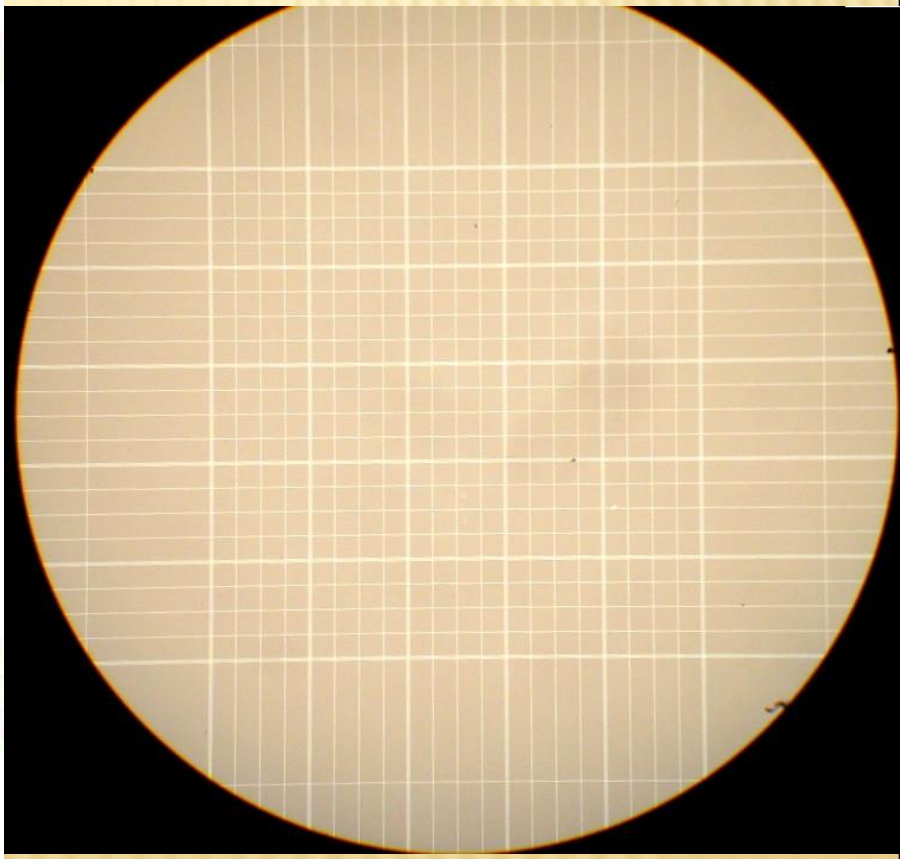
The hemacytometer is divided into sections.

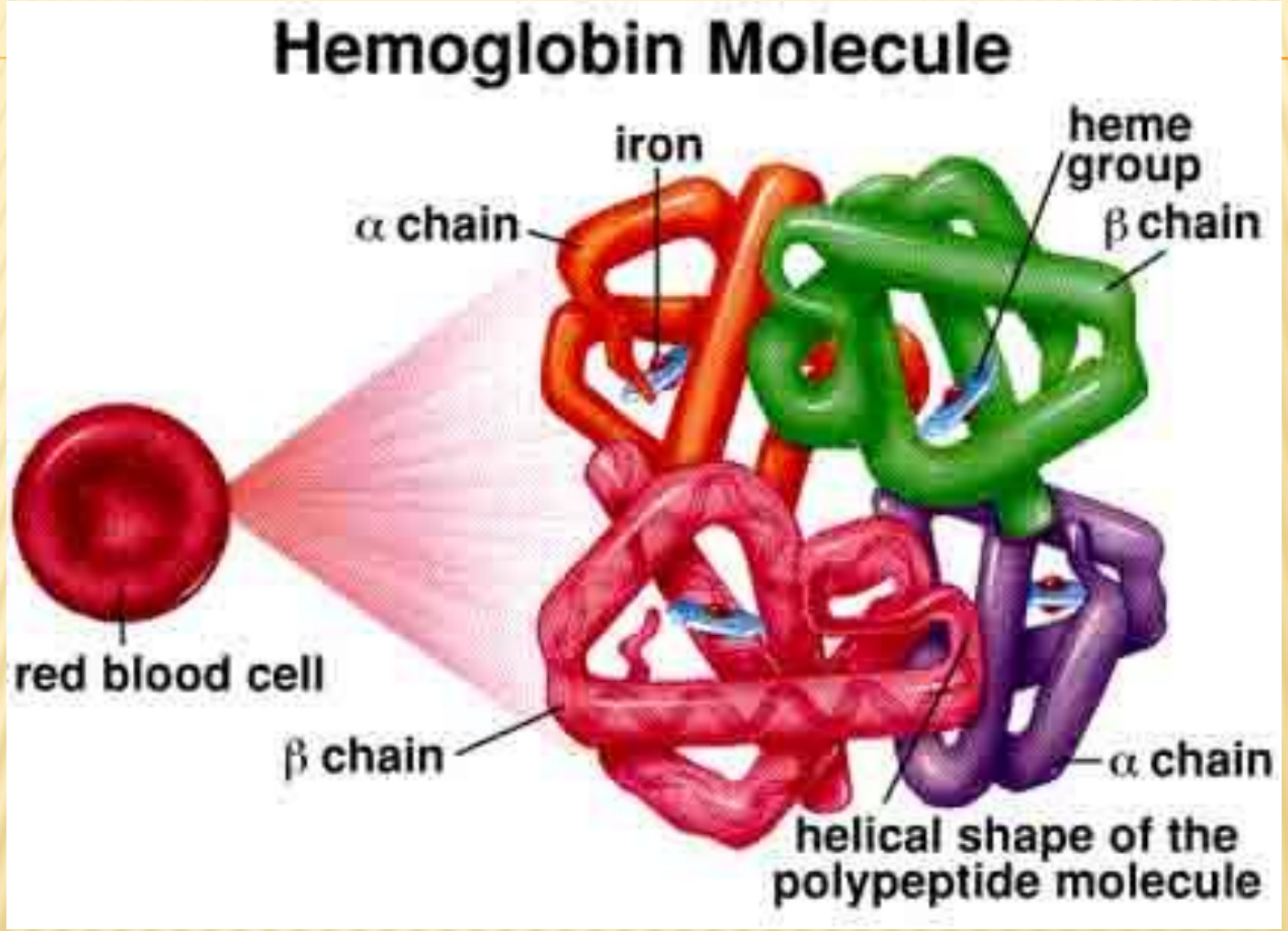
The WBCs are counted in the outer four sections (each section contains a 4x4 grid with 16 total squares in each section).

The center section of the hemacytometer contains a 5x5 grid. The RBCs are counted in the four corner squares and the central square (each square contains a 4x4 grid, also).


Coverslip
0.1 mm depth
Side View
Top View
Ruled Grid Area
1
2
3
4
5
1 mm
0.2 mm
1 mm
0.2 mm
0.25 mm
0.25 mm


WBC
RBC
Blood cells counting chamber




Hemoglobin Molecule
iron
heme group
α chain
β chain
red blood cell
β chain
α chain
helical shape of the polypeptide molecule

Hemoglobin

In adult humans the Hb molecule (**HbA**) consists of four globular polypeptides:

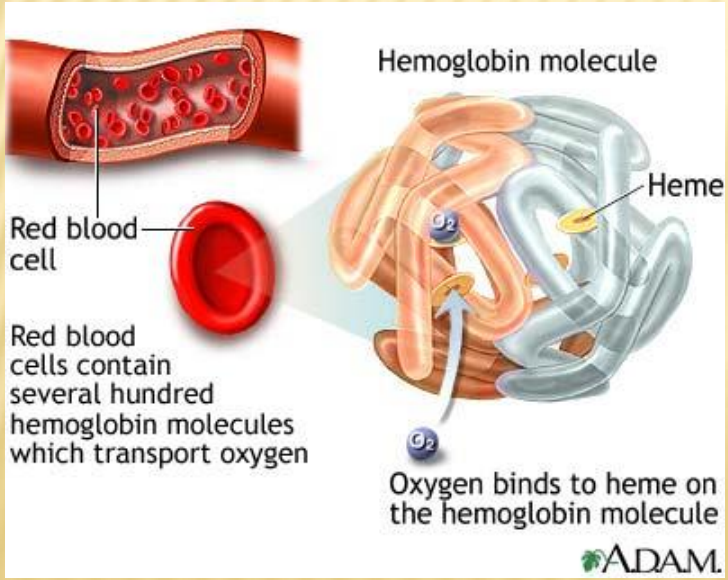
- two ***alpha* (α) chains** of 141 amino acids
- two ***beta* (β) chains** of 146 amino acids

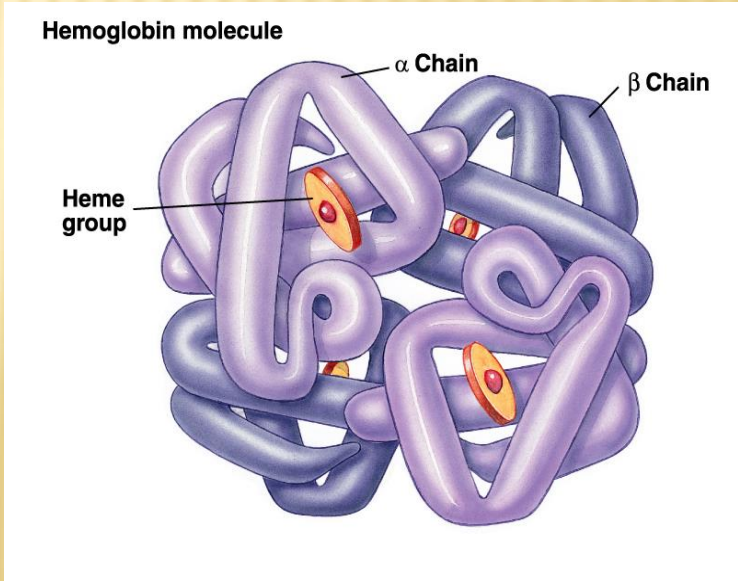
For HbA2 (2.5% in adult): 2 alpha and 2 ***delta*** chains

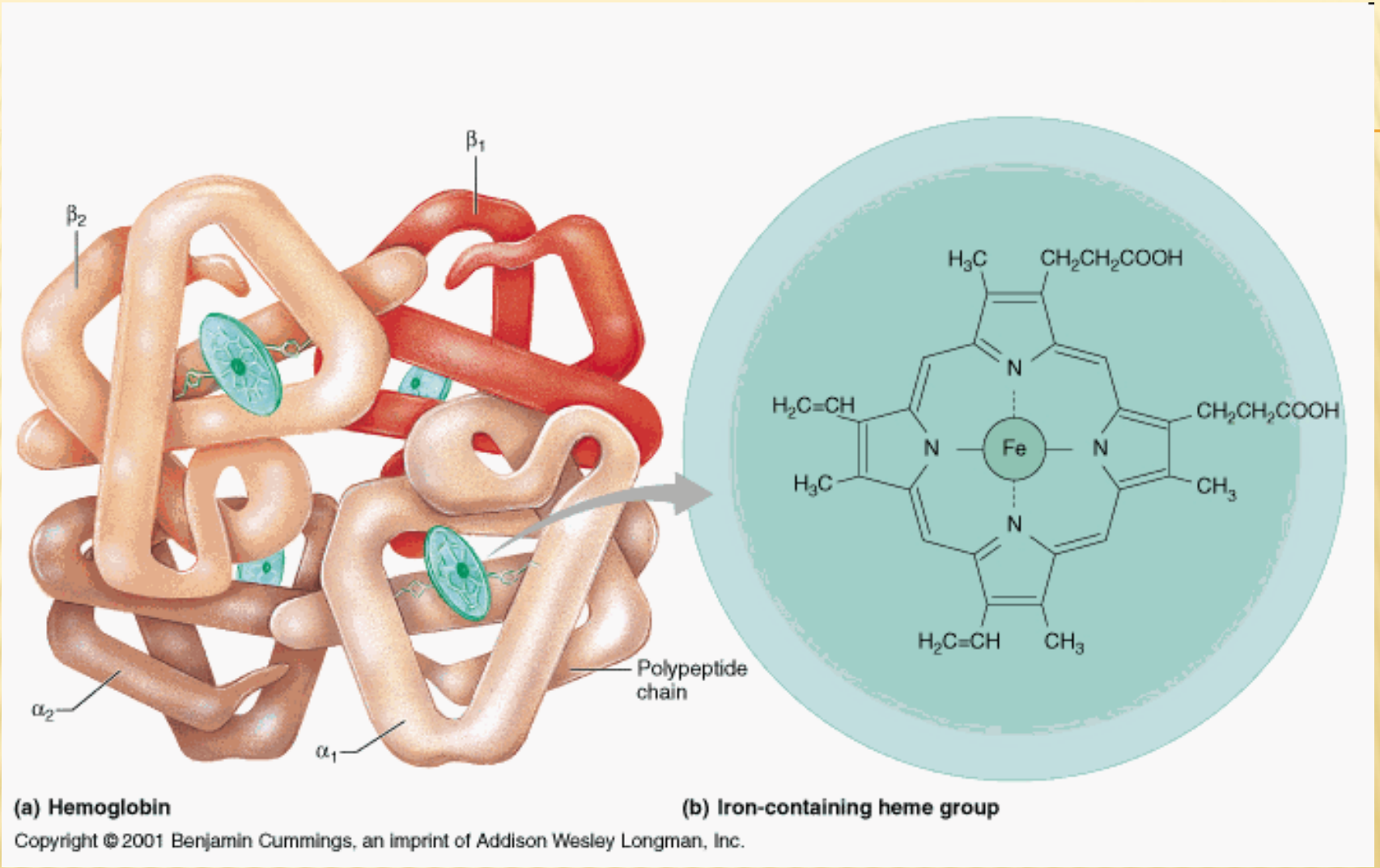
For fetal Hb (HbF): 2 alpha and 2 *gamma* chains

Each of these is attached to a prosthetic group **heme**, with one atom of **iron** at its center. One molecule of O_2 can bind to each heme (reversible reaction, coordination/loose bond).

When Hb is saturated with O_2 it has a bright red color; as it loses oxygen it becomes bluish (cyanosis).



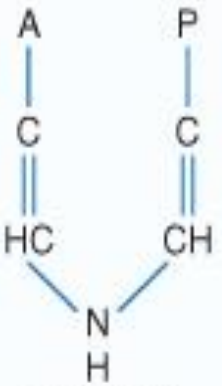




Heme group is a C-H-N porphyrin ring with an iron atom (Fe) in the center (70% of the iron in the body)

SYNTHESIS OF HEMOGLOBIN

I. 2 succinyl-CoA + 2 glycine → (pyrrole)



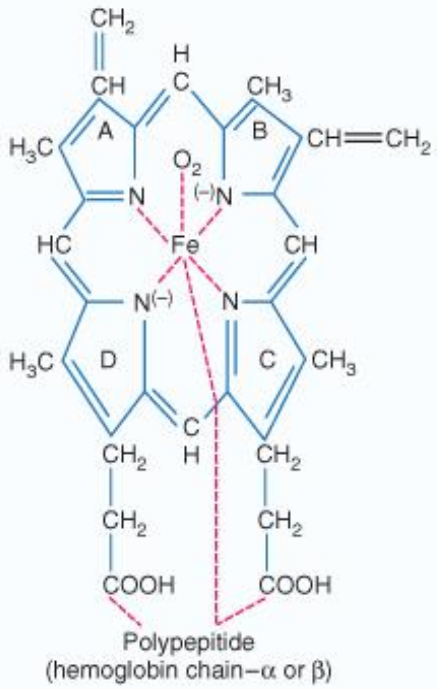
II. 4 pyrrole → protoporphyrin IX

III. protoporphyrin IX + Fe^{++} → heme

IV. heme + polypeptide → hemoglobin chain (α or β)

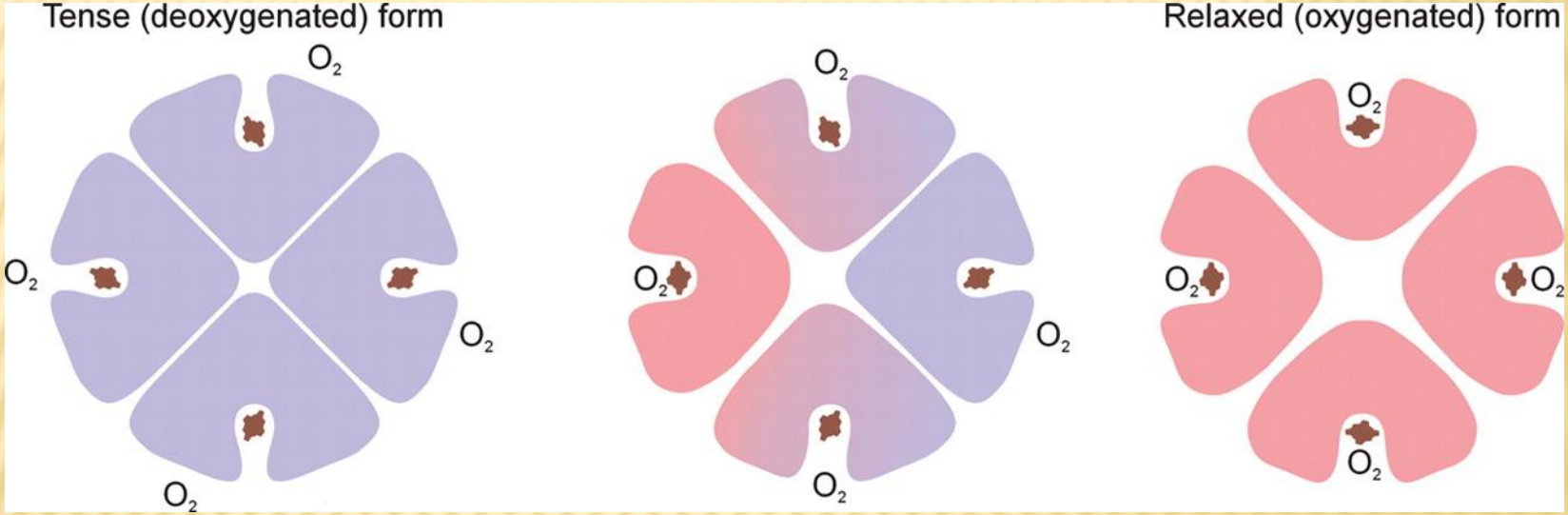
V. 2 α chains + 2 β chains → hemoglobin A

HB MOLECULE



From Guyton, 2006

The transition from ‘tense’ to ‘relaxed’ haemoglobin.



The transition from ‘tense’ to ‘relaxed’ haemoglobin. In its deoxygenated ‘tense’ form, the crevice containing the haem molecule is narrow, restricting the access of oxygen to its binding site. As each oxygen molecule binds, the position of the haem molecule changes which affects the interaction between adjacent globin chains, relaxing the molecule and so allowing easier access of subsequent oxygen molecules to their binding site.

Iron

4-5 g, from which 70% in Hb, 4% myoglobin, 1% other heme compounds, 0.1% combined with transferrin (blood carrier protein)
15-30% stored as ferritin mainly in the reticuloendothelial system and liver

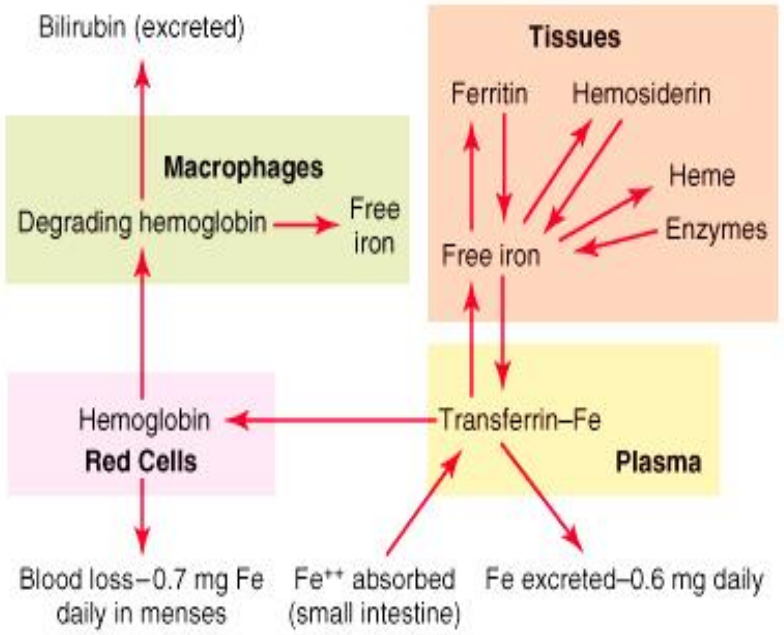
Absorbtion in the small intestine (slow rate, few mg/day) → iron bind loosely to apotransferrin (b-globulin in the bile) → transferrin → rec on the intestinal epithelial cells → absorbtion of transferrin by pinocytosis → plasma transferrin (1/3 saturated) released in the plasma → transport to the tissues → in the cells cytoplasm iron combines mainly with apoferritin (a 460 kda protein) to form ferritin = storage iron

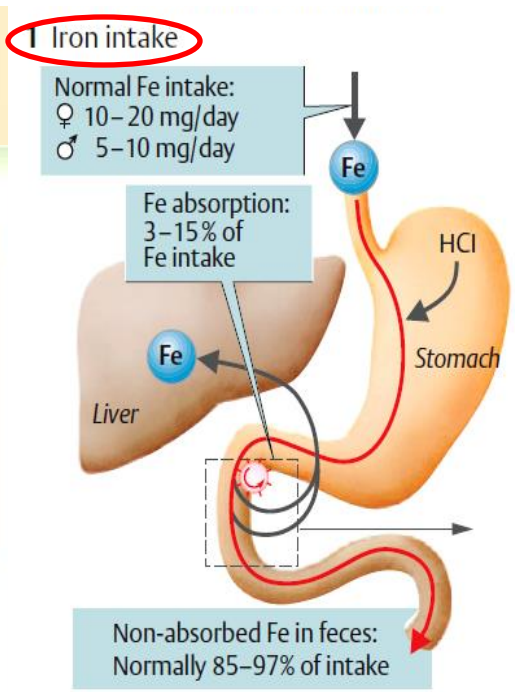
When more iron than apoferritin, then an insoluble storage form = hemosiderin

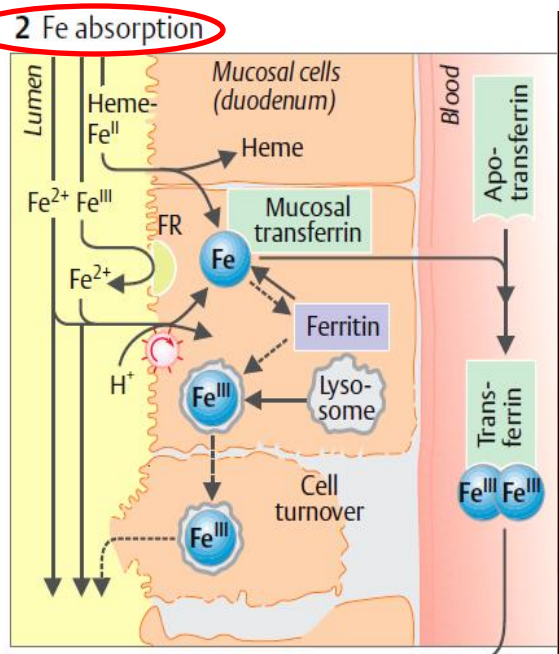
Transferrin binds strongly with receptors on erythroblasts → endocytosis → direct deliver of the iron for heme is synthesis

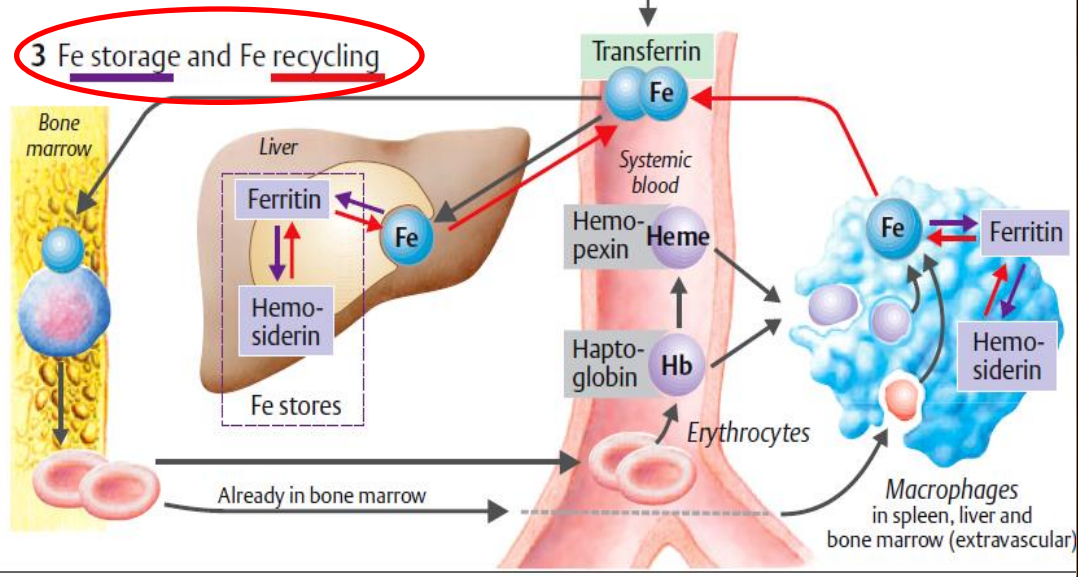
Daily loss of iron: 0.6 – 1.3 mg/day

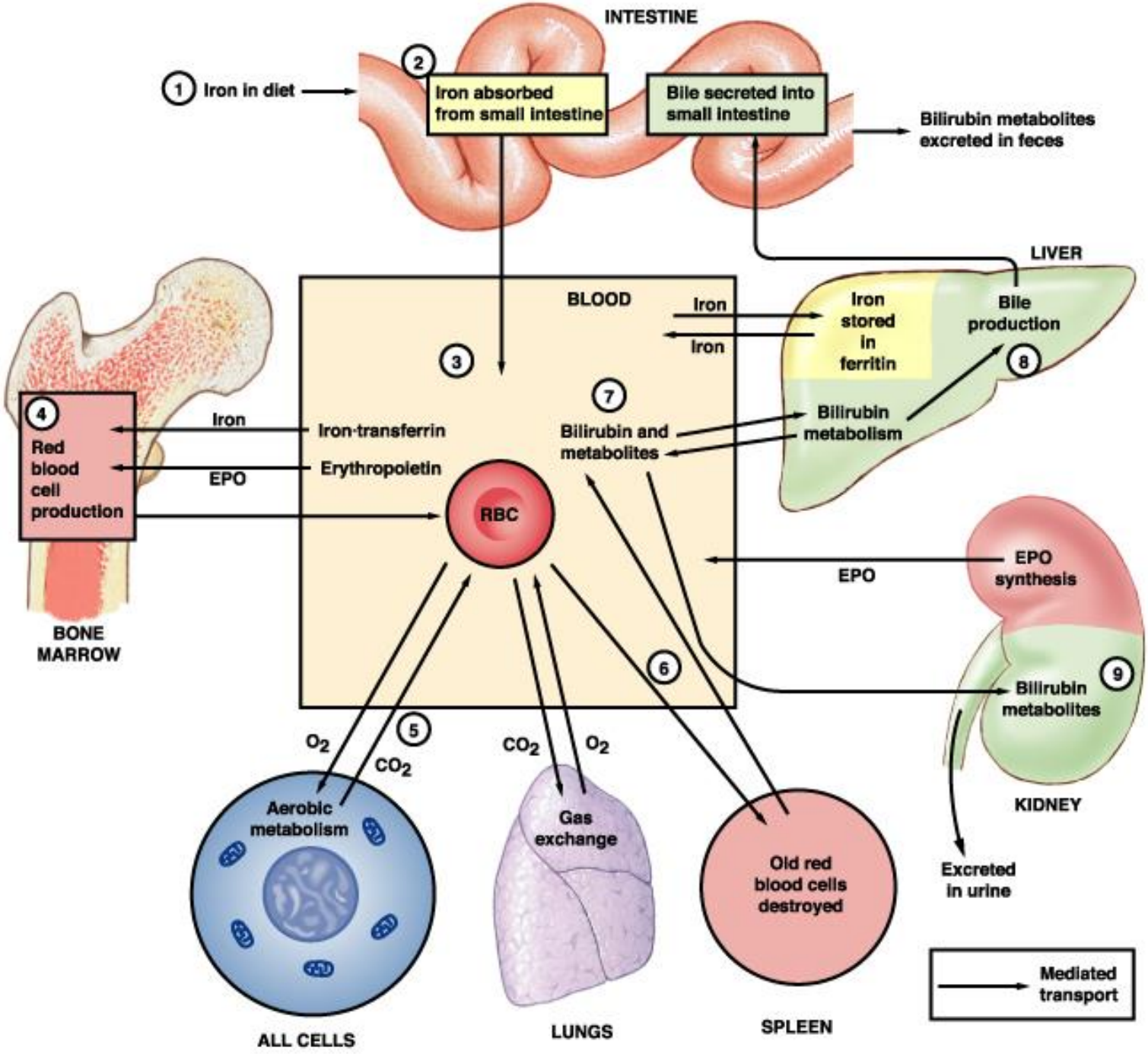
Iron transport and metabolism










INTESTINE
1 Iron in diet
2
Iron absorbed from small intestine
Bile secreted into small intestine
Bilirubin metabolites excreted in feces
LIVER
BLOOD
Iron
Iron
Iron stored in ferritin
Bile production
3
8
7
4
Iron
Iron-transferrin
Bilirubin and metabolites
Bilirubin metabolism
Red blood cell production
EPO
Erythropoietin
RBC
EPO
EPO synthesis
BONE MARROW
6
9
Bilirubin metabolites
5
O_2
CO_2
CO_2
O_2
Aerobic metabolism
Gas exchange
Old red blood cells destroyed
Excreted in urine
KIDNEY
ALL CELLS
LUNGS
SPLEEN
Mediated transport

Iron Profile

Name	Reference Range	Reference SI	Description
Iron	50–170 µg/dL	9–30 µmol/L	Amount of iron bound to transferrin in blood
Ferritin	150–200 ng/mL	15–200 µg/L	Storage form of excess iron
Total Iron-Binding Capacity (TIBC)	252–479 µg/dL	45–86 µmol/L	Amount of iron needed to bind to all transferrin
Transferrin	200–380 mg/dL	2–3.8 g/L	Transferrin which is not bound to iron
Transferrin Saturation (Iron/TIBC)	20%–50%	0.2–0.5	Percentage of transferrin with iron bound to it

FETUSES LIVE IN A LOW OXYGEN ENVIRONMENT AND REQUIRE A SPECIAL HEMOGLOBIN

- The developing fetus cannot breathe and must get all of its blood from the placenta
- Fetal blood has a very low pO2, about 30 mm Hg, equivalent to living at 26,000 feet altitude ("Everest in utero“, Barcroft)
- To extract more oxygen from the mother's blood fetuses make a special hemoglobin (**HbF** - 2 alpha and *2 gamma chains*) which has a **very high affinity for oxygen**:

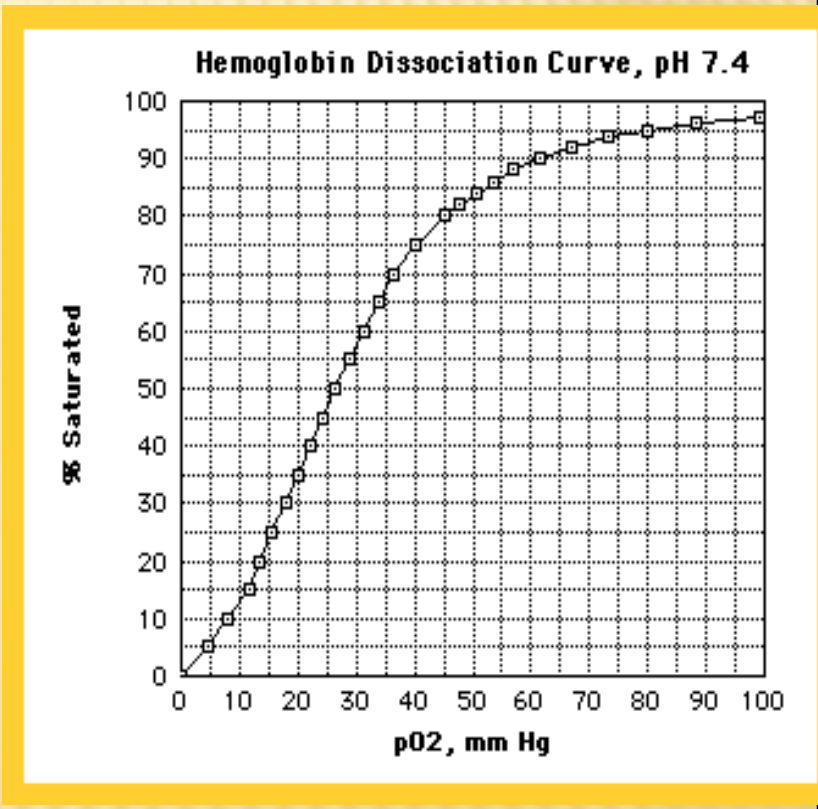
  HbF exhibits a low affinity for 2,3-DPG, lowers affinity of Hb for O_2, by binding to deoxyhemoglobin**.**

  The positive histidine residues of HbA β-subunits that are essential for forming the 2,3-DPG binding pocket are replaced by serine residues in HbF γ-subunits.

PARTIAL PRESSURE OF O_2 IS HIGH ENOUGH TO GIVE NEARLY 100% SATURATION OF HB AT SEA LEVEL

- As the pO2 in the alveoli increases, Hb in the red cells passing through the lungs rises until Hb is ~100% saturated with oxygen
  - at 100% saturation each Hb carries 4 O_2 molecules
  - this is equal to 1.34 mL O_2/g of Hb

- A plot of % saturation vs pO_2 gives an S-shaped curve = "**hemoglobin dissociation curve**"



Gas transport in the blood - Oxygen transport

1.34 mL oxygen / 1 g Hb.

0.03 mL of oxygen dissolved / L / mmHg of PaO_2 of blood

Total blood O_2= O_2 **dissolved in plasma**+O_2 **bound to Hb** (oxyHb)

	2%	98%
200 mL O_2 /**L**	3 mL O_2 /**L**	197 mL O_2 /**L**
	x **5 L of blood**	
1000 mL O_2	15 mL O_2	985 mL O_2

Cells metabolism depends on Hb transport of oxygen:

$$Hb + O_2 \leftrightarrow HbO_2$$

Oxygen consumption at rest ~ 250 mL O_2 / min.

~ ¼ from available O_2

Hb saturation depends on:

- PO_2 (changes with air composition, alveolar ventilation, gas exchange)

- number of potential O_2-binding sites available in RBC (depends on the number of Hb molecules)

  → % saturation of Hb

- relationship between PO_2 and % saturation of Hb:

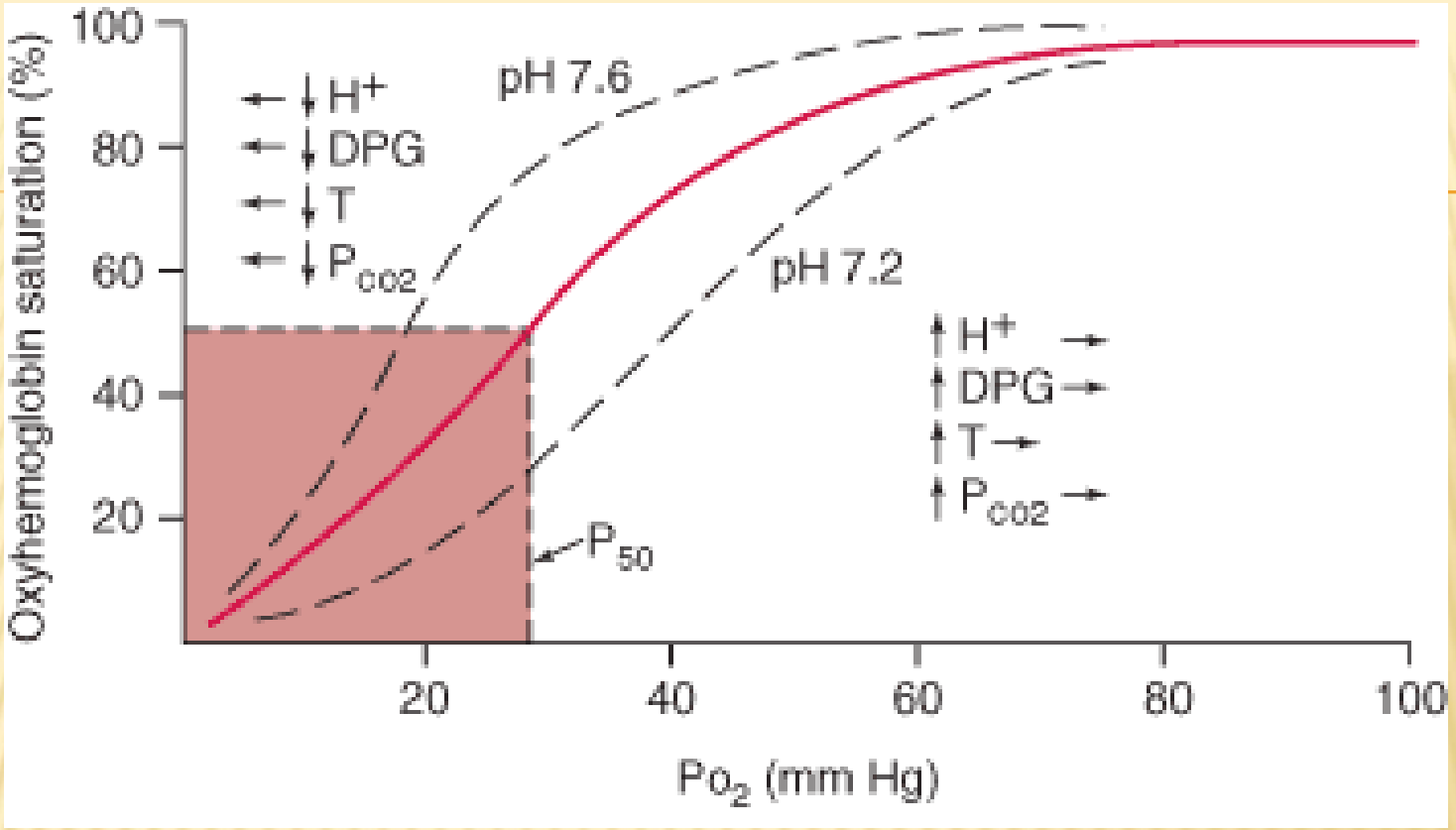
  oxyhemoglobin dissociation curve (*in vitro* determination)

  PO_2 = 100 mmHg → 98% HbO_2 saturation

  PO_2 > 60 mmHg → > 90% HbO_2 saturation

  PO_2 = 45 mmHg → 75% HbO_2 saturation …(reservoir)

  PO_2 = 20 mmHg → 35% HbO_2 saturation (in exercising mm.)

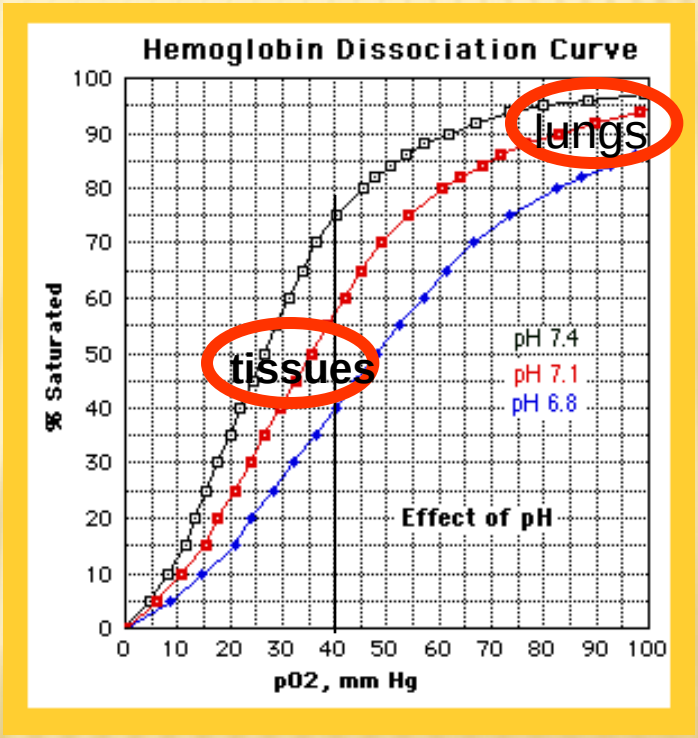


Arterial oxyhemoglobin saturation is related to Po_2. Po_2 at 50% saturation (P_{50}) is normally 27 mm Hg.

The dissociation curve is shifted to the right by increased hydrogen ion (H^+) concentration, increased RBC 2,3-diphosphoglycerate (DPG), increased temperature (T), and increased Pco_2. Decreased levels of H^+, DPG, temperature, and Pco_2 shift the curve to the left. Hb characterized by a rightward shifting of the curve has a decreased affinity for O_2, and Hb characterized by a leftward shifting of the curve has an increased affinity for O_2.

FACTORS INVOLVED IN O_2-HB BINDING

Changes in
- ↑ t (ºC)
- ↑ PCO_2 → Shift to the right of HbO_2 dissociation curve
- ↓ pH



More changes in the steep part of the curve, corresponding to the tissues in the periphery

For PO_2 = 40 mmHg, more O_2 is released from Hb, with decrease in pH

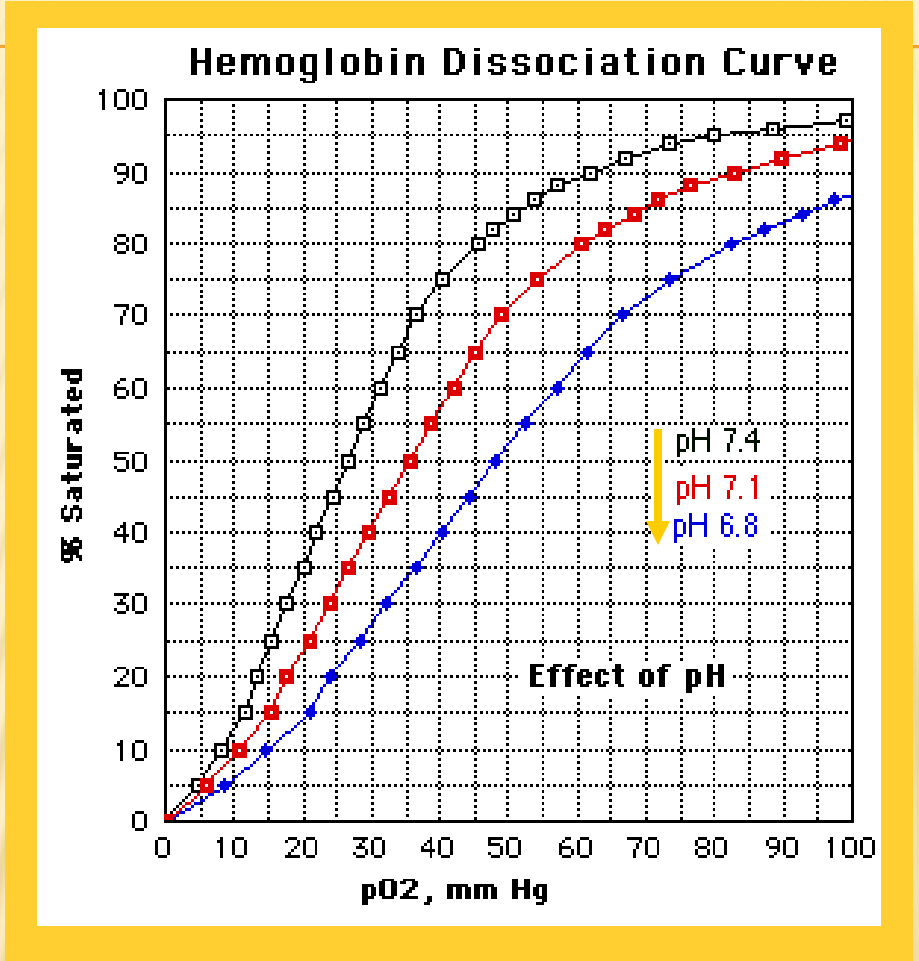
ACID CONDITIONS HELP RELEASE O_2 IN THE TISSUES: BOHR EFFECT

- The **increased unloading/releasing of O_2 at low pH** is known as the **Bohr effect**

- Active tissues make lots of acid (carbonic and lactic)→ the low pH decreases Hb affinity for O_2 and causes more O_2 to come off in the tissues – an effect important during exercise

- H+ stabilise the T form of HB→ lowers the affinity of Hb for O2→ right shift of the OxyHB curve

Bohr effect: decreased pH shift curve to the right



The left (black) curve is at pH 7.4, the middle (red) curve is at pH 7.1, and the right (blue) curve is at pH 6.8. For any pO_2 the hemoglobin is more saturated at pH 7.4 than at pH 6.8.

THE RELATIONSHIP BETWEEN HB- OXYGEN AND CO2- LUNGS LEVEL

HALDANE effect – how O2 determines HB affinity for CO2

CO2 binds to amino groups→ carbaminoHB

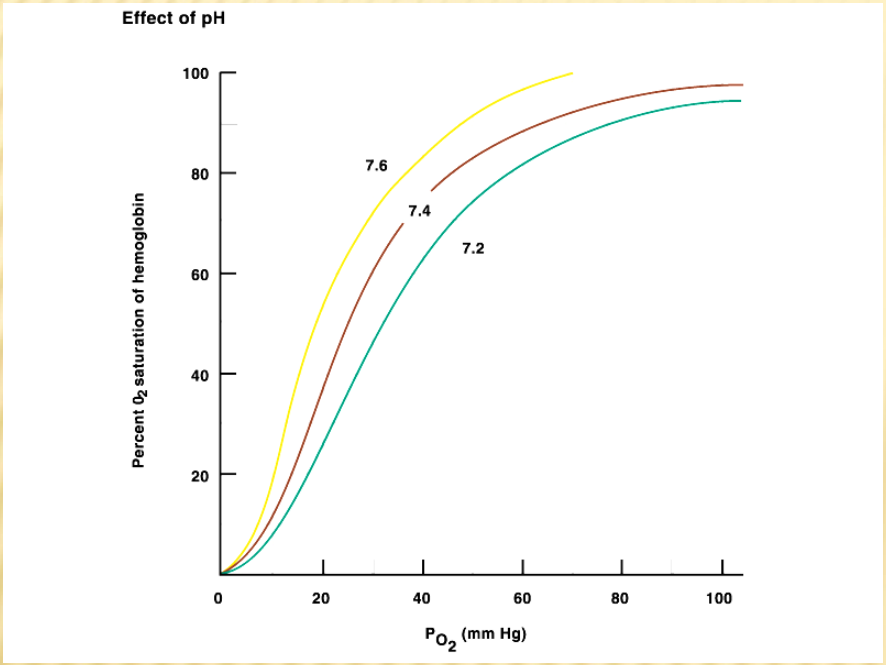
- CO2 binds first the alpha chains (when pCO2 is low)- oxygen and CO2 coexist on the HB molecule

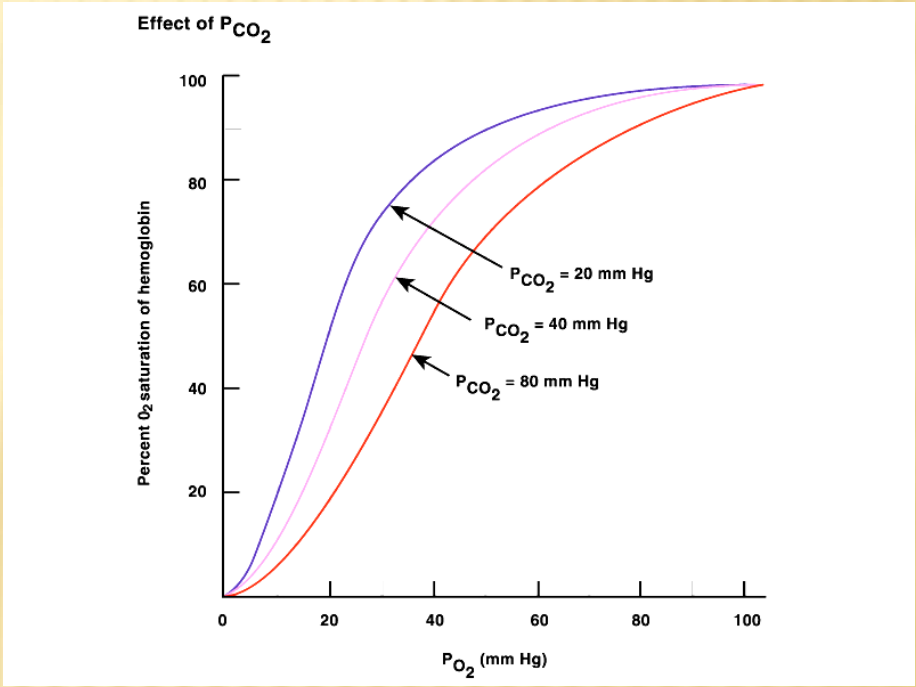
- only after the alpha chains are being saturated that it binds to beta chains (pCO2 is high)- O2 is released

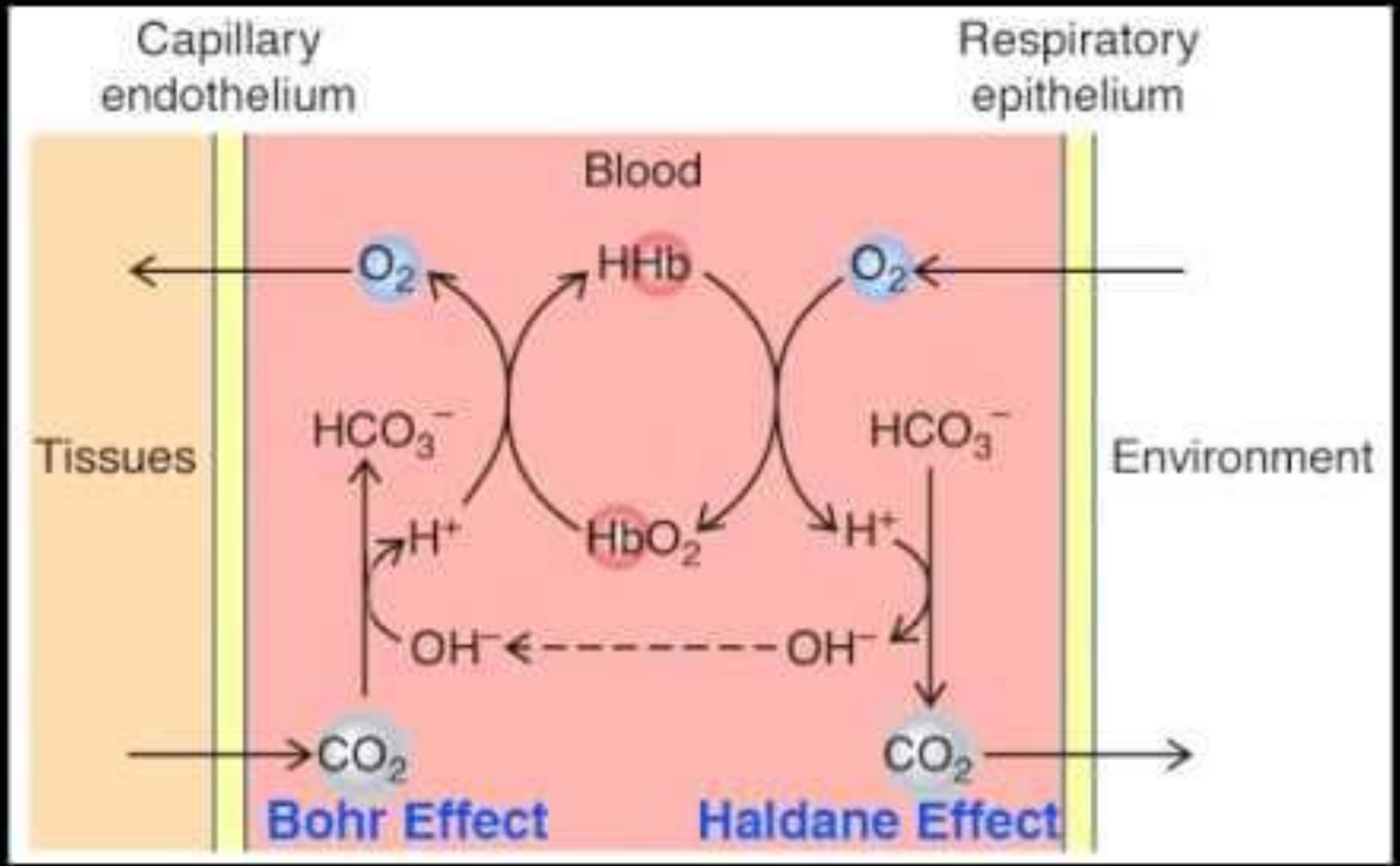
BOHR like effect

O2 with a high pressure→ detaches H+ from HB→ shifts the bicarbonate equillibrum equation to the right→ CO2 formation→ exhaled

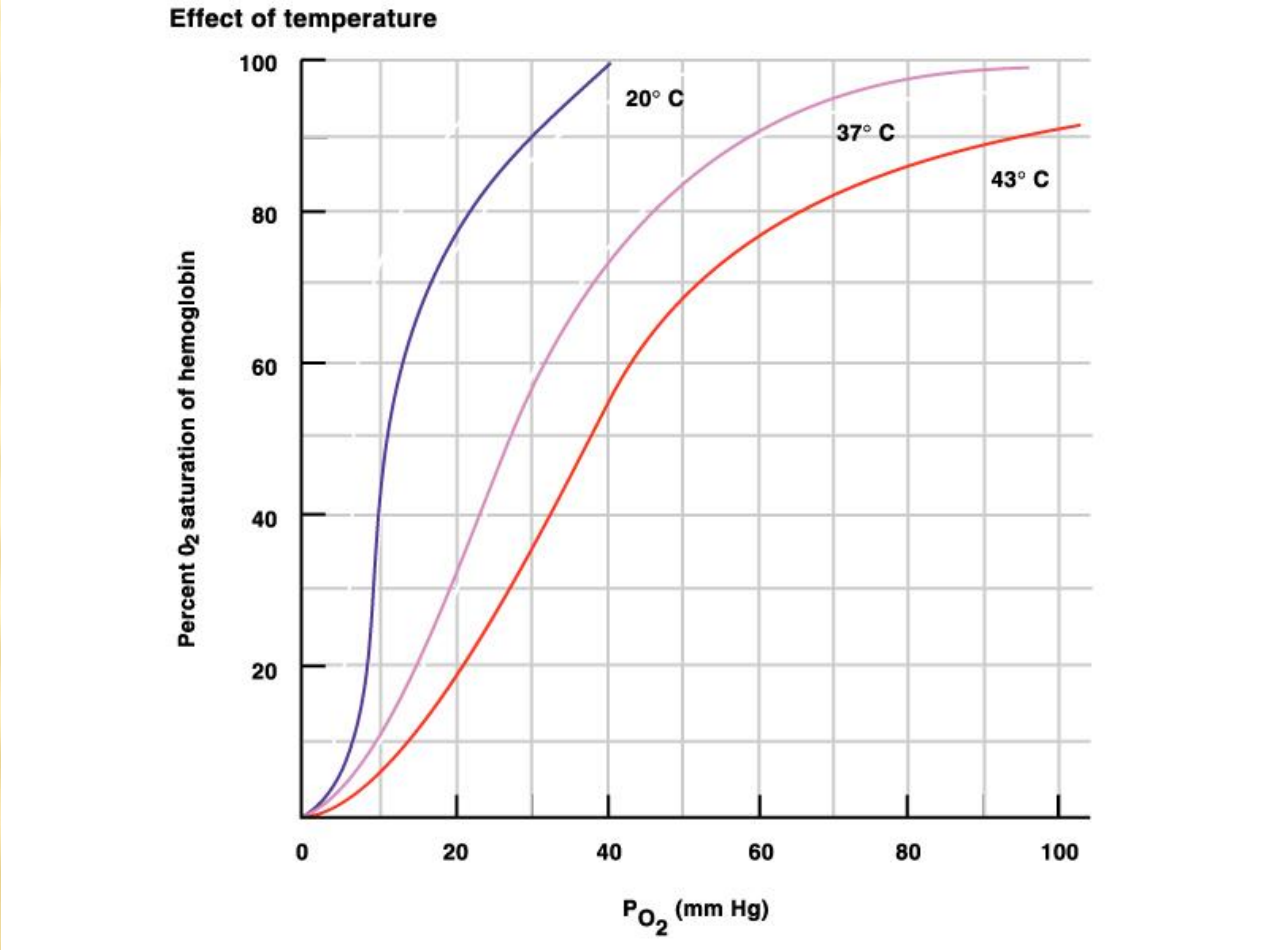
EFFECT OF PH AND PCO_2 ON HBO_2 DISSOCIATION CURVE





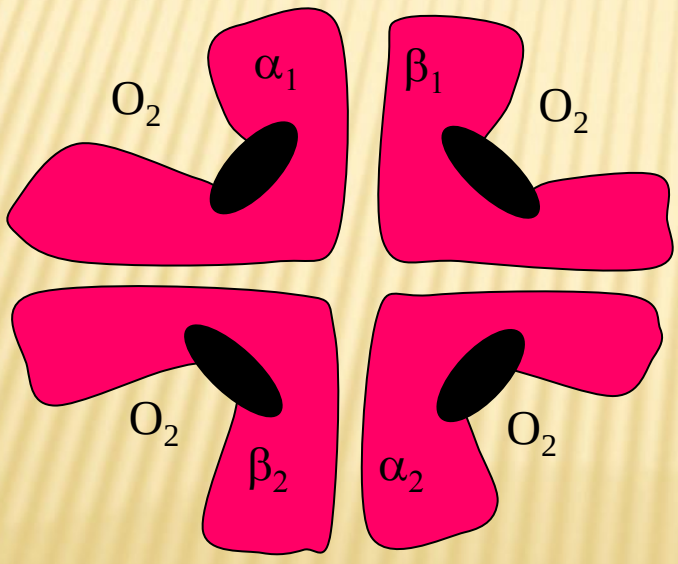

Capillary endothelium
Respiratory epithelium
Blood
Tissues
Environment
O_2
HHb
O_2
HCO_3^-
HCO_3^-
H^+
HbO_2
H^+
OH^-
OH^-
CO_2
CO_2
Bohr Effect
Haldane Effect

EFFECT OF TEMPERATURE ON HBO_2 DISSOCIATION CURVE

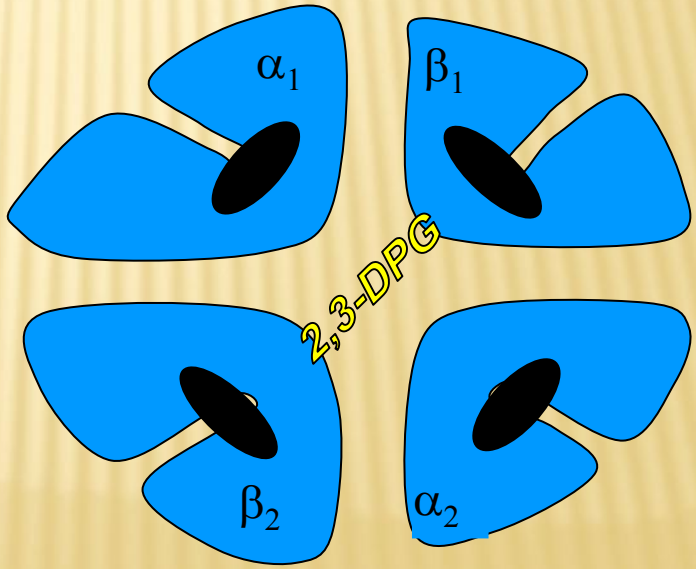


2,3-DPG (2,3-diphosfoglycerate)

Intermediate of glycolysis pathway; Embden Meyerhof pathway.
Increased production in RBC in chronic hypoxia (anemia, high altitude)
Lowers affinity of Hb for O_2, by binding to deoxyhemoglobin
Binds 1:1 with Hb - binds with 3 positively charged groups on each β-chain
Shift HbO_2 dissociation curve to the right
After oxygenation 2,3-DPG is extruded.
HbF binds 2,3-DPG less strongly than HbA (adult Hb).

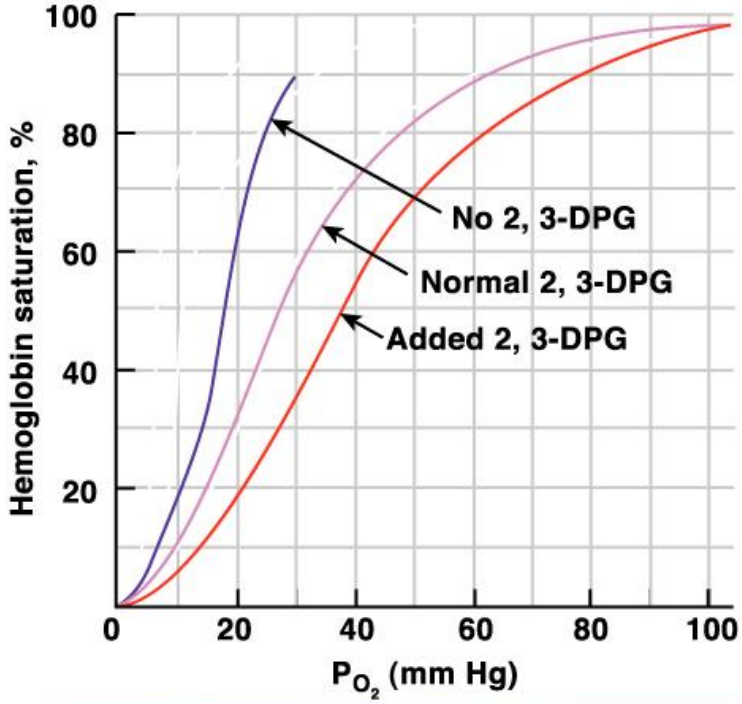


Oxyhemoglobine

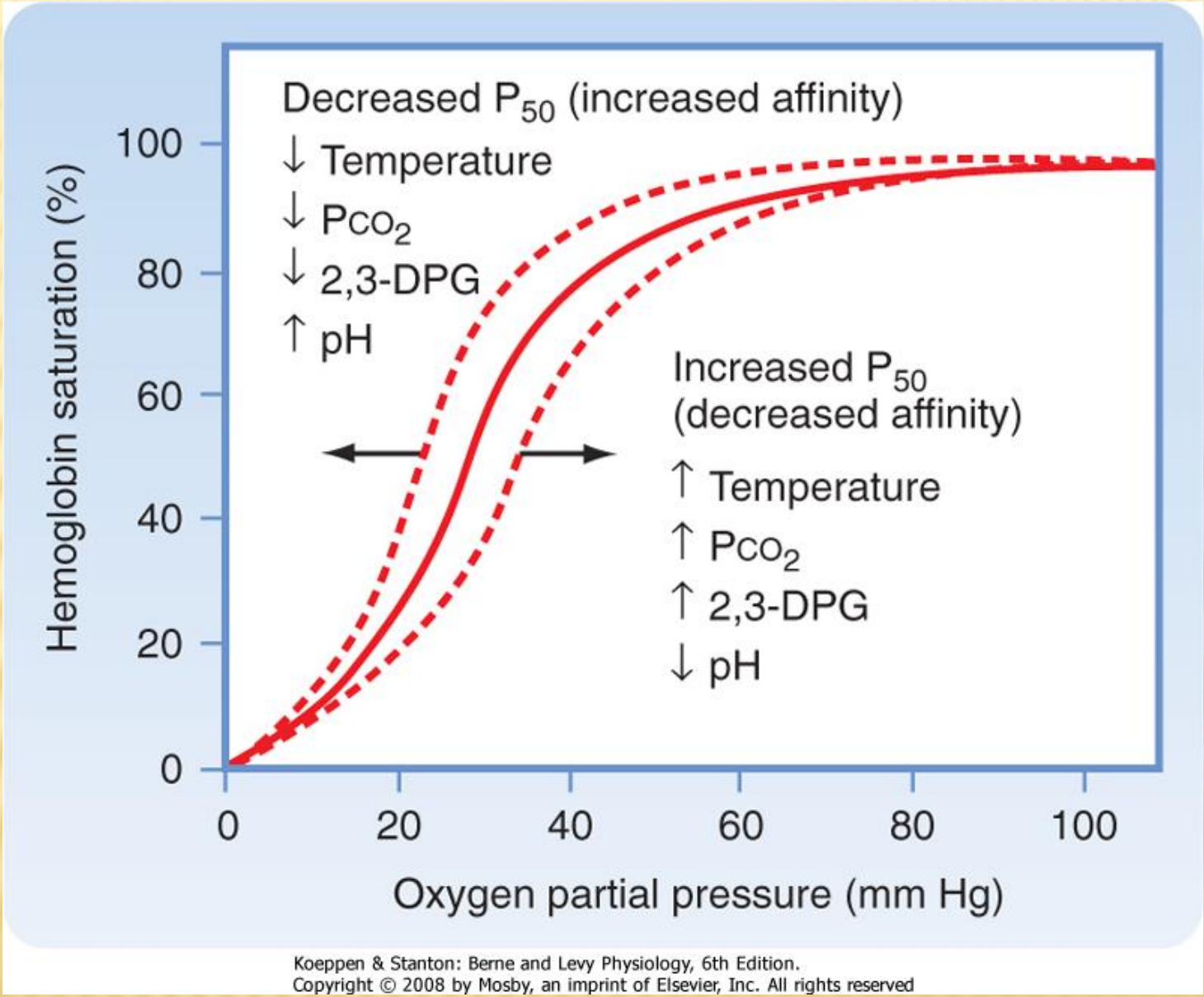


Deoxyhemoglobine

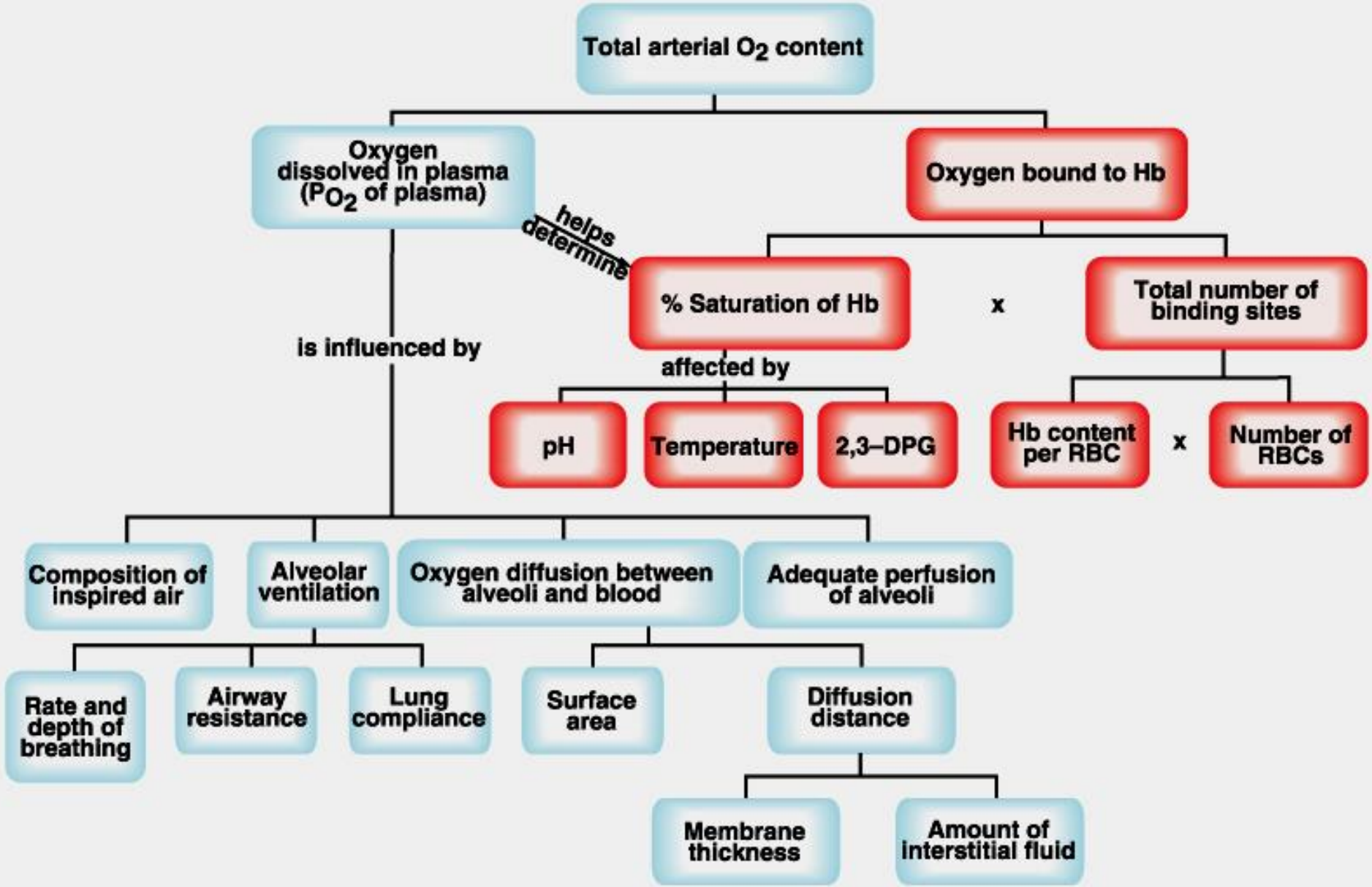
EFFECT OF 2,3-DPG ON HBO_2 DISSOCIATION CURVE



Graph question: Blood stored in blood banks loses its normal content of 2, 3-DPG. Is this good or bad? Explain.



FACTORS CONTRIBUTING TO THE TOTAL O2 CONTENT OF ARTERIAL BLOOD



Gas transport in the blood - CO_2 transport

CO_2 – potential toxic waste: when increase (hypercapnia)
→ acidosis → depression of CNS, etc

Transport / removal of CO_2 from the tissues in 3 ways:

7% dissolved in the plasma (venous blood)

93% diffuses in RBC

→ 70% converted to HCO_3^-

- transported to the lungs & act as a buffer for metabolic acids

- the H^+ concomitantly released is buffered by Hb → **Hb.H**;
- if CO_2 ↑→ H^+ ↑ → no Hb available for H^+ buffering
→ respiratory acidosis

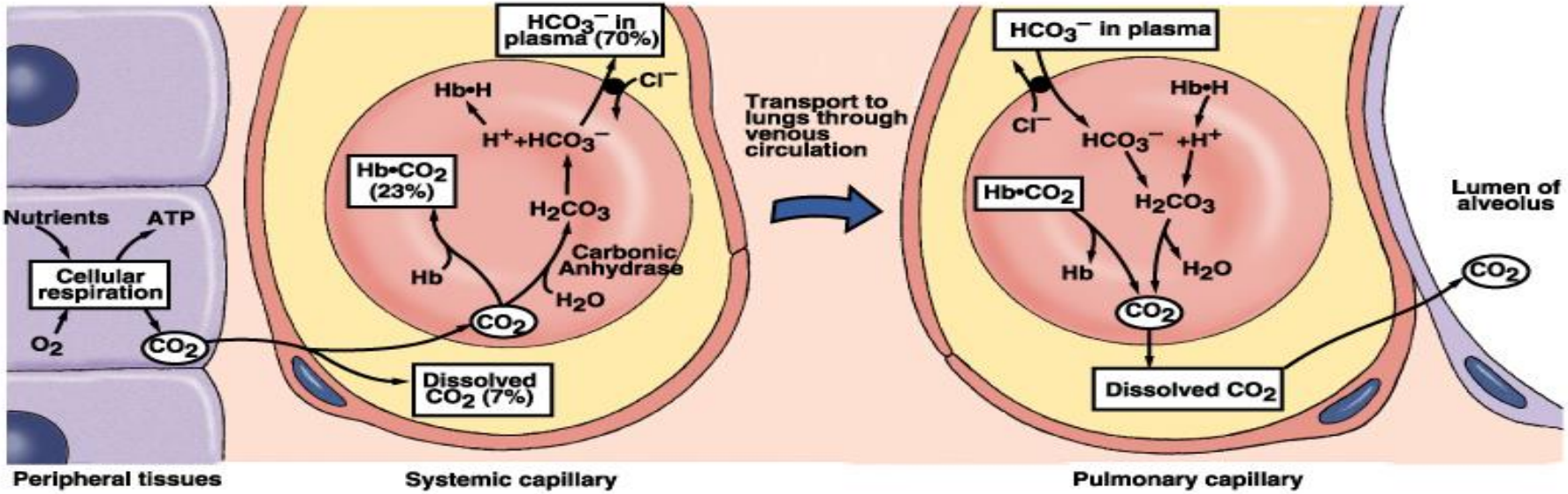
→ 23% binds to Hb → $Hb.CO_2$ (carbaminoHb)

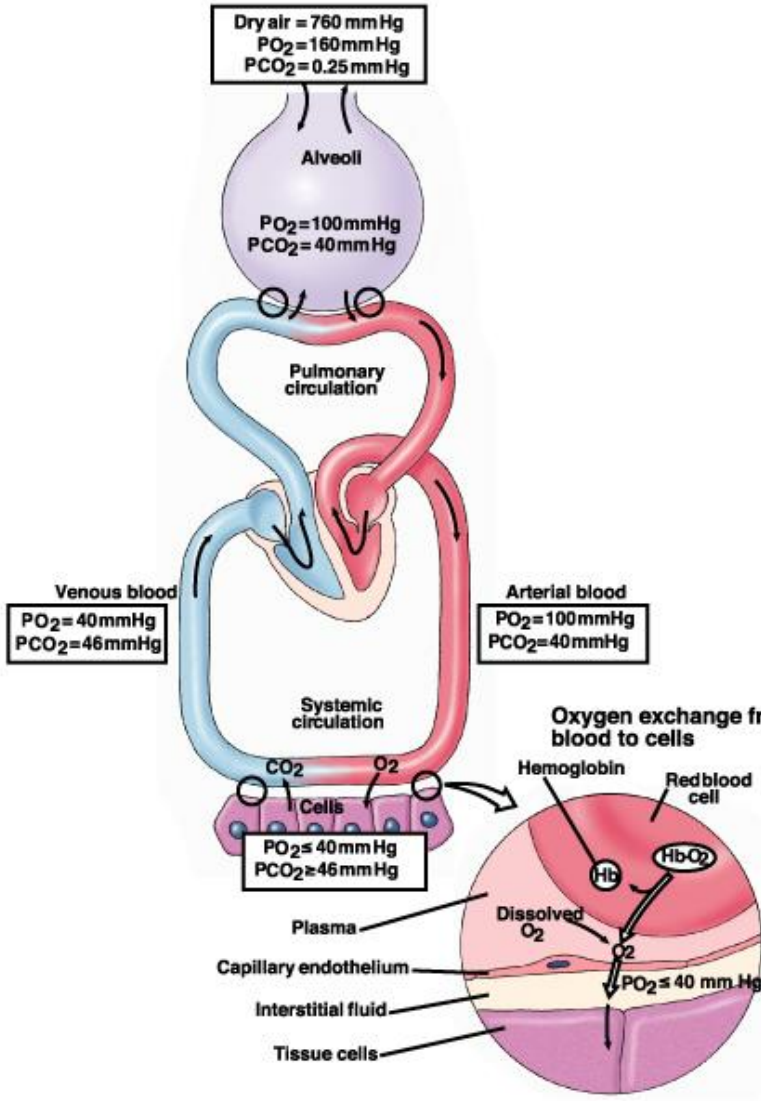
- formation of $Hb.CO_2$ is favoured by the presence of CO_2 and H^+ (decrease Hb affinity for O_2)

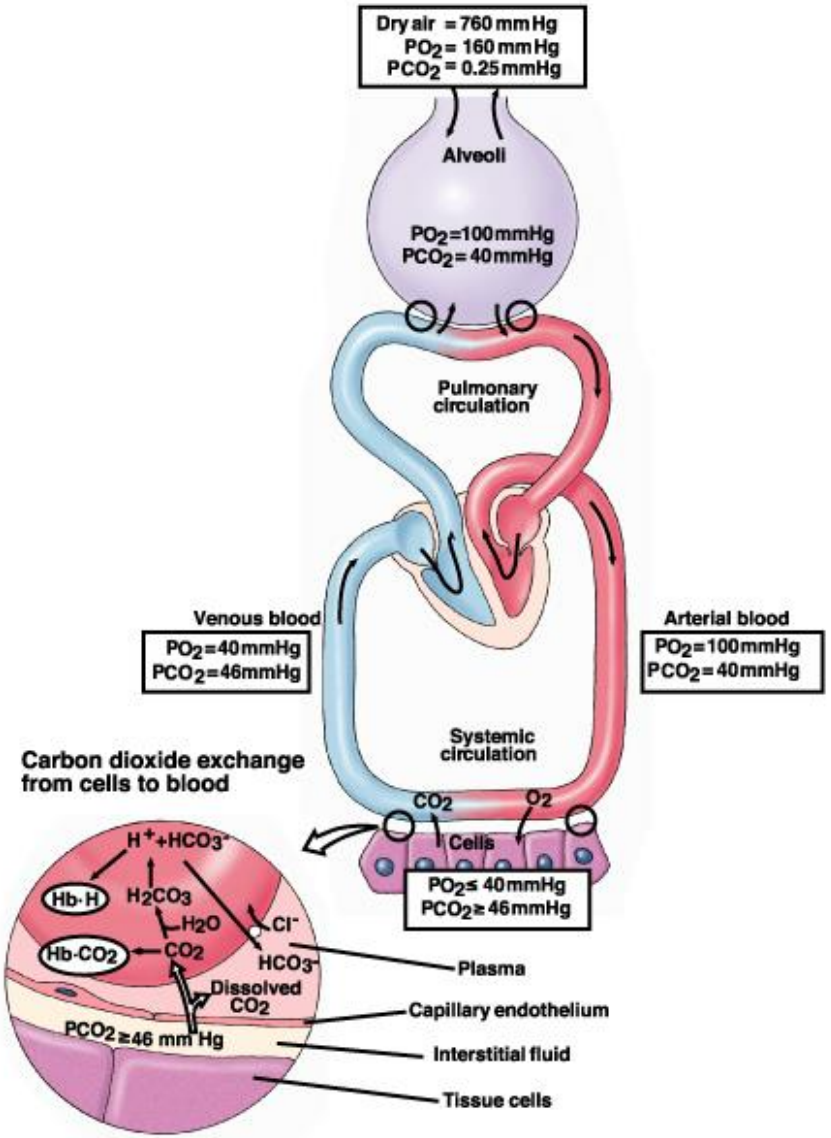
CO_2 removal at the lungs

- ↓PCO_2 → equilibrium of CO_2 - HCO_3^- reaction favours CO_2 release and diffusion into the alveoli
- $H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow \mathbf{CO_2} + H_2O \rightarrow \mathbf{CO_2}$ released
- reverse of HCO_3^- gradient → HCO_3^- enter RBC in exchange with Cl- (chloride shift)

$$CO_2 + H_2O \xleftrightarrow{CA} H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$







Gas transport

Anemia = reduction of Hb conc. in the peripheral blood below the normal for the age and sex

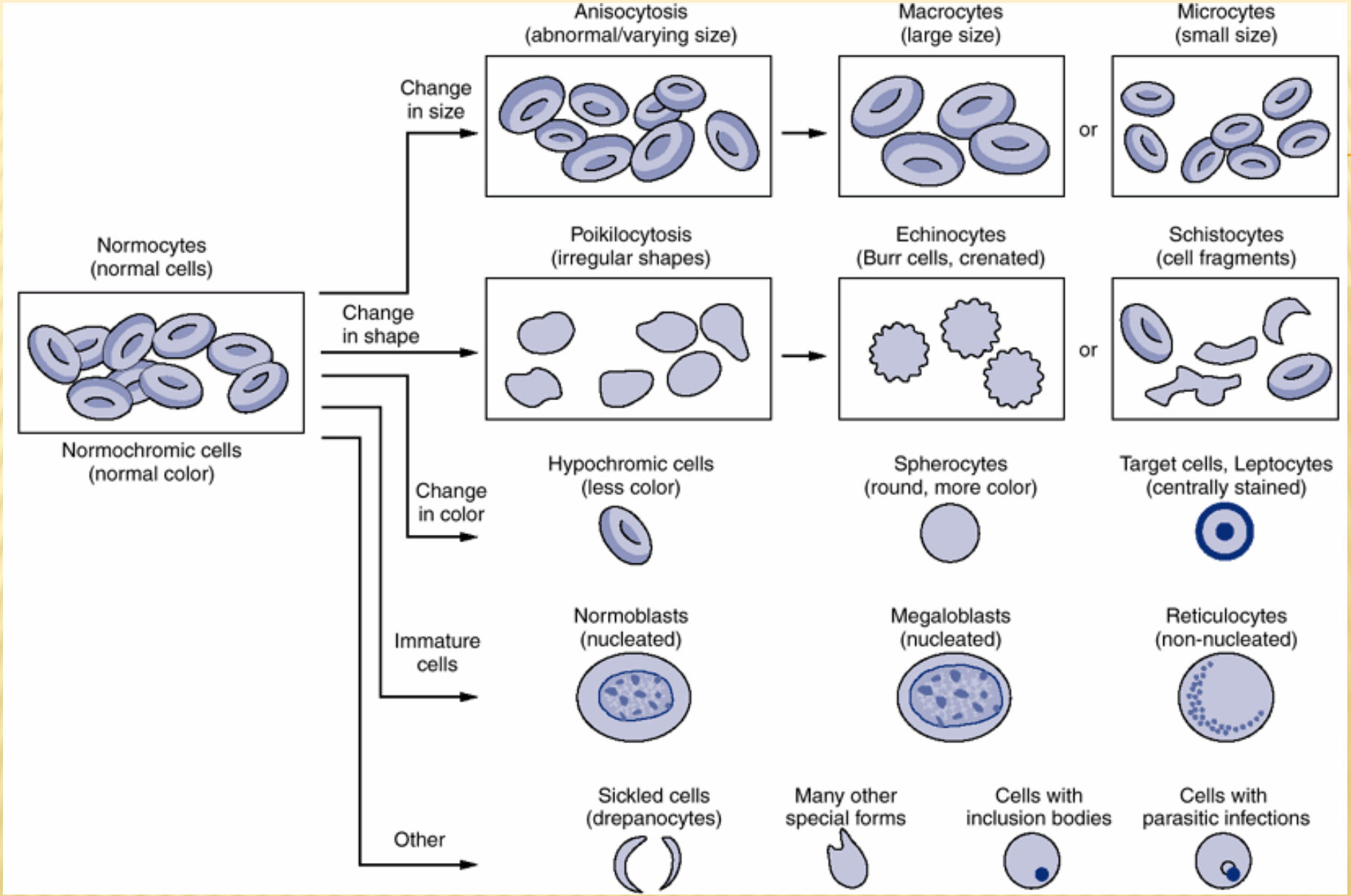
- relation plasmatic volume – Hb conc.
- decrease RBC number and / or decrease Hb
  - Blood loss anemia – hemorrhagic anemia (acute/chronic)

    Acute hemorrhage → plasma is restored much faster than the cellular elements → hemodilution, with normocytic, normochromic anemia

    Chronic blood loss→ microcytic, hypochromic /iron-deficiency anemia
  - Hemolytic anemia (hereditary spherocytosis, sickle cell anemia, erythroblastosis fetalis)
  - Deficiency anemia: Megaloblastic anemia, Iron-deficiency anemia
  - Hemodilutional anemia: hypervolemia due to volume retention
  - Aplastic anemia

Polycythemia

- Secondary polycythemia (hypoxic conditions); physiologic polycythemia for the natives who live at high altitudes
- Polycythemia vera (erythremia) – excess production of RBC



Pathological changes in erythrocyte morphology. The synopsis of erythrocyte abnormalities presents a variety of possible deviations that are helpful for diagnosis of anemias and other diseases (from Rhoades & Bell, 2009).

Blood Groups

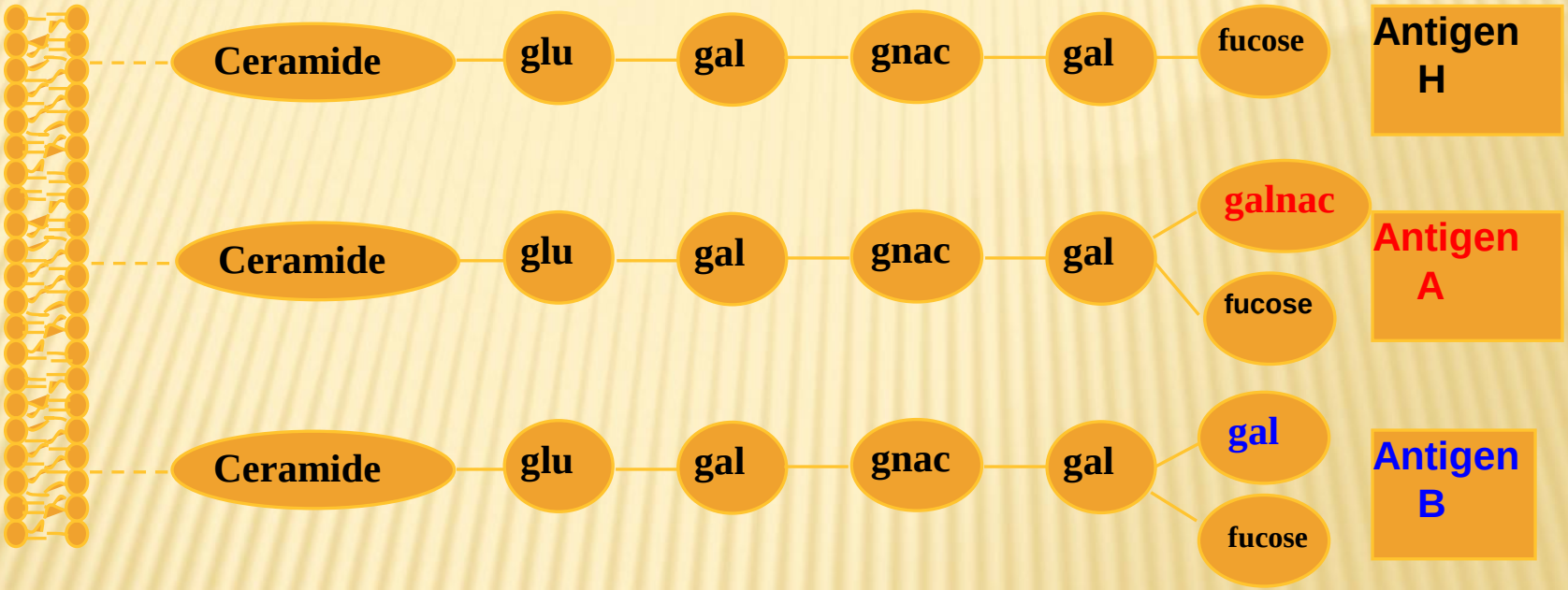
The ABO Blood Groups & The Rh System

Blood groups are created by antigens (AGs) present on the surface of red blood cells.

RBC AGs are membrane proteins/glycoproteins/glycolipids encoded by a number of different genes, but lack the MHC proteins found on nucleated cells.

First discovered in 1900 and from then on, the most important in assuring safe blood transfusions.

The ABO Blood Groups: structure of ABO antigens



O group: H substance with a terminal L-fucose

A group: Galnac = N-acetyl galactosamine

B group: Gal = D-galactose

BLOOD TYPES

- Blood group determination depends on:

1. **Aglutinogens**- antigens on the surface of the RBC- most important A and B
2. **Agglutinins**- antibodies in plasma- alpha and beta

- Agglutinins normally exist in the blood.

- A antigen reacts with alpha antibody and B with beta to form an antigen- antibody complex→ leading to RBC agglutination and hemolysis

- !!! A- alpha and B- beta should never coexist

The ABO Blood Groups

The four ABO phenotypes ("blood groups") present in the human population and the genotypes that give rise to them.

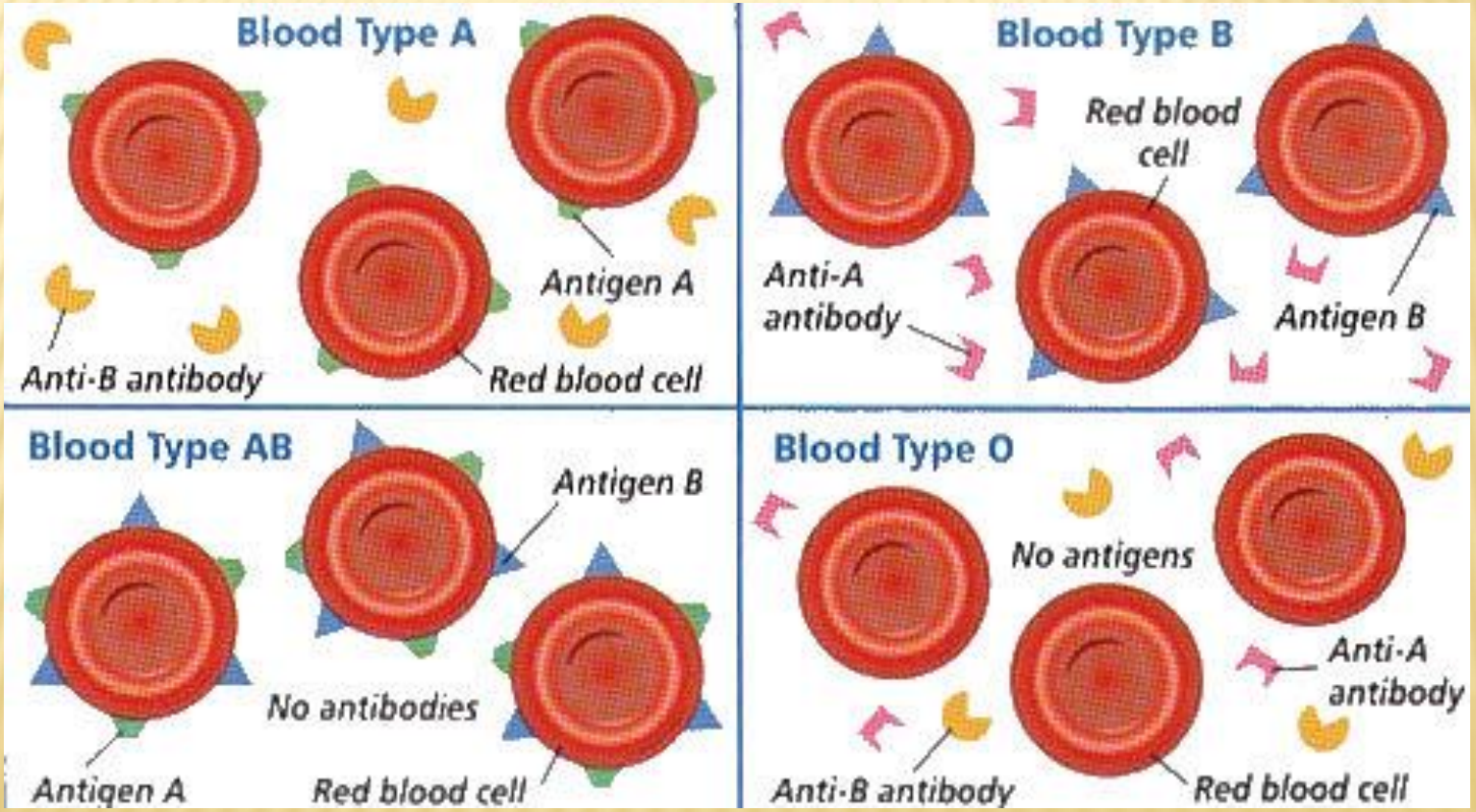
Blood Group	**Antigens on RBCs**	**Antibodies in Serum**	**Genotypes**
A	**A**	Anti-B	*AA* or *AO*
B	**B**	Anti-A	*BB* or *BO*
AB	**A** and **B**	None	*AB*
O	None	Anti-A and anti-B	*OO*

BLOOD TYPES

1. Group A with A antigen on the red cells and anti-B antibodies in the plasma.
2. Group B with B antigen on the red cells and anti-A antibodies in the plasma.
3. Group AB with both A and B antigens on the red cells and neither anti-A nor anti-B in the plasma.
4. Group O with no A or B antigens on the RBC and both anti-A and anti-B antibodies in the plasma.

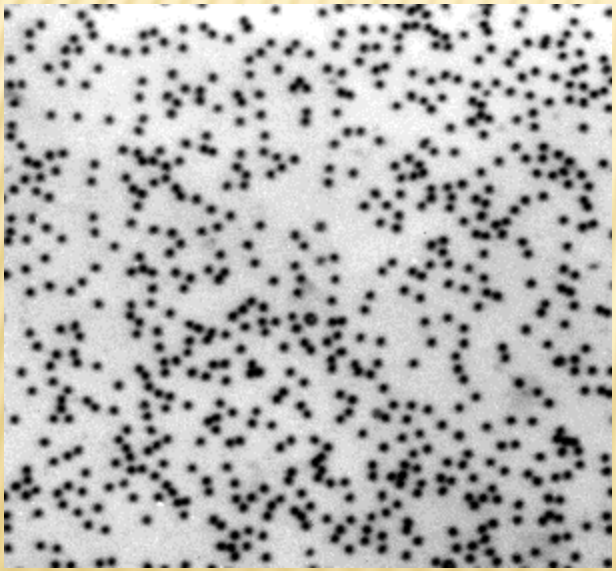
BLOOD TYPES

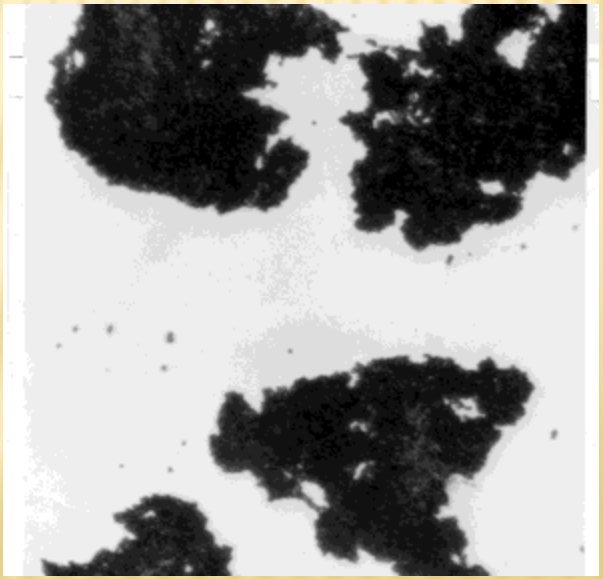
- So… as A and alpha/ B and beta never meet:

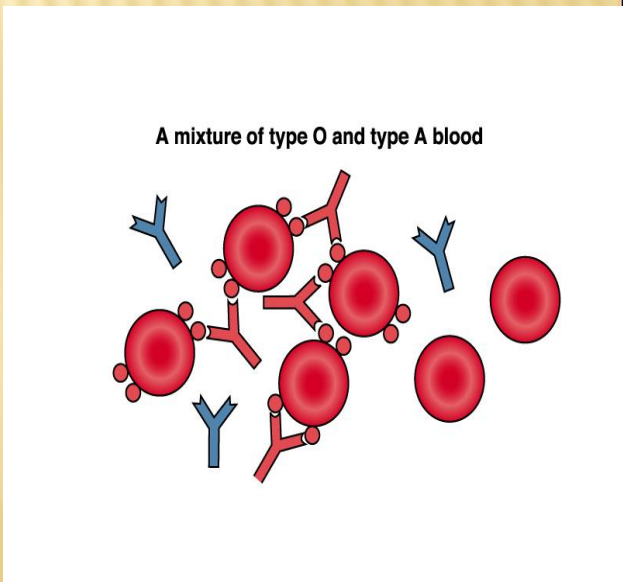


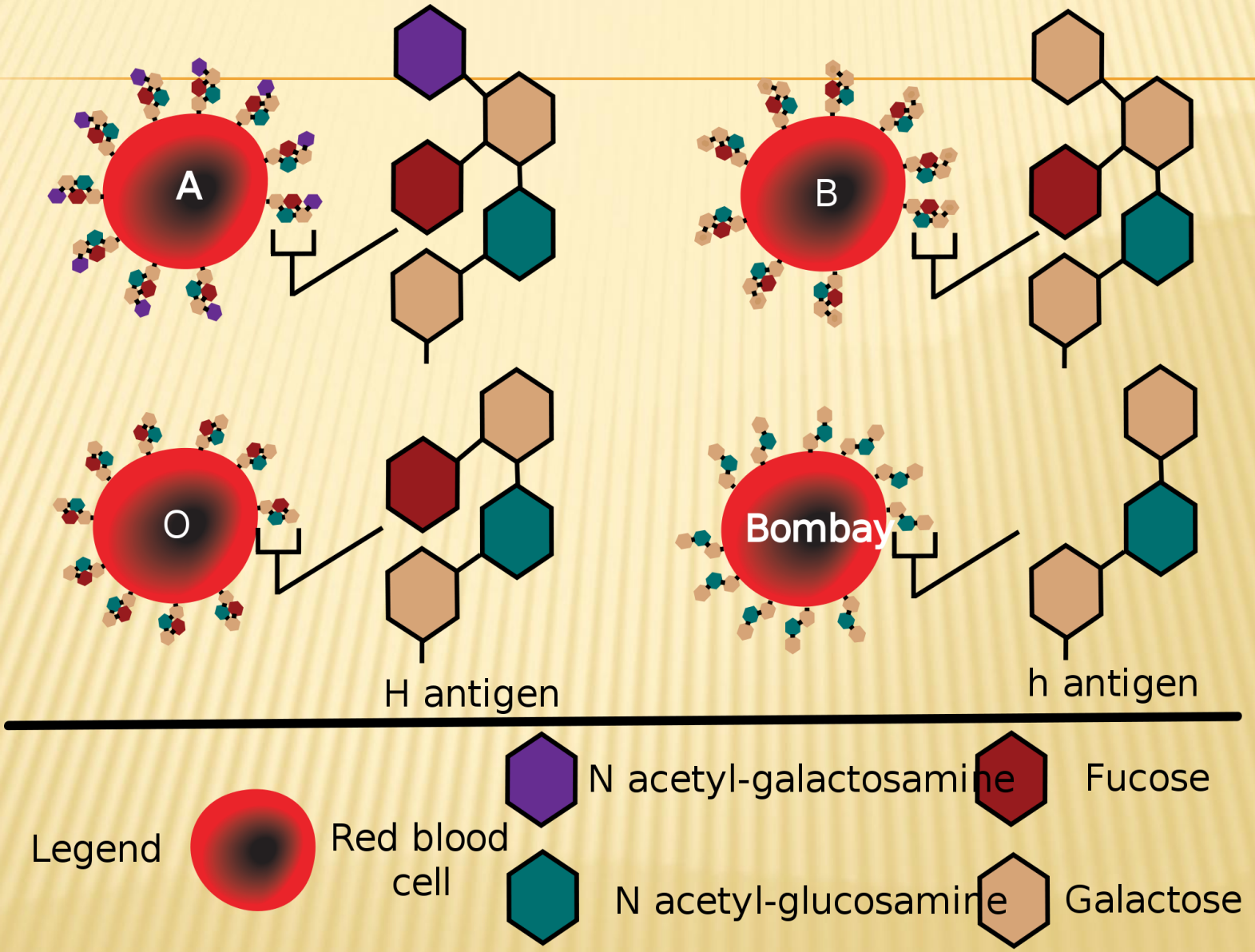
When RBC carrying one or both antigens are exposed to the corresponding antibodies, they agglutinate/clump together. People usually have antibodies against those red cell antigens that they lack.

Human red blood cells before (left) and after (right) adding serum containing anti-A antibodies. The agglutination reaction reveals the presence of the A antigen on the surface of the cells.








A
B
O
Bombay
H antigen
h antigen
Legend
Red blood cell
N acetyl-galactosamine
Fucose
N acetyl-glucosamine
Galactose

- The **ABO system** is the most important blood-group system in human-blood transfusion.
- The associated anti-A and anti-B antibodies are usually IgM

Transfusion…

- Transfused blood must not contain RBC that the recipient's antibodies can clump.
- Although theoretically it is possible to transfuse group O blood into any recipient, the antibodies in the donated plasma can damage the recipient's red cells.
- Thus all transfusions should be done with exactly-matched blood.
- **Why do we have antibodies against red cell antigens that we lack?**

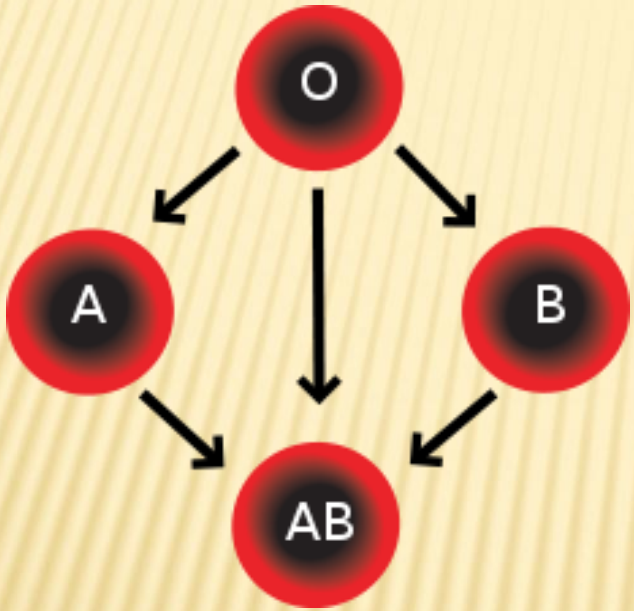
  Bacteria living in our intestine, and probably some foods, express epitopes similar to those on A and B.

  We synthesize antibodies against these if we don’t have the corresponding epitopes (if our immune system sees them as "foreign" rather than "self“).

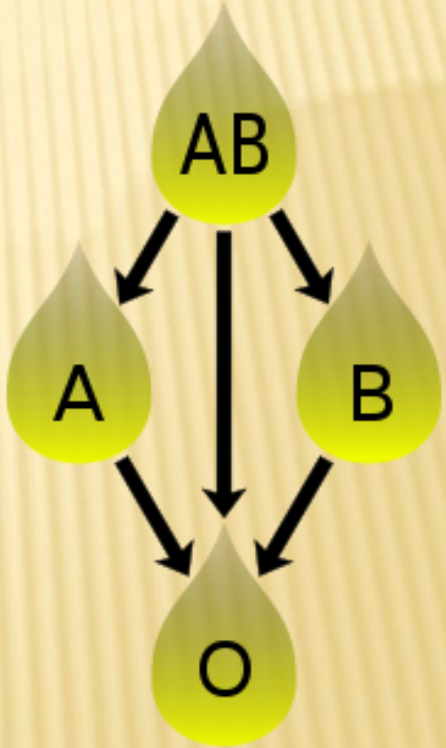
BLOOD TRANSFUSION FOR SMALL QUANTITIES

- Major cross matching occurs when the donor erythrocytes are mixed with the recipient's plasma
- **RULE for small transfusion <500 ml: in the donor blood there should not be any aglutinogen to react with the recipient's aglutinins.**
- O is the universal donor
- AB is the universal recipient

BLOOD AND PLASMA COMPATIBILITY



Red blood cell compatibility chart
In addition to donating to the same blood group; type O blood donors can give to A, B and AB; blood donors of types A and B can give to AB



Plasma compatibility chart
In addition to donating to the same blood group; plasma from type AB can be given to A, B and O; plasma from types A, B and AB can be given to O

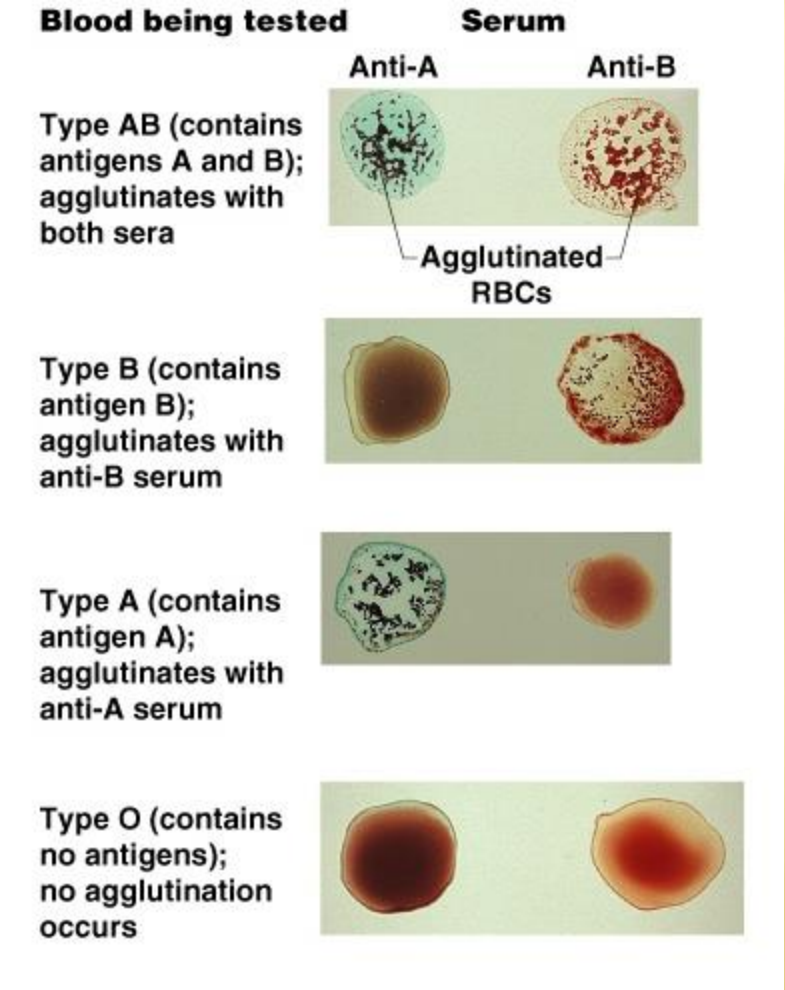
BLOOD TRANSFUSION

- The blood compatibility chart is used only in <500 ml of blood transfusions
- **> 500 ml- same group!!!**
- -because transfused blood also contains plasma (agglutinins) which may destroy recipient's RBC

ABO DETERMINATION TEST

- Beth Vincent method (most used)
- Principle: contact between the erythrocytes to be tested and three types of hemotest serum (that contain known antibodies) will produce agglutination or not, depending on the type of agglutinogen on the cell.
  - Alpha serum
  - Beta serum
  - Alpha + beta serum

BLOOD TYPE DETERMINATION TEST



RH SYSTEM

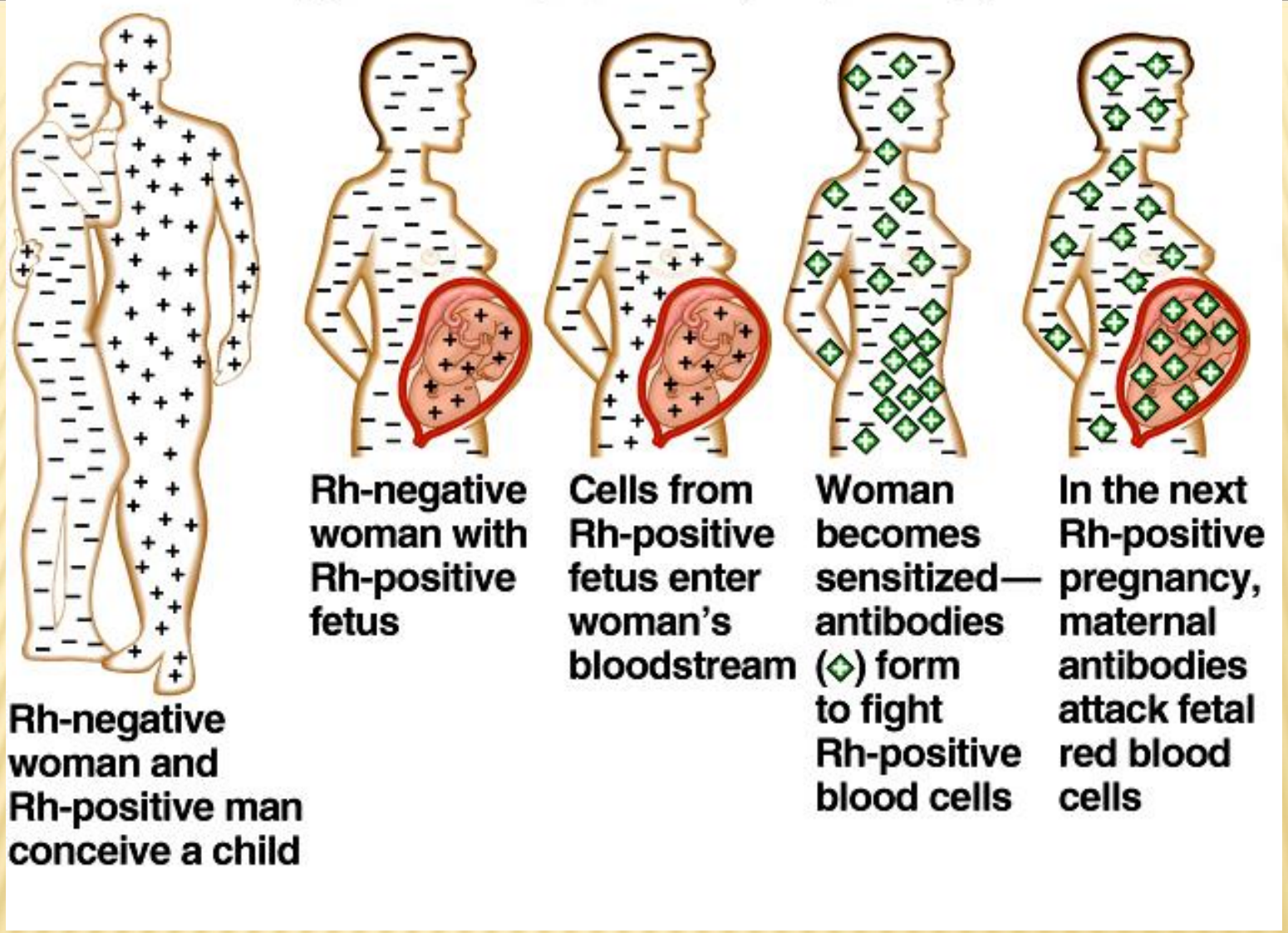
- First detected in 1940 by Landsteiner and Wiener when they injected blood from rhesus monkeys into guinea pigs and rabbits.
- More than 50 antigens have since been discovered
- In routine blood typing and cross-matching tests- the D antigen, (Rh factor or Rho[D])
- If the D antigen is present, that person is Rh-positive (85%); if the D antigen is absent, that person is Rh-negative (15%).
- Unlike the ABO system, **antibodies to Rh antigens don't develop naturally.**
- They develop only as an immune response after a transfusion or during pregnancy.

RH SYSTEM

- Rh system becomes important when one considers the eventuality of **Rh incompatibility between mother and fetus**; in such a case, the antibody-mediated cytotoxicity mechanism involved threatens the fetus/ **or in transfusion**
- During birth, a leakage of the baby's red blood cells often occurs into the mother's circulation. If the baby is Rh positive (inheriting the trait from its father) and the mother is Rh negative, these red cells will cause the mother to manufacture antibodies against the Rh antigen.
- The antibodies (IgG class) do not cause problems for that first born, but can cross the placenta and attack the red cells of a subsequent Rh+ fetus.
- The red cells are destroyed, leading to anemia and jaundice- erythroblastosis fetalis or hemolytic disease of the newborn- may result in fetal death.

RH DETERMINATION

- Similar principle as in blood group typing
- Anti- D serum placed over Rh negative blood- no agglutination
- Anti- D serum placed over Rh positive blood- agglutination



The Rh- mother will produce anti-Rh antibodies.
Causing hemolytic disease of the newborn this can lead to brain damage, mental retardation, and even death.

RHOGAM

- Serum that contains antiRh antibodies
- Prevents isoimmunisation
- It is reccomended to Rh – mothers with Rh + babies:
  - In the 28th week of pregnancy (controversy- neurotoxic preservatives? in the shot)
  - Within the first 72 h after delivery (only if the baby is Rh +)