Unconventional Sources
of Dietary Fiber

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Physiological and in Vitro Functional Properties

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General Mills, Inc.

Based on a symposium

sponsored by the

ACS Division of Agricultural

and Food Chemistry

at the 183rd Meeting of the

American Chemical Society,

Las Vegas, Nevada,

March 28–April 2, 1982

A C S   S Y M P O S I U M   S E R I E S **214**

ACS Symposium Series

M. Joan Comstock, *Series Editor*

FOREWORD

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PREFACE

T HE IDEA OF ORGANIZING THIS SYMPOSIUM was born at the 179th Annual ACS meeting in Houston in 1980, from discussions with the previous chairman of the Agricultural and Food Chemistry Division, Dr. Robert Ory. It was felt that the subject of dietary fiber should be viewed in a broader spectrum that should not cover excessively the current common, almost classical types, of fiber such as wheat and corn bran or cellulose. It should also encompass less conventional sources of dietary fiber which would be the main topic of this symposium. Because soluble nondigestible plant polysaccharides are now included in the definition of dietary fiber, and because numerous nondigestible polymers of nonplant origin exist, greater attention should be paid to these alternatives. These polymers have a variety of unique physiological, chemical, and functional properties that need to be better understood and utilized. This type of understanding is frequently achieved when different points of view are presented and a variety of scientists engage in discussion.

Presentations in this volume come from chemists, medical researchers, and microbiologists, as well as nutritionists and food scientists. In a few cases, rather exotic fiber types such as tobacco fiber, wheat straw lignin, or shellfish aminopolysaccharides are discussed. Other sources include psyllium, different legumes, and vegetable and fruit fibers. There is no doubt that additional unconventional fiber sources will be continuously identified and increasingly used.

It is my hope and belief that this publication will help in the expansion and utilization of fiber sources in foods, as well as in pharmaceutical and medical preparations. After all, we should recognize that the chance to accomplish this is quite favorable because "complex carbohydrates," which are the main constituents of dietary fiber, represent the only group of major food components for which increased daily intake is repeatedly being recommended.

I would like to express my appreciation and thanks to all participants of this symposium and to the writers of the individual chapters for their efforts and contributions. Without them, this undertaking and publication

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would not be possible. Finally, I am grateful to Akiva Pour-El for his administrative help, and to General Mills and The Quaker Oats Company for their generous financial support.

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November 1982

Effect of Conventional and Unconventional Dietary Fibers in Colon Carcinogenesis

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Epidemiologic and animal model studies sug-
gest a protective effect of certain dietary fibers
against colon cancer. This protection by the
dietary fibers may be mediated through (a) the di-
lution of tumorigenic compounds in the gut,(b) the
binding of tumorigenic compounds to the fiber in
the gut, and (c) an indirect effect on the meta-
bolism of carcinogens. Experiments were conducted
in animal models to study the effect of certain
non-conventional dietary fibers on colon carcino-
genesis. Rats were fed the diets containing al-
falfa, citrus pulp, pectin, wheat bran, undegraded
carrageenan or cholestyramine and treated with
azoxymethane (AOM), 3,2'-dimethyl-4-aminobiphenyl
(DMAB), or methylnitrosourea (MNU) to induce
tumors. Animals fed the wheat bran or citrus pulp
and treated with AOM or DMAB had a lower colon
tumor incidence than did those fed the control
diet and treated with respective carcinogens. Al-
falfa diet had no effect on AOM- or MNU-induced
colon tumors, whereas the pectin diet inhibited
AOM-induced but not MNU-induced colon tumors.
Animals fed the diets containing undegraded carra-
geenan or cholestyramine developed more AOM and/or
MNU-induced colon tumors than did the rats fed the
control diet. Thus, the protective effect of var-
ious dietary fibers in colon carcinogenesis de-
pends on the type of fiber fed, as well as the
type of carcinogen used to induce tumors.

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Rapid progress has been made in basic concepts concerning carcinogenesis (1,2). There are agents which are genotoxic, which by definition interact with the gene to yield an abnormal genetic material and would be considered initiating agents (3). The second broad class of agents act by epigenetic mechanisms and tend to increase the development of lesions initiated by genotoxic carcinogens. Thus, this list includes co-carcinogens which operate at the same time as genotoxic carcinogens and can alter the metabolism of a genotoxic agent with an increased ratio of activation/detoxification metabolites. Such agents can also act as more classic tumor promoters which exhibit their effect after the action of a genotoxic carcinogen.

Current concepts of colon cancer etiology stem from multidisciplinary research efforts based on three major approaches, namely: (1) the variation in incidence of colon cancer as a function of area of residence, with particular regard to migrant population; (2) the changes in incidence as a function of time; and (3) detailed laboratory studies in humans, in animal models, and through in vitro systems. The consistency of these findings suggests that environmental factors in general, and dietary factors in particular, play a dominant role in the development of colon cancer in humans.

Epidemiologic studies suggest that diets particularly high in total fat and low in fiber and in certain vegetables as well as high intake of beef are generally associated with an increased incidence of large bowel cancer in man (4-10). Dietary fat may be a risk factor in the absence of factors that are protective, such as use of high fibrous foods and fiber (11,12). As an example, in Finland, where the dietary intake of fat is similar to many of the Western countries and the fiber intake is higher, the incidence of colon cancer is lower than all of the Western countries.

This brief review evaluates current research on the relation between dietary fiber and large bowel cancer in humans, including the use of animal models. This brief review also presents an evaluation of the mechanism whereby certain dietary fibers including conventional and unconventional fibers modify the risk for the development of colon cancer.

Correlation and Case-Control Studies

Cross-national correlations between the incidence of colon cancer and dietary habits have been used to select hypotheses for testing in case-control and cohort studies. These studies have shown that certain food preferences appear to be associated with either a high- or a low-risk for colon cancer. When such correlations are supported by experimental evidence from animal studies, the hypothesis could be attractive.

Burkitt (10,13) first observed the rarity of large bowel cancer in most African populations and suggested that populations consuming a diet rich in fiber have a lower incidence of this type of cancer, while those eating refined carbohydrates

and little fiber have a higher incidence. A recent study com-
paring low-risk populations in Kuopio, Finland, with those at
high risk in Copenhagen indicated that dietary fiber intake is
higher in Finland compared with Copenhagen (14).   The Finnish
population is unique in this respect because in this country the
total dietary fat is similar to countries with high rate of
colon cancer.   The crucial difference in dietary intake between
Finland and Denmark and other Western countries may relate to
dietary fiber and meat.   Our data also suggest that one of the
factors contributing to the low risk of large bowel cancer in
Kuopio appears to be that a high intake of dietary fiber (mainly
cereal fiber) leads to increased stool bulk, in effect diluting
tumorigenic compounds in the colon (12).   The results are con-
sistent with a possible role for dietary fiber in the prevention
of colon cancer in humans.

     Case control studies have been conducted to study the poss-
ible relation of dietary fiber to large bowel cancer. Recently,
Dales et al. (15) found that among American blacks significantly
more colon cancer patients than controls reported that their
diet was high in saturated fat and low in fibrous foods.   In-
vestigating many dietary constituents, Modan et al. (16) dis-
covered that those contributing less to the diets of patients
with colon cancer than to the diets of controls were those con-
taining fiber.   Bjelke (17) who interviewed hospitalized
patients and controls in Minnesota and in Norway, learned that
colorectal cancer patients less frequently ate vegetables, in
particular the Minnesota patients ate less cabbage. Similarly,
Graham et al. (18) found that individuals who ate vegetables
such as cabbage, broccoli and Brussels sprouts had a lower risk
of colon cancer.

     These studies indicate that diets with a high intake of
total fat and a low intake of certain fibers and certain vege-
tables are generally associated with an increased incidence of
colon cancer in humans.   Even in populations consuming high
amount of fat, high dietary fiber acts as a protective factor in
colon carcinogenesis.

Possible Mechanism of Protective Effect of Dietary Fiber In
Colon Carcinogenesis

     Although the concept of fiber involvement in colon carcino-
genesis is attractive, the data often appear contradictory and
confusing. Discrepancies may have arisen from the general mis-
use of fiber terminology.   As well, experimental design has
failed to account for the possible subtle effect of inhibitors,
especially in relation to the promoting process. Evaluations of
the biologic function of dietary fiber have often lacked com-
plete information on the nature of the fiber.

     Dietary fiber comprises a heterogenous group of carbo-
hydrates, including cellulose, hemicellulose and pectin, and a
noncarbohydrate substance, lignin (19).   According to Van Soest
(20), fibers can be classified into three groups: vegetable

fibers, which are highly fermentable and have little indigest-
ible residue; brans, which are less fermentable; and chemically
purified fibers, such as cellulose, which are relatively nonfer-
mentable. Pectins and gums, soluble substances that are not
true fibers, are considered part of the dietary fiber complex
because of the similar effects they can elicit in the diet.
Wheat bran and vegetable and fruit fibers have different per-
centages of cellulose, hemicellulose and lignin.
Carrageenan, a broad generic class of sulfated polysaccharides
derived from a wide range of seaweed species, can be classified
as non-conventional fiber and is used in food as an emulsifier,
stabilizer, thickener and gelling agent.
     The protective effect of dietary fiber may be due to ad-
sorption, dilution or metabolism of cocarcinogens, promoters and
yet-to-be-identified carcinogens by the components of the fiber
(12,21,22). There is evidence that alfalfa, wheat straw and
some other fibers can bind considerable amounts of bile acids in
vitro (23). This indicates that the different types of non-
nutritive fibers possess specific binding properties. Dietary
fiber could also affect the enterohepatic circulation of bile
salts (24) which act as colon tumor promoters (6). Fiber not
only influences bile acid metabolism (12,22) thereby reducing
the formation of potential tumor promoters in the colon, but
also exerts a solvent-like effect in that it dilutes potential
carcinogens and cocarcinogens by its bulking effect (12) and is
able to bind bile acids and certain carcinogenic compounds
(23,25,26). On the other hand, dietary undegraded carrageenan
markedly enhances the bile acid content in the colon, thereby
increasing the potential colon tumor promoters in the gut (6).
     Smith-Barbaro et al. (26) in our laboratory determined the
capacity of various fibers to bind the colon carcinogen 1.2-di-
methylhydrazine (DMH) in vitro. The percent of DMH bound to
wheat bran, corn bran, alfalfa fiber and dehydrated citrus pulp
was dependent on pH of the medium as well as the type of fiber
examined. Results from this study show that at colonic pH, a
greater percent of DMH was bound by wheat bran than by citrus
pulp. Therefore, it is possible that certain fibers bind car-
cinogen at colonic pH, thus making it unavailable for contact
with the colonic mucosa. Other fibers such as pectin (soluble
fiber) do not bind DMH at colonic pH, but may modify the meta-
bolism of carcinogen via activation/deactivation steps either in
the liver and/or in the colonic mucosa.
     Investigations have been carried out in several laborator-
ies to determine whether there are differences in fecal constit-
uents between populations at high and low risk of colon cancer,
and whether changes in the fiber content of the diet would alter
the concentration of fecal bile acids that act as colon tumor
promoters and the activity of fecal microflora. Recently, we
studied healthy individuals in Kuopio, Finland, an area of low
risk for the development of colon cancer (12). Dietary

histories indicated that the total fat consumption is similar to that in the United States but the intake of cereal fiber in Finland is higher and the daily output of feces three times higher than that of healthy individuals in the United States. The concentration of fecal secondary bile acids, mainly deoxycholic acid and lithocholic acid is less in Kuopio than in the United States, but the total daily output is the same in the two populations because of the threefold greater daily output of feces in Kuopio. This suggests that increased fecal bulk dilutes suspected carcinogens and promoters that may be in direct contact with the large bowel mucosa. Cummings (27) demonstrated that fiber from carrot, cabbage, apple, bran and guar gum produces different responses in fecal weight in humans related to the intake of pentose-containing polysaccharides in the fiber. The fecal weight increased by 127% when bran was added to the diet and 20% when guar gum was added; carrot, cabbage and apple produced intermediate changes.

In another study, Cummings (28) reported that an increase in cereal fiber intake from 17 to 45 g/d increased the fecal weight from 79 to 228 g/d and diluted the fecal bile acids. Kay and Truswell (29) showed that adding wheat fiber to the diet decreased the concentration of fecal bile acids and neutral steroids because of the bulking effect of fiber, whereas the addition of pectin to the diet increased the fecal steroid and bile acid output. These results suggest that the effect on fecal bile acid excretion may depend on the type of fiber consumed.

The effect of dietary wheat bran and alfalfa at 15% level on the composition of fecal bile acids was studied in rats fed a semipurified diet (30). Diets containing wheat bran and alfalfa caused a significant increase in stool weight. The concentration of fecal bile acids, particularly hyodeoxycholic acid, β-muricholic acid, deoxycholic acid and lithocholic acid was lower in rats fed wheat bran, compared to those fed a control diet, but the daily output of these bile acids was the same in both groups. Alfalfa had no effect on the concentration of fecal bile acids, but the daily excretion of deoxycholic acid, lithocholic acid and 12-ketolithocholic acid was increased compared to the control diet. It is apparent from this study that the fecal excretion of bile acids varies with the type and amount of dietary fiber.

Until recently, the nature of the carcinogens responsible for colon cancer not only was obscure, but there were no real leads. Because of potential importance of fecal mutagens in the genesis of large bowel cancer and of possible role of dietary factors in the induction of colon cancer, the fecal mutagenic activity of various population groups with distinct dietary habits and varied colon cancer incidences was determined by several investigators. Ehrich et al. (31) have demonstrated that the stools of South African urban whites who consume a high-fat, low-fiber diet and who are at high risk for colon

cancer development were higher (17% of the individuals) in muta-
genic activity with Salmonella typhimurium strains TA98 and
TA100 without microsomal activation compared to South African
urban and rural blacks (0-5% of the individuals) who consume a
low-fat, high-fiber diet and who are at low-risk. Bruce et al.
(32) were the first to show that the feces of some normal humans
consuming a high-fat, low-fiber diet contained compounds that
caused direct mutagenesis of TA98 and TA100 in the Ames assay.
They have also demonstrated that increased dietary fiber,
-tocopherol or vitamin C reduced fecal mutagens. Kuhnlein et
al. (33) compared fecal mutagens (water extracts) from a group
of vegetarians consuming high-fiber diets with those from per-
sons on typical North American diets containing meat. On TA100
and TA98, ovo-lactovegetarians and strict vegetarians had lower
levels of fecal mutagens than non-vegetarians. Correlation
studies between the pH of the fecal homogenate and mutagenicity
indicate the presence of several fecal mutagens.
     Recently, we have studied fecal mutagens of 3 populations
with distinct risk for the development of colon cancer, a high-
risk population in New York (non-SDA) consuming a high-fat, low-
fiber, mixed-Western diet, a low-risk vegetarian SDA (Seventh-
Day Adventists) and a low-risk population in Kuopio, Finland
consuming a high-fat, high-fiber diet (34). Fecal samples of
non-SDA were highly mutagenic in TA98 without microsomal activa-
tion, followed by TA100 without activation and TA100 with acti-
vation. None of the samples of SDA tested showed mutagenic
activity in any of the tester systems, whereas Kuopio samples
exhibited activity only in TA98 with microsomal activation.

Animal Model Studies in Colon Carcinogenesis: Effect of
Conventional and Unconventional Fibers
     Research on the mechanisms of cancer causation in the large
bowel has been assisted by the discovery of several animal
models that mirror the type of lesions seen in man.   These
models include (a) induction of large bowel cancer in rats
through chemicals such as 3-methyl-4-aminobiphenyl, or 3-methyl-
2-naphthylamine; (b) derivatives and analogs of cycasin and
methylazoxymethanol (MAM) such as azoxymethane (AOM and 1,2-di-
methylhydrazine (DMH), which work well in rats and mice of
selected strains; and (c) intrarectal administration of direct-
acting carcinogens of the type of alkylnitrosoureas, such as
methylnitrosourea (MNU) or N-methyl-N'-nitro-N-nitrosoguanidine
(MNNG) which lead to cancer of the descending colon in every
species tested so far.
     The relation between dietary fiber consumption and colon
cancer has been studied in experimental animals. Wilson et al.
(35) found that Sprague-Dawley rats fed a diet containing 20%
corn oil or beef fat and 20% wheat bran had fewer benign colon
tumors induced by DMH given by gastric intubation than rats fed
a control diet containing 20% fat and no bran. It is possible
that with additional time, a number of tumors that were classi-

fied as benign might have developed a more invasive character. There was no difference in the incidence of colon cancer between the rats fed corn oil and those fed beef fat. In another study, Clapp et al. (36) found that DMH-induced colon tumors were increased in BALB/C mice fed a semisynthetic diet containing 5% fat and 20% W/W corn bran, soybean bran and soft winter wheat bran. DMH-induced colon tumor incidence in mice fed the control diet was surprisingly low, about 12%. In these studies, the experimental diets containing high-fat/no bran and high-fat/high bran, (35) or low-fat/no-bran and low-fat/high bran (36) differed substantially in caloric density. Animals on high bran diets might have consumed quantitatively different levels of vitamins and minerals compared to those fed high fat-no bran diet or low fat-no bran diets because the rats adjust their food intake to compensate for non-digestible fiber in high bran diet. It is also possible that certain fibers may cause abnormal histology and damage to colonic mucosa, thereby altering the absorptive capacity of the intestinal tract as well as bile acid-binding capacity (37,38) which have bearing on colon carcinogenesis.

A recent study by Fleiszer et al. (39) indicated that the incidence of colon tumors induced by DMH in rats decreases as the dietary intake of fiber increases. The number of animals used in this study was small. The diets in that study, namely high bran diet (28% fiber), special chow (15% fiber), rat chow (5% fiber) Flexical diet (0% fiber) differed not only in consistency (that is, solid or liquid) but also in the proportions of protein and fats, which have been shown to have an independent effect on colon carcinogenesis induced by DMH. However, the colon cancer incidence in fiber-free (Flexical) group was lower than in rat chow group. Some reduction in tumor incidence in the rats ingesting a high-fiber diet might be expected on the basis of reduced energy intake. Although the study's findings suggest that reduced intake alone cannot account for the significant protective effect of dietary bran, a better experimental design might have strengthened the results.

In another study, Cruse et al. (40) found that a diet containing 20% wheat bran had no effect on colon carcinogenesis induced by DMH in rats. Not only were the number of animals used in this study small (10 rats/group), but also the doses of the chemical in their experiment were so high (40 mg/kg body weight-/week for 13 weeks) that any protective effect of bran might have been unobservable. In a study of the effect of diet on chemical carcinogenesis, it is important to avoid exposing the animal to an excessive level of carcinogen for a long period, as this may obscure more subtle changes induced by certain dietary modifications. In addition, differences in caloric density of experimental diets attributable to the dilutional effect of added fiber further complicated the interpretation of the data. In fact, the data presented by Cruse et al.(40) suggest that a high-fiber diet reduces the frequency of death due to DMH in rats.

The effect of a diet containing 15% alfalfa, pectin or
wheat bran on colon carcinogenesis by MNU or AOM was studied in
F344 rats by Watanabe et al. (41). In this study, the experi-
mental diets were not adjusted isocalorically. The addition of
pectin or wheat bran to the diet greatly inhibited colon tumor
incidence induced by AOM, a carcinogen requiring host-mediated
metabolic activation (Table I). However, the incidence of AOM-
induced colon tumors was not influenced by the addition of
alfalfa to the diet. The diets containing wheat bran and pectin
did not protect against MNU-induced colon carcinogenesis.

Table I

Colon Tumor Incidence in Female F344 Rats Fed Diets
Containing Pectin, Alfalfa, or Wheat Bran and Treated with
Azoxymethane or Methylnitrosourea

| Diet | % Animals Colon Tumors | |
	Azoxymethane treated	Methylnitrosourea treated
Control	57	69
Pectin	10[a]	59
Alfalfa	53	83[b]
Wheat Bran	33[a]	60

[a] Significantly different from the groups fed the control diet
     or alfalfa diet by X^2 test, $P<0.05$.

[b] Significantly different from the other groups, $P<0.05$.

Thus, modifying effect of pectin on AOM-induced colon car-
cinogenesis might be explained in terms of diet-dependent intes-
tinal mucosal, as well as hepatic, microsomal carcinogen-meta-
bolizing enzyme inhibitors or inducers in the diet that modify
the capacity of the animal to metabolize the carcinogen (42).
However, this factor would presumably not play a role in MNU-
induced carcinogenesis because MNU does not require metabolic
activation either in the liver or in the intestine. These re-
sults thus indicate that the protective effect of fiber in colon
carcinogenesis depends on the type of carcinogen and the source
of fiber.
    The effect of alfalfa, wheat bran and cellulose on the in-
cidence of intestinal tumors induced by AOM was further studied
in Sprague-Dawley rats fed diets containing 10% alfalfa, wheat
bran or cellulose and 30% beef fat, 20% alfalfa, bran or cellu-
lose and 6% beef fat, or 30% alfalfa, bran or cellulose and 6%
beef fat (21). The presence of 10% fiber in the high fat diet
did not reduce the frequency of intestinal tumors. However, in
the same study, presence of 20% bran or cellulose or 30% of any

fiber in a diet containing 6% fat significantly reduced the fre-
quency of intestinal tumors. All the groups, except those with
a diet containing 20% alfalfa, had a lower frequency of tumors
in the proximal half of the large bowel than the groups not in-
gesting fiber. The concentration, but not the total daily
excretion, of fecal steroids was significantly lower in the
groups with a lower tumor frequency. Therefore, the inhibition
of tumor formation by dietary fiber may be due to the dilution
of bile steroids in the lumen of the intestine by the additional
bulk (21).

    Bauer et al. (43) have demonstrated that the protective
effect of dietary fiber against colon carcinogenesis probably
occurs at the promotional stage rather than in the initiating
period. Rats were fed a fiber-free diet with 20% corn oil or
diets containing 20% corn oil and 20% wheat bran, 20% carrot
fiber or 6.5% citrus pectin from 3 days before the first injec-
tion of DMH (15 mg/kg body wt/week for 12 weeks) until 14 days
after the last injection. They were then transferred to a stan-
dard rat pellet diet for 10 to 12 weeks and autopsied. The
fiber-containing diets were fed only for about 15 weeks out of a
total experimental period of 25 weeks.There was no difference in
the incidence of colorectal tumors between the groups fed a
fiber-free diet and those fed a diet containing wheat bran or
carrot fiber. The citrus pectin group had a higher incidence of
colorectal tumors. However, it is possible that the high tumor
yield resulting from large doses of the carcinogen in this study
masked any protective effect of dietary fiber. In addition,
these results and those of others reported above have suggested
that the continual feeding of a high fiber diet protects against
colon carcinogenesis, while a switch from a high-fiber to a low-
fiber diet after administration of the carcinogen has no ob-
servable effect. These observations imply that dietary fiber
protects against tumorigenesis during the promotional phase.

    The effect of dietary wheat bran and dehydrated citrus
fiber at 15% level and 5% dietary fat on intestinal carcino-
genesis induced by AOM and DMAB was studied in male F344 rats
(44,45). Composition of diets was adjusted so that all animals
in different experimental groups consumed approximately the same
amount of protein, fat, minerals and vitamins. The animals fed
the wheat bran or citrus fiber and treated with AOM had a lower
incidence (number of animals with tumors) and multiplicity
(number of tumors/tumor bearing rat) of colon tumors and tumors
of the small intestine than did those fed the control diet and
treated with AOM (Table II; 15). Although 15% purified pectin
in the diet (41) inhibited the colon tumor incidence better than
did 15% dehydrated citrus fiber, in this study the inhibition of
colon tumor multiplicity was more pronounced with the dehydrated
citrus fiber compared with purified pectin. Because dehydrated
citrus fiber contains about 20% pectin, the pectin content of
this diet was considerably lower than that of the diet used in

Table II

Colon Tumor Incidence in F344 Male Rats fed Diets Containing Wheat Bran or Citrus Fiber and Treated with Azoxymethane

Diet	Animals with colon tumors						Colon tumors per tumor bearing rat		
	Total		Adenoma		Adenocarcinoma		Total	Adenoma	Adenocarcinoma
	No.	%	No.	%	No.	%			
Control (96)[b]	86	90	83	86	60	63	3.45 ± 0.16[a]	2.37 ± 0.16	1.08 ± 0.18
Wheat Bran (51)	36	71[d]	24	47[d]	20	39[d]	1.55 ± 0.12[e]	0.94 ± 0.13[e]	0.61 ± 0.11[e]
Citrus Pulp (51)	32	63[d]	21	41[d]	20	39[d]	1.67 ± 0.18[e]	0.90 ± 0.14[e]	0.88 ± 0.16

a Total represents animals with adenomas and/or adenocarcinomas

b Effective number of animals in each group is shown in parenthesis

c Mean $\pm$ SEM

d Significantly different from the group fed the control diet by X^2 test (P<0.05 or better)

e Significantly different from the group fed the control diet by Student's t-test (P<0.05 or better)

the study by Watanabe et al. (41). Therefore, the modifying
effect of dehydrated citrus fiber in intestinal tumor incidence
may be due to the pectin content of the citrus fiber, its fecal
bulking effect, and its non-fiber components.

The animals fed the wheat bran and treated with DMAB had a
lower incidence and multiplicity of colon and small intestinal
tumors compared to those fed the control diet and treated with
DMAB (Tables III and IV; 44). Animals fed the diet containing
citrus fiber developed fewer DMAB-induced small intestinal
tumors than did the rats fed the control diet.

Recently, Freeman et al. (46) compared the incidence of
colon tumors induced by DMH in Sprague-Dawley rats fed either a
fiber-free diet or a diet containing 4.5% purified cellulose.
Among the animals ingesting cellulose, fewer had colonic neo-
plasms, and the total number of colon tumors in this group was
lower. This protective effect appeared to be associated with a
shift in tumor distribution from the proximal colon to a more
distal site. Although the mechanism for this apparent redistri-
bution of tumors within the colon remains obscure, some change
in the luminal physiochemical environment or some inherent diff-
erence in the mucosa of the two areas may be responsible.

Carrageenans are complex polysaccharides prepared from the
red marine algae and are widely used in the food industry. A
series of studies with degraded carrageenan showed that this
product could induce ulceration of the cecum and the proximal
colon in laboratory animals (47-49). Although some species of
animals fed undegraded carrageenan appeared to be healthy with
no adverse effect (50,51), guinea pigs had ulceration in the
colon (52). More recently, the carcinogenicity of food-grade
carrageenan has been evaluated in a lifetime animal study in
rats fed 4g/kg body wt. and in guinea pigs fed 3.7g/kg body wt.
(53). There was no significant difference in mammary tumor
yield between the control diet and carrageenan-fed animals.

The effect of dietary undegraded carrageenan on AOM- and
MNU-induced colon carcinogenesis was studied in F344 rats in our
laboratory (54). The animal fed the carrageenan diet and treat-
ed with AOM or MNU had a higher incidence of colon tumors than
did those fed the control diet (Table IV), suggesting a promot-
ing effect of undegraded carrageenan in colon carcinogenesis.

Agar, a sulfated polysaccharide derived from the red-purple
seaweed, is used in food products as a gelling agent and as a
laxative and is not digested by humans. Dietary agar enhances
DMH-induced colon carcinogenesis in male CF_1 mice (55). In an-
other study, cholestyramine added to the diet at 2% level en-
hanced colon tumors induced by AOM (56). The tumors in the
cholestyramine fed rats were larger, appearing more like human
colonic tumors.

Table III

Colon and Small Intestinal Tumor Incidences in F344 Male Rats Fed Diets Containing Wheat Bran or Citrus Fiber and Treated with 3,2'-dimethyl-4-aminobiphenyl

| Diet | Animals with colon tumors (tumor incidence) | | | | | | Animals with small intestinal tumors (tumor incidence) | | | | | |
	Total[a] No.	%	Adenoma No.	%	Adeno-carcinoma No.	%	Total No.	%	Adenoma No.	%	Adenocarcinoma No.	%
Control[b] (94)	43	46	38	40	13	14	52	56	12	13	45	48
Wheat bran (50)	13[d]	26	10[d]	20	4	8	17[d]	34	2	4	15[d]	30
Citrus Pulp (50)	22	44	18	36	7	14	14[d]	28	3	6	12[d]	24

a   Total represents animals with adenomas and/or adenocarcinomas

b   Effective number of animals in each group is shown in parenthesis

c   Mean $\pm$ SEM

d   Significantly different from the group fed the control diet by X^2 test, P<0.05 or better

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The aforementioned limited results suggest that the protection against colon carcinogenesis afforded by dietary fibers, both conventional and unconventional, depends on the source of fiber and the type of carcinogen.

Table IV

Colon Tumor Incidence in Female F344 Rats fed a Control or Undegraded Carrageenan Diet and Treated with AOM or MNU

	Carcinogen	No. of rats	%	Tumors/rat
Control diet	AOM	30	57[a]	1.5
Carrageenan diet	AOM	26	100	11.3
Control diet	MNU	29	69[a]	1.5
Carrageenan diet	MNU	29	100	4.4
Control diet		15	0	0
Carrageenan diet		15	7	1

[a] Significantly different from the carrageenan diet by X^2 test (P<0.01, or better)

Conclusions
During the last decade, as this review indicates, some progress has been made in the understanding of the role played by the dietary constituents in general and specifically the role of fibers in cancer of the colon. The population with high incidences of cancer of the colon are characterized by consumption of a high level of dietary fat. Furthermore, dietary fat may be a risk factor for the colon cancer in the absence of factors that are protective, such as use of certain high fibrous foods and fiber. Thus, alteration of dietary habits leading to a higher intake of certain fibers would be indicated to decrease the risk of this important cancer. Beginning this dietary pattern early in life may prove most beneficial.
The demonstration of two stages in experimentally induced cancer in animal models suggests that there are two stages in environmentally induced cancer in humans (57). Most human cancers probably result from a complex interaction of carcinogens, co-carcinogens, and tumor promoters. Most of nutritional or dietary factors act at the promotional phase of carcinogenesis.

Because promotion is a reversible process, in contrast to the rapid, process of initiation by carcinogens, manipulation of promotion would seem to be the best method of cancer prevention. In addition to pinpointing harmful environmental agents and their elimination, which is also one of the practical methods of preventing cancer, there seems to be on the horizon the promise of select methods of prevention. Although there is much need for further research to understand the modification of carcinogenic process, the successes thus achieved have expanded into some understanding of the numerous steps involved in the activation and detoxification of various chemical carcinogens and promoters by a variety of means. Application of a number of such basic tools, elimination of harmful agents from the environment, reduction of biochemical activation processes and a concomitant increase in detoxification reactions, as well as trapping of active intermediates by harmless nucleophilic reagents point to a promising future for preventive efforts. Thus, there is hope that these important types of cancer can be controlled by modifying not only environment with respect to genotoxic carcinogens, but also that of epigenetic carcinogens and, thus, humans cancer risk feasibly can be reduced (58).

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Leguminous Seed Fiber

Uses in Disease States and Effects on Carbohydrate Digestion in Vitro and Absorption in Vivo

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The fiber hypothesis suggested that diabetes and heart disease are the result, at least in part, of a lack of fiber in the diet. This paper examines some of the recent evidence concerning the mechanism by which dietary fiber affects carbohydrate metabolism, and how this, in turn, may be of use in the treatment of these disorders. It is suggested that the rate of digestion and absorption of carbohydrate from the small intestine is a major determinant of the physiological response to a food. Both the rate of diffusion of glucose in vitro from glucose/fiber mixtures, and the rate of in vitro digestion of foods, are proportional to the in vivo blood glucose responses seen after feeding the fibers or the foods. In this context dietary fiber derived from leguminous seeds, taken either purified, or in its natural state (i.e. eating the bean itself) has been shown to be most effective in reducing blood glucose and serum lipid concentrations. This strongly supports the increased consumption of beans for health reasons.

Current interest in dietary fiber began about ten years ago when Burkitt and Trowell hypothesized that diabetes and heart disease were fiber deficiency disorders (1). Since then there has been increasing research on the effects of dietary fiber on carbohydrate and lipid metabolism. Such work was pioneered and stimulated in large part by Dr. J. Anderson of Lexington, Kentucky who first used high carbohydrate/high fiber diets to treat diabetes (2) and Dr. D. Kritchevsky who has examined the effects of fiber on the metabolism of cholesterol (3).

Our own interest in dietary fiber was stimulated by the originally suggested mechanism by which primitive diets affect carbohydrate metabolism. According to the hypothesis, the

fiber-rich energy dilute primitive diet travelled the full length
of the small intestine releasing its products of digestion slowly
(Fig.1a). By contrast, the refined Western diet was seen to be
rapidly absorbed in the upper small intestine (Fig.1b). The
consequences of a fiber-rich meal, it was supposed, would be a
slow, prolonged and relatively small rise in the blood glucose
level, compared with a rapid rise after a refined, fiber depleted
meal (Fig.2).

Effect of Leguminous Fiber on Gut Physiology

To see whether fiber might slow the absorption of material
from the intestine we used a simple dialysis model. Solutions of
glucose were placed inside semipermeable dialysis bags with and
without the addition of equal amounts of different types of
dietary fiber; wheat bran, pectin, methyl cellulose, gum
tragacanth and guar gum. Compared with the fiber-free control,
all types of fiber produced some impedance to the outward passage
of glucose, but the most effective were the most viscous
substances guar and tragacanth.

When these same five fiber preparations were added in 12 g
dietary fiber equivalents to 50 g oral GTT taken by normal
volunteers there was a flattening of both the glucose and insulin
responses (4). Again guar and tragacanth, the most viscous
materials, were the most effective. The question obviously arose
as to whether the flatter blood glucose responses after guar were
the result of malabsorption of glucose caused by a much reduced
rate of absorption. However studies using urinary xylose
excretion as a measure of carbohydrate absorption suggested that
after a guar containing GTT there was delayed but complete
absorption of the carbohydrate load (4). The lack of symptoms of
carbohydrate malabsorption also strongly supported this.

In addition to slowed absorption in the small intestine, a
reduced rate of gastric emptying and decreased upper
gastrointestinal motility may also be factors responsible for
reduced glucose levels after fiber containing meals. Guar and
pectin have been shown by isotopic studies to delay gastric
emptying in both normals (5) and postgastric surgery patients
(6,7). In addition breath hydrogen studies have demonstrated
that viscous fiber increases the mouth to cecum transit
time (4). However as a model of the effect of fiber in the small
intestine, the % reduction of diffusion of glucose from dialysis
bags by each of the four viscous dietary fibers (guar gum,
tragacanth, pectin and methyl cellulose) was proportional to the
degree to which each fiber reduced the blood glucose response (r
= 0.9938) (Fig.3). The coefficient of determination for this
regression line suggests that over 95% of the reduction in the
blood glucose response by each fiber may be accounted for by
impaired diffusion of glucose from the small intestine (8,9).

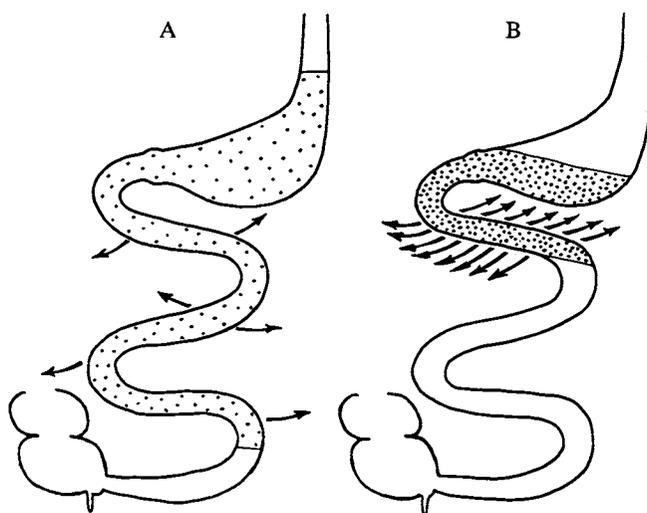The dynamic viscosity of 1% solutions of the fibers were

Figure 1. Hypothetical effect on upper gastrointestinal function of high-fiber diets containing low energy density foods (A) and low-fiber diets of high energy density foods (B).
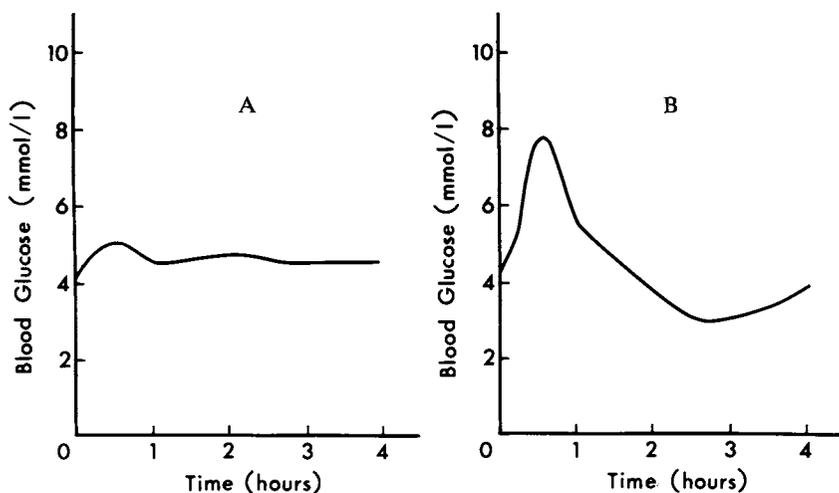
Figure 2.   Hypothetical effect on postprandial blood glucose of high-fiber diets containing low energy density foods (A) and low-fiber diets of high energy density foods (B).
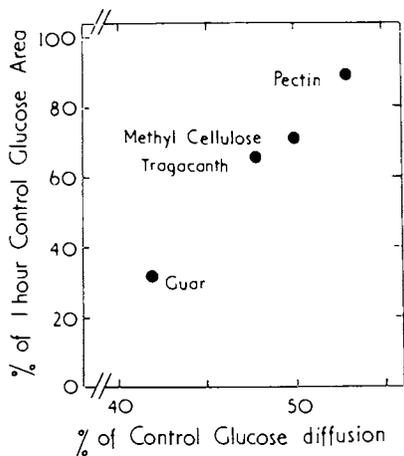

Figure 3.   Mean (n = 6 normal subjects) blood glucose area (calculated geometrically for the 0, 15, 30, 45, and 60 min blood glucose concentrations) of 50 g glucose tolerance tests containing 12 g dietary fiber from guar, gum tragacanth, pectin, or methyl cellulose. The mean blood glucose area is expressed as a percent of the control (fiber-free) blood glucose area and plotted against the concentration at 3 h of glucose in the dialysate surrounding a dialysis bag that contains 30 mL 0.1 M glucose solution and 1 g of guar, gum tragacanth, pectin, or methyl cellulose expressed as a percent of the control (fiber-free) dialysate glucose concentration (r = 0.99, p < 0.01).

measured by Stokes method and were as follows: guar, 1.3
N.sec/m; gum tragacanth, 0.52 N.sec/m; pectin, 0.21 N.sec/m; and
methyl cellulose, 0.07 N.sec/m (4). Because of the small numbers
the relationships between viscosity of the individual fibres and
the in vitro reduction of diffusion of glucose from dialysis bags
(r = 0.917), and viscosity and the in vivo reduction of blood
glucose responses (r = 0.913) did not reach significance.

Use of Fiber in Treatment of Diabetes

Similar reductions of the postprandial glucose rise after a
meal have been seen in diabetics given guar and pectin (10).
Moreover, in metabolic ward studies on 18 diabetics, the
majority of whom were taking insulin, the addition of 14 to 26 g
guar per day to the diet for 5 days resulted in a 50% reduction
in urinary glucose output compared with a 5 day control period
(11) (Fig.4). In these studies guar was given in divided doses
with each meal, mixed into fruit juices, soups and mashed potato,
or baked into breads and crispbreads. Others have also used
viscous fibers to treat diabetics and found decreased fasting
blood glucose levels and urinary glucose excretion with Konjac
Mannan (12) and guar (13).
Particulate fiber (i.e. bran and cellulose) has not produced
such marked or consistent effects. Nevertheless two studies have
shown reduced postprandial blood glucose, daily glycosuria,
insulin requirement and frequency of hypoglycemic episodes in
diabetics under metabolic ward conditions (14,15). In addition
wheatbran has been shown to improve glucose tolerance on longer
term supplementation of newly diagnosed non-insulin requiring
diabetics, (16) and in non-diabetics with diverticular disease
(17). Nevertheless the effects of wheat bran on blood glucose
responses to glucose and starch, glucose kinetics and plasma
insulin concentrations have not always been confirmed (4,18,19).

Importance of Mixing Fiber Supplements with Food

Guar has not always been used successfully in diabetes;
(20,21) the reason here is probably that guar was not given
pre-mixed with the carbohydrate portion of meals. To illustrate
this, we found in a group of healthy volunteers that guar given
just 2 min before a glucose tolerance test was without effect on
the resulting blood glucose response (Fig.5), while the same
amount of guar mixed with the glucose resulted in the expected
flattening (22).

Therapeutic use of Fiber in Diabetes: Need for Fiber Supplemented Foods

Long term use of viscous fiber is only possible if palatable

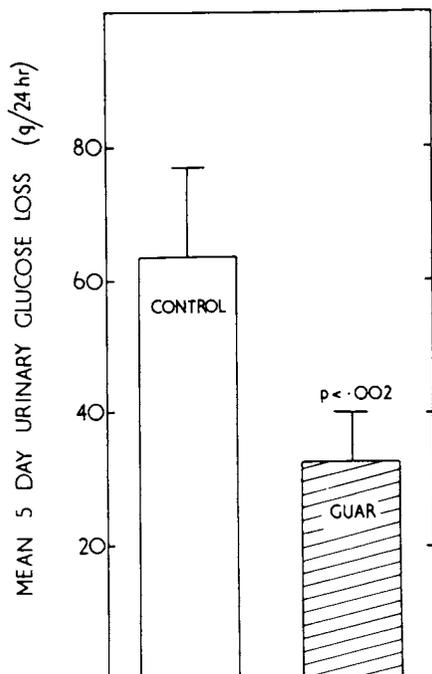*Figure 4.   Overall mean (± SEM) daily urinary glucose loss in 18 diabetics (22 studies) over 5 d without (control) and with 14–26 g guar per day added to metabolically controlled diets.*

supplements are available. A guar crispbread produced by
Speywood Laboratories (Bingham, Nottingham, England) is the only
preparation known to the authors where fiber has been effectively
and palatably pre-mixed with the dietary carbohydrate . Each
slice of crispbread contained 1 g guar, and diabetic patients
consumed 14-26 slices per day depending upon their calorie intake
(1 slice per 100 kcal). In addition to the 50% reduction of
urinary glucose excretion seen using this product in metabolic
ward studies, (23) it has allowed a 20% reduction in insulin dose
over the first three mouths of use to be maintained in a group of
6 insulin requiring diabetics for over a one year period (24).
No reductions in serum Ca^{++}, PO_4, Zn^{++} or Cu^{++} or hemoglobin
concentration were observed in 8 patients over a six month
period (24). On the other hand total and LDL cholesterol were
decreased significantly (5.0 $\pm$ 0.2 to 4.5 $\pm$ 0.3 mmol/l p 0.05;
and 3.2 $\pm$ 0.2 to 2.6 $\pm$ 0.3 mmol/l p 0.01 respectively) and HDL
and TG showed no change (1.2 $\pm$ 0.2 to 1.3 $\pm$ 0.1 mmol/l and 1.2 $\pm$
0.2 to 1.3 $\pm$ 0.3 mmol/l respectively) (24). These findings
confirm similar effects of guar crispbread on serum lipids of
hyperlipidemic patients (25).

Slowly Digested Carbohydrate in Whole Foods

        In the same way that dietary fiber may alter absorption
of carbohydrate from foods with which it is mixed, so may
different foods make their carbohydrate available for absorption
at different rates. When portions of 14 differnet foods
(wholemeal bread, white and wholemeal rice and spaghetti,
millet, buckwheat, instant mashed potato, porridge oats, sweet
potato, kidney and soya beans, marrowfat peas and red lentils)
each containing 2 g carbohydrate were mixed with human digestive
juices (obtained from duodenal aspiration) and placed in
dialysis bags there was a wide spectrum of rates of release of
the carbohydrate they contained (26). Bread and grain products
released their carbohydrate at over twice the rate as did the
leguminous seeds (p < 0.01). When 50 g carbohydrate portions of
these foods were fed to groups of normal subjects the beans gave
49% (p < 0.01) lower blood glucose responses than did the other
foods (26). The estimated % carbohydrate digested *in vitro*
after 5 hr correlated significantly with the total dietary fiber
content of the 14 foods (r = 0.53 p< 0.05). The *in vivo*
glycemic responses of these 14 foods (expressed as the glycemic
index - see below) also correlated significantly with their
fiber content (r = .62 0.02).

The Glycemic Index

        Both fiber and food form can modify the glycemic
response to "available" carbohydrate. Available carbohydrate,
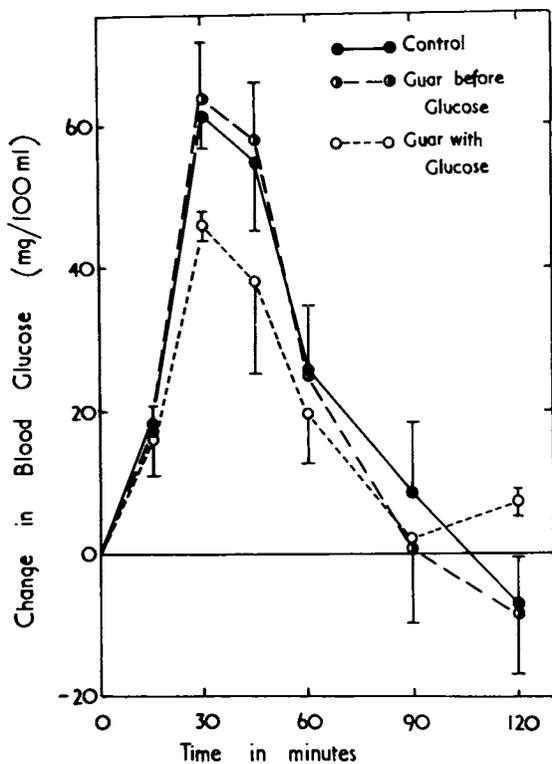as distinguished from unavailable carbohydrate, was first

Figure 5.   Mean change in blood glucose in 5 normal individuals after consuming 50 g glucose in 600 mL lemon flavored water, 50 g glucose and 14.5 g guar in 600 mL lemon flavored water, or 14.5 g guar in 300 mL lemon flavored water followed 2 min later by 50 g glucose in 300 mL lemon flavored water.

determined for a large number of foods by Lawrence and McCance in
1929 in order to accurately determine the carbohydrate content of
Lawrence's diabetic diets (27). It now seems important for
diabetic diets that foods should be classified also in respect of
their rates of biological availability or the extent to which
they raise the blood glucose. We have therefore carried out
tests of this nature on over 60 foods (28). For the purpose of
comparisons between foods we have calculated the glycemic index
as:

$$\frac{\text{area under 2 hr blood glucose curve after food}}{\text{area under the 2 hr blood glucose curve after}}$$
$$\text{equivalent amount of glucose}$$

     With glucose as 100%, common foods varied from a glycemic
index of almost 100% to less than 15% (Fig.6). The importance of
the rate of digestion of food in determining the blood glucose
response is suggested by the significant correlation between rate
of digestion in vitro and the glycemic response in vivo to the 14
foods for which data is available (r = 0.86 p < .001) (Fig.7).
     There was no correlation for 62 foods between glycemic index
and food fiber content (r = 0.220), or between glycemic index and
food sugar content (r = 0.022), suggesting that these were not
major factors responsible for the glycemic response. Stronger
negative correlations were observed between glycemic index and
food fat content (r = 0.386, p < 0.01) and glycemic index and
food protein content (r = 0.532, p < 0.001) (28). The lack of
correlation with fiber may have been due to the small effect
cereal and vegetable fibers had in reducing blood glucose
responses (18). For example there was no difference between
wholemeal and white breads, rice or spaghetti, and despite the
high fiber content of fresh broad beans, carrots and parsnips,
the blood glucose responses of these foods were respectively 79%,
92% and 97% that of glucose (28). Similarly the correlation of
glycemic index with food sugar content may have been due to the
contribution of sugars such as galactose and fructose to the
total food sugar content. These sugars, although presumably
rapidly absorbed, would not appreciably raise the blood glucose
level (28).
     Food tables therefore do not allow one to predict the
glycemic response to a food. For this reason selection of foods
should be based on physiological testing in man.

High Fiber Foods and Diabetic Treatment

     The results of studies where the cereal fiber content of the
diabetic diet were raised have been variable. Their
interpretation is also difficult because in many cases the
carbohydrate intakes were also raised (2,29,30). It has been
suggested that the higher carbohydrate intake may have
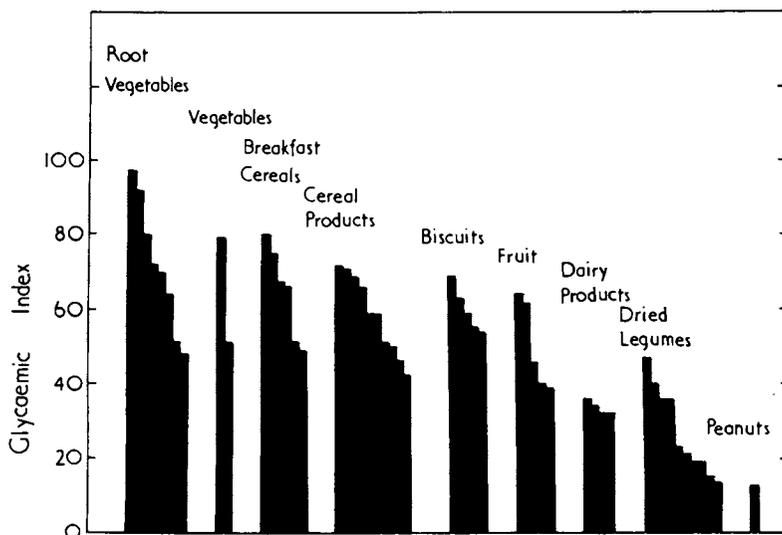contributed to the improved diabetic state, (31-34) however this

Figure 6. Glycemic index of foods (i.e., area under 2 h blood glucose response curve of 50 g carbohydrate food portions, 50 g glucose itself being 100%). Each bar in each block represents the mean result for one food tested by 5–10 normal individuals.
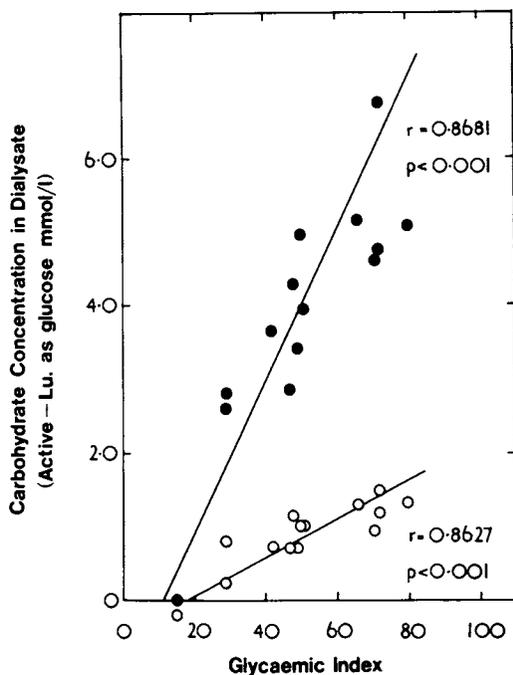
Figure 7. The glycemic index (see text or Figure 6) of 14 foods plotted against the concentration of sugars, in the dialysate at 1 and 5 h, released during in vitro digestion of the same foods.

Carbohydrate concentration in the dialysate is expressed as the difference between the sugar concentration in the dialysate that surrounds the dialysis bag containing the digestive juices plus food (active) and the sugar concentration surrounding the dialysis bag containing the digestive juices (Lu = Lundh juice) alone. Key: 0, 1 h; and ●, 0.5 h.

is still a matter of debate (35). In our metabolic ward studies
with guar 9 patients had less than 40% carbohydrate in the diets
and 14 had over 40%. Supplementation of the diets with guar
produced a significantly greater reduction of the urinary glucose
loss in the high carbohydrate group (64 ± 7% vs. 33 ± 10%
p < 0.02) (36). This improvement in carbohydrate tolerance with
high carbohydrate diets may in part explain the success reported
in some studies using wheat bran (37). The validity of high
carbohydrate/high fiber diets has been established by the pioneer
work of Dr. J. Anderson using diets high in both fiber and
carbohydrate taken in unprocessed cereals, cereal products,
leguminous seeds and leafy vegetables (2,38-40). Of particular
interest to us is the fact that such high carbohydrate diets
might also be classed as low glycemic index diets. Patients
originally consuming 43% carbohydrate diets were switched to 70%
carbohydrate high fiber diets and showed improved control and
greatly reduced insulin requirements. Those on less than 20
units of insulin daily could have it withdrawn. These benefits
were maintained longterm on an outpatient basis if patients kept
to 60% carbohydrate high fiber diets.

    The advantages of low glycemic index diets for diabetics may
not be confined to improved blood glucose control. Beans have
been used successfully to treat hypercholesterolemic individuals
in Sishuan province, China (41). In addition it has been shown
that locust bean gum reduced serum lipids in hyperlipidemic
individuals (42). Reduction of blood lipid levels is important
to diabetic individuals who are at a greatly increased risk of
developing cardiovascular disease than the general population
(43). Effective dietary means for achieving this are welcome at
a time when increasing side effects of hypolipidemic drug therapy
with, for example clofibrate, are being discovered (44-47).

Conclusion

    Dietary fiber is only one of several possible pharmacolog-
ically active substances found in foods, and present in high
concentrations in leguminous seeds which may be responsible for
the different rates of digestion and blood glucose responses of
different meals. Enzyme inhibitors, lectins and saponins are
other so called antinutritional factors, also associated with
dietary fiber which are able to alter small intestinal function
(48). The gastrointestinal tract evolved to deal with these
constituents in foods so that while toxic in large amounts (as in
uncooked beans) small amounts may have beneficial effects.
    These antinutrients have now largely been eliminated from the
Western diet by refining and highly processing our foods.
However, as with dietary fiber, we are likely in the future to
see specific combinations of them reincorporated in small amounts
into our diet for reasons of health. The studies on dietary
fiber have opened the way for this with their demonstrations of

health benefits for diabetics, and possible relevance to the general population for the prevention of disease. It is also likely in the future, as more knowledge becomes available, that we shall see the food industry playing a role in the pharmacological manipulation of the diet for health using plant derived substances.

Acknowledgments

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Structural Chemistry of Some Nonstarchy Polysaccharides of Carrots and Apples

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Problems in the extraction, isolation, fractionation, and subsequent analysis of non-starchy polysaccharides, which may function as dietary fibre components, are discussed for such substances from carrots and apples. Structural studies on polysaccharides from carrots have resulted in the characterization of a branched arabinan, an esterified galacturonan (pectin), and an acidic xylan as individual components, and further evidence points to the presence of a 4-linked galactan and a 4-linked glucomannan.

The non-starchy polysaccharides from plant sources fall into a limited number of structural families (1) which include cellulose, other β-$\underline{D}$-glucans of the 3-linked type (callose) and of the mixed 3- and 4-linked type from cereals, xyloglucans, the various xylans, the pectic substances, i.e. galacturonans and rhamnogalacturonans, and associated arabinans, galactans and arabinogalactans of type I (4-linked), the arabinogalactans of type II (3,6-linked), galactomannans and glucomannans. For investigations on dietary fibre components which seek to establish correlations between individual polysaccharide species and particular physiological and in vitro functional properties, it is essential to establish the structural identities of these substances.

In our studies on the polysaccharide constituents of carrots and apples the following considerations have been borne in mind: (i) that account must be taken of the readily soluble as well as of the more obviously fibrous non-starchy polysaccharides; (ii) that cell wall materials must be prepared with minimum loss of these more readily soluble polysaccharides; (iii) that 'broad spectrum' analyses of 'pectic substances' and 'hemicelluloses', even when supported by determinations of individual sugar components, are rarely sufficient to establish the nature of these substances since the same sugar constituents occur in quite
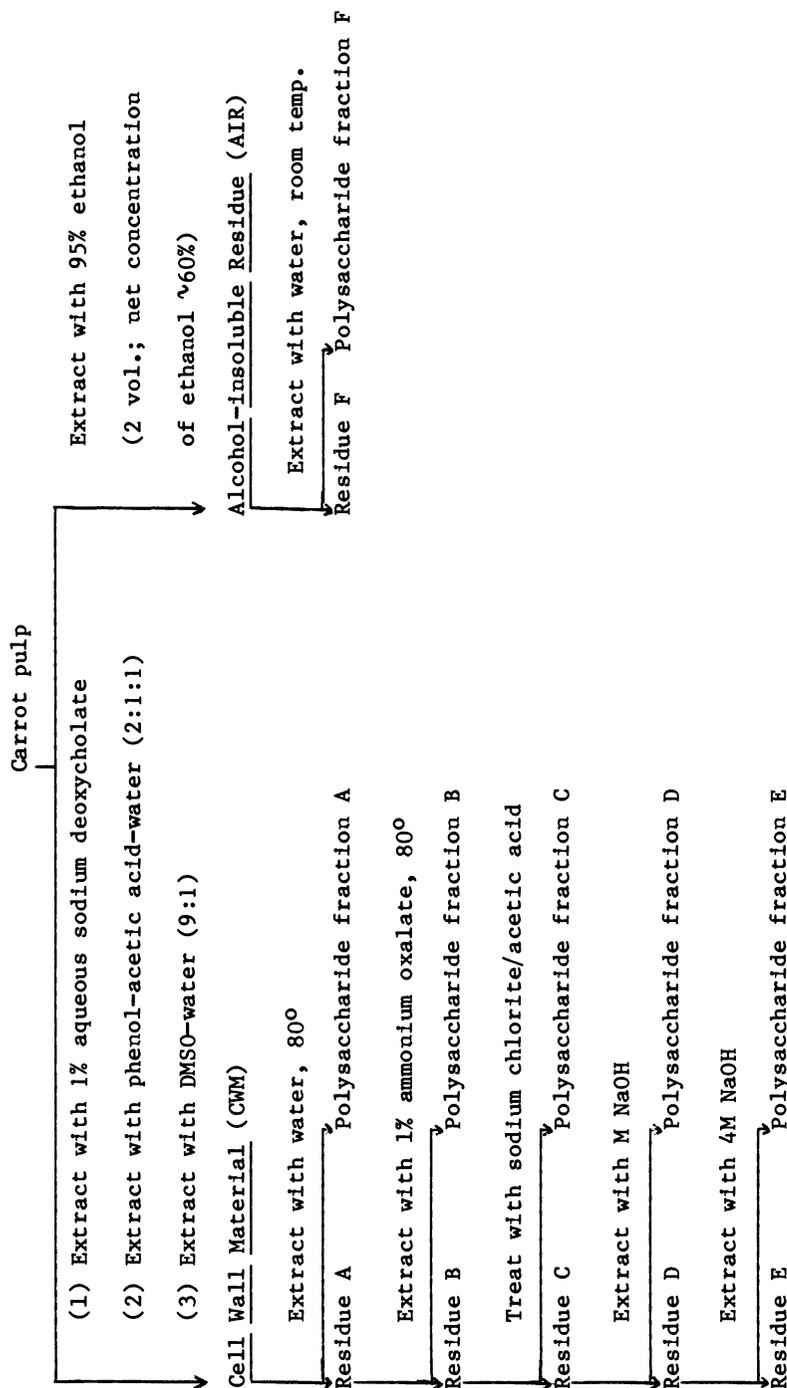
different species; and hence (iv) that individual polysaccharides
should be examined in sufficient detail to establish their
identities.   The fractionation of all polysaccharides from a
single source is not easily achieved, but with information on
characteristic linkage types (from methylation analysis) in
addition to sugar composition it is frequently possible to
identify polysaccharides in incompletely separated mixtures.
    Selvendran and his collaborators (2) in their extensive
studies on dietary fibre polysaccharides have stressed the need
for careful treatment of plant material so as to avoid changes in
the ease of extraction of polysccharides through modifications in
physical properties, and to account for all those polysaccharides
which may survive passage through the upper gastrointestinal
tract regardless of their natural fibrous or non-fibrous nature.
Although no one procedure is likely to prove suitable for all
types of plant material, the aim is to achieve maximum
solubilization of starch and cytoplasmic material with minimum
loss of the non-starchy cell wall polysaccharides. The procedure
developed by Selvendran (2) involves successive extractions with
cold aqueous sodium deoxycholate, phenol-acetic acid-water, and
dimethylsulfoxide (DMSO) containing 10% of water, and has been
used to prepare carrot cell wall material (CWM) (Scheme 1) for
the graded extraction of polysccharide fractions on which
preliminary structural studies have been carried out.   An
alcohol-insoluble residue (AIR) has also been prepared by
extraction of carrot pulp with 95% ethanol (but net concentration
∿60% due to water in carrots) for use as an alternative source
for the more readily soluble polysaccharides.

Results and Discussion

    Sugar analyses for carrot CWM and the corresponding AIR are
shown in Table I and, as appropriate, indicate sugars formed on
hydrolysis with trifluoroacetic acid (3) or after digestion with
72% sulfuric acid followed by dilution (4) [and thus provide by
difference an estimate of the insoluble cellulose component],
together with uronic acid, estimated as galacturonic acid using
the 3-hydroxydiphenyl reagent (5).   The similar relative
proportions of sugars indicate that, aside from the cellulose,
the main polysaccharides are pectins and associated neutral
polysaccharides (arabinans, galactans and/or arabinogalactans).
Compositional data alone do not differentiate between the
differently linked arabinogalactans, nor do they indicate whether
xylose residues occur as pectin constituents [as in soybeans
(6)], or more probably in xylans or xyloglucans.

Carrot pulp

(1) Extract with 1% aqueous sodium deoxycholate

(2) Extract with phenol–acetic acid–water (2:1:1)

(3) Extract with DMSO–water (9:1)

→ Extract with 95% ethanol (2 vol.; net concentration of ethanol ~60%)

Alcohol–Insoluble Residue (AIR)

   Extract with water, room temp.

   Residue F → Polysaccharide fraction F

Cell Wall Material (CWM)

   Extract with water, 80°

Residue A → Polysaccharide fraction A

   Extract with 1% ammonium oxalate, 80°

Residue B → Polysaccharide fraction B

   Treat with sodium chlorite/acetic acid

Residue C → Polysaccharide fraction C

   Extract with M NaOH

Residue D → Polysaccharide fraction D

   Extract with 4M NaOH

Residue E → Polysaccharide fraction E

Scheme 1. Flow sheet for polysaccharide extractions from carrot pulp

TABLE I.   Sugar compositions (%) of carrot alcohol-insoluble
           residue (AIR), cell wall material (CWM) and CWM
           residue E

Sugar	AIR		CWM		CWM residue E	
	TFA[a]	H_2SO_4[b]	TFA	H_2SO_4	TFA	H_2SO_4
Rhamnose	3.5	3.0	2.5	2.0	1	1
Arabinose	6	8	5.5	5.5	1.5	2
Xylose	1.5	2	1	1	0.1	0.3
Mannose	1	2.5	0.5	1	n.d.	0.3
Galactose	11	13.5	9.5	9.5	4	5
Glucose	2	32	1	22	3	33
Uronic acid[c]		19		15		5

[a] Hydrolysis with trifluoroacetic acid.

[b] Digestion with 72% sulfuric acid, followed by dilution and
     hydrolysis.

[c] Estimation with the 3-hydroxydiphenyl reagent.

        CWM was then extracted sequentially with water at 80°
and with ammonium oxalate at 80°, delignified with acidified
sodium chlorite at 70°, and then further extracted with M and
4M sodium hydroxide.   The crude polysaccharide extracts were
likewise analyzed for constituent sugars and uronic acid (Table
II).   In order to obtain defined polysaccharide fractions the
following separations were attempted. Polysaccharide fraction A
was   separated   by   ion-exchange   chromatography   on
diethylaminoethyl(DEAE)-Sephadex A-50 and the neutral fraction
afforded a virtually pure arabinan (1 in Scheme 2) after
selective precipitation with cetyltrimethylammonium hydroxide
(7).   Linkage analysis by methylation confirmed that this
arabinan was of the highly branched type associated with pectins
for which the representative, but not unique, structure (1) may
be advanced.
        Polysaccharide fraction B, isolated from ammonium oxalate
extraction, was a pectin of low degree of esterification.   This
material could not be fractionated satisfactorily by ion-exchange
chromatography since only a small proportion was eluted from
DEAE-Sephadex with neutral buffers. A more characteristic pectin
sample, which was possibly lost during CWM preparation, was

TABLE II.   Sugar composition (%) of polysaccharide fractions from graded extraction of carrot CWM or AIR

Source			CWM			AIR
Polysaccharide fraction	A	B	C	D	E	F
Extractant	H2O	NH4 oxalate	HClO2	M NaOH	4M NaOH	H2O
Sugar[a]						
Rhamnose	4	5	6	1.5	1.5	3
Arabinose	27	8	14	4	6	4
Xylose	0.5	0.5	0.5	19	14	0.2
Mannose	0.5	n.d.	0.2	9	5	n.d.
Galactose	16	12	19	8	10.5	9.5
Glucose	1	0.2	0.5	16.5	7	1
Uronic acid[b]	17	69	27	9.5	8.5	77

[a] Hydrolysis with trifluoroacetic acid.

[b] Estimation with the 3-hydroxydiphenyl reagent.

obtained by extraction of AIR with water. Kirtschev and Kratchanov (8) have reported the isolation of carrot pectin with a variety of aqueous reagents, and our extraction of water-soluble pectin is based on the extraction of a similar carrot AIR with water at room temperature to minimize the possibilities of inadvertent acid hydrolysis or base-catalyzed β-elimination. The aqueous extract afforded a pectin preparation (uronic acid, 77%; degree of esterification, 59%) which was remarkably free from extraneous material and contained the characteristic neutral sugar constituents of many galacturonans, i.e. galactose, arabinose and rhamnose. Two fractions were obtained when this pectin was subjected to ion-exchange chromatography on DEAE-Sephacel using potassium acetate buffered at pH 5. Although neutral sugars predominated in the minor fraction, the major polysaccharide fraction still contained the same neutral sugar constituents, but in reduced proportions.
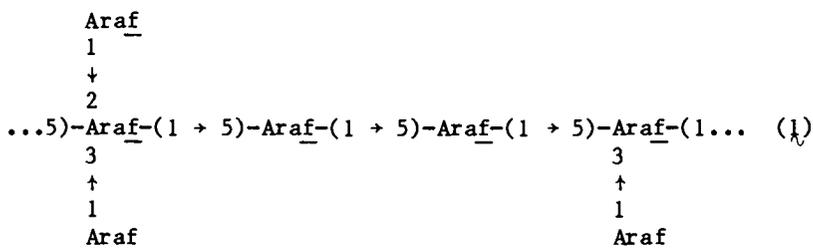
Linkage analysis of pectins by methylation, designed to avoid the twin problems of base-catalyzed degradation during methylation and sugar decomposition under conditions required for

Hydrolysis of methylated arabinan → 2,3,5-Me$_3$ Ara (40)

                                        2,3-Me$_2$ Ara (33)

                                          2-Me Ara (13)

                                              Ara (12)

```
      Ara f
      1
      ↓
      2
...5)-Araf-(1 → 5)-Araf-(1 → 5)-Araf-(1 → 5)-Araf-(1...  (1)
      3                                   3
      ↑                                   ↑
      1                                   1
      Ar af                               Ar af
```

Scheme 2. Arabinan - methylation analysis and structure (1)

complete hydrolysis of the methylated derivative, was performed by (1) methylation using a minimum excess of base and hydrolysing the methylated pectin to identify neutral sugar constituents, and (2) methylation of the carboxyl-reduced polysaccharide (9). The latter procedure is limited only in that most of the galactose derivatives are those arising from carboxyl-reduced galacturonic acid rather than original galactose residues. The results of these analyses are shown qualitatively rather than quantitatively in Table III, and are interpreted in terms of a linear galacturonan structure with deviations arising from arabinose, galactose and rhamnose as minor neutral sugar constituents (1). Rhamnose residues probably interrupt the galacturonan chain at intervals. Arabinofuranose residues are present in branched chains of the same type as in the arabinan (1) and probably constitute a covalently attached sub-unit. In addition to a small proportion of 4-linked galactopyranose units, other residues of this sugar are present as 6- or 3,6-linked units, whose origin is presently unexplained.

The alkali-soluble 'hemicellulose' fractions contained an array of neutral sugars with small proportions of uronic acid residues. Attempts to separate individual polysaccharides from fraction D by ion-exchange chromatography on DEAE-Sepharose CL-6B and by adsorption chromatography on microcrystalline cellulose (10) were only partially successful. Nevertheless, sugar analyses of these sub-fractions (Table IV), coupled with linkage analysis by methylation (Table V), provided evidence for the nature of the main polysaccharide components. Fraction D4 was an acidic xylan with only small proportions of sugar constituents arising from other polysaccharides. Methylation analysis was consistent with the presence of 4-linked β-D-xylopyranose

TABLE III. Methylation analyses of carrot pectin and the
            carboxyl-reduced polysaccharide

Sugar	Methylated pectin	Methylated carboxyl-reduced polysaccharide	
	Sugars detected as alditol acetates	Sugars detected as alditol acetates	Structural unit
Galacturonic acid		$2,3,6\text{-Me}_3$ Gal	-4 GalpA 1-
Galactose	$2,3,4,6\text{-Me}_4$ Gal	$2,3,4,6\text{-Me}_4$ Gal	Galp 1-
	$2,3,6\text{-Me}_3$ Gal	$2,3,6\text{-Me}_3$ Gal	-4 Galp 1-
	$2,3,4\text{-Me}_3$ Gal	$2,3,4\text{-Me}_3$ Gal	-6 Galp 1-
	$2,4\text{-Me}_2$ Gal	$2,4\text{-Me}_2$ Gal	$-3,6$ Galp 1-
Arabinose	$2,3,5\text{-Me}3$ Ara	$2,3,5\text{-Me}3$ Ara	Araf 1-
	$2,3\text{-Me}2$ Ara	$2,3\text{-Me}2$ Ara	-5 Araf 1-
	2-Me Ara	2-Me Ara	$-3,5$ Araf 1-
Rhamnose	$3,4\text{-Me}2$ Rha	$3,4\text{-Me}2$ Rha	-2 Rhap 1-
	3-Me Rha	3-Me Rha	$-2,4$ Rhap 1-

residues   with   attached   side   chains   of   single
4-O-methyl-α-D-glucopyranosyluronic   acid   units   approximately
every 12-15 units (3 in Scheme 3). [Enantiomeric (D or L) and
anomeric (α or β) configurations commonly encountered in plant
polysaccharides are assumed.]   The nature of the side chains was
shown by (a) hydrolysis of the carboxyl-reduced polysaccharide to
give 4-O-methylglucose from the corresponding uronic acid, and
(b) methanolysis of the methylated xylan followed by acetylation
to yield inter alia anomeric acetylated methyl ester methyl
glycosides of the partially methylated aldobiouronic acid (2),
whose   identities   were   established   by   comparison   (glc-mass
spectrometry) with samples similarly generated from methylated
alfalfa xylan (11). Other polysaccharide sub-fractions from the
alkaline extract contined 4-linked residues of galactose, glucose
and manose, together with arabinofuranose residues of the same
type as in the above-mentioned arabinan (1). The association of
4-linked glucose and mannose residues points to the probable
presence of a glucomannan. Linear chains of 4-linked galactose
residues likewise point to the presence of a galactan of the type
frequently associated with pectins.

TABLE IV. Composition of sub-fractions from polysaccharide fraction D

Fractionation		Ion-exchange			Adsorption on cellulose				Cellulose followed by ion-exchange	
Eluant		H₂O	0--0.5M KOAc		0.5M NaOH	H₂O	7M Urea 0.5M NaOH		H₂O	M KOAc
Fraction	D	D1	D2	D3	D4	D5	D6	D7	D7a	D7b
Sugarsᵃ										
Rhamnose	5		3	3	1	2				
Fucose		2								
Arabinose	15	11	22	33	3	28	2	2		2
Xylose	29	8	45	22	80	29	70	47	15	70
Mannose	13	28	1	1	4	1	9	18	38	12
Galactose	17	7	20	16	5	26	1			
Glucose	21	42	4	7	7	13	10	32	48	14
Uronic acidᵇ	10				8					

ᵃ Neutral sugars formed on hydrolysis with trifluoroacetic acid are expressed in relative proportions.

ᵇ Estimation (wt. %) with the 3-hydroxydiphenyl reagent.

TABLE V. Relative proportions of methylated sugars formed on
hydrolysis of methylated polysaccharide fractions and
identified by glc alone or glc-ms* of partially
methylated alditol acetates.

Fraction	D*	D1	D2	D3	D4*	D7b
Sugar[a]						
2,3,5-Me$_3$ Ara	7	5	5	7		
2,3-Me$_2$ Ara	4	1	3	4		
2-Me Ara	3	p	2	5		
Ara	2	p	2	2		
2,3,4-Me$_3$ Xyl	3	3	1	1	3	1
2,3-Me$_2$ Xyl[b]	36	4	47	40	84	25
2/3-Me Xyl					12	3
2,3,6-Me$_3$ Gal	7	6	25	18		
2,3,6-Me$_3$ Man	11	30	2	2		16
2,3,6-Me$_3$ Glc	14	27	2	2		26

[a] A number of minor components whose structural identities
were not confirmed are omitted.

[b] May include small proportions of 2,3,4,6-Me$_4$ Gal.

These results provide evidence for the nature of these
carrot polysaccharides for which reasonably unambiguous
structural information has been obtained. Pectin (with an
associated arabinan) and cellulose are the main non-starchy
polysaccharides. An acidic xylan is present in smaller amounts
together with a 4-linked galactan and probably a 4-linked
glucomannan. The CWM and AIR from different batches of carrots
differed in total carbohydrate composition, although much less in
the relative proportions of sugar constituents, so that only
limited quantitative conclusions may be drawn. However, since
these results were obtained, analyses of CWM and AIR from the
same batch of carrots have shown rather similar carbohydrate
compositions. The relatively small compositional differences
point to some loss of the more soluble pectic substances during

Hydrolysis of methylated xylan → 2,3,4-Me$_3$ Xyl   (1)

2,3-Me$_2$ Xyl (25)

3-(+ some 2-) Me Xyl   (3)

Methanolysis and acetylation to give partially methylated
aldobiouronic acid derivatives (2) - m/e values are shown
for characteristic fragment ions in the mass spectrum
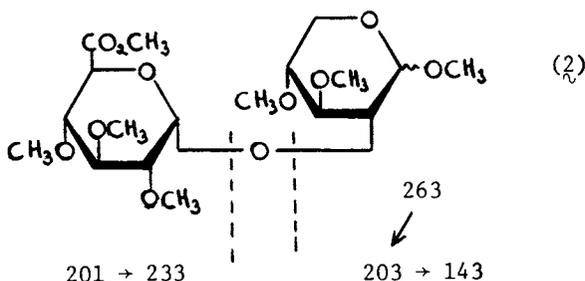
...4)-Xyl$\underline{p}$-(1 → 4)-Xyl$\underline{p}$-(1 → 4)-Xyl$\underline{p}$-(1 → 4)-Xyl$\underline{p}$-(1...   (3)
                              2
                              ↑
                              1
              4-$\underline{O}$-Me-Glc$\underline{p}$A
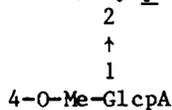
Scheme 3. Acidic Xylan (polysaccharide fraction D4) -
methylation analysis, partial fragmentation to methylated
aldobiouronic acid derivatives (2), and general structure (3)

CWM preparation, a conclusion supported by the isolation of
smaller quantities of these polysaccharides on extraction with
water and aqueous ammonium oxalate.
      The results of sugar analyses of the CWM and AIR (Table VI)
from parallel batches of apples likewise indicate similar overall
carbohydrate compositions with only small losses of pectic
substances from the CWM, a conclusion again supported by the
isolation of smaller quantities of these materials on subsequent
extraction. Structural studies on apple polysaccharides will be
reported elsewhere.

TABLE VI. Sugar composition (%) of apple CWM and AIR, and pectin fractions extracted with (A) cold water, (B) hot water, and (C) ammonium oxalate

Source	CWM				AIR				
Extract			B	C			A	B	C
Hydrolysis[a]	TFA	H_2SO_4	TFA	TFA	TFA	H_2SO_4	TFA	TFA	TFA
Sugar									
Rhamnose	2.5	2.5	3	3.5	2	1	2.5	2.5	3
Fucose	1.5	1.5	n.d.	0.5	1.5	1	1.5	0.5	2
Arabinose	12.5	11.5	23	22	12.5	12.5	9	23.5	21
Xylose	6.5	6	2	3.5	7.5	5	2	1	3
Mannose	1	4.5	n.d.	0.5	1	4	1	0.5	n.d.
Galactose	6	6	5	14	6	4	6	5	8
Glucose	2	23	1	3	2	18.5	2.5	2.5	1
Uronic acid[b]	15	15	55	37		16.5	54	58	64

a Hydrolysis with trifluoroacetic acid or after digestion with 72% sulfuric acid followed by dilution.

b Estimation with the 3-hydroxydiphenyl reagent.

Experimental

General Methods. Gas-liquid chromatography was performed
with a Perkin-Elmer Sigma 3B gas chromatograph fitted with a
flame ionization detector using (a) a packed column of 3% of
silicone gum Silar 10 CP on Chromosorb W-HP (100/200 mesh) or (b)
a S.C.O.T. column coated with silicone gum OV-225. A
Perkin-Elmer Data System Sigma 10 B was used for peak
integration. For glc-mass spectrometry columns (b) or (c)
W.C.O.T. coated with silicone gum SP-2330 were used in a
Pye-Unicam series 204 gas chromatograph connected by a jet
separator to a VG Micromass 16F mass spectrometer and VG data
system 2000, operated with an inlet temperature of $\sim250^{\circ}$, an
ionization potential of 70 eV, and an ion-source temperature of
$\sim250^{\circ}$. Evaporations were carried out under diminished
pressure at temperatures of 40° or less.

Sugar Analyses. Hydrolyses of polysaccharide fractions and
cell wall preparations were performed (a) directly with
trifluoroacetic acid (3) and (b) after digestion with 72%
sulfuric acid and dilution, with M-sulfuric acid (4). D-Allose
was added as an internal standard and the sugar mixtures were
converted into alditol acetates (12) for analysis by glc on
column (a). Uronic acid determinations were carried out
spectrophotometrically with the 3-hydroxydiphenyl reagent (5).
In the case of insoluble materials determinations were performed
after digestion with 72% sulfuric acid and appropriate dilution.

Methylation Analyses. Polysaccharide samples were
methylated by the Hakomori procedure as described by Lindberg
(13). In the case of acidic polysaccharides, especially for
pectins, de-esterification was first carried out at 4° with
sodium hydroxide at pH 12 and, in order to minimize
base-catalyzed degradation, methylations were performed with
restricted proportions of reagents (14). Completeness of
methylation was checked by the absence of hydroxyl bands in the
ir spectra. Neutral methylated sugars formed on hydrolysis were
converted into the derived partially methylated alditol acetates
for analysis by glc alone using column (b) with confirmations of
identities by glc-mass on columns (b) or (c). Methylated acidic
polysaccharides were methanolyzed with methanolic 3% hydrogen
chloride for 12h at 100° in sealed tubes, and the resulting
methyl glycosides were acetylated for glc examination on column
(b).

Preparation of Carrot CWM. Carrots (250 g per batch) were
cut into small pieces and blended with aqueous 1% sodium
deoxycholate (SDC) (200 mL) for 5 min in a Waring blender with
addition of 2-octanol (2 mL) as an anti-foaming agent. The
blended carrot pulp was transferred to a ball-mill container with

1% SDC (550 mL) and ball-milled for 16 h at 4°. Light microscopic examination revealed ∿ 95% rupture of cells. The suspension was then centrifuged at 20,000 $\underline{g}$ for 0.5 h at 4°. The residue was washed twice with distilled water (2 x 200 mL), recovered by centrifugation, and extracted twice with phenol-acetic acid-water (2:1:1,w/v/v, 2 x 500 mL) for periods of 0.5 h after short treatments in a blender to ensure uniform suspensions. The insoluble residue was then blended with DMSO-water (9:1, v/v, 350 mL) and the suspension was sonicated for 30 min whilst maintaining the temperature at ∿ 20°. The suspension was stirred at room temperature for 16 h, the insoluble residue was separated by centrifugation, and the procedure was repeated. The final insoluble residue was washed five times with water (5 x 100 mL), at which stage a starch-iodine test was negative, and the residue (10.8 g) in aqueous suspension was isolated by freeze-drying.

Extraction of Polysaccharides from Carrot CWM. CWM (25 g) was extracted three times by stirring in water (1 L) for 2 h at 80°. After each extraction the suspension was centrifuged and the combined supernatant liquids were freeze-dried to give polysaccharide fraction A (0.5 g).

Residue A was extracted three times with aqueous 1% ammonium oxalate (1 L) for 2 h at 80° with maintenance of pH at 5. The final suspension was removed by centrifugation to give residue B (17.2 g), the combined supernatant liquids were dialyzed against distilled water (with frequent changes) for 2 days, and polysaccharide fraction B (4.4 g) was isolated by freeze-drying.

Residue B (13.5 g) suspended in water (1.35 L) was delignified by treatment with acetic acid (1.7 mL) and sodium chlorite (4.1 g) at 70° in an inert atmosphere, and the suspension was stirred for 15 min. The residue was separated by centrifugation, the procedure was repeated twice, the delignified CWM (residue C) was separated after washing twice with water, and the combined centrifugates and washings were dialyzed against distilled water and freeze-dried to give polysaccharide fraction C (0.9 g).

Residue C (11.5 g) was treated at 4° by stirring with dilute sodium hydroxide (200 mL) at pH 12 in an inert atmosphere in order to effect de-esterification of remaining pectin with minimum base-catalyzed degradation. Sodium hydroxide was then added to obtain a total volume (1 L) of molar reagent, and the suspension was stirred for 2 h at room temperature. The insoluble residue was separated and extracted again, and the combined extracts were neutralized with acetic acid, dialyzed against distilled water, and freeze-dried to give polysaccharide fraction D (0.8 g). The residue from the foregoing extraction was further extracted in a similar manner with 4M sodium hydroxide, and the extracts were worked up to give polysaccharide fraction E (0.25 g), leaving residue E (9.3 g).

Preparation of Carrot AIR and Isolation of Polysccharide
Fraction F.   Carrots (900 g) were cut into small pieces and
blended in 95% ethanol (1.5 L), and the suspension was heated
under reflux for 2 h.   The residue was then extracted
continuously with fresh ethanol until all colour had disappeared
affording carrot AIR (35 g).   Carrot AIR (35 g) was extracted
thrice by stirring vigorously in water (2 L) at room temperature
to give polysaccharide fraction F (5.3 g).

Examination of Polysaccharide Fractions.   Fraction A.
Fraction A (425 mg) was chromatographed on a column (1.6 x 60 cm)
of DEAE-Sephadex A-50 (formate form) which was eluted with water
and then sequentially with 0.2M, 0.4M, 0.6M and M sodium
formate.   Fraction Al (220 mg), eluted with water, gave on
hydrolysis arabinose and galactose in the ratio of 5:1 with
traces of glucose and mannose, but contained no uronic acid.
Cetyltrimethylammonium hydroxide solution (2 mL) and M NaOH (2
mL) were added to fraction Al (160 mg) in water (20 mL).   After
20 min the flocculent white precipitate was removed by
centrifugation.   The precipitate was dissolved in water (20 mL)
containing sufficient acetic acid for neutralization and the
polysaccharide derivative was reprecipitated with M NaOH (2 mL).
A solution of the precipitate in dilute acetic acid was deionized
successively with Dowex 1X4 (HCO_3^-) and 50WX8 (H^+) resins, and
freeze-dried to give arabinan (1) (15 mg).   Hydrolysis of the
polysaccharide gave arabinose (92%) and galactose (7%).   A sample
of arabinan was methylated and hydrolysis of the methylated
polysaccharide, followed by reduction and acetylation gave the
alditol acetates shown in Scheme 2.

Fraction D.   Fraction D (205 mg) (carbohydrate content, 50%)
was chromatographed on a column (1.6 x 50 cm) of DEAE-Sepharose
CL-6B (acetate form) which was eluted with water to give fraction
D1 (22 mg), with a linear gradient of 0--0.5M potassium acetate
(500 mL) to give fractions D2 (12 mg) and D3 (17 mg), and 0.5M
sodium hydroxide (200 mL) to give fraction D4 (33 mg) (total
recovery of carbohydrate, 86%).   A sample of fraction D4 was
converted into the carboxyl-reduced polysaccharide (9),
hydrolysis of which gave 4-O-methylglucose as an additional
neutral sugar constituent.
Aqueous 7% cupric acetate (∿8 mL) was added dropwise to
fraction D (200 mg) in water (60 mL), the precipitate was removed
by centrifugation, the supernatant liquid was concentrated (to 25
mL) and poured into ethanol (100 mL).   The resulting precipitate
was washed with ethanolic 1% hydrogen chloride to remove copper
ions and then with ethanol to remove acid.   The residue in water
was chromatographed on a column (1 x 25 cm) of microcrystalline
cellulose which was eluted successively with water, 7M urea, and

0.5M sodium hydroxide to give fractions D5 (9 mg), and D7 (24 mg). Fraction D7 was further chromatographed on DEAE-Sepharose CL-6B to give fractions D7a and D7b eluted with water and M potassium acetate. The polysaccharide sub-fractions were analyzed for sugar composition (Table IV) and selected samples were subjected to methylation analysis (Table V).

Fraction F. The polysaccharide had $[\alpha]_D + 169°$ (c 0.16) and the methoxyl content (7.6%) corresponded to a degree of esterification of 59%. The crude pectin (210 mg) was chromatographed on a column (1.6 x 40 cm) of DEAE-Sephacel (acetate form) which was eluted with 0.2M and 0.4M potassium acetate buffered at pH 5 to give fractions F1 (22 mg) [Found: rhamnose, 3; arabinose, 13; galactose, 18; uronic acid, 32%] and F2 (135 mg) [Found: rhamnose, 3; arabinose, 3; galactose, 6; uronic acid, 84%].

A sample of fraction F2 was methylated (14, 15). Methanolysis of the methylated polysaccharides followed by acetylation gave a mixture of methyl glycoside acetates amongst which derivatives of 2,3-di-O-methylgalacturonic acid predominated (glc on column b). A further sample of the methylated polysaccharide was hydrolyzed and the derived partially methylated alditol acetates (from neutral sugar constituents only) were examined by glc-ms (Table III). A sample of fraction F2 was converted into the carboxyl-reduced polysaccharide by the procedure of Taylor and Conrad (9). Methylation analysis of the carboxyl-reduced polysaccharide was performed in the usual way (Table III).

Preparation of Apple CWM and AIR and Extraction of Pectin Fractions. Apples ('Granny Smith' variety) were cored and peeled and the resulting pulp (2 kg batches) was stored in ethanol suspension to minimize enzymic oxidation. Separate batches of pulp were processed, as described for carrot preparations, to furnish apple CWM (32.5 g) and apple AIR (39.8 g) for which analytical data are recorded in Table VI. Polysaccharide fractions B (4 g) and C (2.5 g) were extracted from CWM with water at 80° and aqueous 1% ammonium oxalate at 80°. Likewise polysaccharide fractions A (2.1 g), B (5.2 g), and C (4.5 g) were isolated from AIR by extraction with water at room temperature, 80°, and with aqueous 1% ammonium oxalate respectively.

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Legumes and Their Soluble Fiber: Effect on Cholesterol-Rich Lipoproteins

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Water-soluble plant fibers and soluble-fiber rich
foods have distinct hypocholesterolemic effects in
man and experimental animals. We evaluated the
hypocholesterolemic effects of dried beans, rich in
soluble fiber, for ten hypercholesterolemic men on a
metabolic ward. They were fed a control diet for
seven days followed by a bean-supplemented diet for
21 days. The bean-supplemented diet provided 115 g/d
(dry weight) of dried beans but otherwise resembled
the control diet. Control and bean-supplemented
diets had similar energy, carbohydrate, protein, fat
and cholesterol contents but the bean-supplemented
diet had more soluble and total plant fiber. Average
serum total cholesterol concentrations were 19 per
cent lower on bean-supplemented than on control
diets. Bean supplementation lowered low-density
lipoprotein cholesterol more than high-density
lipoprotein cholesterol concentrations resulting in
an increase in the high-density to low-density
ratios. Soluble-fiber rich foods may have an
important role in the long-term management of
patients with hypercholesterolemia.

High-fiber foods or food products have valuable health
promoting effects. Intake of selected high-fiber items can
improve diabetic control (1), lower blood lipid concentrations
(2,3) and perhaps reduce the risk of heart attack. Plant fibers,
defined as the polysaccharide and lignin components of plant
foods which are not digested in the human small intestine, affect
the physiology and influence the metabolism of various nutrients.
For example, certain plant fibers have substantial
hypocholesterolemic effects. Selected water-soluble fibers such
as pectin, guar and oat gum have unique cholesterol-lowering
properties. We will review the effects of selected plant fibers
or fiber-rich foods on serum cholesterol concentrations. We also

will provide a preliminary report on the metabolic effects of
bean-supplemented diets for hypercholesterolemic men.   Current
information indicates that fiber-rich foods have notable
therapeutic potential for treating certain metabolic conditions
and may prevent the development of certain diseases.   However,
since the available information is largely descriptive in nature,
we have little understanding of the mechanisms underlying the
important metabolic effects of plant fibers.   Further work is
required to delineate the mechanisms for plant fibers and to
determine the practical role of fiber-rich foods in the
management or prevention of certain human disorders.

Hypocholesterolemic Effects of Plant Fibers-Rat Studies.   In
Table I we compare the cholesterol-lowering effects of selected
fibers used in experimental studies with rats.   Since many
different experimental designs were used, this collation provides
only general guidelines to the effects of different fiber
sources.

TABLE I.   EFFECTS OF PLANT FIBER SUPPLEMENTS ON SERUM
           CHOLESTEROL CONCENTRATIONS OF RATS.[1]

Ingredient	No. of Studies	Cholesterol % of control
Cellulose	9	96
Wheat bran	6	100
Oat Bran	2	81
Oat gum	1	59
Pectin	13	80
Guar	3	81
Bengal gram	2	72

1. Data from Table I (4) except for oat bran (7) and oat
gum (8).

Laboratories have consistently reported that neither
purified cellulose nor wheat bran have significant
cholesterol-lowering effects (4).   From these observations we
conclude that the water-insoluble types of polysaccharides have
minimal cholesterol-lowering effects.   In sharp contrast,
purified water-soluble fibers such as pectin and guar have
distinct hypocholesterolemic effects.   The cholesterol-lowering
properties of water-soluble fibers are shared by fibers from
quite diverse sources.   Pectins are cell wall constituent of
many plants and usually are extracted from citrus products.
Oat gum is a gum extracted from oat bran while guar is a
storage polysaccharide extracted from the Indian cluster bean.
In addition, these three soluble fibers range in chemical
structures ranging from the beta glucan of oat gum and the
galactamannan of guar to galactouronic acid, the major
component of pectins.   Preliminary studies suggest that the
physiologic properties of fibers can be correlated with their
cholesterol-lowering properties since the viscosity and the

extent of methoxylation of pectin influences the
hypocholesterolemic effect (5).

Hypocholesterolemic Effects of Plant Fibers–Human Studies.
Most of the purified fibers and fiber–rich foods that have been
tested in animals also have been used for human studies (Table
II). These human studies yield similar conclusions.

TABLE II.   EFFECT OF PLANT FIBER SUPPLEMENTS ON SERUM[1]
            CHOLESTEROL CONCENTRATIONS OF HUMANS[1]

Source of Fiber	No. of Studies	Plant Fiber, g/d	Serum Cholesterol % of control
Cellulose	3	16	100
Wheat bran	14	17	101
Whole oats	1	15	89
Oat bran	2	27	83
Pectin	10	25	87
Guar	3	24	84
Bengal gram	1	Unknown	78
Beans	1	30	81

1. Data from Table II (4) except for oat bran (3) and   beans
(current study).

     Although the experimental designs varied, certain
generalizations seem apparent. Cellulose and wheat bran do not
have discernible cholesterol–lowering properties while oat
products and legumes which are rich in water–soluble fibers and
the purified soluble pectins have distinct hypocholesterolemic
effects.   The purified pectin and guar preparations have
practical limitations because these materials develop a gummy,
glue–like material when hydrated and must be ingested rapidly
to avoid nausea and vomiting.   Guar, however, has been
successfully incorporated into a crispbread preparation which
is reasonably palatable (6). Oat bran can be eaten as a hot
cereal or incorporated into a tasty muffin.   As we will
describe later, moderate quantities of beans can be served
without difficulties for most individuals.

METHODS

Overview of Study. We designed this study to determine if
supplementing the diet of hypercholesterolemic men with 115 g
(dry weight) of beans/day was associated with significant
changes in blood lipid concentrations. On a metabolic ward we
fed a control diet for seven days to obtain baseline
measurements and then fed a bean–supplemented diet for 21 days.
For each patient the control and bean diets provided identical
quantities of carbohydrate, protein, fat and cholesterol. Thus

these two diets were designed to provide identical intakes of
energy and nutrients and differed only in the amounts of
soluble and insoluble plant fiber.

Patient Selection. We identified men whose serum total
cholesterol concentration had exceeded 260 mg/dl on at least
two previous measurements. We did not include patients with
secondary causes of hypercholesterolemia (such as
hypothyroidism or renal disease) or with serum triglyceride
concentrations exceeding 500 mg/dl. None had received
lipid-lowering drugs in the last six months. Thus we invited
patients with type IIa or IIb hyperlipoproteinemia to
participate in this study. We obtained informed consent from
each patient in accordance with the Human Investigations
Subcommittee of the University of Kentucky. After admission to
the metabolic ward, patients were randomly allocated to the
bean-diet study or the oat-bran study. All patients enrolled
completed the study. To avoid bias in selection, the principal
investigator did not learn of the diet allocation until after
admission to the hospital. We will summarize the response of
ten consecutive patients to the bean diet since the response to
the oat bran diet will be reported elsewhere.

Diets. The control and bean diets were composed of commonly
available foods (Table III) and were computed to provide
identical amounts of energy, carbohydrate, protein, fat and
cholesterol (Table IV).
The bean diet provided 115 g of dried beans/day served in soups

          TABLE III.   FOODS SERVED ON REPRESENTATIVE DAYS
             CONTROL DIET   BEAN DIET
     Milk 480 g   Milk 400 g
     Eggs 38 g   Eggs 38 g
     Butter 8 g   Butter 20 g
                                    Margarine 8 g
     Ham 95 g   Turkey 48 g
     Ground beef 100 g   Ground beef 48 g
     White bread 85 g   White bread 28 g
     Wheat flake cereal 30 g   Graham crackers 17 g
     Sweet potatoes 110 g   Potatoes 200 g
     Macaroni 92 g   Cabbage 105 g
     Green beans 100 g   Kale 90 g
     Brussel Sprouts 100 g   Tomatoes 110 g
     Peaches 100 g   Peaches 112 g
     Apricots 130 g   Orange 220 g
     Apple juice 100 g   Orange juice 110 g
                                    Navy beans 50 g (dry)
                                    Pinto beans 65 g (dry)

and as cooked beans. To incorporate beans into the diet we had
to reduce the intake of beef and pork from approximately 200 to
100 g/day and increase the intake of butter and margarine to
replace the fat content of these meats. The average control
diet provided approximately 20 grams of total plant fiber and 5
grams of soluble fiber/day whereas the average bean diet
provided approximately 50 grams of total and 20 grams of
soluble fiber/day. Beans provided approximately 30 grams of
total and 13 grams of soluble fiber/day. The nutrient and
fiber content of each diet was measured, the actual food
consumption was measured and the energy, nutrient and fiber
intake was computed as previously described (1-3).

TABLE IV.   COMPOSITION OF FOOD SERVED TO REPRESENTATIVE PATIENT

	Control	Bean Diet
Kcalorie	1715	1711
Carbohydrate, total (g)	182.1	183.4
Simple	91.1	91.3
Complex	90.8	91.9
Protein (g)	85.2	85.8
Fat, total (g)	70.3	70.5
Saturated	33.2	30.6
Monounsaturated	28.7	26.6
Polyunsaturated	6.6	10.6
Cholesterol (mg)	447.4	449.0
Plant fiber, total (g)	19.8	49.1
Soluble	4.5	19.4
Insoluble	15.3	29.7

Measurements. We weighed patients daily. Each morning we
collected blood after a 10-hr fast and measured the serum total
cholesterol, triglycerides and glucose. On the last three days
of each diet we measured serum high density lipoprotein (HDL)
cholesterol concentrations and calculated low density
lipoprotein (LDL) cholesterol values. We collected all stools
and measured frequency, wet and dry weights. Selected stool
samples will be analyzed later for neutral steroids,
cholesterol and bile acids. We used the paired T-test for
statistical comparisons.

RESULTS

Serum Cholesterol Concentrations. Bean-supplemented diets
effectively lowered serum cholesterol concentrations by an
average of 19%. Since the low density lipoproteins (LDL) are
considered to be atherogenic and the high density lipoproteins
(HDL) may protect against the development of atherosclerosis,
we were interested in the effects of bean diets on these

lipoproteins. Average serum total cholesterol concentrations
decreased from 299 mg/dl on the control diet to 242 mg/dl on
the bean diet (Table V). Most of this reduction was due to the
reduction in LDL cholesterol concentrations which decreased in
each individual; average values dropped from 219 to 169 mg/dl,
a 23% reduction. Average serum HDl cholesterol concentrations
also decreased on the bean diet although the 15% reduction was
smaller than observed for LDL cholesterol concentrations. The
ratio of HDL:LDL cholesterol concentrations, considered to be
an indicator of the atherogenic potential of serum, changed
favorably with a slight, but insignificant, increase form 0.15
on the control to 0.17 on the bean diet.

TABLE V. RESPONSE OF FASTING SERUM CHOLESTEROL, TRIGLYCERIDES
          AND PLASMA GLUCOSE CONCENTRATIONS TO BEAN DIET.
          VALUES FOR 10 MEN

	Control Diet	Bean Diet	% Change
Serum cholesterol, mg/dl			
Total	299+14	242+12	-19
LDL	219+15	169+11	-23
HDL	33+3	28+2	-15
Serum triglyceride, mg/dl	238+31	221+38	-7
Plasma glucose, mg/dl	98+4	95+3	-3

     Four patients had type IIa hyperlipoproteinemia
characterized by an elevation of serum cholesterol
concentrations with normal serum triglyceride concentrations;
this represents a selective elevation of the LDL. These
patients with hypercholesterolemia alone responded better to
the bean diet than did six other patients. These six patients
had type IIb hyperlipoproteinemia characterized by an elevation
of both serum cholesterol and triglyceride concentrations
reflecting abnormally high LDL and very low density
lipoproteins (VLDL) concentrations. Thus the four patients
with type IIa hyperlipoproteinemia had a 24% reduction in serum
total cholesterol concentration with values decreasing from 314
to 239 mg/dl; their LDL cholesterol concentrations decreased by
27%. The six patients with type IIb hyperlipoproteinemia had a
16% decrease in serum total and a 19% decrease in LDL
cholesterol concentrations. We will need to study more
patients to determine if these different responses are
consistent and have important therapeutic implications.

Serum Triglyceride Concentrations. The bean diet did not have
a significant effect on serum triglyceride concentrations.
While average serum triglyceride concentrations decreased by
7%, values decreased in seven patients and increased in three
patients. This confirms our earlier experience with oat bran

(<u>3</u>) and indicates that diets which are selectively enriched with soluble plant fibers are not effective in lowering serum triglyceride concentrations. High fiber diets which provide a mixture of insoluble and soluble fibers from a variety of different foods, however, are effective in lowering serum triglyceride concentrations (<u>2</u>).

Serum Glucose Concentrations. Bean diets did not have a significant influence of fasting serum glucose concentrations; values decreased in seven men and increased in three men. However, all three men who had average serum glucose concentrations above 100 mg/dl (suggesting a diathesis to diabetes) on the control diet, showed a reduction with the bean diet. Previous studies (<u>4</u>) have indicated that soluble fiber supplements have a greater impact on postprandial plasma glucose concentrations than on fasting glucose concentrations. In these studies we did not measure postprandial glucose concentrations or do glucose tolerance tests; these studies may have demonstrated an effect of bean-supplemented diets on glucose metabolism.

Body Weights. Over a four week period, average weights decreased from 76.4 + 4.2 kg (mean + SEM) on control diets to 75.3 + 4.4 kg on the bean diet. This reduction in body weight is largely atrributable to the fact that most of these patients were overweight and wanted to lose weight in the hospital. Although we strongly encouraged patients to eat all of the food that was served, most did not consistently eat everything that was served.   Thus their caloric intake was lower than anticipated. Careful analysis of their intake indicates that there was a proportional decrease in energy, nutrients and fiber without selective changes in any one item.   Changes in body weight, however, were not correlated with changes in serum cholesterol concentrations.   Using a tertile analysis, we observed for three patients who lost the most weight that average reductions in serum cholesterol concentrations were identical to average reductions for three patients who lost the least amount of weight.

Other Effects. We carefully monitored these inpatients daily for side effects related to the increase in total plant fiber intake from an average of 20 to 50 grams/day.   Only two patients reported gastrointestinal side effects with bean diets.   Both noted abdominal distention and discomfort during the first ten days on the bean diet. These side effects were tolerable and neither patients requested that we modify or discontinue the bean diets; the diet was well tolerated by one patient during the last week of observation and was reasonably well tolerated by the other patient during the last week. With direct questioning we learned that all patients has more gas production with the bean diet. They had more flatulence and

more eructations (belching). None of these patients developed
diarrhea or other side effects.

DISCUSSION

    Our current studies confirm previous reports (4) that
supplementing the diet with soluble fibers or fiber-rich foods
significantly lowers serum cholesterol concentrations in man.
We documented that supplementing the diet with 115 g of dried
beans lowered serum total cholesterol concentrations by an
average of 19%. Bean supplements were reasonably well
tolerated and appear to offer promise as a practical measure
for the long-term management of hypercholesterolemia. While
bean-supplemented diets were as effective as oat-bran
supplemented diets in lowering serum cholesterol
concentrations, oat bran was better tolerated by our patients.
Nevertheless, because beans are widely available and are a
commonly used food, they offer promise as an adjunct in the
long-term management of patients with hypercholesterolemia.
Based on reported side effects associated with guar and pectin
supplements and on discussions with investigators who have used
these purified fibers, we feel that bean supplements are better
tolerated and associated with fewer side effects than are these
purified fibers.
    Earlier we reported (3) that supplementing the diet with
100 grams of oat bran/day selectively lowered LDL cholesterol
concentrations and did not alter HDL cholesterol concentrations
of hypercholesterolemic men. In this study, we compared the
effects of oat bran with that of beans using the same protocol;
hypercholesterolemic patients were randomly allocated to either
bean or oat-bran supplemented diets. We have not completed our
analysis of the oat-bran branch of the study. However,
preliminary observations suggests that beans and oat bran have
similar effects on LDL and HDL cholesterol concentration over a
21-day period of study. Our current studies do indicate that
bean supplemented diets favorably influence the ratio of HDL to
LDL cholesterol concentrations and thus reduce the theoretical
diathesis to atherosclerosis. Currently we are examining the
long-term effects of diets supplemented with either beans or
oat bran to assess this question in a more practical fashion.
    The lack of an effect of bean-supplemented diets on serum
triglyceride concentrations is consistent with our previous
experience and that of others (4). While a mixture of soluble
and insoluble fibers from a wide variety of foods has a
significant triglyceride-lowering effect, (2) we have not
identified specific types of fibers or fiber-rich foods which
are effective in lowering serum triglyceride concentrations in
humans or experimental animals. Nevertheless, we have
documented that weight-maintaining, high-fiber diets
dramatically lower serum triglyceride concentrations of
hyperetriglyceridemic patients. When we treated ten patients

with serum triglyceride concentrations exceeding 1000 mg/dl
(six-fold higher than normal) with high-fiber diets, we
observed an 80% reduction in serum triglyceride concentrations
while these patients were hospitalized. With longer term
follow-up we have noted that high-fiber diets are accompanied
by an even further reduction in serum triglyceride
concentrations which now average only 10% of values initially
observed (Anderson, J.W. unpublished observations). Further
studies are required to determine the type of fibers or
fiber-rich foods which act to lower serum triglyceride
concentrations. The mechanisms responsible for the reduction
in serum triglyceride concentrations associated with high-fiber
diets have not been determined.

     The influence of high fiber diets on glucose metabolism
has been reviewed in detail elsewhere (4). Our studies of
these patients with a diabetic diathesis suggest that
bean-supplemented diets improve glucose metabolism but we did
not rigorously examine this question in this study. In a
previous study where we fed similar amounts of soluble fiber
from an oat bran source, we observed a significant improvement
in oral glucose tolerance tests and a significant reduction in
average insulin concentrations in response to oral glucose
administration (unpublished observations, see Ref 10). Other
workers have documented that supplementing the diet with
soluble fibers improves the disposal of glucose after meals.

     The mechanisms responsible for the cholesterol-lowering
effects of soluble fibers are reviewed elsewhere (4). Our
studies and those of others suggest that two mechanisms may
play a major role in the cholesterol-lowering effects of
soluble-fiber supplemented diets. First, these diets are
accompanied by significant increases in fecal bile acid
excretion (3). This may divert cholesterol which is
synthesized in the liver from cholesterol-containing
lipoprotein particles into the synthesis of bile acids.
Furthermore, we have documented that biliary bile acid
excretion is significantly higher in oat bran treated rats than
in control rats (11). Second, short chain fatty acid
metabolites resulting from the fermentation of plant fiber in
the colon may influence cholesterol synthesis. Soluble fibers
are almost completely hydrolyzed to short chain fatty acids
such as acetate, butyrate and propionate in addition to other
products in the right side of the colon. Our preliminary
studies indicate that these short chain fatty acids in
physiologic concentration are able to significantly inhibit
hepatic cholesterol synthesis utilizing rat hepatocytes
preparations (12). These studies suggest that plant fibers not
only deplete the body of bile acids, but they also act to
attenuate hepatic cholesterol synthesis. Further studies are
required to evaluate the physiologic importance of these
observations and to delineate other mechanisms by which plant
fibers lower serum cholesterol concentrations.

CONCLUDING COMMENTS

Fiber-rich foods offer many health-promoting benefits for humans. High fiber diets lower insulin requirements of diabetic individuals, lower serum cholesterol concentrations of hypercholesterolemic individuals, dramatically lower fasting serum triglyceride concentrations of patients with severe hypertriglyceridemia, offer therapeutic advantages for obese patients because of their high satiety value (9), and alleviate symptoms of hypoglycemia of patients with reactive hypoglycemia (13). High-fiber or fiber-supplemented diets have the potential to lower the risk for atherosclerosis or even reverse atherosclerosis in humans and reduce the risk for certain types of cancer.

The medicinal value or therapeutic potential of high-fiber food supplements has not been examined carefully. Studies of the cholesterol-lowering properties of high-fiber foods have provided the strongest evidence that selected foods may have potential therapeutic effects. Additional studies in diabetic patients have documented the therapeutic effects of fiber supplements. Our studies with oat bran indicate that high fiber foods can effectively and safely lower serum cholesterol concentrations of hypercholesterolemic men. Our current studies document that the intake of 115 g of dried beans daily lowers average serum cholesterol concentrations by 19%. These results clearly demonstrate the need to examine the effectiveness, palatability, practicality and safety of other high-fiber or fiber-supplemented food items for a variety of metabolic derangements in humans.

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Purified Psyllium Seed Fiber, Human Gastrointestinal Tract Function, and Nutritional Status of Humans

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Because of its high hemicellulose content, purified
psyllium seed fiber has excellent hydrophilic qual-
ities which account for its effectiveness as a
bulking-type laxative. In spite of this long usage,
purified psyllium seed fiber has not been popularly
suggested as a source of dietary fiber in the supp-
lementation of food products. In a series of
studies conducted in this laboratory, purified
psyllium seed fiber was added as a fiber supple-
ment to bread and to peanut butter. Products
were fed to human subjects as part of a mixed
food diet in which the psyllium seed provided
the major source of dietary fiber. Different
studies were designed to compare effects of
psyllium seed fiber with other purified and food
fiber sources. Results indicated that while pos-
itive effects associated with increased feeding
of fiber tended to be greater with psyllium than
with other test fibers employed, the negative
effects associated with decreased protein and
mineral utilization also tended to be greater.

Recent concern for increasing dietary fiber intake of Amer-
icans has generated interest in a multitude of plant products
which theoretically might be of use as fiber supplements to foods
destined for consumption by humans. Often suggestions for the
use of these products seem to be promted on the basis of econo-
mical criteria rather than by using desired human physiological
response as a justification. For example, many currently-used
or projected, fiber-rich ingredients are by-products from estab-
lished industries; hence, are readily available at relatively
low cost. Since all forms of dietary fiber do not elicit similar
physiological effects in the human, this approach may lead to
false expectations on the part of both the food processor and the
consumer when the fiber enriched product is actually produced,
sold and used.

Ground psyllium seed husks have been fed to humans for gener-
ations as a pharmaceutical to increase stool bulk and to achieve
and/or maintain gastrointestinal tract motility. Some of the
many bulk laxative products sold in the United States which are
based on psyllium seed fiber for their functions effects include
the following: Mucilose Flakes, Effersyllium, Mutamucil, Konsyl,
L.A. Formula, Syllact, and Syllamalt (1). Sometimes these pro-
ducts are mixed with sweetening agents or flavorings to increase
palatability. Warnings to drink the products suspended in water
or with water are also included to avoid intestinal blockage
problems. While it may not be part of the popularly accepted
image of dietary fiber, the desired physiological effect of fiber
from laxatives or less directly from food resources is the same.
    Psyllium is a member of the genus plantage. Psyllium or
more correctly, Plantago psyllium, is also referred to as
fleawort. It has been cultivated for many years in Spain and
France expressively for its mucilaginous seed coatings for use
as a laxative. Its laxative properties are associated with its
high content of various hemicelluloses. Hemicelluloses in compar-
ison to the other major fiber classes are notable for their
hydrophilic and, hence, bulking properties.
    At the University of Nebraska, Department of Human Nutrition
and Food Service Management, a commercial psyllium seed fiber
laxative product, Mucilose Flakes (Winthrop Laboratories,
Sterling Drug Co.) has been used for more than 30 years as a
fiber source for human subjects maintained on synthetic and
semi-synthetic research diets. The usual carrying agents used
in these studies were starch breads and starch cookies. Several
years ago when this laboratory first became interested in effects
of dietary fiber on gastrointestinal tract function and on
nutrient utilization, it appeared rational to continue to use
the same fiber source in the initial studies and as comparative
fiber to other fiber sources in later studies. The purpose of
this paper will be to review some of the results of these
studies (2-20). In order to simplify presentation, results of
several studies involving a total of 85 adult subjects have been
grouped together. Not all subjects received all experimental
treatments; however, the same basic control diet was used in all
studies. This basal diet was based on ordinary foods and
included milk, corn flakes, rice, noodles, ground beef, tunafish,
peanut butter, peaches, pears, jelly and sugar. Fiber supple-
mentation levels were moderately high ranging from 14 to 20 g/
subject/day. Bread or peanut butter were used as the carrying
agent. Data from all studies were pooled. Statistical analyses
(Biometrics Laboratory, University of Nebraska) included Analysis
of Variance and Least Square Means test.

Effect on Gastrointestinal Tract Function and Fecal Bulk

Psyllium fiber is composed of several hemicelluloses, which are complex polymers of pentosans, hexosans and galactans. Hemicelluloses are noted for their hydrophilic properties which make them excellent bulking agents. Many studies have been conducted seeking to define relationships among fiber intake, fecal bulk, and fecal transit time times. Results of these studies have been confusing in that different fibers seem to elicit different degrees of response (21-25). Furthermore, individual subject response often is tremendously variable. For example, increased dietary fiber has been found to be useful in relieving symptoms of both diarrhea by decreasing stool bulk and increasing fecal time as well as constipation by increasing stool bulk and decreasing fecal time. Therefore, mean values sometimes involve individual values changing not only to different degrees but actually in opposite directions depending upon the subjects involved.

In the current project, fecal transit times were measured by calculating time required for colored glass bead markers to pass through the gastrointestinal tract (solid phase markers). Fecal transit times of subjects were not significantly affected due to the feeding of dietary fiber in comparison to the fecal transit times when no fiber supplements were given with the laboratory controlled diet. However, numerically, the feeding of psyllium resulted in the shortest fecal transit time. All subjects in this study were assumed to have normal gastrointestinal tract function so it is perhaps not surprising that fecal transit times were not significantly affected as a result of feeding the fiber supplements (Table I).

Feces of subjects were divided into period lots by use of fecal dye markers. Fecal wet weights were significantly affected as a result of feeding the fiber supplements (Table I). When psyllium was the fiber supplement used, fecal wet weights were double those when no fiber supplement was given. These values were numerically higher than those obtained when other test fiber sources were used. Fecal excretion on a dry weight basis were also increased when psyllium was used as a fiber source in comparison to when no fiber supplements were employed; however, these values were not as high as those obtained when such fiber supplements as cellulose or corn bran were employed suggesting that water bulking was the primary cause of increased fecal wet weight when psyllium was fed.

Effect on Proximal Composition of Feces

Feeding of psyllium fiber resulted in a significant increase in percentage of water contained in feces during this project in comparison to values obtained when the laboratory controlled diet was fed without fiber supplementation or when other sources

Table I

Effect of Psyllium and Other Fiber Sources on Various
Parameters of Gastrointestinal Tract Activity, Fece
Composition, and Blood Lipid Patterns of Humans

Parameter	Fiber source						
	Psyll-ium	Cellu-lose	Pectin	Wheat Bran	Rice Bran	Corn Bran	None
Fecal transit time (hr)	36.1	39.1	39.7	40.8	41.6	45.2	45.3
Fecal weight-wet (g/day)	154.9^c	114.1^{bc}	96.6^b	105.5^b	99.2^b	96.0^b	69.8^a
Fecal weight-dry (g/day)	30.5^b	34.8^b	25.2^{ab}	29.0^{ab}	30.4^b	37.2^b	20.3^a
Fecal water (%)	80.3^c	69.5^b	73.9^b	72.5^b	69.2^b	61.2^a	70.9^b
Fecal fat (%)	2.03^c	1.62^b	1.42^{ab}	1.97^c	2.04^c	1.74^b	1.30^a
Fecal protein[1,2] (g/day)	18.8^c	15.7^b	15.9^b	18.7^c	17.8^{bc}	20.0^c	10.7^a
Fecal cellulose (g/day)	4.0^{ab}	12.6^c	2.8^a	3.4^a	3.3^a	4.7^b	2.2^a
Fecal hemicell-ulose (g/day)	7.5^b	3.8^a	3.1^a	9.0^{bc}	5.4^a	11.5^c	2.5^a
Fecal lignin (g/day)	0.86^{ab}	0.95^b	0.68^a	0.69^a	1.02^b	0.98^b	0.60^a
Fecal copper[3] (mg/day)	5.04^b	4.07^a	3.91^a	5.81^b	5.55^b	5.07^b	3.78^a
Fecal zinc[4] (mg/day)	13.32^b	10.26^a	9.63^a	12.13^b	11.13^{ab}	10.03^a	9.64^a
Fecal magne-sium[5] (mg/day)	379^b	264^a	278^a	450^c	375^b	403^{bc}	252^a
Fecal calcium[6] (mg/day)	899^b	873^b	752^a	910^b	827^{ab}	799^a	796^a
Blood serum cholesterol (mg/dl)	158^a	168^b	172^b	173^b	172^b	179^b	172^b
Blood serum triglyceride (mg/dl)	91^a	105^b	105^b	102^b	122^c	117^c	103^b

Table I (cont)

Parameter	Fiber source						
	Psyllium	Cellulose	Pectin	Wheat Bran	Rice Bran	Corn Bran	None
Blood serum phospholipid (mg/dl)	210[a]	209[a]	202[a]	195[a]	189[a]	205[a]	220[a]

[1] Protein calculated by multiplying nitrogen x 6.25. Since not all fecal nitrogen is from protein, these values are probably over-estimations.

[2] Protein intake was 65 g/subject/day during all periods except when bran supplements were used. Amount of additional protein/subject/day during these periods were as follows: wheat bran, 2.01; corn bran, 2.93; and rice bran, 2.62.

[3] Copper intake was 4 mg during all periods except when bran supplements were used. Amounts of additional copper/subject/day during these periods were as follows: wheat bran, 0.053; corn bran, 0.007; and rice bran, 0.008.

[4] Zinc intake was 10 mg/subject/day during all periods except when bran supplements were used. Amounts of additional zinc/subject/day during these periods were as follows: wheat bran, 0.270; corn bran, 0.066; and rice bran, 0.213.

[5] Magnesium intake was 1450 mg/subject/day during all periods except when bran supplements were used. Amounts of additional magnesium during these periods were as follows: wheat bran, 21.28; corn bran, 30.32; and rice bran, 42.13.

[6] Calcium intake was 1450 mg/subject/day during all periods except when bran supplements were used. Amounts of additional calcium/subject/day during these periods were as follows: wheat bran, 25.32; corn bran, 20.15; and rice bran, 12.12.

Values with different letter superscripts are significantly different from one another ($P < 0.05$).

of fiber were fed. In spite of the high water content, stools
were well-formed during periods when the psyllium fiber was used.
This attests to the water holding capacity of this fiber source.
    Fecal fat loss (Goldfisch method) was also increased when
psyllium fiber was fed in comparison to losses when no fiber
supplements were employed (Table I). However, these losses were
no greater than when several of the other test fiber supplements
were used. Loss of fat in feces may be considered either an
advantage or a disadvantage depending upon whether problems of
obesity and atherosclerosis are being considered or whether
malabsorption syndromes are the topic in question.
    Fecal nitrogen losses (Kjeldahl method) were significantly
increased as a result of feeding any of the test fiber sources
in this project (Table I). Psyllium feeding resulted in higher
fecal nitrogen losses than did the feeding of the other two
"purified" fiber sources, cellulose and pectin, but no higher
losses than the feeding of the three test brans. Nitrogen
values have been converted to protein (N x 6.25) in this project.
Since all fecal nitrogen is not protein nitrogen, these values
are undoubtedly falsely high but can be used for comparative
purposes.

Effect on Fecal Fiber Composition

    Since psyllium fiber is composed of several hemicelluloses,
it might be supposed that feces produced when high levels of
psyllium are fed would reflect this high content of hemicellu-
loses. Similarly, it might be expected that feces produced when
other sources of dietary fiber are fed would reflect the fiber
composition of the fiber source. A complexing factor to this
simplistic approach is that the feeding of dietary fiber may
result in changes in lower gut microflora and that microflora
residue also will influence fecal fiber composition. Furthermore,
about half of the hemicellulose is known to undergo degradation
in the lower gut. In addition, chemical methodology for class-
ification of fiber classes is rather inexact when applied to the
clinical laboratory situation. Methods of Goering and Van Soest
were used in the present study.
    In the current project, the cellulose fraction in feces was
increased only when the cellulose supplement was given. Numer-
ically, the feeding of psyllium resulted in some increase in
this fraction but not at the statistically significant level.
Hemicellulose content of feces was significantly increased as a
result of feeding psyllium; however, these values were not as
high as those achieved when wheat bran or corn bran was fed.
Corn and wheat bran contain considerable amounts of hemicellulose
but not at the high levels found in psyllium. Furthermore, the
water-holding capacity of these two fiber sources were not as
high as those for psyllium. Hence, it would appear that fecal
residual fiber is perhaps not a good index of functionality of

the fiber. Fecal lignin levels (Table I) were increased as a
result of fiber feeding but these were not statistically differ-
ent from one another.

Effect on Mineral Utilization

    Considerable investigation has been reported on the effects
of increasing dietary fiber on utilization of various minerals.
Fiber supplementation may dilute the concentration of minerals
by increasing fecal bulk, may lessen the absorption by decreasing
fecal transit time, may encourage absorption of minerals upon
fiber residues or trap minerals within residues, and/or may
complex with phytate and other inhibitors of mineral utilization.
However, results of studies investigating utilization of differ-
ent minerals under conditions of additions of different levels
and sources of fiber have in no way been conclusive (26-29).
    In the present project, fecal copper losses were significantly
increased when psyllium fiber was used as a fiber source in com-
parison to levels when no fiber supplements were given (Table I).
These losses were significantly greater than when the cellulose
and pectin supplements were given but not greater than when the
bran supplements were used. Fecal zinc loss was also signifi-
cantly increased when psyllium supplements were used as well as
losses of calcium and magnesium. It is important to remember,
however, that increased fecal mineral losses are of nutritional
significance only if the subjects have marginal or inadequate
intakes of the minerals in question. When diets supply adequate
amounts of these nutrients, this negative effect is of no physio-
logical importance.

Effect on Blood Serum Lipid Patterns

    One of the proposed advantages of inclusion of dietary fiber
in foods and eating patterns is the decrease in blood serum
lipids which may occur since increased circulating blood serum
lipids (lipidemias) have been identified as risk factors for
development of atherosclerosis and coronary heart disease. It is
thought that not only will dietary fiber inhibit the absorption
of dietary fat by dilution of fat in the chyme and/or through
absorption of fat in the fiber itself but will also inhibit the
absorption of bile salts and thus lower the absorption of endo-
genous cholesterol. While survey studies indicate that vegetar-
ians and other groups customarily consuming high fiber diets have
low levels of circulating blood lipids and a lower incidence of
atherosclerosis and coronary heart disease than do people consum-
ing typical American or westernized diets, it is difficult to
separate the variables of fiber from those of total dietary fat,
dietary cholesterol, unsaturated to saturated fatty acid ratio,
and sucrose intake,all of which are known to affect circulating
blood lipids (30-34). In laboratory controlled studies with

humans, these effects of dietary fiber on circulating blood lipids
have not been consistently demonstrated (35-40).

    In the present series of studies, blood serum lipids were
determined by a commercial laboratory (Stat Laboratory, Wichita,
Kansas). Blood cholesterol levels of subjects were lower when
subjects received the experimental diets than when subjects con-
sumed their self-selected diets. The mean cholesterol level of
subjects while on the self-selected diets was 193 mg/dl and while
receiving the controlled diet alone or with psyllium, cellulose,
pectin, wheat bran, rice bran or corn bran were 172, 158, 168,
172, 173 and 179 mg/dl, respectively. The general lowering of
blood cholesterol levels due to feeding of the experimental diet
was probably due to its low fat (30% of energy) and low choles-
terol (less than 100 mg/day) levels. Psyllium fiber feeding
resulted in significantly lower blood serum cholesterol levels
than did the feeding of other test dietary fibers or than the
feeding of the basal diet without fiber supplementation.

    Blood serum triglyceride levels were also depressed in the
current project in comparison to normal mean values of subjects
on self-selected diets (133 mg/dl) as a result of feeding the
laboratory controlled diet whether or not fiber supplements were
included. However, as shown on Table I, psyllium feeding resulted
in a statistically significant further decrease in blood serum
triglyceride levels in comparison to the other test sources of
fiber or when no test fiber was given. Blood serum phospholipid
levels were also measured; however, no statistically significant
changes in this serum lipid fraction were observed either as a
result of feeding the experimental control diet or as a result
of fiber supplementation of this diet.

Conclusion

    Fiber derived from psyllium seed has a long history of use as
a pharmaceutical to produce laxation. It's high bulking proper-
ties are due to its high content of hemicellulose. When incor-
porated into food products and fed to humans, it produces the
desirable effects of increased stool bulk, decreased fecal
transit time, and decreased blood serum cholesterol and trigly-
ceride levels. However, concurrently psyllium fiber supplemen-
tation results in some depression in absorption of selected
minerals, fat and protein. This was also true of some but not
all of the other fiber sources tested.

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Locust Bean Gum in Food Products Fed to Familial Hypercholesterolemic Families and a Type II$_B$ Patient

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Familial hypercholesterolemia (FHC) is associated with severe premature coronary artery disease and death and is resistant to conventional dietary treatment. Locust bean gum (LBG) incorporated into food products was fed to adults and children with FHC. Twenty-eight patients, 18 adults and 10 children were divided into two groups (Group A and Group B), and using a cross-over design were fed identical food products with and without LBG (8-30 gm/d) as outpatients in four-week periods. Three additional adults with different lipid abnormalities were studied using the same protocol. Diet records, food product consumption, lipids, and lipoproteins were analyzed every two weeks. Total cholesterol (C) was lowered 11% (275 to 246 mg%) in Group A at four weeks (p < .001) and 17% (255 to 215 mg%) in Group B at eight weeks of LBG diet (p < .001). Low density lipoprotein cholesterol (LDL) fell 10% at four weeks in Group A (p < .001) and 17% at eight weeks in Group B (p < .001). HDL/LDL ratios increased in both groups. Two patients with FHC, one with a partial ileal bypass and one taking lipid-lowering medications, experienced no further lowering of serum lipids or lipoproteins. A patient with elevations in cholesterol and triglycerides (Type II$_B$) had a 22% drop in both. LBG food acceptance was good. There were no significant side effects of the diet. LBG was more effective than some pharmacological agents in lowering lipids, and in outpatients over a two-month period appears to be one of the most effective dietary treatments of FHC. LBG in food products appears to be a safe, effective means of lowering serum lipids in normal and hypercholesterolemic adults and children with FHC.

FHC patients are often resistant to standard dietary management of their hyperlipidemia. Pharmacological treatment in children who will require long-term management involves problems with safety, expense, effectiveness, and may not be acceptable to the child or the parents. For these reasons, drugs are seldom used in the management of hyperlidemic children.[1] Atherosclerosis has is origins in children and potentially may be moderated by lowering serum cholesterol and LDL cholesterol. The prevention of atherosclerosis should begin in children with FHC at an early age, safe and effective treatment is needed, and dietary fiber is of potential use in these patients.

The introduction of fiber into the field of lipid metabolism stems from epidemiological data which compares the incidence of diseases common in the Western world with native African populations.[2-4] Specifically, hyperlipidemia and FHC are associated with a striking incidence of coronary artery disease in the U.S.[5-8]

It is often difficult to manage hyperlipidemia in FHC with standard dietary management. Specific fibers in appropriate amounts exert a pronounced hypolipidemic effect in man and animals.[9] Pectin and Guar have been especially effective in lowering cholesterol in normal and hyperlipidemic patients.[10-11] However, not all fibers are effective in lowering lipids. No two sources of dietary fiber have the same effect on lipid metabolism.[12] Some dietary fibers, although effective as hypolipidemic agents, are not palatable or are difficult to incorporate into food because of increased viscosity or other chemical or taste properties.

LBG comes from the endosperm of the locust bean,[13] which the first-century Greeks used as a laxative and as a paste to bind mummies.[14] It is a white, odorless powder without a distinctive taste and is not to be confused with carob, which is a chocolate substitute which comes from the locust bean husk.

LBG has previously only been used in trace amounts in food products and has not been evaluated in treating hyperlipidemia. It is a hydrophylic compound that in an unhydrated form could create a bolus-like obstruction of the bowel. Bowel obstruction has been reported with the use of a similar hydrophylic compound,[15] although Kahn has recently reported feeding subjects 9 gm/day of Guar in capsules which were not hydrated without side effects.[16]

Study Design and Population: Thirty-two patients were selected from the Hennepin County Medical Center's Hyperlipidemia Clinic on the basis of their willingness to cooperate and make frequent clinic visits. One patient, a four-year-old child, withdrew from the study before the food products were fed because of fear of blood drawing. The FHC patients were defined by published criteria which include: elevation of total cholesterol and LDL cholesterol, normal levels of VLDL cholesterol and triglycerides, one parent and one child, and at least one first-degree relative, with FHC. The families also often had individuals by family history with premature cardiovascular disease. Twenty-one adults (aged 22-53) and 10 children (aged 10-18) completed

the study. Three of the adults were analyzed separately because of the different lipid abnormaliites.

Twenty-eight patients were divided to attempt to balance FHC and N, adults and children, males and females, and baseline lipid levels into two groups, A and B. The study was a modified cross-over design (see Table 1). This allowed a simultaneous comparison of two nearly identical diets with and without LBG, and also provided information after two months of LBG food products in Group B. At Week 16, subjects returned for lipid measurements while back on the normal baseline diet for 4 weeks, .

Normals were included with their hyperlipidemic spouses and siblings because entire families followed the diet and thus compliance was improved. Also, there is speculation that normals might respond differently from FHC patients to dietary management and the normals served as an additional control. Of interest, Group A had more FHC patients (11 vs 7) and Group B had no FHC females (Table 2).

The total cholesterol, VLDL-C, and triglycerides were similar in both groups. Group A had higher LDL-C and lower HDL-C levels, reflecting perhaps the greater number of FHC patients in Group A (Table 2). It is interesting that Group A, which had 8 women versus 5 in Group B, had lower HDL-C levels. This may be explained by the absence of FHC females in Group B (Table 3).

Three patients with different lipid abnormalities were studied simultaneously using the same protocol. One patient was a 53-year-old man with FHC who had a partial ileal bypass two years prior to the study. The second patient was a 49-year-old Type II_B man with elevations of both his cholesterol and triglycerides. A third patient, a 51-year-old man with FHC, was on cholestyramine, nicotinic acid, and atromid during the entire study.

Identical-appearing food products were fed with or without locust bean gum. The food products were bread, crackers, cookies, and a potato product. The food products contained 8-10% LBG by dry weight. Each portion, i.e. slice of bread, one cookie, two crackers, six

TABLE 1

DIET STUDY DESIGN

FEEDING PERIOD		I	II	III	
WEEK NUMBER	-2,0	2,4	6,8	10,12	16
GROUP A	BL	E	C	E	BL
GROUP B	BL	C	E	E	BL

BL - BASELINE DIET
C - CONTROL WITHOUT LBG
E - EXPERIMENT WITH LBG

TABLE 2

SEX, LIPID TYPE, AND AGE

| | | GROUP A (ECE) | | GROUP B (CEE) | |
		YOUNG	ADULT	YOUNG	ADULT
FHC	MALE	2	3	3	4
	FEMALE	1	5	0	0
	TOTAL	3	8	3	4
NORMAL					
	MALE	2	0	1	0
	FEMALE	1	1	1	4
	TOTAL	3	1	2	4

ECE   EXPERIMENTAL, CONTROL, EXPERIMENTAL DIET
CEE   CONTROL, EXPERIMENTAL, EXPERIMENTAL DIET

TABLE 3

PATIENT LIPID BASELINE VALUES - MG%, (SD)

	GROUP A (ECE)	GROUP B (CEE)
CHOLESTEROL	265 (82)	255 (83)
LDL CHOLESTEROL	200 (82)	177 (87)
HDL CHOLESTEROL	46 (11)	55 (18)
VLDL CHOLESTEROL	20 (12)	24 (17)
TRIGLYCERIDE	89 (57)	101 (57)

potato pieces, contained between 1.7 and 2.0 gm of LBG. Food products with and without LBG were analyzed in duplicate. The products with LBG contained higher amounts of total and soluble dietary fiber (using the revised Furda Method).[17] The amount of insoluble dietary fiber was the same for products with and without LBG. There were no significant differences in carbohydrate, protein, and fat content between LBG and non-LBG containing products. Fat analysis was performed for palmitic, oleic, linoleic, linolenic, stearic, arachidic, and arachidonic acid.[18-19] No significant differences were found between products with and without LBG (Table 4).

The families were seen at two-week intervals to collect three-day diet histories and two-week daily dietary records for the food products. The study families had been followed in the lipid clinic and given extensive dietary instruction over a period of six months to seven years before the study was initiated. The above data was recorded on nine consecutive visits at two-week intervals. The first two and the last visits were obtained while the patients were on their normal baseline diets. The feeding periods were four weeks in

TABLE 4

PRODUCT ANALYSIS

	BREAD		COOKIES		CRACKERS		POTATO	
	LBG	C	LBG	C	LBG	C	LBG	C
PROTEIN	10.6	12.6	4.9	5.4	12.5	13.0	2.6	2.5
CARBOHYDRATE	49	55	62	64	67	69	23	21
MOISTURE	36	29	8	4.7	4.7	3.1	67	65
FAT	2.4	2.7	21	23.7	11	11.2	4.5	9
LINOLEIC	37	37	13.9	14.3	55	55	58	57.5
OLEIC	36	35	59	59	29	28	25.7	26.2
PALMITIC	17	16	13.5	13.5	11	11.2	11.6	11.3
T.D.F.	20	11.5	16	4.2	24	17	25	12
I.D.F.	9	8	3.5	1.6	11	11	12	9
S.D.F.	11	3.5	12.5	2.6	13	6	13	3

T.D.F. = TOTAL DIETARY FIBER
I.D.F. = INSOLUBLE DIETARY FIBER
S.D.F. = SOLUBLE DIETARY FIBER
LINOLENIC, STEARIC, AND ARACHIDIC WERE IN LESSER AMOUNTS (0-10%)
RALTEC ANALYSIS
C = CONTROL DIET
UNITS VARY DEPENDING UPON TYPE OF ASSAY

duration. Total calories, cholesterol, saturated fat, unsaturated fat, protein, carbohydrate, crude fiber, vitamin and mineral intake were coded using the USDA Handbook 456[20] and scored by the USDA Handbook 8[21] on a Digital Equipment Corporation computer PDP 11/34. Using the Keys, Anderson, Grande equation, the dietary intake of saturated and polyunsaturated fat and cholesterol was used to estimate the change in serum cholesterol due to non-LBG changes in the diet.[22] This was termed the dietary effect.

The food products were distributed to the families at their two-week clinic visits. The families were instructed not to change their diets during the study period. Exchanges were arranged within food preferences so that protein, carbohydrate, and fat consumption changes would be minimized. Because the food products primarily contained carbohydrates, carbohydrate-caloric exchanges were planned individually to minimize changes from the patients' baseline diets.

LBG in food products was prescribed to the subjects on the basis of body size. Adult males ate 25-35 gm/day, adult females age 10-25 gm/day, and children ate 10-20 gm/day of LBG in the food products. Most subjects ate selections from all four of the food products.

Laboratory and Exercise Evaluation: Laboratory evaluations performed at each two-week visit included plasma total cholesterol, LDL-C, HDL-C, VLDL-C, and plasma total triglycerides.[23] The lipoprotein determinations, LDL-C, HDL-C, and VLDL-C were not performed at Week 16. White blood cell counts, calcium, and serum glutamic oxaloacetic-transaminase (SGOT) were done before and after feeding LBG products. Weight was recorded at each visit.

Glycosylated hemoglobin determinations were performed on stored frozen hemolysates from eight Group B subjects at the end of the four-week control period and again at the end of the eight-week LBG feeding period. All eight subjects demonstrated a decrease in total cholesterol and LDL cholesterol in response to LBG. None of the subjects were diabetic or had symptoms or a past medical history of carbohydrate intolerance.

Glycosylated hemoglobin was determined by modification of a colorimetric assay which measures stable ketoamine-linked sugar.[24] Mild acid hydrolysis of the sugar linked to hemoglobin yields 5-hydroxymethyfurfural which reacts with thiobarbituric acid to form a colored product. The procedure was performed on globin extracts to prevent colorimetric interference by heme. Chromatographically purified HbA_aC was used as the standard for the method. Glycosylated hemoglobin was expressed as relative precentage of the total hemoglobin.

Exercise forms were filled out at the end of the control and experimental periods for 65 different types of exercise activity. This data was recorded for each month of the study and included the number of times the activity was performed and the duration per occasion.[25] The study was performed during the months of February,

March, and April, when outdoor activities remained similar. Subjects were asked to maintain their normal physical activity. No apparent change of exercise as determined by these forms was observed during the study, and thus no correlation could be made between exercise and lipid changes.

The study was approved by the Human Volunteer Committee at the Hennepin County Medical Center to include adults and children.

Statistical Methodology: The relationships between LBG intake and lipid changes were analyzed by regression analysis which allowed for adjustment for various covariates.   The within-person correlation coefficients between LBG and specific lipid determinations were calculated.   The means of these correlation coefficients are approximately normally distributed, so they were tested by the t-test, using the empiric standard error. To ensure that other dietary changes did not explain lipid changes, the correlations between lipid changes and the dietary effect were similary tested.   The regressions were carried out for all persons together and for Group A and B separatedly. Conditional F-tests were applied to see if Group A and B differed in the lipid (or lipid-fraction) response to LBG.   The significance of a covariate was tested by the t-test on the regression coefficient.

All statistical analysis was carried out on the PDP 11/70 computer at the Laboratory of Physiological Hygiene using a Fortran program for the correlation analysis and the Biomedical Data Processing statistical package program BMDP1R for the regression analyses.[26]

Results

Thirty-one patients completed the study.   The lipid and lipoprotein levels were averaged for Group A and B for each of three periods and the baseline levels and are presented with standard deviations in Table 5.   Three patients were evaluated separately because of the different nature of their lipid disorder and surgical or medical treatment.

Total Cholesterol: Comparing the control diet (Period II) to the experimental diet (Period III), Group A decreased their total cholesterol from 276 to 246 mg%, an 11% decrease ($p < .001$).   The cholesterol and triglyceride values returned to baseline levels at Week 16, four weeks after resuming the normal baseline diet. Group A did not significantly decrease their cholesterol levels during the first experimental feeding period (Period I) when compared to baseline values; this could not be explained on the basis of LBG consumed, exercise, weight change, or any dietary variable evaluated.

Group B, comparing the control diet (Period I) to the experimental diet (Period II and III), decreased their total cholesterol from 255 to 215 mg%, a decrease of 16% ($p < .001$) (Table 5).

TABLE 5

LBG, CHOLESTEROL, LIPOPROTEIN CHOLESTEROL, TRIGLYCERIDE LEVELS BY GROUP FOR EACH TWO-WEEK PERIOD (MG/DL) (S.D.)

WEEK	DIET	CHOL	LDL-C	HDL-C	VLDL-C	TG	LBG
GROUP A							
-2	BL	263.5(78)	199.5(78)	45.9(12.6)	18.1(12.9)	80.5(49.7)	0
0		266.3(88)	200.7(88)	45.3(10.7)	21.3(12.4)	99.2(69.0)	0
PERIOD I	E						
2		257.5(87)	195.9(87)	45.5(9.9)	16.3(9.4)	94.3(60.8)	15.4(6.4)
4		261.7(89)	202.6(89)	43.7(10.3)	15.3(9.0)	91.5(61.1)	15.1(4.6)
PERIOD II	C						
6		277.9(96)	217.3(94)	48.1(10.6)	12.9(11.3)	88.6(58.2)	0
8		272.7(93)	209.1(89)	45.1(10.1)	18.9(13.8)	100.8(72.4)	0
PERIOD III	E						
10		253.3(88)	191.4(85)	43.6(11.1)	15.4(11.9)	95.9(85.1)	12.2(4.3)
12		246.3(86)	191.7(86)	42.9(10.2)	11.7(12.2)	82.9(57.9)	11.5(6.3)
16	BL	266.9(92)	----	----	----	84.6(58.0)	0

GROUP B							
-2	BL	251.8(86)	174.9(87)	53.5(16.4)	23.8(17.1)	107.0(56.4)	0
0		257.2(18)	177.6(89)	55.1(20.1)	23.7(18.3)	108.4(61.0)	0
PERIOD I							
2	C	262.5(82)	183.4(93)	55.2(18.1)	23.8(19.1)	116.8(64.5)	0
4		249.2(84)	176.8(90)	52.0(16.3)	20.4(19.2)	109.3(54.8)	0
PERIOD II							
6	E	229.5(73)	160.6(83)	51.7(18.4)	17.2(12.7)	93.2(44.9)	14.4(5.5)
8		221.2(66)	151.7(75)	52.6(15.9)	17.5(11.5)	80.9(40.9)	13.4(7.3)
PERIOD III							
10	E	219.2(61)	151.3(69)	47.6(16.8)	20.2(15.2)	102.9(49.5)	12.0(5.4)
12		214.7(67)	150.0(70)	48.5(13.8)	16.2(6.3)	92.5(27.2)	11.9(7.3)
16	BL	251.6(82)	----	----	----	102.8(58.0)	0

E - FOOD PRODUCTS WITH LBG
C - FOOD PRODUCTS WITHOUT LBG
BL - BASELINE DIET WITHOUT FOOD PRODUCTS

LDL Cholesterol: Comparing control to experimental feeding periods, Group A LDL-C was lowered 10% from 211 to 191 mg% (p<.001). Comparing Period I experimental diet to baseline diet, there was no significant change in LDL-C in Group A (Table 5).
        Group B lowered their LDL-C 17% from 181 mg% during control diet (Period I) to 141 mg% on the experimental diet (Period II and III) (p<.001) (Table 5).

HDL Cholesterol: In Group A, comparing control to experimental diet periods, HDL-C levels fell 7%. In Group B, HDL-C levels fell 10%. The HDL/LDL ratio in Group A increased 4% (N.S.), while in Group B the ratio increased 12% from .29 to .33 (p<.001.) (Table 5).

VLDL Cholesterol: The association between VLDL-C changes and LBG was just significant. Both groups demonstrated an erratic response in VLDL-C levels to LBG, but by the end of the feeding experiment (Week 12), both groups were over 30% lower in VLDL-C than at baseline (Table 5).

Triglycerides: All subjects had normal triglyceride levels before, during, and after the feeding studies. And there was no change during LBG feeding of significance (Table 5).

Males vs Females, FHC vs Normal, and Children vs Adults: All subgroups demonstarted a lowering of both total cholesterol and LDL-C. There was no difference in the cholesterol or LDL-C response to LBG between males and females of either group. FHC patients consistently had a greater lowering of cholesterol and LDL-C than normals in both groups (p<.05). Cholesterol and LDL-C in adults were consistently lowered more than in children (Table 6). This was not significant when adjusted for weight.

Patient with Elevations in Triglycerides and Cholesterol: The patient with Type II$_B$ hyperlipidemia had a 22% and 18% decrease in cholesterol and 24% and 22% decrease in triglycerides at 4 and 8 weeks during the experimental LBG feeding periods. VLDL cholesterol fell 24% at 4 and 8 weeks.

Partial Ileal Bypass and LBG: The patient with the partial ileal bypass was unusual in that he had consistently elevated HDL levels. This patient had no significant lipid lowering on the experimental LBG diet. It is interesting that the HDL cholesterol increased on the LBG diet and fell during the control diet.

LBG Plus Pharmacological Treatment: To help understand the mechanism of action, we also fed the LBG food products to a 52-year-old man with FHC who was on three lipid lowering medications during

TABLE 6

LIPID RESULTS BY PERIOD, SEX, LIPID TYPE, AND AGE - MG%

GROUP		BASELINE A	B	PERIOD 1 A	B	PERIOD 2 A	B	PERIOD 3 A	B
				E	C	C	E	E	E
CHOL	M	264	296	265	296	281	258	256	253
	F	266	188	255	184	270	173	244	159
	FHC	304	313	300	316	320	272	289	261
	N	156	185	147	184	150	170	141	164
	Y	233	252	232	250	234	230	216	227
	A	286	256	278	259	303	222	272	210
LDL	M	200	226	208	231	222	199	200	193
	F	200	97	191	99	206	88	185	83
	FHC	237	244	238	252	256	214	230	203
	N	96	97	90	96	94	88	85	90
	Y	173	180	178	186	179	164	167	163
	A	217	174	214	176	236	151	208	143
HDL	M	36	44	37	44	38	42	35	40
	F	54	70	51	69	54	68	50	61
	FHC	45	41	44	41	46	39	42	38
	N	48	70	48	67	48	67	47	60
	Y	42	54	42	52	41	53	40	50
	A	48	55	48	55	50	52	46	47

Y = CHILDREN M = MALE   E = EXPERIMENTAL DIET
A = ADULT   F = FEMALE   C = CONTROL DIET

the study, cholestyramine (24 gm/day), nicotinic acid (4 gm/day), and chlofibrate (2 gm/day). His usual cholesterol levels were in the 400's mg% range when not on medication and were in the low 200's mg% range when on the medications. There was no change in serum lipids on the medications with the addition of LBG. In evaluating these two patients, it would seem that the bile acid loss by ileal bypass or cholestyramine may have been maximized, thus there was no additional effect in bile acid binding and loss with the LBG.

Glycosylated Hemoglobin: The incorporation of LBG into a controlled diet for eight weeks did not change glycosylated hemoglobin levels.

Dietary Data: The amount of LBG was analyzed for each group and subgroup - males versus females, adults versus children, and FHC versus normals - for each of the three periods. Amounts of LBG were calculated by body weight of the subjects and by kilocalories. No significant differences were seen between groups or subgroups. Group A children consumed less LBG than adults in Period I; this difference persisted after adjustment for kilocalories, but not after adjustment for body weight (Table 7).

Diet analysis of both groups at the two baseline clinic visits revealed children ate 161 (+/- .46) mg/day of cholesterol and P/S ratio of .58 (.14). Adults in our study ate 242 (145) mg/day of cholesterol and the P/S ratio was .56 (.20).

The intake of protein, carbohydrate, total fat, saturated fat, monounsaturated fat, and polyunsaturated fat did not change significantly during the study (Table 8). Separate analysis of these dietary factors did not correlate with the changes seen in the serum lipids.

Total calories for both groups averaged 1700 at baseline, increased slightly and then decreased to 70 less than baseline during Period III. The subjects were 2.0 pounds above baseline weight at Period I, and 1.8 pounds above baseline at Period III. Non-LBG fiber intake was analyzed from the dietary records and did not differ or change over the study from group to group. There were no significant difference between or within groups at baseline or during any of the three feeding periods for total calories, non-LBG fiber, or weight change (Table 9).

There were preferences for the individual food products which remained quite constant over the study. The bread and cookies were the food products consumed more often. The potato product was the least used.

Side Effects of LBG Intake: No serious side effects were reported during the feeding of either the control or experimental food products. Subjects reported increased flatulence while on the LBG-containing products. Flatulence decreased and at about one to weeks the amount

TABLE 7

LBG MG/DAY

	PERIOD 1 A	B	PERIOD 2 A	B	PERIOD 3 A	B
AMOUNT LBG						
M	14.5 (6.3)	0	0	15.6 (6.1)	13.0 (6.6)	13.7 (5.5)
F	16.1 (3.2)	0	0	11.2 (5.8)	10.8 (3.4)	9.1 (4.3)
Y	12.4 (2.1)*	0	0	13.5 (1.5)	9.2 (2.6)	10.6 (2.2)
A	17.3 (5.1)*	0	0	14.2 (8.0)	13.6 (5.7)	12.8 (6.8)
FHC	15.3 (5.2)	0	0	16.0 (6.5)	12.2 (5.8)	13.6 (6.0)
N	15.3 (4.0)	0	0	11.6 (5.3)	10.9 (2.9)	10.0 (4.5)
LBG/WEIGHT						
M	12.3 (4.2)	0	0	12.5 (5.3)	10.6 (3.7)	10.8 (3.9)
F	11.7 (2.6)	0	0	9.7 (4.6)	7.9 (2.7)	8.0 (4.0)
Y	12.5 (3.7)	0	0	14.7 (4.5)*	9.4 (3.8)	11.5 (3.7)
A	11.5 (3.3)	0	0	9.4 (4.4)	9.0 (3.3)	8.6 (4.0)
FHC	11.2 (3.2)	0	0	12.2 (5.7)	8.7 (3.2)	10.1 (3.6)
N	14.0 (3.4)	0	0	10.5 (4.5)	10.5 (4.1)	9.3 (4.8)
LBG/KCALS						
M	6.1 (2.7)	0	0	7.2 (2.1)	5.7 (2.0)	6.3 (1.5)
F	8.3 (2.3)	0	0	7.3 (3.3)	7.4 (2.1)	5.6 (1.7)
Y	5.2 (0.7)*	0	0	6.6 (1.3)	4.8 (1.5)*	5.1 (0.6)
A	8.7 (2.6)	0	0	7.7 (3.0)	7.8 (1.6)	6.6 (1.8)
FHC	7.6 (2.9)	0	0	7.5 (2.1)	7.0 (2.3)	6.4 (1.7)
N	6.5 (1.9)	0	0	7.0 (3.1)	5.7 (1.6)	5.6 (1.5)

*SIGNIFICANT DIFFERENCE ($<.05$)

TABLE 8

DIET ANALYSIS BY GROUP AND PERIOD (S.D.)

		PROT	CHO	FAT	SAFA	MUFA	PUFA
BL −2,0	A	16.0(2.9)	56.2(6.5)	29.1(4.0)	9.0(1.3)	12.0(1.5)	4.7(1.5)
	B	15.9(1.2)	51.2(12.3)	32.4(9.9)	10.4(4.5)	13.4(3.9)	5.7(1.5)
PERIOD 1 +2,+4	A	13.8(2.5)	55.7(5.6)	31.8(4.0)	8.9(1.6)	13.6(1.6)	6.1(1.7)
	B	15.1(2.4)	43.3(4.9)	30.8(2.8)	9.0(1.2)	12.0(2.6)	5.8(1.0)
PERIOD 2 +6,+8	A	14.6(2.6)	55.0(7.5)	32.8(4.8)	9.1(1.7)	13.5(1.4)	6.2(2.1)
	B	16.9(3.8)	53.9(9.1)	28.1(4.1)	8.8(1.9)	11.5(3.3)	5.1(1.3)
PERIOD 3 +10,+12	A	15.1(2.1)	54.4(7.0)	31.7(4.4)	8.4(1.4)	13.4(1.8)	6.3(1.6)
	B	16.3(2.6)	50.6(7.8)	34.2(4.3)	10.3(2.4)	13.8(3.6)	5.5(1.1)

SAFA - SATURATED FATTY ACIDS
MUFA - MONOUNSATURATED FATTY ACIDS
PUFA - POLYUNSATURATED FATTY ACIDS

TABLE 9

CALORIES NON-LBG FIBER AND WEIGHT CHANGE

(S.D.)

		CALORIES	FIBER (GM/DAY)	WEIGHT (LBS)
BL	A	1845 (686)	5.0 (2.0)	130.6 (35)
	B	2075 (430)	4.3 (1.3)	126.7 (41)
PERIOD 1	A	2248 (610)	2.3 (2.4)	133.0 (36)
	B	2215 (351)	2.7 (0.6)	128.3 (41)
PERIOD 2	A	1964 (704)	3.1 (0.9)	133.0 (36)
	B	1903 (506)	2.4 (1.0)	128.5 (41)
PERIOD 3	A	1878 (706)	3.3 (3.5)	133.0 (36)
	B	1945 (533)	2.3 (1.4)	127.6 (40)

reported was usually not a problem. No one had diarrhea, but stool
size increased.   One patient had significant constipation which
remitted after discontinuing the diet temporarily. After three days
this person was able to resume the diet without further problem.
There was no change observed in the WBC counts, serum calcium, or
SGOT determinations.

Discussion

        LBG food products successfully lowered serum cholesterol and
LDL-C. In Group A (comparing control to experimental diet feeding
periods), serum cholesterol decreased 11% and LDL-C decreased 10%.
In Group B, serum cholesterol decreased by 13% at 4 weeks and 17% at
8 weeks of LBG feeding, and LDL cholesterol decreased 17% at 4 and
8 weeks.   HDL/LDL ratios increased in both groups, but not
significantly in Group A. Group A cholesterol and LDL-C at 4 weeks
of LBG feeding did not change from the baseline diet.   Group A
cholesterol and lipoprotein values increased while on the control diet,
suggesting that there was a suppresion effect on cholesterol levels
during LBG feeding.

Evaluation by Subgroup: Group A and B patients were evaluated by
subgroup for their response to LBG over the three feeding periods.
Both groups and subgroups experienced a decrease in their total
cholesterol, LDL-C, and to a lesser extent HDL-C comparing control
to LBG feeding periods. Both males and females decreased their total
cholesterol, LDL-C, and HDL-C. Both FHC and normal lipid patients,
children and adults, decreased their lipid levels.   Contrary to
Tuomilehto's report,[27] the FHC females in our study decreased their
serum cholesterol on LBG by 10% from 319 to 288 mg% (p< .001).

Control of Dietary Variables: It is necessary to analyze and compare
as many of the dietary variables as possible[28]; the food products with
and without LBG contained similar amounts of carbohydrate, protein,
and fat. Diet analysis at baseline and at two-week intervals did not
reveal significant differences for dietary intake between groups during
the control and experimental feeding periods.   There were no
significant differences during the various feeding periods or between
groups for total fat, saturated fatty acids, monounsaturated fatty
acids, polyunsaturated fatty acids, total calories, non LBG fiber, or
weight change (Tables 8 & 9). In fact, both groups, in face of serum
cholesterol and LDL-C lowering, gained a slight amount of weight,
which often is associated with a rise in serum lipids.
        Analysis of our data revealed that Group A responded to LBG
only after being fed the control diet; no change was observed in lipids
in Period I when compared to the baseline diet, which did not contain
the same food products. Group B responded after they had been fed

control food products. These observations highlight the necessity of controlling as many dietary variables as possible.

Dietary Cholesterol and P/S Ratio: The low dietary cholesterol and modest P/S ratio in our patients provided a reasonable but not maximal conventional dietary approach to controlling hypercholesterolemia. The mean intake in children of 161 mg/day and P/S of .58 compares to USDA figures of 370 mg/day of cholesterol and P/S of .36 for children. Adults consumed 242 mg/day and P/S of .56 as compared to national norms for adults of 451 mg/day of cholesterol and P/S ratio of .22.[28-29] The changes in cholesterol, LDL-C, HDL-C, and VLDL-C attributed to the LBG food products were in addition to the presumed effect of the baseline diet which was not altered significantly during the study. It is not known what the effect of LBG food products would be on a similar group whose baseline diet included average cholesterol and P/S intake.

Glycosylated Hemoglobin: Kiehm[31] and Anderson[32] found that when diabetic patients on small doses of insulin were treated with high-carbohydrate, high-fiber diets, insulin requirements were reduced. Jenkins demonstrated that Guar gum lessened glycosuria in diabetics.[33] Miranda found bread with high content of cellulose flattened the glucose tolerance curve.[34] Jenkins found that viscosity was important in flattening the glucose tolerance curve in his work with Guar and tragacanth. There was a slowing of absorption of xylose from the small intestine.[35]
    None of the subjects that we report have carbohydrate intolerance and to our knowledge glycosylated hemoglobin has not been evaluated in normoglycemic patients on fiber diets.
    LBG did not influence carbohydrate metabolism sufficiently to alter glycosylated hemoglobin levels despite lowering blood lipids.[36] More sensitive techniques of protein glycosylation have recently become available[37] and glycosylation of short-lived proteins might be useful in retrospective evaluation of possible recent hypoglycemic episodes.

Potential Role of LBG in Treating Hypercholesterolemia: Standard dietary treatment of hypercholesterolemia by decreasing saturated fat, increasing polyunsaturated fat, and decreasing animal protein and cholesterol intake remain the mainstay of therapy. Free living, non-institutionalized patients, for reasons of compliance, individual response, or dietary preference, may not successfully lower their serum lipids or the result may be suboptimal. Thus, the significance of the lipid lowering observed in this outpatient study may be greater than similar results with institutionalized patients where compliance is greater. Some individuals in the study, despite comparable LBG intake, did not lower their lipids, perhaps just as feeding monkeys

dietary cholesterol indicates that there are diet responders and diet non-responders.[38] Adults in an outpatient setting, after initiation of standard dietary treatment, usually do not respond by more than a 5-10% lowering of serum cholesterol. Of a group of FHC children evaluated at age 2, only 40% could maintain normal serum cholesterol levels despite near maximal dietary treatment.[39] Prevention of heart disease and/or regression of atherosclerotic lesions may require a decrease of serum lipids to near normal levels. If the dietary response is suboptimal, pharmacological management of hyperlipidemia usually is necessary. In children, pharmacological agents are often not accepted because of their potential for side effects, expense, and the long duration with which they must be consumed.[40] Specific dietary fibers, such as LBG, may enhance current treatment regimens, if they can tastefully be incorporated into foods.

Physiology of LBG: LBG was used in the present study because of its similarity to Guar gum, which in food products has proved to be an effective means of lowering serum lipids. Guar gum is similar to LBG in molecular weight, galactomannan content, moisture, protein, and ash. Although both gums are viscous, LBG is less viscous than Guar. A 1% addition of Guar gum to distilled water develops a viscosity of 6000 CPS as compared to LBG, which develops a viscosity of 3000 CPS measured on a Brookfield RVF spindle No. 3 at 20 rpm at 25º C. Guar has one D-Galacto pyranose for every two D-Manno pyranose units, while LBG has one D-Galacto pyranose for unit for every four D-Manno pyranose units.[41-42] Because LBG is less viscous than Guar, its incorporation into food products is less difficult. The lower viscosity of LBG food products may decrease the gumminess or stickiness, and thus improve palatability. In small doses (6.7 gm/day), LBG increased stool weight by 10%[43]. Guar in comparable doses to the present study increased stool weight by 20%.[44]

Fiber Analysis: The revised Furda method of fiber analysis used on our food products determines soluble and insoluble fractions as well as the total fiber.[17,44] The lipid lowering effect of fiber appears to be in the soluble portion of the fiber because the amount of insoluble fiber was the same for food products with and without LBG. This study does not, however, isolate a lipid-specific portion of the fiber, nor can one determine whether the lipid effect is restricted to the soluble or the insoluble fiber fraction.

Mechanism of Action: Dietary fibers such as LBG are thought to lower serum lipids by binding bile acids and increasing bile acid excretion. Micellular formation which is essential for cholesterol absorption is interefered with. LBG fiber may act as a water-holding and cation-exchange agent, increasing total fecal bile acid and neutral sterol output because of the increased water-holding capacity. Fiber

in the large intestine may act as a physiological chromatography column, whose gel filtration and acid ion-exchange properties decrease bile acid absorption and hence lower serum lipids.[45]

Conclusions

LBG food products provided a safe, effective means to lower serum lipids in hypercholesterolemic and normal adults and children over a three-month feeding study. Serum cholesterol was lowered 11-16%, LDL-C was lowered 10-17%, and HDL/LDL ratios increased (4-12%). The LBG food products were consumed without significant side effects and were well accepted.

It is appealing to develop safe, effective, bio-engineered foods which can be consumed by asymptomatic children and adults over a long period of time. Utilizing standard hypolipidemic dietary therapy, fiber-enriched foods can be used either as a primary treatment or as an adjunct to pharmacological management and prevention of premature atherosclerosis can be approached in the young and asymptomatic. It appears possible that bio-engineered food products can displace the pills and powders which often serve as negative reinforcements to prolonged treatment programs.

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Pectins and Guar Gum: Effect on Plasma Lipoproteins and Tissue Lipoprotein Lipase Activity in Rats

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Various dietary fiber supplementations were given
to rats fed semisynthetic diets with 20% casein and
20% peanut oil for 3-6 months. When added to cholest-
erol free diets, 5% guar gum slightly decreased
total plasma and HDL cholesterol, whereas 5% low or
high methoxylated pectin had no significant effect
or a slightly elevating effect. When 0.5% cholest-
erol was included in the diets, 5% pectin decreased
total cholesterol and increased HDL cholesterol,
thus increasing substantially the relative HDL con-
centration. Wheat bran increased total and HDL
cholesterol, and there seemed to be a dose-response
relationship. Differences in plasma triglycerides
were generally insignificant. Lipoprotein lipase
activity was slightly elevated in heart tissue
after feeding wheat bran and slightly lowered in
adipose tissue after feeding high methoxylated
pectin.

Dietary fiber might be a factor protecting against athero-
sclerotic disease (1). This hypothesis is based on epidemiological
data linking a large number of "Western" diseases with a low
intake of dietary fiber (2). It has got experimental support,
however in that certain types of dietary fiber might alter plasma
lipoprotein concentrations.
    The relationship between the plasma lipoprotein profile and
clinical appearance of atherosclerotic heart disease is now well
established. During the latest decade increased interest has
focused on the differential effect of various lipoprotein classes;
specifically the protective function of high-density lipoproteins

(HDL) against atherosclerosis has been firmly established (3), whereas increased levels of low-density lipoproteins (LDL) are regarded as a major risk factor for cardiovascular disease.

Effects of dietary fiber on plasma lipids have been most pronounced and most consistent in studies using purified, viscous types of fiber, such as pectin and guar gum. The decrease in total and LDL cholesterol when feeding these fibers is accompanied by an increased fecal bile-acid excretion. Increased bile-acid loss with increased de novo synthesis from cholesterol is usually con- sidered the main mechanism by which dietary fiber may alter plasma cholesterol. However, decreased absorption of dietary cholesterol or altered cholesterol and lipoprotein metabolism by other mechanisms may also be important (for review see for instance 4).

Wheat bran binds bile-acids in vitro (5) but in vivo effects on plasma cholesterol have been almost uniformly negative (6). Some studies on small groups of humans (7,8), however, indicate that bran might increase HDL cholesterol, although unchanged (9) or even decreased (10) levels have also been reported.

Colon cancer is the form of cancer most strongly linked to dietary factors, especially fat and fiber. It is suggested that the bulking effect of dietary fiber would dilute carcinogens and diminish the time during which the colonic mucosa is exposed to carcinogens (11). Fibers such as pectin and guar gum, however, increase the flow of bile salts to the large bowel and have poor bulking effect as they are readily degraded by the intestinal microflora. Since bile salts and/or their microbial degradation products are cocarcinogens, this complicates the dietary fiber/ colon cancer hypothesis.

Experiments with chemically induced colon cancer in the rat have partially, but not consistently, supported the dietary fiber hypothesis (12). Thus, Bauer et al. (13,14) recently reported that both high- and low-methoxylated pectin (6.5 or 5% in a diet containing 20% casein, 20% fat and no other dietary fiber) given during induction of colon cancer with subcutaneous 1.2-dimethyl- hydrazine, enhanced the yield of tumors. Guar gum, 5%, also tended to increase the tumor yield, although not significantly compared with controls on a virtually fiber-free diet. Others(15) however reported a protective effect of 15% pectin.

With this background, the present investigation was under- taken. The effect on plasma total and HDL cholesterol, and tri- glycerides of two kinds of pectin with different degrees of methoxylation, and of guar gum was studied in 3-6 months feeding experiments with rats. Wheat bran and oat bran were also studied for comparison. Pectin and wheat bran was fed both with and without dietary cholesterol.

To throw some light upon mechanisms by which dietary fiber could influence plasma lipoproteins, heart and adipose tissue lipoprotein lipase activities were measured after feeding some of the dietary fiber containing diets. This key enzyme in lipoprotein metabolism is one of the most important factors determining plasma triglyceride and plasma HDL concentrations (16).

Materials and Methods

    Animals. Male SPF Sprague-Dawley rats (Anticimex, Stockholm,
Sweden), weighing approximately 135 g at the start of the experi-
ment, were used. They were kept individually in steel-wire cages
with free access to water and were adapted to a 12 h light and
dark shift. The room was air-conditioned, maintained at 23° and
5o-60% relative humidity. The different diets were fed ad lib. in
metal containers designed to give minimal losses.

    Diets. A basal diet with 20% casein, 20% fat, wheat starch
as main carbohydrate source and proper amounts of vitamins and
minerals was prepared as described earlier (17). The various
dietary fiber containing diets were prepared by substituting
dietary fiber preparations for wheat starch.

    Analysis of dietary fiber. Total dietary fiber including
both water-soluble and water-insoluble components was analysed
with an enzymic method as described by Asp et al. (18). The die-
tary fiber was characterized by gas-liquid chromatographic assay
of monosaccharides after acid-hydrolysis and gravimetric deter-
mination of acid insoluble lignin. Uronic acids were assayed with
a decarboxylation method. These analyses were performed as
described by Theander and Åman (19).

    Analysis of plasma lipids. HDL was separated by selective
precipitation of very-low-density lipoprotein (VLDL) and low-
density lipoprotein (LDL) by dextran sulphate and manganese
chloride (20), and HDL cholesterol was measured by cholesterol
determination of the clear supernatant fraction. Cholesterol and
triglycerides were analysed by enzymic procedures (21,22).

    Experiment 1. Diets containing 5% high-methoxylated (HM)
pectin, 5% low-methoxylated (LM) pectin, 5% guar gum or 20% wheat
bran (corresponding to about 10% bran fiber) were fed for 6 months
to rats (15 in each group) and compared with the basal diet.
After 6, 12 and 26 weeks, 5 rats in each group were killed after 16
hours fasting by carbon dioxide narcosis. Blood was withdrawn by
cardiac puncture (17).

    Experiment 2. In this experiment the basal diet, a 5% HM-
pectin diet and a 10% wheat bran diet (corresponding to 5% bran
fiber) were fed to groups of 7 rats both with and without 0.5%
dietary cholesterol. For comparison, 10% wheat bran and 10% oat
bran diets were also studied (without cholesterol).
    Plasma lipids were analysed as in Experiment 1. Lipoprotein
lipase activities in adipose tissue and heart muscle was assayed
with tri (^{3}H) oleoylglycerol emulsion as substrate (23). Activities
are expressed as nmol of fatty acid released per minute per mg
protein.

Statistical evaluation. Student´s t-test (two-tailed) was used for weight of rats and food intake. Differences in plasma lipids, lipoproteins and lipoprotein lipase activities were evaluated with Wilcoxon´s test (two-tailed).

Results and Discussion

Experiment 1. The dietary fiber content and composition of the various dietary fiber preparations used have been reported earlier (17). The HM and LM pectin preparations had 74 and 37% respectively of their carboxyl groups methoxylated. The uronic acid content was 80 and 86% respectively (dry weight basis). The content of neutral sugars, mainly galactose, was 6 and 4% respectively. The guar gum preparation contained 89% neutral sugars (dry weight basis), mainly mannose and galactose. The wheat bran contained 52% dietary fiber (dry weight basis) with a typical composition dominated by arabinoxylan and cellulose. The acid insoluble lignin content was 5%.

Figure 1 shows the growth curves in the first experiment. The guar gum group (G) had slightly slower weight development than the other groups. When the weight increase was calculated per unit energy consumed, the guar gum group had a significantly lower value, in spite of an 8% increase in the food intake. Fecal losses could not explain this lower weight development since fecal dry weight increased only 0.3 g/day and most of this increase can be expected to represent undegraded guar gum. It is interesting in this context to recall an investigation (24) showing uncoupling of oxidative phosphorylation in liver and heart mitochondria of rats given small doses of gum arabic, gum tragacanth or modified celluloses.

Total plasma cholesterol (Figure 2) was significantly lowered by guar gum after 12 weeks but the pectins did not significantly alter the level. Wheat bran increased total cholesterol at all the time points studied (17).

HDL cholesterol (Figure 3) was also lowered by guar gum but increased by wheat bran. The relative HDL concentration (percent of total cholesterol) was close to 0.7 in all cases.

Plasma triglycerides decreased with age but were similar in all groups. On gel electrophoresis, no chylomicrons were demonstrable, indicating that plasma triglyceride concentrations essentially corresponded to VLDL concentrations.

Experiment 2. As in the first experiment, weight development and food intake was similar in the different gorups.

Figure 4 shows total plasma cholesterol after 3 months on the different diets. In the groups not fed dietary cholesterol pectin gave a slight but significant plasma cholesterol increase. As in Experiment 1, wheat bran also increased the total cholesterol and there seemed to be a dose-response relationship.
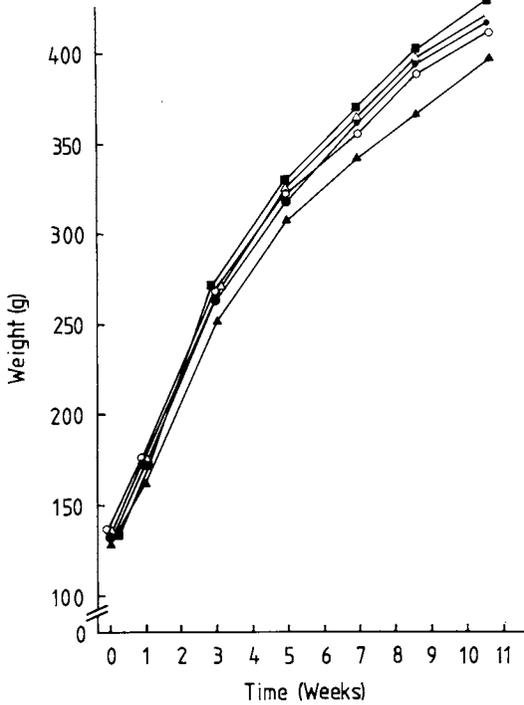
Figure 1.   Growth curves of rats fed various dietary fiber containing diets. Key:
●, *HM pectin, 5%;* △, *LM pectin, 5%;* ▲, *guar gum, 5%;* ■, *wheat bran, 20%,*
corresponding to 10% bran fiber; and ○, *basal diet.*
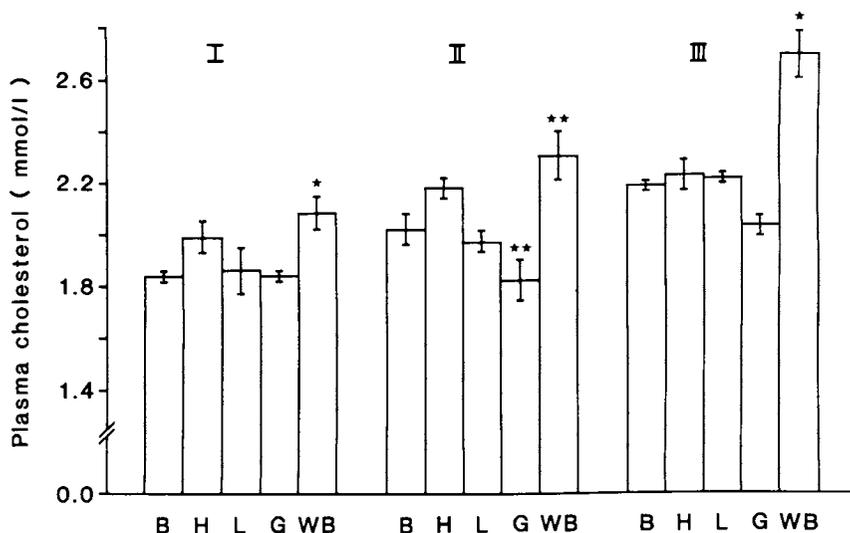
Figure 2.   Total plasma cholesterol after 6 (I), 12 (II), and 26 (III) weeks in rats
given diets containing different dietary fibers. Key: Group B, basal diet; Group H,
high-methoxyl pectin diet; Group L, low-methoxyl pectin diet; Group G, guar gum
diet; Group WB, wheat bran diet; *, P < 0.05; and **, P < 0.01. Mean values
are given with their standard errors represented by vertical bars. (Reproduced with
permission from Ref. 17. Copyright 1981, British Journal of Nutrition.)
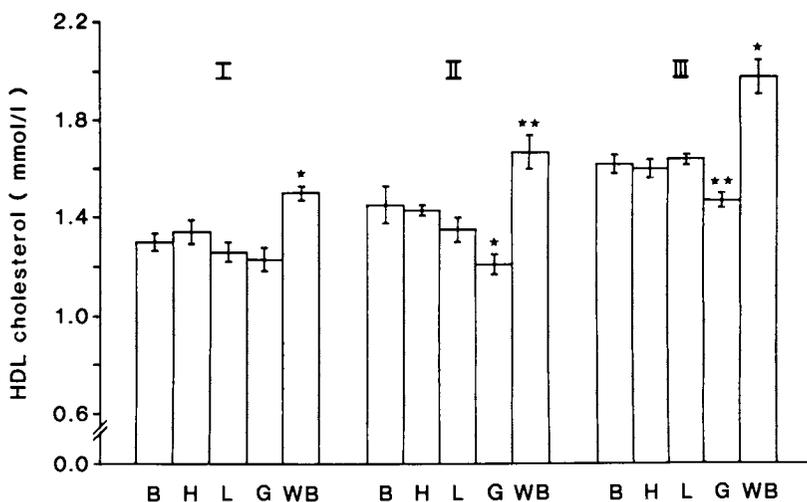
Figure 3.   High-density-lipoprotein cholesterol after 6 (I), 12 (II) and 26 (III)
weeks in rats given diets containing different dietary fibers. Key is the same as in
Figure 2. Mean values are given with their standard errors represented by vertical
bars. (Reproduced with permission from Ref. 17. Copyright 1981, British Journal
of Nutrition.)
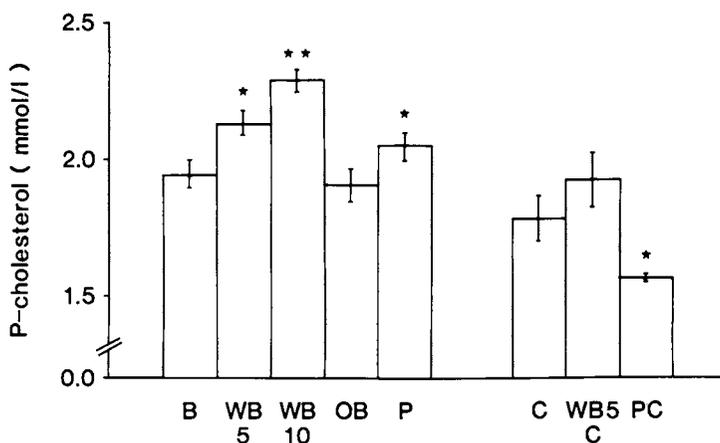
Figure 4.   Total plasma cholesterol after 3 months in rats given diets containing different dietary fibers, without cholesterol (left) and with 0.5% cholesterol (right).
Key: Group B, basal diet; group WB5 and WB10, wheat bran diets with 5 or 10% wheat bran fiber; Group OB, oat bran diet with 10% fiber; Group P, pectin diet with 5% high-methoxylated pectin; Group C, basal diet with 0.5% cholesterol; Group WB5C, wheat bran diet (5% fiber) with 0.5% cholesterol; Group PC, pectin diet, 5%, with 0.5% cholesterol; *, P < 0.05; and **, P < 0.01. Mean values are given with their standard errors indicated by vertical bars.*

When 0.5% cholesterol was added to the diets, total cholesterol
levels decreased slightly whereas HDL cholesterol concentrations
fell by about 30%. When added to the cholesterol containing diet,
5% pectin significantly decreased the total plasma cholesterol
and increased the HDL cholesterol level (Figure 5). Wheat bran
increased HDL cholesterol both with and without dietary choles-
terol.
      These changes demonstrate that the relative HDL concentration
i.e. HDL cholesterol as percent of total cholesterol, was strongly
decreased by dietary cholesterol despite the slight reduction of
the total plasma cholesterol levels (Figure 4, 5). Despite its
lack of effect in rats fed without cholesterol, pectin could part-
ly reverse the negative effect of dietary cholesterol on the
Total/HDL cholesterol ratio. Wheat bran did not significantly
change this ratio since it led to similar elevations in both total
and HDL cholesterol.
      Oat bran did not change plasma cholesterol levels. Others
(25) have shown that oat bran decreases total plasma cholesterol
and increases HDL in cholesterol fed rats. The effect seems to be
related to soluble oat gums. The lack of effect of oat bran in our
experiment might be due to the fact that we studied this fiber
only in cholesterol-free diets. Soluble gums may also have been
lost in preparing this fiber enriched oat bran (81% dietary fiber).
      Plasma triglycerides were not altered by pectin but signi-
ficantly lowered in the wheat bran group with 5% bran fiber and in
the oat bran group.
      Lipoprotein lipase activity in heart tissue was not altered
by pectin but slightly increased in the wheat bran group. In adi-
pose tissue the activity was significantly lowered in the group
fed pectin.

General Discussion

      The evaluation of effects of dietary constituents on plasma
lipid and lipoproteins requires selective measurements of indivi-
dual lipoprotein classes. For example, the addition of cholesterol
to the diet in the rat does not increase total plasma cholesterol
levels; in the present study a slight reduction in plasma choles-
terol was in fact seen. However, the HDL cholesterol fraction was
markedly reduced, and the VLDL and/or LDL cholesterol level must
therefore have been increased. Extrapolation from data in humans
would indicate that this shift in lipoprotein profile is disadvan-
tageous from the atherogenic point of view. The increase in VLDL/
LDL cholesterol may readily be explained by the dietary load of
cholesterol which is transported to peripheral tissues by these
lipoprotein particles (16). The reduction in HDL which are con-
sidered to be involved in cholesterol transport from peripheral
tissues to the liver, is however an interesting but so far un-
explained phenomenon.
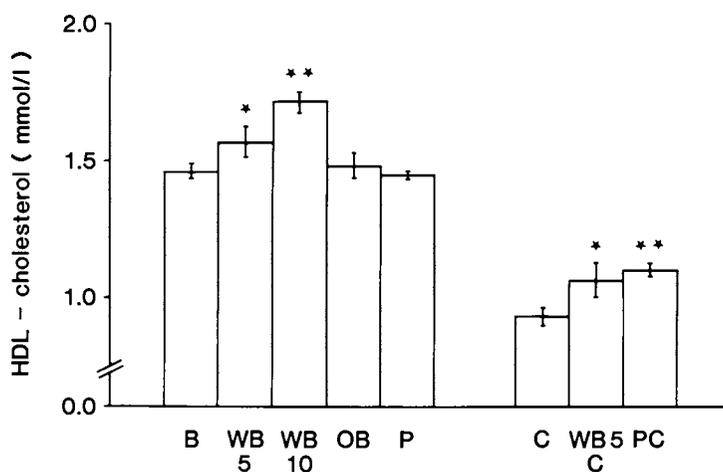
Figure 5.   High-density lipoprotein cholesterol after 3 months in rats given diets containing different dietary fibers, without cholesterol (left) or with 0.5% cholesterol (right). Key is the same as in Figure 4.

The effects of dietary fiber on plasma lipoproteins are   in
some cases, strongly affected by the presence of cholesterol in
the diet. This study confirms that dietary cholesterol is essen-
tial for the plasma cholesterol lowering effect of pectin. In fact
no such effect was obtained in the absence of cholesterol in our
high protein/high fat diets, and the effects of pectin might be
interpreted as a partial reversal of the disadvantageous effects
of dietary cholesterol on the plasma lipoprotein profile.

Interference with the reabsorption of bile salts leading to
increased fecal excretion and de novo synthesis from cholesterol
is generally regarded to be the main mechanism by which pectin
and other types of viscous fiber affect plasma lipid concentra-
tions. Some studies indicate, however, that the presence of die-
tary cholesterol might be important (26,27). This is strongly
supported by our data. The mechanism might be related to disturbed
micellar formation affecting intestinal cholesterol absorption.
The highly significant favourable effect of pectin (in the
cholesterol containing diets) on the HDL/total cholesterol
ratio may be related to altered endogenous cholesterol synthesis
due to decreased absorption of dietary cholesterol.

Several mechanisms are involved in the regulation of plasma
HDL levels. Besides the rate of production of HDL in the liver
and intestine and the rate of elimination in peripheral tissues,
the pattern of intravascular metabolism is also important in the
setting of HDL concentrations. LPL catalyzes the rate-limiting
step in the lipolytic degradation of triglyceride-rich lipopro-
teins; since this reaction is also associated with a transfer of
amphipathic surface components (e.g. cholesterol, phospholipids
and apoproteins) to the HDL particles, a high LPL activity will
generally be associated with high plasma levels of HDL (16).

The increase in HDL and total cholesterol in the group fed
bran did not seem related to dietary cholesterol. The increase in
heart tissue lipoprotein lipase activity indicates an increased
peripheral elimination of triglycerides in muscular tissue which
is consistent with an elevation in HDL cholesterol. This increase,
however, is small compared with changes in lipoprotein lipase
activity induced by for instance ethanol (28). It is therefore
more probable that the effect of wheat bran on HDL cholesterol is
also related to altered rate or site of lipid absorption in the
intestine.

Acknowledgments

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Aminopolysaccharides—Their Potential as Dietary Fiber

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Aminopolysaccharides could serve as a new source
of dietary fiber having special physiological and
in vitro functional properties. The fact that
they are of animal and microbial origin and cur-
rently not approved for use in foods may be
reasons why they have not been considered within
the context of dietary fiber. Chitin and chitosan,
two major readily available aminopolysaccharides
are described in terms of their functional
properties in foods, and in their possible role
as potential pharmaceutical or food additives.
Special attention is paid to their physiological
effects in rats. The interaction of chitin and
chitosan with lipids and cholesterol, and their
effect on lipid absorption suggest that some
aminopolysaccharides show strong binding activity
towards specific lipids. This activity seems to
be a function of the density of positive charge
in the aminopolysaccharide molecule where electro-
static forces between lipids and aminopoly-
saccharides play a greater role than hydrophobic
interactions. Since aminopolysaccharides are
nondigestible and some reduce lipid absorption in
rats, they could be considered as ingredients
having "negative" calorie value. The hypocholes-
terolemic activity of chitosan and its side
effects are compared with currently available
hypocholesterolemic agents such as cholestyramine.

The widely accepted definition of dietary fiber as pro-
posed by Trowell (1) refers to "plant polysaccharides and
lignin which are resistant to hydrolysis by digestive enzymes
of man" as the sole constituents of dietary fiber. Being
limited to the plant polymers only, this definition does not
include undigestible polysaccharides from other sources such

as those of animal origin, or those which are prepared
synthetically. There are several food-approved cellulose
derivatives and synthetic gums, and recently polydextrose
which "are resistant to hydrolysis by digestive enzymes of
man" and therefore, they should be considered dietary fiber
as well. Animal aminopolysaccharides which are part of the
traditional diet of the Eskimos offer another example (2).
     Godding (2) spelled out the limitations of Trowell's
definition and proposed a modified definition of so called
"edible fiber", which consists of "polysaccharides, related
polymers and lignin, which are resistant to hydrolysis by the
digestive enzymes of man". It seems logical that all non-
digestible edible polymers, regardless of their origin,
should be considered dietary or edible fiber because of two
common denominators - their polymeric nature and their
resistance to hydrolysis by the digestive enzymes of man.

General Properties

     The common natural aminopolysaccharides include chitin,
chitosan, keratan sulphate, hyaluronic acid, chondroitin and
dermatan sulphates, heparin and blood group substances.
While chitin and chitosan contain hydroxyl, amino and acetyl
groups, the others contain also carboxyl and sulphate groups.
In this paper, attention is paid only to chitin and chitosan.
Chitin is a cellulose like polymer which is present in fungal
cell walls and exoskeletons of arthropods such as insects,
crabs, shrimps, lobsters and others. Chemically, chitin and
chitosan are polyglucosamines which are differentiated only
by the extent of acetylation of amino groups as shown in
Figure 1. Although there is no clear distinction between
chitin and chitosan, it is generally accepted that chitin is
extensively acetylated, while chitosan is virtually deacetyl-
ated. The typical chitins have usually 70-95% degree of
acetylation (DA) which corresponds to 15-20.7% acetyl content
while chitosans commonly have 15-25% DA corresponding to
3.2-5.3% acetyl content (3). The degree of acetylation is
probably the most important parameter in these polysaccharides
and it greatly determines their physiological and in vitro
functional properties.
     Another important parameter is the molecular weight (MW)
and its associated viscosity. The average MW of some chitins
can exceed 10^6. Chitosan, since it is prepared from chitin
by alkaline deacetylation has a lower number average MW
ranging usually between $1x10^5$ - $3x10^5$. Chitosan displays a
wide range of viscosities in diluted acid media which depend
mainly on its MW (3). The relative viscosity of high viscosity
chitosans is comparable with the viscosity of guar or traga-
canth gums. The viscosity of chitosan likely plays an
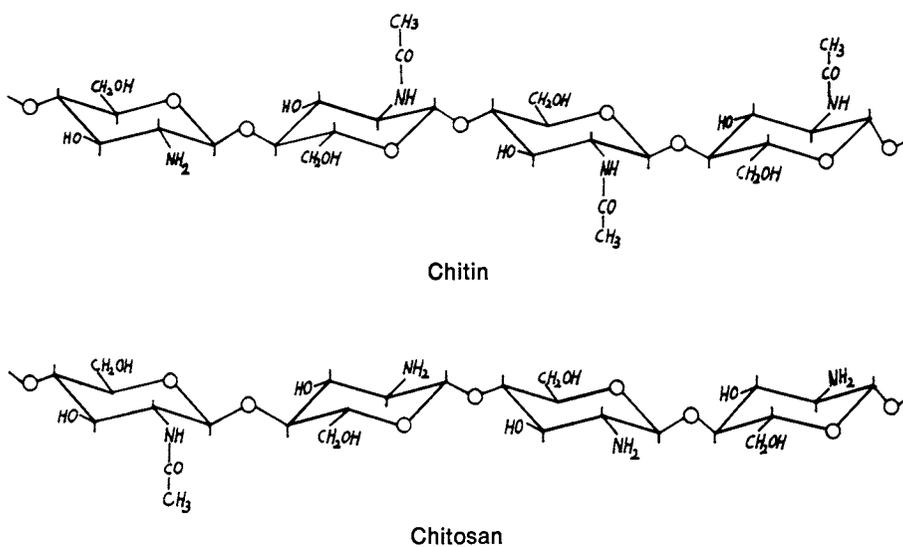
Chitin

Chitosan

Figure 1. Chemical structure of chitin and chitosan.

important role in its physiological properties later discussed.
Only chemically treated (acid hydrolyzed) chitin forms
viscous solutions (4).
    While chitin is insoluble in common solvents (is soluble
in concentrated mineral and formic acids and some special
solvents), chitosan is soluble in diluted mineral and organic
acids (3). Chitosan is not soluble at pH > 6.0 and it func-
tions only in acid systems, which is relevant to its food
applicability. Due to the high density of the positive charges
chitosan behaves in aqueous acid solutions as a polycationic
molecule. This behavior is not typical for chitin because of
its high degree of acetylation. Chitosan in the polycationic
form binds various anions forming salts or complexes with
them. It can be considered a weak anion exchange resin.
Chitosan is a strong base toward hydrochloric acid, since
the primary amino groups with pK$_a$ 6.3, easily form quaternary
nitrogen salts at low pH. At high pH, however, the amino
groups of chitosan are weak bases and therefore do not
interract with anions and do not dissociate neutral salts.

Potential Applications in Foods

    Chitin and chitosan have been recommended as potential
feed and food additives because of their useful functional
properties. Though officially not approved for use in foods
in the U.S., some aminopolysaccharides are part of the
traditional diet of Eskimos as well as being present in
different Oriental foods such as tempeh, sufu and even aged
beef (5). In the nonpurified form, chitin is present in
oyster shell powder which is used in animal feed as a source
of calcium. Glucosamine, the monomeric unit of chitosan is
used extensively in oral pharmaceutical preparations.
    Microcrystalline chitin produced by controlled acid
hydrolysis may be suitable for use as food thickener and
stabilizer (4). The viscosity and emulsion stability of
microcrystalline chitin is 10 to 20 times higher than that of
crystalline cellulose making it suitable for applications in
mayonnaise, peanut butter and other emulsion-type foods (6).
Addition of microcrystalline chitin to white and protein
fortified breads has been shown to increase specific loaf
volume (7).
    Chitosan due to its high viscosity in systems of pH < 5.5
can function as thickener, stabilizer or dispersing agent
(6). Other applications include gel and film formation
(8,9), encapsulation and inclusion in packaging materials
(10). Bough (11) recommended chitosan as a coagulating agent
for food processing wastes with its subsequent utilization as
animal feed. Recently, Rha and Sanchez (12) prepared and
described chitosan globules which simulate the cellular

texture of cooked rice and may have other specific texture applications in foods.

Physiological Activity

Chitin and chitosan are believed to be of low toxicity. According to Arai (13), the LD_{50} of chitosan in laboratory mice is 16g/kg of body weight. This is similar to that of salt or sugar. In an eight weeks feeding study (14), it has been suggested that chitosan is safe in rats up to 10% in the diet. At 15% level, enlargement of liver and kidneys with a few other changes have been observed. While chitin is believed to be virtually inert in the gastrointestinal tract of mammals, chitosan has been recently associated with strong hypocholesterolemic activity in rats.

Sugano et al (15), compared chitosan, cholestyramine, cellulose and different brown algae at 5% level in rat diets containing cholesterol. Besides showing lowest weight gain and highest fecal weight, rats fed chitosan also had the lowest plasma cholesterol and showed its greatest excretion. The excretion of bile acids was only slightly enhanced by chitosan as compared to cholestyramine. The results of twenty days feeding experiment are shown in Table I. Of particular interest was the observation that chitosan caused repression of microbial transformation of cholesterol to coprostanol. Cholestryamine, on the other hand, was more effective in reducing liver cholesterol.

Kobayashi et al (16), compared the effectiveness of chitosan with konjac flour and two hypocholesterolemic drugs. Their experiment included one week feeding of cholesterol-containing diet to rats with 4% level of test materials. They observed that chitosan caused the greatest depression of serum cholesterol level and was superior to konjac flour used at the same level and to moristerol and benikol used at 0.5% and 0.8% levels respectively. While konjac flour and benikol caused severe diarrhea, chitosan did not affect the feces.

Nagyvary et al. (17), studied high viscosity (4,000 c.p.) chitosan in relation with cellulose, citrus pectin (9% CH_3O) and Al salt of pectin at 4% level in rat diet containing cholesterol. The feeding period lasted four weeks. Their observations are summarized in Table II. The level of serum cholesterol in chitosan fed group was approximately 44% lower than in cellulose fed group, 22% lower than in pectin group and about 16% lower than in Al-Pectin fed group. The liver cholesterol was about 60% lower in chitosan-fed group than in the three other groups. There was also a pronounced hypo-lipidemic effect of chitosan on serum and liver as compared to the other fibers. What seems to be noticeable is the fact that both cholesterol and triglycerides were lowered even more dramatically in liver than in serum.

Table I.

PLASMA AND LIVER LIPID CONCENTRATIONS AND FECAL OUTPUT OF NEUTRAL STEROLS*

DIETARY REGIMENS 5%	PLASMA LIPIDS (MG/DL)		LIVER LIPIDS (MG/G)	
	CHOLESTEROL	TRIGLYCERIDES	CHOLESTEROL	TRIGLYCERIDES
CELLULOSE	162±11[a]	110±6[a]	33.3±2.6[a]	42.2±1.7[a]
CHOLESTYRAMINE	107±7[b]	146±13[a]	5.8±0.7[b]	24.3±1.8[b]
CHITOSAN	94±12[b]	123±9[a]	11.0±1.7[c]	30.3±2.5[b]
UMITORANOO ALGAE	143±16[a]	125±7[a]	36.0±1.9[a]	49.5±3.0[a]

DIETARY REGIMENS	CHOLESTEROL EXCRETED (MG/DAY)	COMPOSITION OF FECAL STEROLS (% OF TOTAL)		
		COPROSTANOL	CHOLESTEROL	PHYTOSTEROLS
CELLULOSE	45.3±2.2[a]	28.6±3.2[a]	68.4±3.5[a]	3.0±0.8[ac]
CHOLESTYRAMINE	50.7±4.1[a]	39.0±3.1[b]	54.4±2.7[b]	6.6±1.4[b]
CHITOSAN	68.7±2.4[b]	18.3±3.0[c]	79.2±3.3[c]	2.5±0.7[c]
UMITORANOO ALGAE	44.5±5.3[a]	45.1±2.0[bd]	49.9±2.4[bd]	5.0±0.8[abd]

a,b,c,d: Values represent the mean ± SEM of 6 rats per group. Values within same column not sharing a common superscript letter are significant at $p < 0.01 - 0.05$
*Sugano et al. (15)

Table II.

EFFECT OF FIBER ADDITIVES ON CHOLESTEROL AND
TRIGLYCERIDE LEVELS*

	CHOLESTEROL LEVELS		TRYGLYCERIDE LEVELS	
ADDITIVE (4%)	SERUM MG/DL	LIVER MG/G	SERUM MG/DL	LIVER MG/G
CELLULOSE	244 ± 35	44 ± 11	563 ± 72	172 ± 33
PECTIN	175 ± 13^{a}	42 ± 7	404 ± 78^{a}	156 ± 17
Al-PECTIN[f]	164 ± 10^{a}	36 ± 6^{c}	382 ± 58^{a}	138 ± 12^{b}
CHITOSAN	$137\pm9^{a,d}$	$15\pm4^{a,d}$	$303\pm33^{a,e}$	$87\pm20^{a,d}$

[a] $p<0.01$ vs Cellulose; [b] $p<0.05$ vs Cellulose;
[c] $p<0.01$ vs Cellulose; [d] $p\lessgtr0.01$ vs Al-Pectin;
[e] $p<0.02$ vs Al-Pectin; [f] Al content of this diet was 0.2%.

*Nagyvary <u>et al</u>. <u>(17)</u>

Recently Sugano et al (18) published a more comprehensive
paper on this subject. They observed that on feeding a high
cholesterol (0.5%) diet to male rats for three weeks, addi-
tion of 2-5% chitosan resulted in a significant reduction of
plasma cholesterol, e.g., 25-30%, without influencing either
food intake or growth. Concentrations of liver cholesterol
and triglycerides were also decreased significantly. Sugano's
results are shown in Table III. Plasma cholesterol lowering
by chitosan was similar to that of cholestyramine while the
liver cholesterol lowering was less extensive, but still
showed an approximate 50% reduction. Chitosan used at 10%
level in the diet showed additional reduction in plasma cho-
lesterol which was accompanied by growth reduction. The
growth reduction was also observed with fine particles of
chitosan at 2% level in the diet. Chitosan increased signifi-
cantly the excretion of neutral sterols, but not acidic
sterols. Chitin, which was also used in this study, did not
show any physiological activity.
     Of particular importance was the long term (81 days)
feeding study of a diet containing no cholesterol and a very
low (0.5%) level of chitosan, which resulted in the occurence
of more cholesterol as high density lipoproteins (HDL) and
less as very low density lipoproteins (VLDL) as compared to
the basal diet (18).
     Unpublished experiments (19) using rats with a cannulated
left thoracic lymphatic chanel (20) indicated that during
acute conditions, there was a massive reduction in absorption
of cholesterol as well as oleic acid in the presence of
chitosan. In this experiment, rats were given by gastric
intubation, tritiated cholesterol, C^{14} oleic acid and sodium
taurocholate to which either chitosan or cholestyramine were
added. The lymph was collected for twenty-four hours. The
results shown in Table IV indicate that chitosan inhibits the
absorption of cholesterol and oleic acid at least as effec-
tively as cholestyramine. In chronic studies (19), when rats
were fed diet containing 1% and 5% levels of chitosan and
cholestyramine respectively for 4 weeks, the same inhibition
of absorption of cholesterol and oleic acid between chitosan
and cholestyramine was observed.
     Recently, the hypocholesterolemic effect of chitosan in
Guinea pigs has been reported (21).

Possible Mechanism of Hypocholesterolemic and Hypolipidemic Activity of Chitosan

     The mechanism of hypocholesterolemic and hypolipidemic
action is not universal and it frequently varies from one
compound to another. However, compounds having chemical and
other similarities may also display a similar mechanism of
action.

Table III.

EFFECTS OF CHITOSAN AND CHITIN AND RELATED COMPOUNDS ON PLASMA AND LIVER LIPIDS AND FECAL EXCRETION OF STEROLS[a],*

EXPERIMENT[b]	DIETARY MANIPULATIONS	PLASMA		LIVER		STEROID EXCRETION	
		CHOLESTEROL	TRIGLYCERIDE	CHOLESTEROL	TRIGLYCERIDE	NEUTRAL	ACIDIC
	%	MG/DL		MG/G		MG/DAY	
1	CONTROL	127 ± 5^d	104 ± 10^d	35 ± 0.9^d	53.7 ± 6.2^d	49.6 ± 3.7^c	13.1 ± 1.1^c
	CHITOSAN, 5	93 ± 5^e	110 ± 12^d	18.3 ± 2.9^e	31.9 ± 3.3^e	67.7 ± 2.4^d	13.6 ± 0.4^c
	CHITOSAN, 10	79 ± 6^e	106 ± 10^d	11.1 ± 2.0^f	$26.8\pm3.0^{e,f}$	73.6 ± 3.4^d	13.9 ± 0.8^c
	CHOLESTYRAMINE, 5	86 ± 11^e	114 ± 9^d	4.9 ± 0.4^f	19.3 ± 1.5^f	45.2 ± 5.4^c	30.3 ± 1.0^d
3	CONTROL	114 ± 4^e	125 ± 11^d	41.0 ± 2.8^e	53.1 ± 5^e	50.1 ± 5^d	17.0 ± 2.7^d
	CHITIN, 2	106 ± 3^e	132 ± 12^d	37.8 ± 3.9^e	52.5 ± 4.1^e	50.6 ± 4.3^d	16.6 ± 1.3^d
	CHITOSAN, 2	76 ± 4^d	137 ± 9^d	11.3 ± 3.0^f	33.7 ± 4.9^d	74.7 ± 4.6^c	17.2 ± 1.2^d

[a] Mean ± SEM. [b] The age of animals at the start of experiment was: 52 days (experiment 1), 37 days (experiment 3). Feeding period: 20 days. All diets contained 0.5% cholesterol. [d-f],[c-e] Values in the same column in each experiment not sharing common superscript letters are significantly different at p<0.05 to 0.01.

*Sugano et al. (18).

Table IV.

CHOLESTEROL AND OLEIC ACID ABSORPTION IN RATS
WITH CANNULATED THORACIC LYMPHATIC CHANEL[a]

MEASUREMENT (NO. OF RATS)	CONTROL (3)	50 MG CHOLESTYRAMINE (5)	50 MG CHITOSAN (7)
CHOLESTEROL			
% ABSORPTION	59.5 ±3.8	31.7 ±5.6	29.4 ±9.4
MG ABSORBED	29.8 ±1.9	15.9 ±4.7	14.7 ±7.9
% INHIBITION	----	46.7 ±9.4	50.6 ±15.8
OLEIC ACID			
% ABSORPTION	69.1 ±2.2	46.9 ±5.2	41.4 ±12.6
MG ABSORBED	194.9 ±6.2	131.3 ±14.6	115.1 ±35.3
% INHIBITION	----	32.1 ±7.6	40.9 ±18.1

[a]Vahouny et al. (19), unpublished results.

Among the typical food ingredients which have been shown
to be hypocholesterolemic is the group of specific natural
polysaccharides. This includes food gums such as guar gum,
locust bean gum, oat B-glucan, konjac mannan, some pectins
and most recently chitosan. In spite of their different
chemical structure, these substances have a few common
denominators of which some may be responsible for their
hypocholesterolemic and hypolipidemic action. These proper-
ties include nondigestibility in the upper GI tract, high
viscosity, polymeric nature and high water binding in vitro
and low water binding in the lower GI tract. Their nondigesti-
bility and high viscosity in the upper GI tract are believed
by some researchers to be primarily responsible for their
hypocholesterolemic action. The high viscosity of undigestible
polymers likely reduces diffusion and it may result in
coating of the inner walls of the gut and thus prevent or
delay the absorption of nutriens.
Chitosan, while possessing most of these properties, has
an additional highly relevant characteristic resulting from
its chemical structure. It is the ability to bind different
anions, i.e., bile acids or free fatty acids at low pH by
ionic bonds (18,22). The in vitro binding of taurocholate by
chitosan was observed (18) and was comparable in magnitude to
that of cholestyramine. In vivo however, the excretion of
acidic sterols after feeding chitosan was the same as the
control and it was approximately 2x lower than that corre-
sponding to cholestyramine (18). This could be possibly
explained by the fact that chitosan as a weak basic anion
exchange resin, effectively binds anions such as bile acids
only in low pH environment. At pH > 6.0, the ion exchange
capacity is greatly reduced, the binding is not strong enough
and bile acids can be released and reabsorbed in the lower GI
tract. Cholestyramine, colestipol and DEAE Sephadex on the
other hand are strong basic anion exchangers from which the
bile acids are difficult to be released. This hypothesis is
supported by the fact that feeding the cholestyramine resulted
in reduction of liver cholesterol significantly more than
feeding of chitosan (18). The unique ability of chitosan to
reduce serum cholesterol without increasing the fecal acidic
sterols may have a great significance since the increased
binding and excretion of bile acids caused by some fibers and
drugs, specifically by cholestyramine, has been implicated in
the possible enhancement of cancer of the colon (23,24).
Cassidy et al (25) have observed that a six week ingestion of
diets containing 2% levels of cholestyramine, colestipol or
DEAE Sephadex, or 15% levels of alfalfa or pectin resulted in
considerable changes in ultrastructural topography of rat
jejunum and colon. The authors hypothesized that the observed
accumulation of lipids in the small intestinal mucosa suggested
an interference with lipid transport from the cell (25).

Chitosan appears to have a stronger affinity toward
lipids than cholestyramine (26). According to Nagyvary (26),
the maximum binding capacity of chitosan is about 12 mg
lipids/mg of chitosan. His in vitro experiments indicate
that chitosan binds lipid droplets in toto unlike cholestyra-
mine which is more selective for bile salts. Vahouny et al.
(27) have observed however, that cholestyramine has an
extremely high binding capacity toward individual micellar
components in vitro including bile salts, lecithin, choles-
terol, fatty acids and monolein.

It is noteworthy to mention that unabsorbable antibiotics
such as neomycin, tetracyclines and others are known to
possess hypocholesterolemic properties and they can induce
steatorrhea (28,29). Although not polymeric, these substances
are polycationics having several free amino groups, mainly
primary, but also secondary and tertiary. Eyssen et al (30)
studied the cholesterol lowering effect of basic antibiotics
in chicks fed cholesterol containing diet. They concluded
that the hypocholesterolemic activity may not be caused by
antibiotic properties of these drugs, but rather by the
polybasic nature of these molecules. One of the reasons
which supported this conclusion was that N-methylated neomycin
which is a basic neomycin derivative without antibiotic
properties, was also an effective cholesterol lowering agent.
The effectiveness of tested basic antibiotics depended on
number of basic amino groups in their molecules since neither
N-acetylated neomycin nor streptomycin with only three basic
amino groups were effective. A condensation product of two
molecules of streptomycin, bearing seven basic groups,
however, was hypocholesterolemic. The authors suggested that
the ability of polybasic antibiotics to form insoluble salts
with bile acids in the gut and their increased excretion is
likely responsible for serum cholesterol lowering effect.
There seems to be an analogy between the non-effective chitin
and N-acetylated neomycin versus highly effective chitosan
and neomycin in ability to reduce the serum cholesterol.
Chitosan, however, does not seem to possess a variety of
intestinal functions such as antibiotic and other properties
specific for neomycin.

The available data indicate that the hypocholesterolemic
and hypolipidemic activity of chitosan is probably due to
disruption and/or inhibition of micelle formation. At pH
6.0-6.5 chitosan begins to precipitate and as the linear
chains of the polysaccharide start to aggregate, they can
entrap the whole micelles. The entrapped cholesterol, fatty
acids and monoglycerides thus escape absorption. Such "polar
entrapment," shown in Figure 2, can occur in the duodenum.
Another mode of action could be the "disintegration" of mixed
micelles, which can start before the precipitation of chitosan,
and in which the free fatty acids and bile acids are selec-
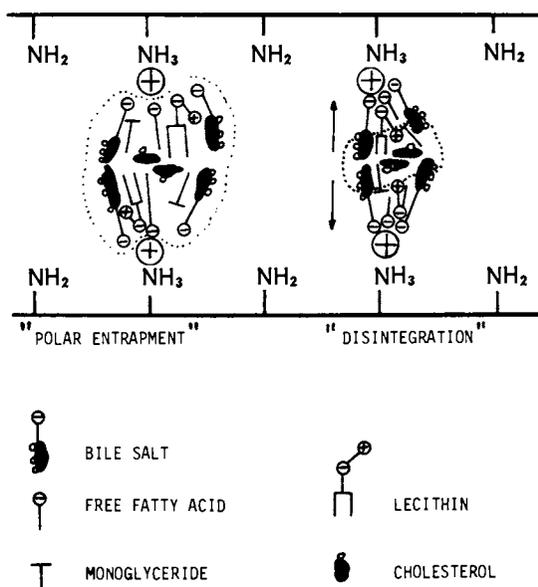
Figure 2. *Possible mode of interaction of chitosan with mixed micelles.*

tively removed from mixed micelles by forming electrostatic bonds with the protonized amino groups of chitosan. Yet another possibility may exist in which the mixed micelles are not formed at all, since the protonized amino groups can immediately bind negatively charged free fatty acids and bile acids prior the micelle formation can take place.

An alternative possible mechanism of hypolipidemic and hypocholesterolemic action of chitosan could be the inhibition of intestinal lipases. A limited work on inhibition of pancreatic lipase by neomycin in humans (31) and in vitro (32), and by cellulose and xylan (32) also in vitro have been reported. No work on the possible inhibition of intestinal lipases by chitosan has been published yet.

Though chitosan may have some similarity with cholestyramine and other hypocholesterolemic/hypolipidemic drugs having character of basic anion exchangers, there seem to be several significant differences in the mechanism of their action as well as in their side effects. The latest results indicate that chitosan is equally effective as cholestyramine in reducing the absorption of dietary cholesterol and lipids however, it does not cause the structural deviations in the intestine of rats when used at 1-5% levels (19). The more detailed comparison of hypocholesterolemic resins, specifically cholestyramine with chitosan is shown in Tables V and VI.

Due to these unique properties, the relative safety and availability, some amino-polysaccharides will likely find, sooner or later, specific applications either in processed foods, special dietary or medical foods or in pharmaceutical preparations.

Table V.

COMPARISON OF HYPOCHOLESTEROLEMIC RESINS WITH CHITOSAN

AGENT	FUNCTIONAL GROUPS	AVERAGE BILE ACID BINDING CAPACITY %	INTESTINAL VILLI OR COLONIC RIDGES WITH STRUCTURAL DEVIATIONS %		EFFECT ON BILE ACIDS EXCRETION
			JEJUNUM	COLON	
CHOLESTYRAMINE	QUATERNARY AMINES	80-100[b]	64.2[a]	39.5[a]	INCREASE ($\leq$x 10)
COLESTIPOL	SECONDARY AND TERTIARY AMINES	50-60[b]	35.9[a]	55.0[a]	INCREASE (x 2-5)
DEAE SEPHADEX	TERTIARY AND QUATERNARY AMINES	30-40[b]	13.0[a]	40.6[a]	INCREASE (x 3)
CHITOSAN	PRIMARY AMINES	PROBABLY LOW	VERY SMALL[c]	NO EFFECT[c]	NO EFFECT

[a] 2% agent in rat diet; [b] in vitro binding at pH 7.0; Cassidy et al. (25)

[c] 1% and 5% agent in rat diet; Vahouny et al. (19)

Table VI.

COMPARISON OF IN VITRO AND IN VIVO
PROPERTIES OF CHITOSAN AND CHOLESTYRAMINE

	CHITOSAN	CHOLESTYRAMINE
ANION EXCHANGER	WEAK	STRONG
VISCOSITY	HIGH	NONE
SOLUBILITY	AT pH < 6.0	INSOLUBLE
LIPID BINDING	HIGH	MODERATE
BILE ACIDS BINDING	?	VERY HIGH
SERUM CHOLESTEROL LOWERING	HIGHLY EFFECTIVE	HIGHLY EFFECTIVE
LIVER CHOLESTEROL LOWERING	HIGHLY EFFECTIVE	EXTREMELY EFFECTIVE
SERUM TRIGLYCERIDES LOWERING	NOT CLEAR	SLIGHT INCREASE
LIVER TRIGLYCERIDES LOWERING	EFFECTIVE	HIGHLY EFFECTIVE
HDL,LDL	INCR. CHOL.IN HDL	LOWERS LDL
LIPID AND CHOLESTEROL LYMPHATIC ABSORTPION	HIGHLY INHIBITED	HIGHLY INHIBITED
NEUTRAL FECAL STEROLS	HIGH	LOW
ACIDIC FECAL STEROLS	LOW	HIGH
FECAL CHOLESTEROL/ COPROSTANOL	HIGH	LOW
CONSTIPATION, DIARRHEA	NONE	CONSTIPATION
FECAL VOLUME	LARGE	SMALL
GUT MORPHOLOGY	NEGLIGIBLE EFFECT	ADVERSE EFFECT

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Catabolism of Mucopolysaccharides, Plant Gums, and Maillard Products by Human Colonic *Bacteroides*

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The mixture of complex carbohydrates which
enters the human colon includes not only plant
cell wall polysaccharides but also a variety of
other types of carbohydrate such as mucopolysac-
charides (from dietary meat or host secretions),
plant gums which are added to foods as emulsifi-
ers, and Maillard reaction products. Some strains
of colonic Bacteroides which can degrade plant
cell wall polysaccharides can also degrade one or
more of these other types of carbohydrates.
Bacterial strategies for degrading these substan-
ces differ. For example, the enzymes responsible
for catabolism of mucopolysaccharides by
Bacteroides are cell-associated rather than extra-
cellular and are not associated with the
bacterial membranes. By contrast, the catabolism
of guar gum, a viscous plant galactomannan,
involves both an extracellular enzyme and at least
one membrane-bound enzyme. Breakdown of poly-
galacturonic acid involves a membrane-bound
enzyme. A feature which is common to all three of
these polysaccharide-degrading systems is that the
enzymes are inducible, that is they are produced
only when the appropriate substrate is encountered
by the bacteria. The Maillard product, fructosyl-
glycine, is not utilized by strains of Bacteroides
which can ferment fructose and fructosyl compounds
such as sucrose, presumably because of diffi-
culties in hydrolyzing the C-N bond which binds
fructose to glycine. Another Maillard product,
isomaltol-galactoside, which contains a glycosidic
bond, can be degraded and the galactose moeity
utilized. Utilization of this compound, which is
an analogue of lactose, appears to occur via the
lactose system. The enzyme which degrades

isomaltolgalactoside, unlike the polysaccharidases which
are produced by the same organism, is constitutive
rather then inducible.

    Nutritional studies in humans have demonstrated quite
clearly that some components of dietary fiber are degraded
extensively during passage through the intestinal tract (1).
Degradation of dietary fiber appears to occur in the colon where
component polysaccharides are fermented by saccharolytic
bacteria which reside there. Although this fermentation occurs
in the colon, it may contribute to human nutrition since a
significant percentage of the bacterial fermentation products
can be absorbed through the colonic mucosa (1, 2).   Because of
the potential contribution of dietary fiber components to host
nutrition and also because of an increased awareness of the role
of the bacterial flora of the colon in human disease, consider-
able attention has been devoted in recent years to the possible
effects of dietary fiber on the colonic flora.
    The focus on bacterial fermentation of components of diet-
ary fiber from conventional sources such as bran should not pre-
vent us from considering other types of carbohydrate which may
also be fermented by colon bacteria. For example, the host
itself produces a variety of polysaccharides which reach the
colon. These include mucins from the secretions which lubricate
the intestinal tract and mucopolysaccharides from sloughed
epithelial cells. It is difficult to obtain precise information
concerning how much of this material is produced daily, but the
amount could easily be comparable to the amount of poly-
saccharide which is ingested in the diet. Some of these host-
produced polysaccharides, particularly the mucopolysaccharides,
are readily utilizable by some of the same bacterial species
which ferment plant polysaccharides (3).
    Another potential source of carbohydrate for colon bacteria
consists of polysaccharides which are added to foods as emulsi-
fiers (e.g. guar gum, alginate) or which are used as stool
softeners (e.g. psyllium). Plant polysaccharides such as guar
gum are also being evaluated for use in the treatment of
diabetes because they retard glucose absorption (4, 5). The
amount of polysaccharide from these sources will vary from
person to person but could be appreciable in some cases.
    Maillard products, associated with the browning of foods,
could also provide some carbohydrate for intestinal bacteria.
At present, this possibility is merely speculative. Many
possible Maillard products exist (6, 7). The Maillard products
which are most likely to be utilizable by bacteria are the early
products, such as glycosylamines and aryl-glycosides, which
resemble fermentable disaccharides. Some Maillard products such
as catechols or substituted pyridines could actually be toxic to

colon bacteria. No estimates are yet available as to the amounts
of these substances which actually reach the colon. Moreover,
the number and variety of Maillard products which can be formed
in foods makes it difficult to decide which compounds to inves-
tigate. Some Maillard products such as aryl-glycosides could be
cleaved by enzymes in the host's small intestine, although this
might not be the case for individuals with digestive enzyme
deficiencies such as lactose intolerance. Moreover, some recent
nutritional studies have shown that varying amounts of compounds
such as sucrose or starch,which are generally considered to be
completely digestible, can escape digestion in the small intes-
tine and thus reach the colon (8, 9). Accordingly, the possi-
bility that colon bacteria are exposed to Maillard products
should be considered.

Polysaccharide Utilization by Colon Bacteria

     From these examples, it can be seen that studies of the
effect of diet on the intestinal flora must take into account
the availability of a variety of polysaccharides. Information
about in vitro fermentation of different polysaccharides by
colon bacteria can help us to make predictions about what sorts
of carbohydrate may be utilizable in vivo, but this information
raises still other questions. To see this, consider some of the
results of surveys of polysaccharide fermentation by numerically
predominant species of colon bacteria (3, 10). Some of these
results are summarized in Table 1. One thing that is immediate-
ly apparent from Table 1 is that the most versatile fermenters
of polysaccharides are Bacteroides species. Moreover, many of
these species can ferment several types of polysaccharides.
This raises the question of which type of polysaccharide would
be utilized preferentially in vivo if these bacteria were to be
confronted by several utilizable polysaccharides at the same
time. Not only do individual strains or species exhibit the
ability to ferment a variety of polysaccharides, but it is also
the case that each polysaccharide can be fermented by more than
one species. Do all of these species actually participate in
degrading a particular polysaccharide in vivo or does one
species predominate due to a more efficient utilization system?
In short, results of surveys of polysaccharide fermentation
allow us to predict that many types of carbohydrate (including
dietary fiber components) will be fermented in the colon, but
these results do not provide answers to other questions such as
which organisms actually degrade a polysaccharide in vivo or
whether a particular dietary carbohydrate (e.g. an emulsifier
such as guar gum) might upset the normal patterns of poly-
saccharide catabolism in the colon if it were present in high
enough concentrations in the diet.

Table 1. Species of intestinal bacteria which can degrade
various indigestible polysaccharides (3, 10)

Polysaccharide	Species of colonic bacteria which can degrade it
Chondroitin sulfate (mucopolysaccharide)	Bacteroides thetaiotaomicron Bacteroides ovatus Bacteroides '3452A'* Bacteroides fragilis subsp.a Bacteroides uniformis
Guar gum, locust bean gum (galactomannan)	Bacteroides ovatus Bacteroides uniformis Ruminococcus albus
Xylan	Bacteroides ovatus Bacteroides fragilis subsp.a Bacteroides eggerthii Bacteroides vulgatus Bifidobacterium adolescentis Bifidobacterium infantis
Pectin, polygalacturonic acid	Bacteroides thetaiotaomicron Bacteroides ovatus Bacteroides '3452A'* Bacteroides fragilis subsp.a Bacteroides vulgatus Eubacterium eligens
Arabinogalactan	Bacteroides thetaiotaomicron Bacteroides ovatus Bacteroides '3452A'* Bacteroides uniformis Bacteroides vulgatus Bacteroides 'T4-1'*

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* Unnamed DNA homology groups (11).

Our approach to these and similar question has been to investigate the mechanisms by which pure cultures of colon bacteria utilize individual polysaccharides in vitro, with a view to determining what factors affect the organism's decision to utilize a particular type of carbohydrate. This approach is based on the assumption that information about the specific features and limitations of polysaccharide-catabolizing systems in colon bacteria will permit us either to make predictions about the extent to which catabolism of a particular class of polysaccharides can occur in vivo or to develop specific methods for detecting metabolic states in bacteria in vivo.

Strategies for Polysaccharide Metabolism

To illustrate some characteristrics of polysaccharide utilization systems of Bacteroides species, we will compare the systems involved in utilization of a host-produced mucopoly-saccharide (chondroitin sulfate), a plant galactomannan (guar gum) and polygalactaronic acid. Bacteroides thetaiotaomicron ferments not only chondroitin sulfate but also structurally similar mucopolysaccharides such as hyaluronic acid and heparin. The degradative enzymes are inducible, i.e. they are only produced in appreciable amounts only when the organism is exposed to the appropriate substrate (12). The rate at which the enzymes are produced following exposure to the polysaccha-ride and the amount of enzyme produced are both proportional to the concentration of polysaccharide in the medium. Thus the higher the concentration of a polysaccharide the more likely it is to be utilized. Induction of enzyme activity is fairly specific. Growth on chondroitin sulfate is accompanied by pro-duction of enzymes which attack chondroitin sulfate and those mucopolysaccharides which closely resemble chondroitin sulfate (e.g. hyaluronic acid). Enzymes for degrading heparin, a muco-polysaccharide which contains a different glycosidic linkage, are not induced by chondroitin sulfate. Bacteria which are growing on heparin produce enzymes which attack heparin but not chondroitin sulfate or hyaluronic acid (K. Voss and A. Salyers, unpublished results).

None of the enzymes which are involved in the breakdown of chondroitin sulfate by B. thetaiotaomicron are extracellular. In fact, the first degradative enzyme, chondroitin lyase, appears to be located in the periplasmic space between the inner and outer membranes of these gram negative organisms (13). Chondroitin lyase breaks chondroitin sulfate into disaccharides and these disaccharides are further degraded by enzymes which are located in the cytoplasm. None of these enzymes is membrane-bound. However there are probably some membrane proteins involved, e.g. receptors on the cell surface which bring the chondroitin sulfate into contact with the lyase and

proteins in the cytoplasmic membrane which transport the
disaccharides into the cytoplasm.

    The chondroitin sulfate-degrading systems of two species of
colon bacteria which are closely related to B. thetaiotao-
micron, Bacteroides ovatus and Bacteroides '3452A'(an unnamed
DNA homology group of Bacteroides), appear to be similar to the
chondroitin sulfate-degrading system of B. thetaiotaomicron in
that the degradative enzymes are inducible and are cell-asso-
ciated rather than extracellular (A. Salyers and M. O'Brien,
unpublished data). Moreover, none of these enzymes in B.
ovatus or B. '3452A' are membrane-bound. It is interesting to
note that DNA homology data indicates that B. ovatus and B.
'3452A' have 30-45% cross homology with B. thetaiotaomicron
(11). We are currently investigating the possibility that some
of this shared genetic material contains the genes for chondroi-
tin sulfate utilization, i.e. that these three species have
identical systems for degrading chondroitin sulfate.

    Guar gum, a galactomannan which is used as an emulsifier in
processed foods, is utilized by a different sort of system.
When B. ovatus is growing on guar gum, there is an enzymatic
activity in the extracellular fluid which decreases the
viscosity of guar gum (14). This enzyme reduces the molecular
weight of guar gum from 10^6 to 4×10^5, but does not produce
small carbohydrate fragments. In addition to this enzyme, there
is also cell-associated galactomannanase activity, most of which
stays with the cell membranes even after treatment with 0.5M
NaCl. This indicates that the enzyme is an integral membrane
protein. The product of this galactomannanase appears to be a
trisaccharide (15). Thus the galactomannan-degrading system
differs from the chondroitin sulfate-degrading system in the
location of the initial degradative enzymes. However, these two
systems resemble each other in that the degradative enzymes are
inducible.

    B. thetaiotaomicron does not ferment guar gum. However it
does ferment another viscous polysaccharide, polygalacturonic
acid. Recent investigations of polygalacturonic acid breakdown
by B. thetaiotaomicron have shown that this organism produces an
inducible membrane-bound polygalacturonase (J. Leedle and A.
Salyers, unpublished results). No extracellular enzyme was
detected.

    Thus colonic Bacteriodes appear to have different strate-
gies for degrading different polysaccharides. It may be the
case that in general the type of mechanisms used for degrading a
particular polysaccharide depends on the type of polysaccharide
being utilized rather than on the species of the organism.

Utilization of Maillard Products

    At present there is little information concerning what
types of Maillard products reach the colon. The lignin-like

polymers which are the end products of the Maillard reaction
reach the colon but are not degraded by colon bacteria (Van
Soest, private communication). It is not known whether earlier
products in the Maillard reaction (e.g. aryl-glycosides, glyco-
sylamines) are digested in the small intestine. However, we can
determine whether these Maillard products could be utilized by
saccharolytic bacteria if they were to reach the colon.
    We have investigated the utilization of two Maillard com-
pounds, fructosyl-glycine and isomaltol-galactoside, by Bacter-
oides species. Fructosyl-glycine is formed during condensation
of glucose with glycine (7, 16). Isomaltol-galactoside is
formed as a consequence of the interaction of an amine with
lactose. The resulting reactions culminate in dehydration of
the glucose moeity to produce isomaltol (17). Isomaltol and
isomaltol-galactoside are found in baked breads and contribute
to the characteristic odor and flavor of these foods (18).
    The structures of fructosyl-glycine and isomaltol-galacto-
side are shown in Figure 1. Fructosyl-glycine could be consid-
ered a structural analogue of sucrose or of other fructosyl com-
pounds such as inulin. We tested two species of colonic Bacte-
roides (B. ovatus and B. thetaiotaomicron) for the ability to
grow in medium which contained fructosyl-glycine as the sole
carbon source. Both of these species ferment sucrose and B.
ovatus also ferments inulin, but neither could utilize the fruc-
tose in fructosyl-glycine. Moreover, crude extracts from sonic-
ally disrupted B. ovatus or B. thetaiotaomicron (grown on fruc-
tose, sucrose or inulin) did not degrade fructosyl-glycine. The
inability of these Bacteroides species to utilize fructoryl-gly-
cine as a carbon source does not rule out the possiblity that
other intestinal becteria might be able to utilize it.
    Although fructosyl-glycine was not utilized as a carbon
source by B. ovatus, it did appear to inhibit growth of B.
ovatus on other carbohydrates (Figure 2). This inhibitory
effect occurred with glucose as well as with fructose and fruc-
tosyl compounds. Note that this effect was not very pronounced
and that it required high concentrations of fructosyl-glycine.
Thus it is not likely to be important in the colon where concen-
trations of compounds such as fructosyl-glycine would be expect-
ed to be much lower. Fructosyl-glycine did not inhibit the
growth of B. thetaiotaomicron on fructose, glucose or sucrose.
So the effect of fructosylglycine on growth may be limited to
certain species.
    Investigations, such as those described above, of the
effect of compounds like fructosyl-glycine on growth rate of
bacteria have a major drawback. The compound being tested must
be incubated for 10-20h at 37°C in growth medium. Although
fructosyl-glycine is relatively stable and there was no visible
darkening of the medium during the incubation period, we were
able to detect by thin layer chomatography traces of compounds

Fructosyl - glycine

$$H_2C - NH - CH_2 - CO_2H$$
$$|$$
$$C = O$$
$$|$$
$$HOCH$$
$$|$$
$$HCOH$$
$$|$$
$$HCOH$$
$$|$$
$$CH_2OH$$
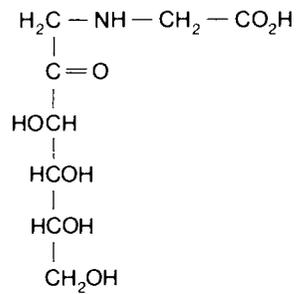
Isomaltol galactoside
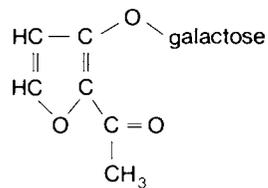
Figure 1.   Structures of fructosyl–glycine and isomaltol galactoside.
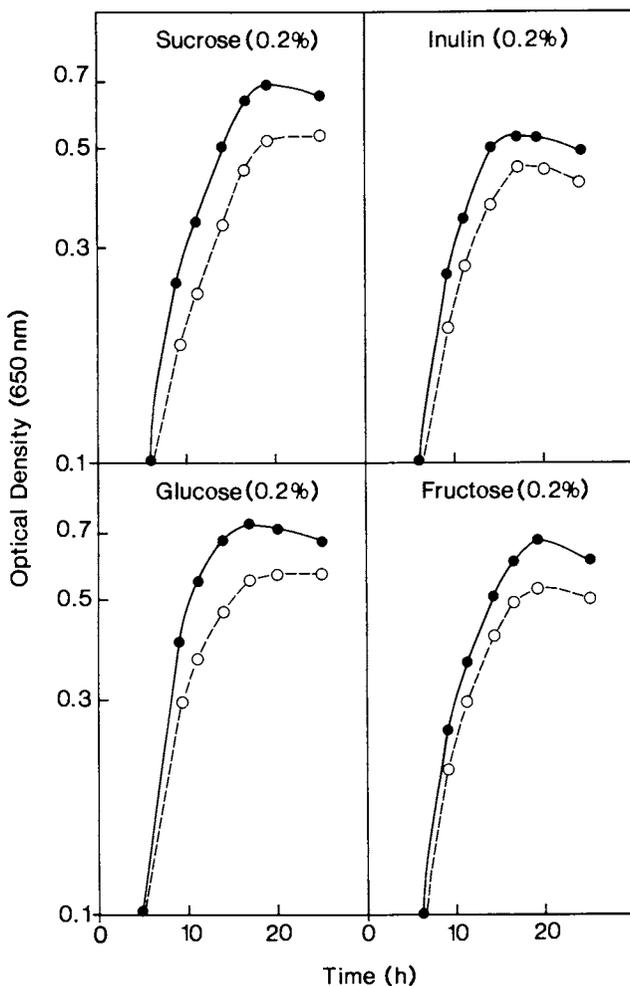
Figure 2. *Effect of fructosyl–glycine on the growth of* B. ovatus *in a medium that contained glucose, fructose, sucrose, or inulin as the sole source of carbohydrate. The basal medium has been described previously (12).*

The carbohydrate and fructosyl–glycine were filter-sterilized and added to the basal medium after autoclaving. The initial concentration of both the carbohydrate and fructosyl–glycine was 2 mg/mL. Cultures grew more slowly in the medium that contained fructosyl–glycine plus the fermentable sugar (○) than in the medium that contained only the fermentable sugar (●).

other than fructosyl-glycine in uninoculated medium which had
been incubated for 12h at 37°C. This raises the question of
whether fructosyl-glycine itself or a further reaction product
was in fact responsible for the effect on B. ovatus.

We tested the effect of fructosyl-glycine on uptake of
glucose, fructose and sucrose by B. ovatus. We found no signi-
ficant effect of fructosyl-glycine on uptake of any of these
compounds. However, the uptake of [^{3}H]sucrose was so slow
that we could have failed to detect reductions in the rate of
uptake which were on the order of 20% or less. We also tested
the effect of fructosyl-glycine on sucrase activity in crude
extracts from disrupted bacteria. Fructosyl-glycine did inhibit
sucrase activity. Further investigations along these lines are
needed to establish whether fructosyl-glycine has any other
effects on bacterial metabolism which might explain why it
inhibits growth.

The results of our investigations of the effects of another
Maillard product, isomaltol-galactoside, can be summarized as
follows (B. Schmetter and A. Salyers, unpublished data): B.
thetaiotaomicron utilized the galactose in isomaltol-galacto-
side. During utilization, isomaltol accumulated in the extra-
cellular fluid. Isomaltol was not utilized, but at high enough
concentrations (4-5 mg/ml) it inhibited growth. Thus isomaltol
is toxic to Bacteriodes, but only at concentrations much higher
than those which are likely to be found in vivo. Isomaltol did
not affect uptake of simple sugars on amino acids. However, it
stopped bacterial synthesis of protein and DNA. Since
[^{3}H]-labeled isomaltol was not taken up by the bacteria in
detectable quantities, it is not clear how isomaltol exerts this
effect. One possibility is that it binds to membranes.

Isomaltol-galactoside appears to be utilized via the
lactose system of B. thetaiotaomicron. Isomaltolgalactoside
inhibited uptake of [^{14}C]-lactose. Moreover mutants which
were deficient in lactose uptake did not utilize isomaltol-
galactoside. Isomaltol-galactoside was hydrolyzed almost as
rapidly by bacterial enzymes as lactose. Whether or not
isomaltol-galactoside itself is actually present in significant
quantities in the colon, it is interesting to note that lactose
analogues of this sort are readily utilized by a lactose-fer-
menting organism. Other Maillard aryl-glycosides may also be
utilized by bacteria which normally ferment structurally similar
saccharides.

The enzyme activity which was associated with the breakdown
of isomaltol-galactoside was constitutive. In this respect, the
catabolic system for isomaltol-galactoside differs from the
catabolic systems for degrading poysaccharides. It also differs
from the lactose system in E. coli which is an inducible system.
The fact that isomaltol-galactoside breakdown can occur without

prior adaptation of the bacteria indicates that these substances
would be rapidly utilized in vivo.

Conclusions

Human colonic bacteria are quite versatile with respect to their
ability to ferment polysaccharides. Moreover, individual
species do not seem to have a consistent strategy for degrading
polysaccharides. In some cases, cell-associated periplasmic
enzymes are used. In other cases the enzymes are extracellular
or even membrane-bound. It may turn out to be the case that the
catabolic strategy is specific for the type polysaccharide
rather than for the species, i.e. different species may utilize
a particular type of polysaccharide in the same way.

Because of the variety of polysaccharides which can be
fermented by some Bacteroides species, it is difficult to pre-
dict with certainty which polysaccharides in the complex mixture
of dietary and host-produced carbohydrates that enter the colon
will be degraded most rapidly and most extensively. Further
information about how these organisms make choices between
different polysaccharides in vitro may help to clarify this
issue. However, nutritionists who are interested in catabolism
of dietary fiber components in vivo should be aware that the
bacteria may prefer other sources of carbohydrate, such as
mucopolysaccharides from host secretions or even Maillard
products, to the dietary polysaccharide under study, and that
this preference may influence catabolism of a particular
polysaccharide in ways which we cannot at present predict.
Effects of this sort may be responsible for some of the
individual-to-individual variation which is encountered in
nutritional studies of dietary fiber utilization.

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Some in Vitro and in Vivo Properties of Dietary Fibers from Noncereal Sources

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Non-cereal dietary fiber sources are compared and
evaluated for cation exchange, water holding
capacity and in vitro fermentability by gut
microflora. Fiber sources studied include
Psyllium gum, alfalfa, purified wood cellulose,
pectin, cabbage, synthetic Maillard product,
soybean hulls and propol. Sources are compared to
the standard AACC wheat bran. Fermentability of
the substrates alfalfa, wood cellulose and wheat
bran show the largest unfermentable residue and
the greatest differences between inocula sources
(rumen inocula greater than human fecal inocula in
all cases). Water-soluble sources (pectins) are
completely fermentable, the exception of Psyllium
gum which contains about 44% of an unfermentable
residue. Cabbage pectin is unusual in having
considerable cold water (50%) solubility. Cation
exchange binding measured with copper are the
highest for pectin, cabbage, bran, Maillard
product = alfalfa and soybean hulls descending in
that order. Wood cellulose, propol and Psyllium
gum have essentially no metal binding capacity.
Dietary fibers of high prophylactic value appear
to have substantial metal binding capacity and are
divisible into two classes based on
fermentability. Human feeding studies indicate
that the fiber sources containing unfermentable
residues will contribute to fecal bulk and,
therefore, transit and passage, while the more
fermentable have their major effects upon gut
microflora. These two aspects are complementary
relative to the effectiveness of dietary fiber.

The identification of dietary fibers as an overlooked factor
in human health and disease has promoted a great deal of interest

in the character and quality of food fiber sources. While
considerable attention has been given to cereal brans, other
sources of dietary fiber exist that offer a variety of qualities.
These include vegetables, gums, wood products and synthetic
polymers.

Dietary studies with humans and other animal models have
emphasized the varied nature of responses to different fiber
sources. Transit and passage depend on the bulk of colonic
contents which in turn appears to depend upon lignified
unfermentable residues as found in wheat bran. The particle size
of bran is an important factor influencing its bulking effect,
coarser residues having more effect (1). In general unlignified
sources such as vegetables and pectin have less effect upon
transit and influence gut microflora more because of their high
fermentability. These residues being less rigid structurally may
have greater swelling and hydration characteristics (2, 3).

Hypotheses to account for the effects of dietary fiber have
emphasized the bulking and binding capacities, in the argument
that faster transit reduces exposure to hazardous substances,
while the binding effect might account for the specific binding
of bile salt and other organic substances and the reported
negative effects upon mineral availability (4). A less
recognized factor is that of the gut microflora which if
stimulated may incorporate ammonia, carbon skeletons and minerals
into the cellular residue (5, 6). This incorporation into
cellular mass would provide an alternative unavailable sink for
these substances.

The purpose of this investigation is to present data on the
variety of dietary fiber sources and characterize them relative
to the physico-chemical characteristics supporting metal ion
binding, hydration and fermentability.

Fiber Sources

The following dietary fiber sources were studied: Psyllium
gum, propol (a glucomannan gum), commercial pectin, solka floc (a
wood cellulose), cabbage, soybean hulls, alfalfa, Maillard
product and standard AACC wheat bran. The Psyllium and propol
gum were donated by the Searle Company, Chicago, IL; other
sources were purchased with the exception of the Maillard product
which was prepared according to the procedure of Olson et al.
(7) from sucrose and glycine. The insoluble fibers from wheat
bran, alfalfa, and soybean hulls were prepared by amylase
digestion and neutral detergent extraction. The cabbage was
converted to a coarse powder by a threefold extraction with 95%
ethanol and passing the alcohol slurry through a meat grinder.
This treatment resulted in a 30-fold concentration of the dietary
fiber over that in raw cabbage.

Water Holding Capacity by Filtration.   The samples were
added to crucibles that had been weighed at room temperature and
hot (100° C).   Deionized distilled H_2O was added to the sample
with backpressure for 2 hrs, then filtered under full vacuum for
1 minute; finally the crucible was wiped to remove excess water
and weighed.   The hydrated sample was dried at 100° C overnight
and weighed.   Grams of water per dry gram of sample was
determined.

Water Holding Capacity by Osmotic Suction.   Water was
removed from hydrated samples by creating an osmotic suction
using a modification of the Robertson and Eastwood method (8).
Dialysis tubing (Mwt cutoff = 2000) was cut into 10 cm lengths
and one end tied.   These bags were soaked in a 0.1% w/v sodium
azide solution overnight.   Sample was placed in the bags and 5 ml
of azide solution was added.   The open end was tied and the
filled bag rested in a 150 ml beaker within a dessicator at a
relative humidity of 100% for 24 hrs. for hydration.   Then 50 ml
of an azide solution (0.1%) with 8.75 g of PEG (3350 Molecular
weight) was placed in the beaker.   This concentration of PEG will
approximate a suction pressure of 270 osmol across the dialysis
membrane after the sample has lost it's nonassociated water.
Readings of the osmolarity were made at 6, 12, 24 and 48 hrs to
determine equilibrium.
     After 24 or 48 hrs, the tube was cut open.   A subsample of
the hydrated material was removed and weighed.   A dry weight of
the fiber subsample was obtained after overnight drying at 100°
C.   Grams of water per gram of sample was then calculated.

Measurement of Cation Exchange Capacity.   Cation exchange
capacity (CEC) was measured based on the high affinity of Cu^{2+}
for carboxylic acid groups (9, 10).   Samples were weighed in 50
ml coarse Gooch crucibles, placed inside a 100 ml beaker and
incubated with 50 ml 1 M copper sulfate for 2-3 hours.   These
crucibles were removed and washed with distilled, deionized water
to remove unbound copper ions.   Then the samples were washed 3
times (25 ml/wash) with 0.6 N hydrochloric acid in 70% (v/v)
propanol-2.   The washes were collected and brought to a known
volume.   Aliquots were brought to pH 8-9 with 2 N ammonium
hydroxide.   A copper indicator, cuprizon, was added, again
brought to a known volume and the absorbance at 590 nm was
measured after 30 minutes to allow for complete color reaction.
Copper nitrate standards were prepared ranging from 0 to 8 PPM.

Fermentation Technique.   A human batch culture technique was
used to measure in vitro fermentability of the samples (11).   The
technique was developed to utilize microflora present in human
fecal matter.   Maintenance of an anaerobic environment during
collecting, processing and inoculating was important.   The use of
a modified camping toilet allowed collection while maintaining

anaerobiosis. A receiving medium was used in the collection vessel. The culture medium and determination of fermentability were the same as the rumen in vitro procedure (12).

Composition of Fibers

Composition of the dietary fiber sources is shown in Table I. The gum sources generally show no insoluble fiber (as NDF) with the exception of the glucomannan source which contains about 5% of a white fibrous residue. Cabbage powder is intermediate with about a third of its dietary fiber in the form of pectin. This pectin is unusual in that it is not precipitated by quaternary detergent and also posseses substantial cold water-solubility in the absence of chelating agents. This characteristic has been mentioned by Bailey et al. (13). Soybean hulls contain significant amounts of galactan and other pectin related carbohydrates (14) in addition to the insoluble NDF fraction.

Table I. Composition of Dietary Fiber Sources

Fiber Source	Dietary Fiber	NDF	HC[1]	Cell	Lignin	Nitrogen
	- - - - - - - - - - - % DM - - - - - - - - - - -					
Psyllium, whole seed	87.7	52.6	23.7	25.4	3.5	–
Psyllium, gum	93.5	ND	ND	4.1	0.3	–
Propol	99.0	4.6	0	0	0	0
Pectin	100.0	0	0	0	0	0
Wood Cellulose	100.0	100.0	2.6	94.5	2.8	0.2
Maillard polymer	ND[2]	74.2	0	0	72.7	8.6
Alfalfa NDF	100.0	53.0	21.6	23.9	7.5	3.2
Cabbage	73.0	44.3	7.3	31.1	1.0	2.9
Soy bean hulls NDF	100.0	56.0	10.5	37.0	2.0	3.2
Wheat bran AACC	44.1	44.1	30.9	9.3	3.9	2.8

[1]Hemicellulose
[2]ND, Not Determined

Fermentability and Water Holding Capacity

The dietary fiber in alfalfa and wheat bran is primarily in the forms of insoluble NDF, and these sources are significantly lignified and therefore contain substantial amounts of unfermentable carbohydrate (Table II). Soybean hulls, cabbage, wood cellulose are less lignified and about 90% fermentable. The wood source is not a pure cellulose and contains significant amounts of hemicellulose and lignin. The hemicellulose fraction is large enough to obtain a digestion balance with human subjects. Hemicellulose in Solka floc is substantially more fermentable than its cellulose (6).

Table II shows a comparison between fermentability values utilizing rumen and human inocula on the same substrates. A comparison of this nature may have inherent inequalities due to differences in inoculum source and collection procedures. These parameters have been evaluated by a number of workers (11, 17, 18). The differences in nutritional schemes between animal species have an effect on microbial populations. Fermentability estimates of a particular substrate are biologically acceptable with respect to the inoculum source.

Table II. Physicochemical and Biological Properties of Dietary Fiber Sources

| | Water Holding Capacity | | Exchange | Fermentability | |
	Filtration	Osmotic suction, 0.3 osmol	meq Cu/ 100 g	Human Fecal	Rumen
	- -g H_2O/g Fiber- -			- - - % - - -	
Psyllium gum	-[1]	3.2	3	-[1]	56
Propol	-[1]	2.5	0[1]	-[1]	100
Pectin	-[1]	5.0	227	90	98
Wood Cellulose	1.4	1.0	5	23	94
Maillard product	2.4	1.7	37	(0)[2]	(0)[2]
Alfalfa NDF	5.8	1.8	36	46[2]	57[2]
Cabbage	20.7	3.0	92	91	91
Soybean Hulls NDF	5.9	1.7	18	-	89
Coarse Bran, NDF	3.5	1.3	87	53[2]	71[2]

[1]Not determined--see discussion.
[2]Used whole plant tissue.

Fermentability using human fecal inoculum ranges from a high of 91% (cabbage) to a low of 0% (Maillard product) demonstrating the sensitivity of human microflora to different fiber sources. Fermentability of cellulose by human fecal microflora (23%) is substantially less than rumen microflora (94%). The microflora in the human intestine may be more affected by fiber composition than rumen microflora. Work by Bryant (19) and Jeraci (11) leads to speculation that variation among inoculum sources in humans on a particular substrate could be greater than in other species. The water holding capacity (WHC) of dietary fiber could have important relationships to many other fiber characteristics (15, 16). A new technique is utilized so that water soluble components of dietary fiber can be measured for WHC. This method differs significantly from previous techniques (8) in that the dialysis tubing had a molecular weight cutoff of 2000. The WHC measured by filtration is consistantly higher for the insoluble fibers. Wood cellulose shows the lowest value by both methods. The three water soluble polysaccharides are among the highest values measured by osmotic suction with pectin holding 5.0 g H_2O

per gram of fiber. Cabbage holds 3.0 g water per gram of fiber.
This is probably due to the large amount of pectin in this fiber
source. The Maillard product is unusual in that it has the
smallest change in WHC between the methods.

    The fermentability of these fibers, the microbial mass
produced and the properties of the microbial products and fiber
residues may be of greater importance in the large intestine than
the characteristics of the unfermented dietary fiber.

Cation Exchange Capacities

    Cation adsorption appears to be important in the formation
of cationic bridges as a mechanism for bile acid, fatty acid and
mineral adsorption in the upper intestine (20). The affinity of
copper for carboxylic acid groups has been employed in pectin
precipitation (10, 21) and in determinations of cation exchange
capacity (CEC) of dietary fiber (9). Table II contains
estimates of cation exchange capacity with pectin having the
greatest amount of copper adsorption per unit weight. Cabbage,
with one third of its dietary fiber value deriving from pectin,
similarily has a substantial exchange value (92 meq/100 g). The
coarse bran, alfalfa and soybean hulls were neutral detergent
fiber preparations using the amylase modification of Robertson
and Van Soest (22) and were thus free of pectin and starch.
Under these conditions lignin with both carboxyl and hydroxyl
groups on the phenylpropane units has a predominant role in CEC.
Differences among dietary fibers are also derived from the
hemicellulose fraction including the glucuronic and galacturonic
acid content. Sugar residues of the glucuronic acid become
available for methylation, amidation or cationic complexes
dependent on the degree of oxidation of the terminal hydroxyl.
This fraction appears to be the primary source of copper
adsorption in wheat bran. Alfalfa and soybean hulls have more
moderate exchange values of 36 and 18 meq/100 g respectively.
The glycine Maillard product exchange value of 37 meq/100 g is
primarily a lignin-like effect. Wood cellulose and Psyllium are
relatively inert polysaccharides with very little copper
adsorption. Propol could not be accurately measured since it
forms a gel. This prevents removal of excess copper. Addition
of ytterbium with the propanol does not cause precipitation.
This is supportive evidence that the exchange would be near zero.

Summary

    Data have been presented emphasizing the variability of
dietary fiber sources. Water holding capacity estimates differed
by technique as well as source. The most important advantage of
the osmotic suction technique is that the measurement includes
the water soluble dietary fiber components. This is a more
realistic model of the digestive tract. The human fecal batch in

vitro fermentation method can be used to study fiber digestion. Variation in degree of fermentation are due to differences in fiber composition and/or inoculum source. Cation exchange capacity estimates also emphasize chemical composition differences with lignin and uronic acids being the primary adsorption sites.

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Dietary Pectin: Effect on Metabolic Processes in Rats

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Dietary pectin affects lipid metabolism, especially
that of cholesterol. One of the explanations pro-
posed to explain an action of pectin on cholesterol
metabolism is through its ability to bind bile acids
and bile salts. However, pectin also has the prop-
erty of forming a gel in water. This gel lowers the
intestinal absorption of cholesterol and thereby
decreases liver cholesterol. Recently, evidence has
been obtained that the presence of pectin in a
cholesterol diet increases the excretion of choles-
terol esters. Results from the administration of
cholesterol-4-^{14}C in the diet showed that the
presence of pectin slows gastric emptying and
results in more labeled cholesterol as well as
cholesterol esters in all segments of the gut.
These observations indicate that the higher levels
of cholesterol esters were mainly exogenous. The
functional role of pectin can be influenced by
many factors such as its degree of esterification,
its chain length and also the quantity and quality
of other dietary ingredients. The effect of pectin
on lowering of liver cholesterol in rats receiving
diets containing corn oil or lard with or without
cholesterol was greater with lard-pectin than with
corn oil-pectin. The excretion of fecal free fatty
acids was markedly greater; among them oleic acid
was the highest in rats fed the pectin as compared
to the pectin-free diet. Pectin also affects carbo-
hydrate metabolism, reducing the postprandial rise
in blood glucose and serum insulin. These effects
of pectin could be beneficial in reducing risk
factors associated with heart disease and diabetes.

        Metabolic processes affected by dietary pectin have been
recognized for more than 2 decades. Effects of dietary pectin on
lipid and carbohydrate metabolism are important and beneficial to
our health.
        Pectin lowers serum cholesterol in humans (1-6) and serum and
liver cholesterol in rats (7, 8) as well as in many other species
(9-13). The ingestion of cholesterol increases serum cholesterol
levels (14, 15). A high level of serum cholesterol is generally
considered a risk factor associated with coronary heart disease
(16, 17). The cholesterol-lowering property of dietary pectin
would thus be expected to reduce the incidence of heart problems.
One of the early studies on the effect of dietary pectin on
cholesterol metabolism in rats was carried out by Lin et al. (18).
They found that more cholesterol was excreted in rats fed a
cholesterol diet containing pectin than in rats fed a control diet
and suggested that pectin inhibited exogenous cholesterol absorp-
tion. Later, Hyun et al. (19), by using a cannulated thoracic
duct technique with rats, measured the quantitative absorption of
cholesterol-4-^{14}C from test emulsions in the intestine and
observed that the absorption of labeled cholesterol was signifi-
cantly depressed by the presence of pectin in the emulsion and the
lowering was due primarily to a decrease in esterified choles-
terol. This finding supported the early results (18). However,
the mechanism involved in the action of pectin on inhibiting
cholesterol absorption is not clear at this time.
        Many investigators have reported that dietary pectin
increased fecal excretion of bile acids as well as neutral sterol
(8, 20). The hypocholesterolemic properties of pectin may be due
to its ability to bind bile acids (21-24). Leveille and Sauber-
lich (21), using everted intestinal sacs from the rat, reported
that pectin depressed taurocholate absorption. They suggested
that a possible mechanism for the hypolipemic action of pectin
involved the binding of bile salts or bile acids which would
result in reduced absorption of cholesterol and in turn in lower
serum cholesterol levels. In addition, the binding of bile acids
and an increased bile acid excretion would increase turnover of
cholesterol into bile acids and provide less cholesterol to the
circulation (24). Jenkins (25) postulated that gums and viscous
fibers may reduce serum cholesterol by mechanisms other than
simply increasing loss of bile salt.
        In our laboratory, we have observed that pectin increased
cholesterol ester excretion in rats fed a cholesterol-containing
diet (26). An early report (27) indicated that fecal excretion of
endogenous cholesterol ester could be influenced by the type of
fat. Polyunsaturated fat (corn oil) as compared with saturated
fat (lard) accelerated cholesterol ester, but not total choles-
terol excretion (27). It was suggested that, under certain
circumstances, cholesterol ester excretion was one of the major
pathways of cholesterol catabolism and a process for lowering body
cholesterol and might explain why corn oil lowers serum

cholesterol level.   Pectin as well as corn oil is considered to be
an hypocholesterolemic agent.   Recently, we have carried out a
study with young rats to elucidate how pectin affects cholesterol
ester excretion.   Cholesterol-4-^{14}C was used as a tracer in the
diet during the last meals following 3-week feeding of unlabeled
diets.   The diet contained 0.5% cholesterol, 10% corn oil, either
with 5% pectin or 5% cellulose (control diet).   Rats were killed
4 hours after the last 2-hour meal feeding.   The general status of
the experimental rats is shown in Table I.   The food intake in the
last feeding was slightly more in the pectin group than in the
cellulose group.   The percent of the consumed ^{14}C-cholesterol
which passed from the stomach into the intestine was 28.6 and 34.8
in the pectin and cellulose groups, respectively (Table II), and
the actual amount was 63.0 mg in the pectin group and 65.3 mg in
the cellulose group.   Thus, the difference of cholesterol levels
in the contents of intestinal sections between diet groups was not
attributed to the intake.   The low percentage of cholesterol that
passed into the intestine in pectin-fed rats at this period is
consistent with the reports that pectin delays the gastric
emptying rate (28).   The contents found in the duodenum and
jejunum were very small.   Therefore, the samples from the same
diet group were pooled.   All data were from pooled samples.   The
ingested labeled cholesterol, both free and esterified, retained
in the duodenum, jejunum, ileum, and cecum (Table II) was higher
in pectin-fed than in cellulose-fed rats.   Similarly, the non-
labeled total cholesterol esters in these sections was also higher
in the pectin-fed than in the cellulose-fed rats.   The results

Table I
General Status of Experimental Rats

	Cellulose diet	Pectin diet
Body weight, g		
Initial	160 + 4	159 + 3
Final	249 + 5	243 + 4
Liver size, g/100 g body weight	4.15 + 0.10	4.08 + 0.47
Food intake, g (last meal)	9.38 + 0.55	10.99 + 0.47
Total labeled cholesterol intake,		
mg/rat	46.9 + 2.8	50.0 + 2.3
Serum cholesterol, mg/100 ml	256 + 14	235 + 14
Serum glucose, mg/100 ml	166 + 6	144 + 9
Fecal esterified cholesterol,		
mg/g (pooled sample)	0.66	13.5
Fecal neutral cholesterol (free),		
mg/g (pooled sample)	32.0	50.0

Table II

Cholesterol Contents of Rat Digestive Tract Segments 4 Hours After a 2-Hour-Meal-Feeding of a Cholesterol-4-^{14}C Diet Containing Either Cellulose or Pectin

	Cellulose diet - cholesterol content,* mg				Pectin diet - cholesterol content,* mg			
	Total cholesterol	^{14}C-cholesterol	Total esterified cholesterol	^{14}C-esterified cholesterol	Total cholesterol	^{14}C-cholesterol	Total esterified cholesterol	^{14}C-esterified cholesterol
Consumed	187.6				219.9			
Stomach retained	122.3 (65.2%)				156.9 (71.4%)			
Stomach emptying	65.3 (34.8%)				63.0 (28.6%)			
Duodenum	0.26	0.029	0.114	0.02	0.864	0.311	0.149	0.06
Jejunum	0.63	0.26	0.147	0.07	1.464	0.839	0.784	0.41
Ileum	11.85	7.88	1.744	1.18	25.38	17.700	7.876	4.108
Cecum**	49.64	18.05	1.709	0.49	75.84	24.366	5.997	1.573
Total (from duodenum to cecum)	62.38	26.219	3.714	1.76	103.548	43.216	14.806	6.151

* All values are of pooled groups of 4 rats.   ** One rat in cellulose diet group had little cecum content, which might have passed into colon or as feces.

provided positive evidence supporting the contention that pectin
lowers cholesterol absorption and that cholesterol ester is one of
the fractions involved. The origin of labeled cholesterol esters
in the lumen was not clear, since labeled cholesterol in the diet
was in the free form. The conditions in the lumen generally favor
hydrolysis of cholesterol esters and there is little, if any,
intraluminal esterification (29). Sterol ester hydrolase (choles-
terol esterase, EC 3.1.1.13) systems are pH-dependent. The
optimal pH for hydrolysis is 6.7 and for esterification is 6.2
(30). Therefore, a possible explanation for the presence of
labeled cholesterol esters in the intestinal lumen was due to a pH
environment favoring synthesis. The gel-forming property of
pectin might maintain a more acidic pH of the chyme as it leaves
the stomach and enters the upper part of the proximal intestine,
thus favoring cholesterol ester synthesis. Cholesterol esters
cannot be directly absorbed by the mucosal cell (29). The source
of the nonlabeled cholesterol ester in the lumen could not be
determined under present experimental conditions. The present
data showed that cholesterol esters were mainly in the ileal
section of the small intestine. This could be due to the unab-
sorbed cholesterol from the upper proximal region passing down to
the ileum where the absorption of cholesterol is less efficient
(31, 32).
       The profile of fatty acids in cholesterol esters showed a
difference between the two diet groups (Table III). The high
levels of palmitate and stearate esters presumably originated from
the dietary fat. Since the absorption of palmitate and stearate
is low, more of these fatty acids were available in the intestine
for esterification. The percentage of oleate was three times
greater in the cellulose-diet group than in the pectin-diet group.

Table III
The Profile of Fatty Acid Esters in the Crystallized Fecal
Cholesterol Esters

| | Diet | |
	Cellulose	Pectin
Fatty acids, %		
Palmitate	30	43
Stearate	32	19
Transoctadecenate	6	21
Oleate	23	7
Linoleate	8	4
Arachidate	--	2

It is possible that the source (endogenous versus endogenous +
exogenous) of the esters may be responsible for the difference.
It has been reported that oleic acid is most readily esterified
with cholesterol during its absorption (33, 34). Furthermore,
Swell and Treadwell (34) also reported that the cholesterol ester
which accumulated to the greatest extent, irrespective of the
fatty acid fed, was cholesterol oleate. Thus, the endogenous
cholesterol esters may be rich in oleic acid.

The results obtained indicate that dietary pectin influenced
the synthesis of cholesterol esters in the intestinal lumen,
thereby providing a possible mechanism by which pectin can
influence cholesterol metabolism.

The effectiveness of pectin in lowering cholesterol in
tissues can be influenced by many factors: (1) The type of pec-
tin - that with methoxy groups and with high viscosity (35, 36)
was reported to be more effective. The results from a comparative
study of several types of pectin by Mokady (36) are shown in Table
IV. Neither a high degree of methylation nor high-viscosity alone
was very effective; (2) the level of dietary fat - with a high-fat
diet (2% corn oil plus 18% beef tallow), pectin has no effect on
serum cholesterol level (37). Tsai et al. also reported that with

Table IV*
Degree of Methylation and Relative Viscosity of Test Substances
and Comparison of Blood Cholesterol with Excretion of Lipids in
the Feces

	Degree of methylation	Relative viscosity	Total blood cholesterol	Fecal lipids[1]	Total fecal sterols
	(%)	(%)	(mg %)	(g/rat)	(mg/rat)
Control			87 ± 5.2^2	0.272	30.4
Pectin 1	7.8	2.9	$79 \pm 5.2_3$	1.245	84.6
Pectin 2	8.2	6.0	66 ± 2.8^3	2.000	113.3
Pectin 3	4.1	23.5	75 ± 3.0	1.060	67.6
Pectin S	7.7	2.4	72 ± 2.8	1.503	82.0
Pectin MR	7.8	2.6	72 ± 2.8	1.300	89.0
Algin	---	10.2	77 ± 3.2	1.367	38.0

[1] Pooled mean value of 8 animals for the entire period of 12 days.

[2] Mean for 8 animals ± SE.

[3] $P < 0.05$.

* After Mokady (36).

as much as 15% corn oil, pectin showed no apparent cholesterol lowering properties (38); (3) type of dietary fat - corn oil versus lard in a cholesterol-pectin diet. Pectin eliminated the elevation of liver cholesterol in the rats fed ad libitum for 4 weeks by decreasing cholesterol absorption with corn oil and by decreasing absorption and increasing disappearance with lard (39) (Table V). In this experiment, all diets for the last 24 hours of feeding contained the same specific activity of cholesterol-4-^{14}C. The total food intake was carefully measured. Then rats were continuously fed their respective diet without ^{14}C-cholesterol for another 24-hour or 48-hour period, designated as 48-hour and 72-hour feeding periods, respectively. The radioactive cholesterol level (% of intake) in the liver was markedly lower in the pectin-fed than in the cellulose-fed rats, regardless of the type of fat. However, pectin accelerated the disappearance of ^{14}C-cholesterol during the 48-hour to 72-hour period only in the lard-containing diet (39). When the rats fed the control diet were shifted to a

Table V
The Effect of Pectin and Fat on Serum and Liver Cholesterol Levels
and the Recovery of Radioactive Cholesterol (% Intake) in the
Liver of Rats 48-Hour and 72-Hour After Feeding Cholesterol-4-^{14}C
Diet for 24 Hours

| | Corn oil | | Lard | |
	Cellulose	Pectin	Cellulose	Pectin
Serum cholesterol mg/dl (10)[1]	134 ± 3^b	115 ± 5^a	160 ± 13^c	147 ± 16^c
Liver cholesterol mg/100 g body weight (10)	65 ± 7^c	34 ± 4^b	70 ± 5^c	21 ± 3^a
Liver Radioactivity % intake 48-hour (5)	16.7 ± 1.6^a	8.3 ± 1.7^b	15.3 ± 0.9^a	6.4 ± 1.2^b
72-hour (5)	14.5 ± 1.2^a	6.3 ± 1.2^b	16.0 ± 0.6^a	4.6 ± 1.2^b

[1] Numbers in the parentheses represent number of animals used.

Means having different superscript letters are significantly different ($P < 0.05$).

cholesterol-free diet for 2 or 4 days, pectin lowered liver cholesterol and total lipids in the lard diet but not in the corn oil diet (Table VI). Fecal steroid excretion on day 4 of feeding agreed with the degree of reduction of liver cholesterol (Table VII). There were significantly more neutral, free, and esterified cholesterol and bile acids in the feces of pectin-fed rats than in the feces of cellulose-fed rats.

Pectin or other mucilagenous fibers could play an important role in carbohydrate metabolism. The addition of gel-forming fiber in the test meal or in the standard glucose tolerance test solution flattens blood glucose and insulin responses in man (40-42) as well as in rats (28). It was suggested that these actions of fiber are due to its reduction of the gastric emptying rate and decrease in the intestinal absorption rate of the carbohydrates by formation of an additional diffusion barrier at the intestinal surface through the ability of the fibers to produce viscous solutions. Recently in our laboratory (Li and Chang, unpublished observation), we have done preliminary work on the effect of pectin or guar gum on sucrose absorption and observed that there was more fructose, glucose, and sucrose remaining in the lower intestinal content of rats 4 hours after a 2-hour meal feeding with a sucrose diet (61.2% w/w) containing either 5% pectin or 5% guar as compared with 5% cellulose (Table VIII). It seems that these fibers reduced the absorption rate of these simple sugars, especially fructose. At present, we are repeating the experiment and appear to be obtaining confirmatory results.

Table VI

Effect of Pectin and Type of Fat on the Removal of Accumulated Liver Lipids in Rats

Type of dietary fat	Liver lipids mg/100 g body wt.	Initial[1]	2-day of feeding Cellulose	2-day of feeding Pectin	4-day of feeding Cellulose	4-day of feeding Pectin
Corn oil	Total lipid	594 ± 39 (10)[2]	599 ± 66	557 ± 41	366 ± 63	485 ± 25
	Triglyceride	73 ± 4 (5)	63 ± 13	71 ± 2	30 ± 4*	55 ± 13
	Total cholesterol	65 ± 7 (10)	67 ± 7	46 ± 9	38 ± 7*	34 ± 7*
Lard	Total lipid	483 ± 25 (10)	460 ± 27	384 ± 28*	517 ± 61	271 ± 36*
	Triglyceride	79 ± 4 (5)	63 ± 7	56 ± 9	82 ± 20	46 ± 12
	Total cholesterol	70 ± 5 (10)	76 ± 12	28 ± 4*	75 ± 16	29 ± 17*

[1] All rats had been fed cholesterol-cellulose diet either with corn oil or lard for 4 weeks.

[2] Number in parentheses represents number of animals.

* Statistical difference from the initial value is significant at 5% level.

Table VII
Fecal Steroid Excretion of Rats at 4th Day Refeeding Period on
Cholesterol-Free Diet

| | Corn oil | | Lard | |
	Cellulose	Pectin	Cellulose	Pectin
Neutral sterol (mg/day) Total	34.7 ± 1.6^{b}[1]	44.9 ± 4.6^{b}	19.6 ± 1.4^{a}	34.2 ± 4.3^{b}
Esterified	7.3 ± 0.4^{b}	18.5 ± 3.4^{c}	4.6 ± 0.9^{a}	11.2 ± 1.7^{b}
Bile acids (mg/day)	13.1 ± 1.2^{a}	31.8 ± 6.1^{b}	11.3 ± 1.5^{a}	23.4 ± 4.6^{b}

[1] Mean $\pm$ SE of 5 rats.

Table VIII
Intestinal Contents of Carbohydrate of Rats Fed a Diet Containing
61.3% Sucrose 4 Hours After 2-Hour Meal Feeding (Data are from a
Pooled Sample of Three Rats from the Same Diet Group)

Carbohydrate*	Intestinal Section**	Diet Cellulose	Pectin	Guar Gum
Fructose	U	1.77	1.64	1.80
	L	0.13	1.29	1.70
Glucose	U	1.28	1.42	1.14
	L	0.05	0.38	0.31
Sucrose	U	4.56	6.06	5.36
	L	0.02	0.99	0.71

*   % = percent of total dry matter

** U = upper half of the intestine; L = lower half of the
    intestine.

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Chitin and Chitosan: Influence on Element Absorption in Rats

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Chitin and chitosan are reported to bind essential elements in vitro. To evaluate these unconventional sources of dietary fiber in vivo, the apparent absorption of six (6) elements (i.e., P, Ca, Mg, Fe, Zn and Cu) from diets containing chitin and chitosan were measured by balance trials in growing rats. At the 5% level, and at three particle sizes, neither chitin nor chitosan affected animal growth or food consumption over a 3 week period compared to a 5% cellulose control diet. Apparent absorption of the six elements were positive in all animals. Increasing both chitin and chitosan to 10% and 20% levels in the diet depressed Fe absorption except in animals consuming 10% chitosan. There was no difference in growth or food consumption between animals groups receiving higher fiber levels. Animals were in positive balance for P, Ca, Mg, Zn and Cu. Greater morphological changes were observed histologically in the small intestine and cecum of animals consuming chitosan and chitin compared to animals consuming cellulose. Increased numbers of neutrophils were seen in the lamina propria of the small intestine and cecum. More crypt epithelial cells of the small intestine were undergoing mitosis. The inability of rats to utilize dietary Fe and lose body Fe is believed to be due to the inflammatory state of the small intestine, caused by chitosan, and to a lesser degree chitin. Reasons for mortality in rats consuming high levels of chitosan, >10%, are also discussed.

Dietary fiber (DF) has taken on a larger meaning than its original definition of plant cell wall material resistant to the digestive process (1) in animals or man.

Many non-metabolizable materials not obtained from plant
sources that are consumed in trace and possibly bulk amounts
both intentionally and unintentionally are now recognized.
Examples include mucopolysaccharides from animal tissue (2)
microbial cell walls (3) exoskeletons of arthopods (4) and
synthetic bulking agents (5). Consideration must also be given
to intestinal tract residues derived from the processing of
foods which Maillard reaction products (6) are an excellent
example.

Early in the last decade, two, now almost classic publi-
cations, (1, 7) caused a reinvestigation of thought as to the
importance of fiber in man's diet. This was not a new revel-
ation (8). What was new, was the recognition of the multipli-
city of health-related disorders attributed to the lack of DF
in the diet (9). Increased DF consumption is advocated (10),
but there may be adverse consequences with excessive intake.
The biochemical and/or physiological action of DF in the gut
have not been clearly delineated.

One of the benefits attributed to DF has been its ability
to sequester bile acids preventing their reabsorption (11).
The objective of decreasing plasma cholesterol by altering bile
acid homeostasis is strongly championed. However, DF has been
implicated in the impairment of essential element absorption
(12). A possible nutritional or health status trade-off exists
for the host consuming DF.

Two unconventional, but potential sources of DF are chitin
and chitosan. Chitin, along with cellulose and collagen, are
the principal skeletal or support matrices in all living
systems. Reviews of the chitin system in animals and plants
(13) and the chemistry of chitin (14) are available.

Commercial chitin is mainly derived from waste products of
crustacea (i.e. crab and shrimp) processed for human food.
Chitin is similar to cellulose, but is a polymer of N-acetyl-D-
glycosamine units, linked by β (1 → 4) glycosidic bonds. It
may have potential for use as DF (15), but it does not have
wide application and has not been approved for use in foods.
At present, ingestion of chitin by man is only incidental.

Treatment of chitin with strong base causes deacylation,
resulting in the product chitosan (Figure 1). This compound
reportedly has potential for lowering blood cholesterol in rats
(4, 16, 17). Chitosan is being compared to and suggested as a
substitute for cholestyramine; this latter compound has proved
to be a very effective agent in the treatment of patients with
elevated serum cholesterol (18). However, the mechanisms of
action may be different. The safety of chitosan or chitin has
not been thoroughly established. Both chitin and chitosan have
been reported to bind essential elements from solution (19) and
chitosan is used to remove suspended organic materials in
waste treatment (20). These observations again suggest a recip-
rocal relationship between cholesterol and essential element
status may occur with the ingestion of chitosan.

CH₂OH / O label structures

1. dilute HCl
2. dilute ⁻OH
3. conc hot ⁻OH
4. wash
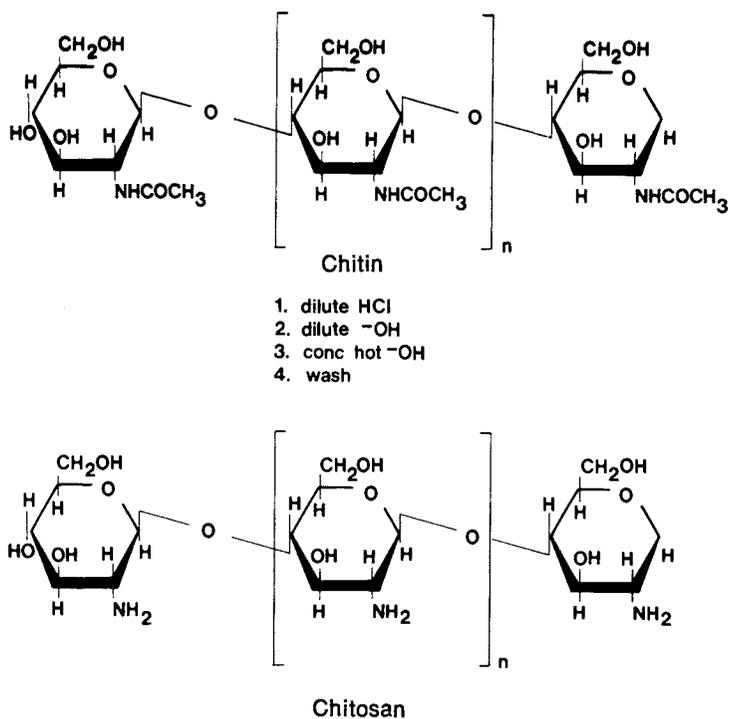
Figure 1.   The chemical structures of chitin and chitosan.

The primary objective of this investigation was to examine the effect of chitin and chitosan on essential element absorption in rats. Since previous reports (4, 21) and unpublished observations by one of the authors (D.T.G.) have indicated high levels of chitosan impair growth and cause death in rats, a second objective was established to more thoroughly explain these findings.

Materials and Methods

Chitin and Chitosan. Chitin and chitosan were received through the courtesy of Kypro Co., Seattle, Washington. After sifting to remove fines and extraneous non-shell flakes, the remaining material was ground in a Thomas Wiley laboratory mill (Model 4, A. H. Thomas Co., Philadelphia, PA) to pass a 2.00 mm screen. Using a Ro Tap Testing Sieve Shaker (Model B, W. S. Tyler, Inc., Mentor, OH), three fractions of each material were collected: <1.00-0.50 mm; <0.50-0.25 mm and <0.25 mm.

Diets. Semi-purified diets were prepared as recommended by the American Institute of Nutrition-AIN (22), and are described in Table 1. Two animal feeding experiments were carried out. In the first experiment (Expt. 1), chitin and chitosan were fed at the 5% level in three particle size ranges. A commercial preparation of the AIN salt mixture was added (U.S. Biochem. Corp., Cleveland, OH) to diets fed in Expt. 1. In the second experiment (Expt. 2), chitin and chitosan of one particle size range 0.50-0.25 mm, were fed at levels of 10 and 20%. The salt mixture used in Expt. 2 was formulated to meet AIN specifications (22). Substitution of increasing amounts of DF in Expt. 2 was made at the expense of dietary carbohydrate. Hydrolyzed cellulose (Celufil; U.S. Biochem. Corp., Cleveland, OH) was incorporated into control diets at levels of 5% (Expt. 1) and 10 and 20% (Expt. 2).

Animals and balance protocal. Male weanling rats, 21 days old, of the Sprague Dawley strain were used in both experiments, 5 animals per diet group. Animals were individually housed in suspended stainless steel cages with wire bottoms in a room maintained at 23°C with a 12 hr light-dark cycle. Glass distilled water and food were provided ad libitum except for food during pre- and post-balance periods. Ten hr before each period, food was removed. Prior to receiving food again, each animal was administered 0.2 ml 0.03% brilliant blue dye in distilled water by gavage. Food consumption was allowed for 60 hr and then food was removed for another 10 hr. Dye was again administered and the procedure repeated twice more in Expt. 2. Only one balance trial was conducted in Expt. 1. Apparent element absorption was calculated from the equation:

Table 1. Composition of Diets.

Nutrient	Experiment 1	Experiment 2	
	--------------- % ---------------		
Casein, vitamin free	20.0	20.0	20.0
Corn oil (0.01% BHT & 0.01% BHA)	5.0	5.0	5.0
Dietary fiber	5.0_1	10.0_1	20.0_1
chitin	——1	——1	——1
chitosan	——1	——1	——1
cellulose	——2	——1	——2
Vitamin premix - AIN-76	1.0^3	1.0	1.0
Mineral premix	3.5	3.5	3.5
Choline bitartrate	0.2	0.2	0.2
DL-methionine	0.3	0.3	0.3
Dextrose-monohydrate	65.0	60.0	55.0

[1]See Material and Methods for particle sizes.

[2]See Material and Methods for type and source of cellulose.

[3]U.S. Biochemical Corp., Cleveland, OH.

Apparent element   diet element concentration   fecal element
absorption: = mg, μg/g X   - concentration
mg, μg/60 hr   g diet consumed/60 hr   mg, μg/60 hr

Phosphorus, Ca and Mg are reported in mg/60 hr; Fe, Zn and Cu
are reported in μg/60 hr. Food efficiency is the ratio of food
intake (g) divided by animal weight gain (g).

    Analytical. Chitin and chitosan samples, diets, fecal
samples and animal carcasses were wet digested in HNO_3-$HClO_4$
prior to element analysis. Determination of Ca, Mg, Fe, Zn and
Cu were by atomic absorption spectrophotometry (23). Phos-
phorus was measured by the method of Fiske-Subbarow (24).
Fecal matter was freeze-dried prior to digestion and analyzed
in duplicate. Animal carcasses were heated to 115°C (15 PSI)
for 2 hr in sealed Mason jars containing one weight volume of
of glass distilled water. After homogenization, the total
contents were made up to an appropriate volume from which 25 ml
aliquots were withdrawn for digestion and element analysis in
duplicate. Hemoglobin was determined spectrophotometrically
(25) as cyanmethemoglobin.

    Statistics. Data for Expt. 1 were analyzed by one-way
analysis of variance (26). Differences among means were
checked by least significant difference. In Expt. 2 the repeated
measurement design (i.e. three balance periods) was subjected to
a split-plot analysis of variance as outlined by Gill and Hafs
(27). Differences among pooled means were verified by least
significant difference.

    Histopathology. Three animals from each group receiving
10 and 20% chitin, chitosan or cellulose were histologically
examined upon termination of Expt. 2. Rats were on test diets
23 days. Animals were assigned a code number prior to necro-
psy. Immediately after euthanization with CO_2, the abdominal
cavity was incised and the intestinal tract examined in situ
along with the other organs. The entire gastrointestinal tract
was removed, perfused with 10% formalin phosphate (0.1M-pH 7.0)
buffer for immediate preservation and then placed in buffered
formalin solution for complete fixation. Samples of liver,
kidney, lung and salivary gland were also preserved in the for-
malin solution. After 24 hr of fixation, the gut and other
tissues were embedded in paraffin and sectioned parallel to the
long axis of the gut in 5 μ slices. The tissues were routinely
stained with hematoxylin and eosin and were permanently mounted.

    Sections of the gut examined histopathologically included:
stomach, duodenum, jejunum, ileum, cecum and colon. A scoring
system was used to quantify the changes observed in these
organs. The two criteria were: (1) inflammatory cell infil-
trate--the number of neutrophils in the lamina propria of the
intestine and (2) mitotic activity--the number of mitotic fig-
ures in crypt cells (i.e. number of crypt cells in metaphase).
    Each mounted tissue was examined at ten random locations

with a light microscope at 400 power magnification (x400). Each
location was referred to as a high-powered field (HPF).
Numbers of mitotic figures are reported as the mean value ob-
served in 10 HPF of each tissue from 3 animals per group.
Quantification of neutrophils were enumerated using the same
counting protocol. Histological lesions observed in the in-
testines were recorded.

Microbiology. Culture specimens of cecal contents for
microbiological examination of the gut flora were aseptically
obtained prior to fixing the entire intestinal tract. Cecal
flora was inoculated onto MacConkeys (BBC, Cockeysville, MD) and
brilliant green agars (Difco Laboratories, Detroit, MI) and sus-
pended in gram negative broth (Difco). After 24 hr incubation
at $37^{o}C$, colony morphology, carbohydrate utilization, catalase
and oxidase production, gram stain reaction and cellular mor-
phology were evaluated (28). Specific organisms tested for
included: Actinobacillus sp, Bardetella bronchiseptica, Citro-
bacter freundii, Escherichia coli, Enterobacter sp, Klebsiella
pneumoniae, Protus sp, Pseudomonas aerugenosa and Salmonella sp.

Results

Experiment 1. At the 5% level of DF consumption, among
seven groups of animals only one group showed a lower weight
gain (Table 2). Pooled weight gains for the 3 groups of animals
(15 rats) consuming one of three particle sizes of chitosan
(103.8±15.6 g) were lower, but not significantly, compared to
the same number of animals ingesting chitin (113.3±11.8 g); the
5 animals fed cellulose had intermediate weight gains (107.9±
7.2 g). It has been reported that finely ground chitosan
restrained growth when fed at the 2% level (4).
There were significant differences in apparent element
absorption values among animal groups consuming chitin and
chitosan in three ranges of particle size (Table 3). A trend
toward lower absorption would appear to result with decreasing
particle size. Significantly lower apparent absorption of P
(P<0.01), Ca (P<0.01), Fe (P<0.05) and Cu (P<0.05) were found in
animals fed chitosan compared to animals consuming chitin.
Only Ca apparent absorption was significantly lower (P<0.01) in
animals fed chitosan versus cellulose controls. Although
differences occurred in apparent element absorption among groups
of animals, all animals were in positive balance for all six
elements with two exceptions. Two groups of animals consuming
chitosan were in negative apparent Cu balance.
The carcasses of the three groups of animals consuming
chitin or chitosan of equivalent particle sizes (0.50-0.25 mm)
or cellulose were analyzed for element concentration (Table 4).
Significantly lower (P<0.05) carcass levels of Ca, Fe, Zn and
Cu were observed in animals fed chitosan as compared to those
fed chitin or cellulose. However, this chitosan diet group

Table 2. Weight gain and efficiency of food utilization among animals fed chitin, chitosan or cellulose in two experiments[1].

Experiment 1[2]

Dietary fiber	Particle size (range) mm	Level %	No. of animal	Initial weight	Weight gain (g)	Food efficiency
Chitin	1.00 – 0.50	5	5	58.4±2.0	108±3.0[a]	2.65±0.11[a]
	0.50 – 0.25	5	5	58.6±1.9	111.2±7.7[a]	2.37±0.01[b]
	<0.25	5	5	59.3±1.7	120.8±2.7[a]	2.47±0.09[a]
Chitosan	1.00 – 0.50	5	5	58.5±1.9	110.3±4.4[a]	2.57±0.06[a]
	0.50 – 0.25	5	5	59.2±1.6	90.2±6.2[b]	2.70±0.14[c]
	<0.25	5	5	59.5±1.8	110.7±6.7[a]	2.54±0.05[a]
Cellulose		5	5	60.2±2.0	107.9±7.2[a]	2.50±0.97[a]

Experiment 2[3]

Chitin	0.50 – 0.25	10	5	58.6±1.3	122.8±7.0	2.54±0.11
		20	5	59.2±1.3	117.0±7.0	2.57±0.11
Chitosan	0.50 – 0.25	10	4	59.1±1.5	113.4±7.9	2.54±0.13
		20	3	59.9±1.5	102.4±9.0	2.75±0.14
Cellulose		10	4	58.8±1.3	123.4±7.0	2.45±0.11
		20	5	58.2±1.5	117.3±7.8	2.69±0.13

[1] Mean ± S.D.; values in same column in Expt. 1 not sharing a common superscript are significantly different (P 0.05).

[2] Growth on diets for 22 days (total animal age 43 days).

[3] Growth on diets for 23 days (total animal age 44 days).

Table 3. Apparent element absorption among animal groups
consuming chitin, chitosan or cellulose at the 5% level.

	Particle size (range) mm	Number of animals	P
Chitin	1.00 – 0.50	5	$190.9 \pm 7.0^{a,b}$
	0.50 – 0.25	5	193.5 ± 11.4^{a}
	<0.25	5	195.1 ± 6.5^{a}
Chitosan	1.00 – 0.50	5	$166.0 \pm 9.0^{b,c,d}$
	0.50 – 0.25	5	$143.5 \pm 10.0^{d,e}$
	<0.25	5	$161.2 \pm 6.0^{c,d}$
Cellulose		5	$174.4 \pm 8.8^{a,b,c}$

[1]Mean ± S.D.; values in same column not sharing a common
   superscript are significantly different (P<0.05).

[2]Days 19–21.5 (60 hr) during 22 day growth period.

[3]Values in parenthesis are negative.

Ca	Mg	Fe	Zn	Cu
mg/60 hr[1,2] ---------------		----------- ug/60 hr[1,2,3] -------------		
110.8±10.3[a,b]	13.8±1.2	1,310±225[a]	536±121	83.0±34.9[a]
120.0±9.4[a]	13.9±1.1	1,305±299[a]	459±61	13.8±7.7[b,c]
88.6±10.3[b,c]	10.7±1.6	812±297[a,b]	454±56	26.1±15.7[b,c]
101.0±12.4[a,b]	14.4±1.4	788±215[a,b]	312±30	(9.2)±11.2[c]
41.7±9.0[d]	9.9±2.6	337±41[b]	357±62	(2.2)±10.0[b,c]
61.1±9.7	12.5±1.7	715±200[a,b]	447±26	41.0±10.6[a,b]
112.8±7.6[a,b]	14.4±1.3	956±84[a]	498±59	44.8±8.8[a,b]

Table 4. Whole body element concentration per g animal weight of
rats maintained on diets containing chitin, chitosan or cellulose
at the 5% level for 22 days (animal age 43 days).

Dietary fiber	Animal weight g	Number of animals	P	Ca
			---------------- mg/g^2 --	
Chitin[1]	178.3±19.1	5	5.19±0.40	8.42±0.49^a
Chitosan[1]	160.8±15.1	5	4.94±0.26	7.01±0.19^b
Cellulose	175.7±20.3	5	5.42±0.58	9.15±0.77^a

[1]Particle size range: 0.50 - 0.25 mm (see Table 2).

[2]Mean ± S.D., values in same column not sharing a common
 superscript are significantly different (P<0.05).

Mg	Fe	Zn	Cu
------------	------------- ug/g^2 -------------		
0.41±0.05	57.5±9.2[a]	26.3±1.5[a]	5.4±1.9[a]
0.34±0.05	41.9±4.0[b]	24.5±1.1[b]	3.1±0.2[b]
0.41±0.06	44.4±3.6[b]	25.6±3.4[c]	3.8±0.4[b]

also exhibited significantly lower weight gains (Table 3)
which would account for the lower element values derived from
carcass analysis.

   Experiment 2. There were no significant differences in
animal weight gains or efficiency of food utilization among
animal groups fed chitin, chitosan or cellulose at levels of
10% and 20% (Table 2). In each DF diet group, weight gains
were lower in animals at the 20% DF level than at the 10% DF
level, but not significantly.

   Apparent element absorption values for three, 60 hr bal-
ance periods for P, Ca, Mg, Fe, Zn and Cu in the six diets are
illustrated in Figures 2-7, respectively. Among animal groups
fed the different DF diets at two levels, the amount of each
element absorbed increased from balance period 1 to balance per-
iod 3 with a few exceptions. Animals consuming 20% chitin in
their diets had declining apparent absorption values for Mg
(Figure 4) and Cu (Figure 7) over 23 days. Animals fed 10 and
20% chitin and 20% chitosan were in constant negative Fe bal-
ance; the magnitude of which increased in each successive bal-
ance period (Figure 5). Apparent Cu absorption in animals con-
suming 20% chitosan was very high and especially during the
3rd balance period. This high apparent absorption can be
explained in part by the high level of Cu in this diet, 11 µg/g,
versus a mean of 7.4 µg/g in the 5 other diets.

   Using data illustrated in Figures 2-7, the apparent element
absorption levels in each period for each animal group were
pooled and are shown in Table 5.

   A second criteria resulting from histopathological examin-
ation of tissues altered by DF was the number of mitotic fig-
ures in crypt epithelial cells which may indicate cell turnover
from functional or morphological cell loss. Animals on chitosan
diets had the largest number of cells in metaphase transition.
An example of increased number of mitotic figures found in
crypts of the duodenum section of the small intestine of ani-
mals on a 20% chitosan diet is shown in Figure 8B. An increased
nuclear division of crypt epithelial cells can only suggest early
loss of cells with production and movement of less mature epith-
elial cells along the villus. Villi of the duodenum from rats
consuming chitosan had the appearance under a HPF of being blunt
or flattened compared to villi from animals ingesting cellulose
(Figure 8A).

   By combining results of inflammatory response (Table 6) and
the number of mitotic figures (Table 7) found in intestinal
tracts, a more realistic comparison of histopathological obser-
vation and possible physiological action caused by DF is best
quantified. Using both indices, total abnormalities in animal
intestines caused by DF can be arranged in the decreasing order
of insult: 20% chitosan > 10% chitosan > 20% cellulose > 10%
chitin > 10% cellulose > 10% chitin. Another morphological
change observed in animals consuming 20% chitosan included
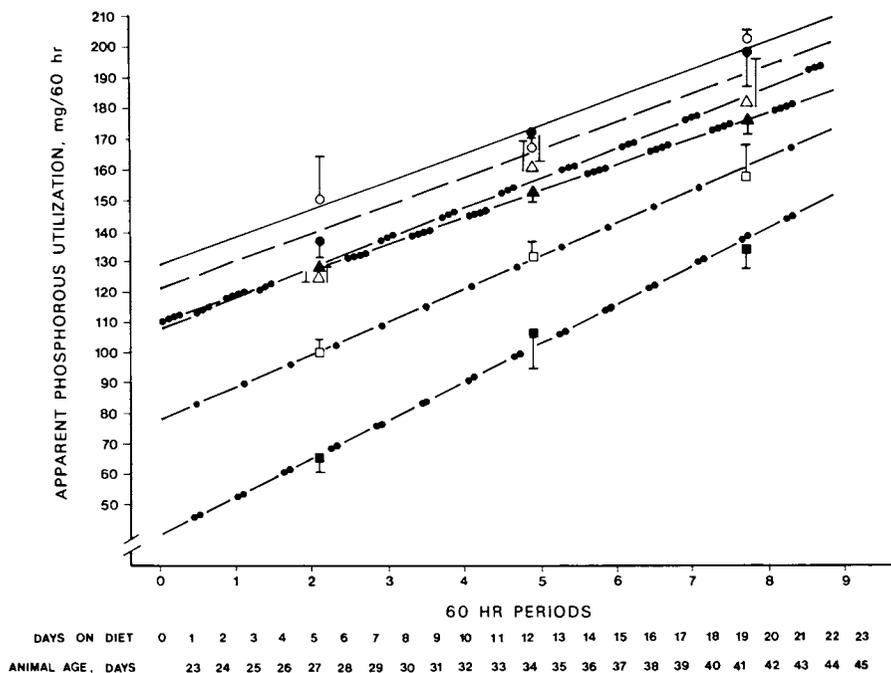
Figure 2. Apparent utilization (i.e., absorption) of P by rats consuming three dietary fibers at two levels. Key O–O, 10% chitin; ●–●, 20% chitin; □–•–□, 10% chitosan; ■–•••–■, 20% chitosan; △–•••–△, 10% cellulose; and ▲–•••–▲, 20% cellulose. Each point represents the mean absorption ± SEM for a 60 h period. Days on the diet and animal age (in days) are also indicated on the x-axis.
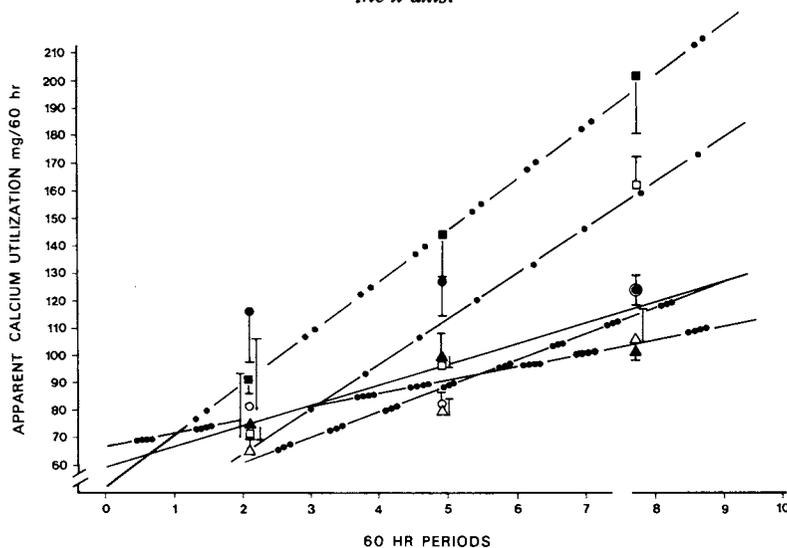
Figure 3. Apparent utilization of Ca by rats consuming three dietary fibers at two levels. Key is the same as in Figure 2.
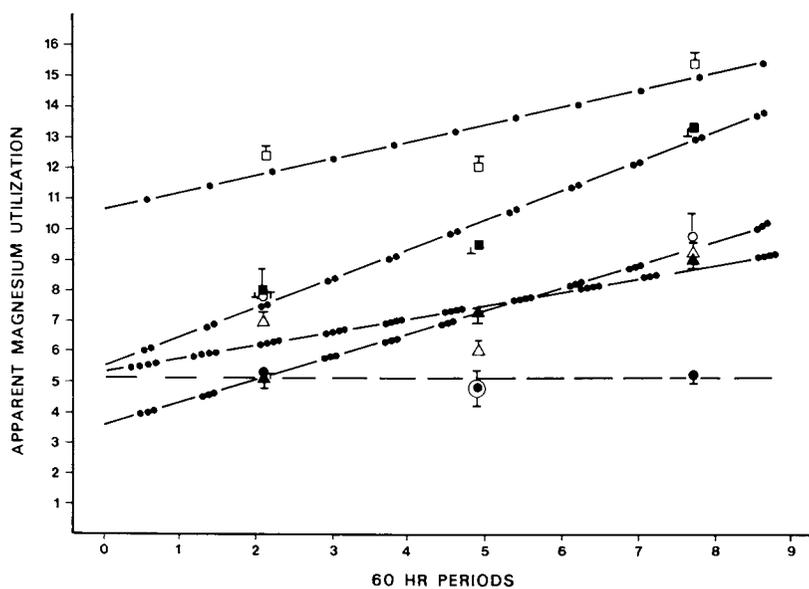
Figure 4. *Apparent utilization of Mg by rats consuming three dietary fibers at two levels. Key is the same as in Figure 2.*
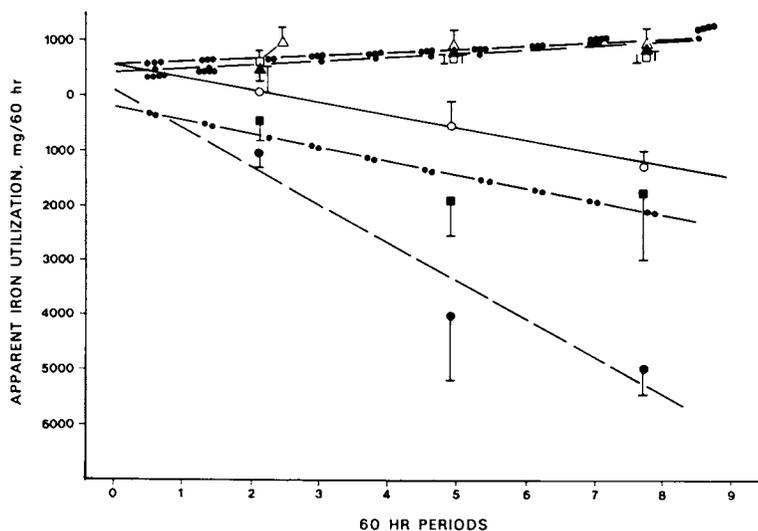
Figure 5. *Apparent utilization of Fe by rats consuming three dietary fibers at two levels. Key is the same as in Figure 2.*
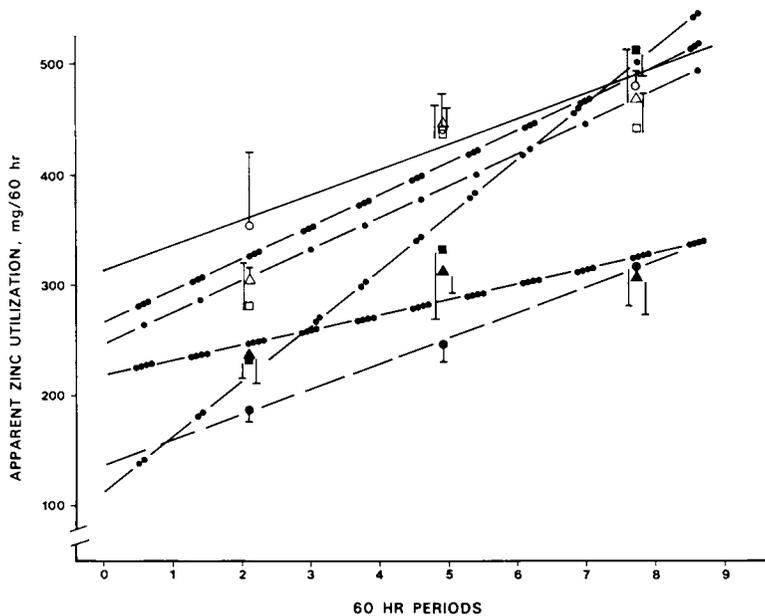
Figure 6. Apparent utilization of Zn by rats consuming three dietary fibers at two levels. Key is the same as in Figure 2.
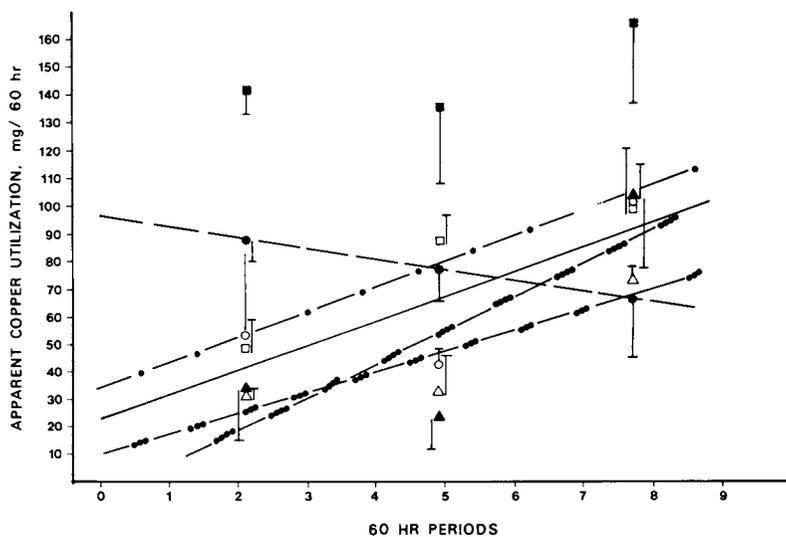
Figure 7. Apparent utilization of Cu by rats consuming three dietary fibers at two levels. Key is the same as in Figure 2.

Table 5. Mean apparent element absorption among animal groups
consuming chitin, chitosan or cellulose at levels of 10 and 20%.[1]

Dietary fiber	Level %	Number of animals	P	Ca
			--------------- mg/60 hr^2 ---	
Chitin	10	5	$174.1 \pm 3.6^{a,b}$	$96.0 \pm 4.8^{a,e,f}$
	20	5	$166.3 \pm 3.4^{a,b}$	$122.2 \pm 4.5^{b,c}$
Chitosan	10	4	130.0 ± 3.8^{b}	$110.7 \pm 5.1^{b,c}$
	20	3	101.8 ± 5.0^{c}	$141.3 \pm 6.5^{c,d}$
Cellulose	10	4	156.0 ± 3.4^{d}	$87.2 \pm 4.5^{a,e,f}$
	20	5	152.0 ± 3.8^{d}	$90.5 \pm 5.1^{a,e,f}$
Chitin vs chitosan[4]			**	*
Chitin vs cellulose[4]			**	*
Chitosan vs cellulose[4]			**	**

[1] Mean of three balance periods.

[2] Mean ± S.D.; values in same column not sharing a common superscript
are significantly different (P 0.05).

[3] Values in parenthesis are negative.

[4] Pooled diet comparisons; *(P<0.05); **(P<0.01).

Mg	Fe	Zn	Cu
-----------------	-------------------- ug/60 hr^{2,3} ---------------------		
7.5±0.5^{a,e,f}	(556)±285^{a,d}	424±14^{a,c,e}	66±9^{a,b,c,e,f}
5.1±0.5^{b}	(3,341)±2,063^{b}	249±13^{b,e,f}	77±9^{a,b,c,f}
13.3±0.5^{c}	771±300^{c}	385±15^{a,c,d,e}	79±10^{a,b,c,f}
10.2±0.7^{d}	(1,374)±388^{a,d,e,f}	358±19^{c,d}	196±13^{d}
7.5±0.5^{a,e,f}	843±269^{c,e,f}	407±13^{a,c,e}	46±9^{a,e,f}
7.2±0.5^{a,e,f}	791±301^{c,e,f}	284±15^{b,f}	54±9^{a,b,c,e,f}
**	**	*	**
N.S.	**	N.S.	**
**	**	N.S.	**
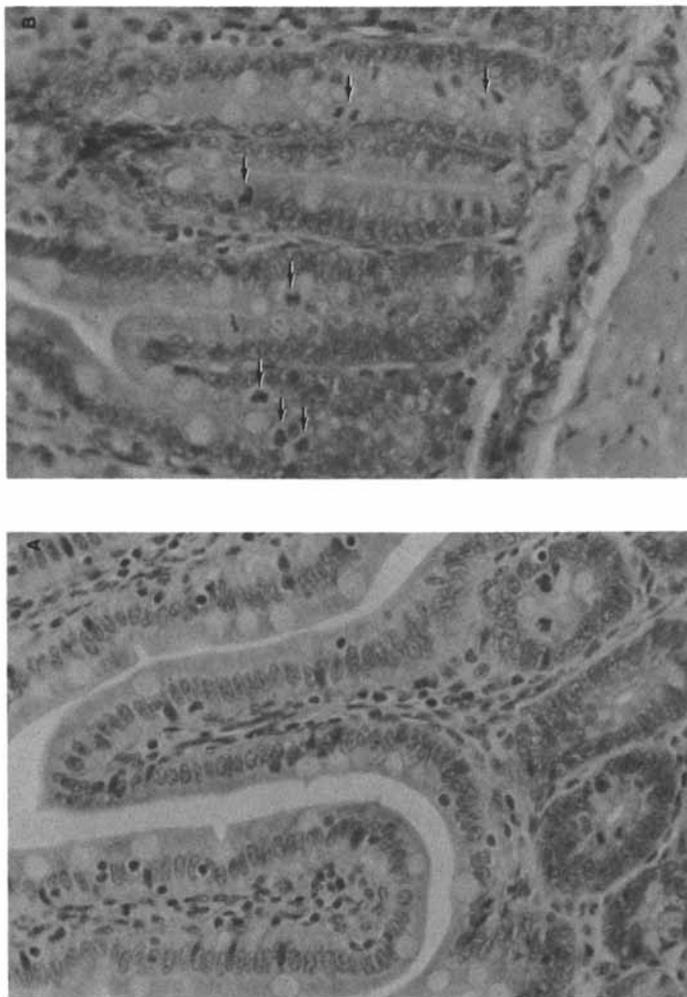
Figure 8. Photomicroscopy of duodenum sections from two rats, one consuming 20% cellulose (A) and the second, 20% chitosan (B) 220×. Cross-section of crypt and longitudinal section of villus illustrated in A, and longitudinal section of crypt area only illustrated in B. Arrows indicate mitotic figures.

Table 6. Inflammatory cell infiltrate of intestinal tracts from
rats consuming chitin, chitosan or cellulose at levels of 10 and 20%.

Dietary fiber	Level %	Duodenum	Jejunum	Ileum	Cecum
		---------- Neutrophils/HPF[1,2,3] -------------			
Chitin	10	3.7±1.1	3.6±0.8	5.4±1.4	4.9±2.1
	20	2.7±1.3	3.3±1.4	3.5±1.4	2.5±1.0
Chitosan	10	6.3±2.3	5.8±1.1	4.9±1.1	4.4±1.4E
	20	3.3±1.1	4.6±1.6	6.1±1.6	8.0±2.6E
Cellulose	10	3.7±1.3	3.7±1.1	3.3±0.8	4.1±1.2
	20	7.1±1.4	3.6±1.3	2.8±1.0	3.7±1.4

[1]See Material and Methods for explanation of determining number of
neutrophiles per high powered field (HPF).

[2]Mean ± S.D.; differences between means were not statistically
tested.

[3]E = edema.

Table 7. Mitotic activity in crypts of intestinal tracts from
rats consuming chitin, chitosan or cellulose at levels of 10 and 20%.

Dietary fiber	Level %	Duodenum	Jejunum	Ileum	Cecum
		------- Mitotic figures/HPF[1,2,3] ----------			
Chitin	10	3.0±1.0	3.2±1.0	3.4±1.0	1.1±0.6
	20	4.1±1.4	3.7±1.3	3.4±1.3	0.7±0.6
Chitosan	10	6.5±1.2	6.7±1.1	3.9±0.9	1.3±0.9E
	20	6.1±1.5	5.0±1.5	6.9±1.9	1.0±0.7E
Cellulose	10	2.8±1.0	3.5±0.9	3.2±1.2	0.7±0.7
	20	4.7±1.4	4.5±1.3	3.3±0.9	1.0±0.7

[1]See Material and Methods for explanation of determining number of
mitotic figures per high powered field (HPF).

[2]Mean ± S.D.; differences between means were not statistically tested.

[3]E = edema.

lacteal dilation or edema of the lamina propria of the cecum
(Figure 9B). This lesion was not observed in ceca of the ani-
mals in the 20% chitin or cellulose groups (Figure 9A). For
all animals, the entire small intestine showed the greatest
changes with respect to dietary fiber.

Two bacteria isolated from the ceca of all test animals
were Escherichia coli and Enterobacter sp. No pathogenic bac-
teria (e.g. Salmonella typhimurium) were identified. The
absence of this latter organism helped rule out enterotoxemia
as a cause of death in one animal and impaired growth in a
second animal consuming 20% chitosan in their diets. Chitosan
and chitin did not appear to change the intestinal flora pop-
ulation either quantitatively or qualitatively. Screening of
anaerobic bacteria was not performed.

Gross and histopathological examination of lungs, kidneys,
livers and salivary glands from all animal groups revealed no
evidence of naturally occurring infectious diseases.

Discussion

Chitosan is being considered as a possible blood choles-
terol lowering agent. Only at levels above 5% in the diet of
rats does chitosan and its parent compound chitin appear to
cause harmful effects. As commercially available, neither com-
pound can be considered homogenous. Extensive cleaning and
standardization of both materials, and specifically chitosan,
may be necessary for future evaluation. Results observed in
this study can possibly be attributed to some unknown consti-
tuent(s) in chitin or chitosan, as may be the case when using
natural products.

Animals ingesting chitin and chitosan at levels of 5, 10
and 20% did not show any significant decrease in growth after
23 days compared to animals fed cellulose at equivalent levels.
Earlier reports have indicated impaired growth in rats when
levels of chitosan in the diet exceeded 10% (4, 21).

At levels of 5%, different particle sizes of chitin or
chitosan did not alter element absorption to any significant
degree. Animals were in positive balance for each of the six
elements examined. Body element composition varied, but, again,
differences were small. At the 5% level in the diet and below,
further long term studies are needed to establish if chitin or
chitosan have any adverse effects on animal health.

Originally this experiment was designed to determine the
degree of positive or negative absorption of elements as
influenced by chitin and chitosan. Dietary element levels
could not be standardized among diets because of the variation
in amounts provided by the test DF sources. Although diets
were adequate in all elements, differences observed in positive
balance values may only reflect differences in amounts con-
sumed. An example of the difference between dietary levels and
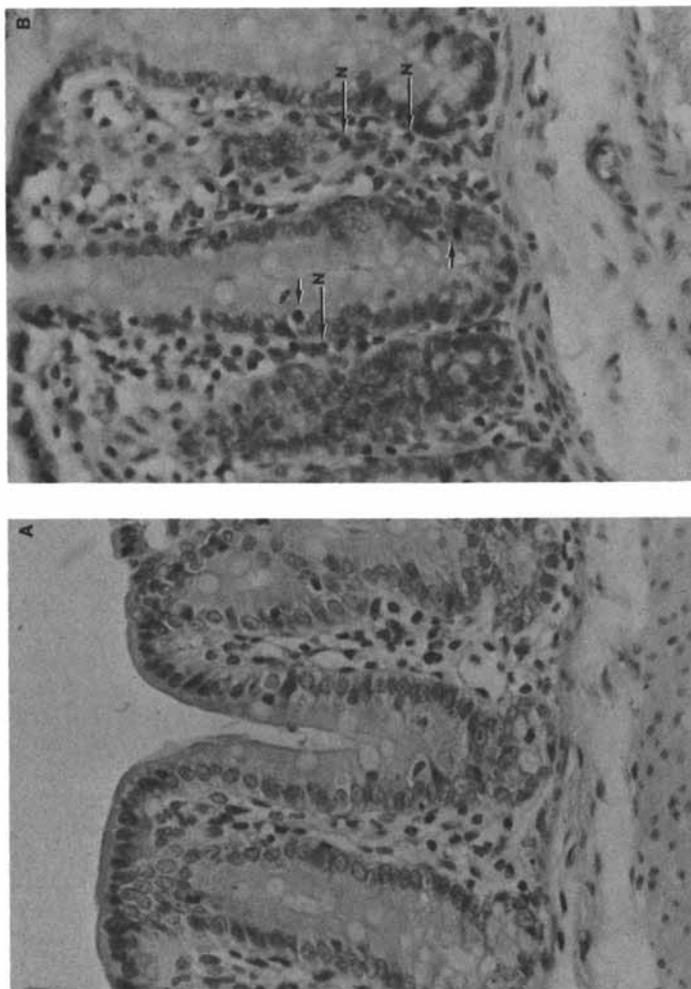
Figure 9. Photomicroscopy of longitudinal sections of cecum from two rats, one consuming 20% cellulose (A) and the second, 20% chitosan (B) 220×. Arrows with N indicate neutrophils, short arrows indicate mitotic figures.

absorption is discussed for Ca. Animals consuming 20% chitosan had the lowest mean absorption of Ca (102 mg/60 hr) while dietary levels were the highest (11.24 mg/g). Dietary Ca levels in the 20% cellulose control diets were lowest (4.95 mg/g), but absorption was significantly higher (152 mg/60 hr) than in the 20% chitosan fed animals. Since both diets contained the same amount of AIN salt mixture, it appears that chitosan may impair Ca absorption. However, caution must be exercised in making this assumption. Growth was lower in animals fed chitosan (20%) and this may account for the lower needs as measured by balance in this study. Further work is needed to evaluate the accuracy of balance techniques in measuring element absorption at low, adequate and excessive element intakes as employed in this study.

For the elements P, Ca, Mg, Zn and Cu, variation in apparent absorption levels existed among animal groups ingesting chitin, chitosan or cellulose at various levels. A clear trend of one DF source exhibiting an inhibitory or enhancing effect on the absorption of any one of these 5 elements was not clearly delineated. Differences existed in absorption of these 5 elements, but none of these changes resulted in animals being in marginal balance or even near negative balance.

The most striking result of this study was the consistently negative Fe balance over time observed in three of the four animal groups fed chitin or chitosan. The positive Fe balance in which animals consuming 10% chitosan maintained themselves contradicts the observations found with the other three diets. This cannot be explained considering other information obtained from the same group. Since all test animal groups had lower hemoglobin levels than cellulose controls (Table 8), the 10% chitosan fed animals would also have been expected to be in negative Fe balance.

Conflicting results were also observed in animals consuming 20% chitin. Their hemoglobin value (13.7±1.1 g Hb/dl) would have been expected to be lower considering the manner in which animals voided Fe via fecal excretion over time (Figure 5). At the rate of Fe loss observed, total depletion of Fe was probable after 23 days on the diet or shortly thereafter.

During the course of Expt. 2, one animal on a chitosan diet at the 20% level died on day 6. Histological observations of the intestinal tract were inconclusive because of the time delay between death and examination (ca. 12 hr). Microbiological survey of internal organs indicated the presence of Proteus sp in the lung and extensive septicemia. This latter condition was diagnosed as the cause of death. A second animal in this group began to show signs of decreased food intake and growth. The animal was maintained on the diet until euthanized and immediately necropsied on day 9. Gross pathology indicated the intestine to be greatly distended with very firm luminal contents appearing to produce constipation. Mild enteritis and

Table 8. Final hemoglobin level in animals
consuming chitin, chitosan or cellulose at levels of
10 and 20% for 23 days.

Dietary fiber	Level %	No. of animals	Hemoglobin g/dl[1]
Chitin	10	5	12.7 ± 0.6^a
	20	5	13.7 ± 1.1^a
Chitosan	10	4	13.5 ± 0.9^a
	20	3	13.5 ± 0.9^a
Cellulose	10	4	14.5 ± 0.7^b
	20	5	14.4 ± 1.1^b

[1]Mean ± S.D.; values not sharing a common superscript
are significantly different ($P < 0.05$).

cecitis were observed, apparently caused by the physical nature of the diet. The swelling and viscous properties of chitosan in acid have been documented (29) and are considered to be some of the major factors contributing to the morbidity and mortality in animals eating this compound. Gross and histological changes observed in the animal examined on day 9 were similar to those seen in animals sacrificed on day 23.

It is not known when, or at what rate, morphological changes take place in the intestine upon ingestion of any DF. However, adaption of the gut to the type and level of DF may account for some of the conflicting results in Fe balance and hemoglobin levels previously mentioned. Early insult to the intestinal wall could lead to immediate interference with Fe absorption and negative balance as was observed with three test diets but not the fourth (i.e. 10% chitosan). Longer feeding trials with balance measurements may be necessary to ascertain if Fe status will be compromised in animals consuming cellulose at high levels. It would appear advantageous to examine the rate of intestinal cellular change in greater detail as well as the ability of this animal model to adapt to type and level of DF.

Histopathologic examination of the intestinal tract of all animals, but especially those consuming chitosan, indicates a possible mechanism to explain the results observed with Fe absorption. The physical nature of chitosan, and to a lesser degree, chitin, when ingested at levels above 5%, seemed to produce a mechanical abrasive effect on the intestinal mucosa. In addition to the increased number of neutrophils in the intestinal lamina propria, the active mitoses of the crypt epithelial cells suggested premature loss of cells from the villi. If the "intestinal mucosal block" (30, 31) mechanism was impaired, this could explain both the animals inability to absorb dietary Fe plus the negative Fe balance observed. Immature mucosal cells do not have sufficient Fe levels to regulate Fe intake from the gut lumen and their early sloughing leads to continued loss of Fe supplied from body stores. In support of this theory, the results of a scanning electron microscopy study which evaluated the topographical changes of the jejunum and colon of rats consuming various types of fiber suggested that alterations in the gut ultrastructure caused by DF may be associated with altered bile acid reabsorption (32).

The chemical properties of chitosan and chitin may provide another mechanism for decreased element absorption and specifically Fe. Chitosan differs from chitin in having free amino groups causing partial solubilization in stomach acid and resulting in viscous intestinal lumen contents. This chemical change does not result with chitin ingestion to any significant degree. However, since chitin may contain some free amino groups, the action of this functional group on the gut needs to be examined further. The free amino groups of chitosan are

believed to bind bile acid and/or alter micelle formation (4).
Through one or both of these mechanisms, consumption of chito-
san by rats lowers their blood cholesterol (4, 16, 17). Be-
cause of the viscous nature of the intestinal contents when
chitosan is partially dissolved with stomach acid, some type of
element "trapping" could be envisioned in the intestine. In
this respect, Fe may be prevented from reaching absorptive sites.
However, Fe would have to be selectively "trapped" or chelated
since the utilization of other elements were not impaired to the
same degree as observed with Fe. An interpretive finding of
this study may be supportive evidence of how certain fibers, at
high levels of consumption, are responsible for impaired Fe
uptake observed in dogs (33) and human experiments (34, 35, 36).

Conclusion

Chitin and chitosan at very high levels of intake may cause
physical insult to the intestinal tract of rats. Also,
apparent absorption of Fe is impaired when chitin and chitosan
are fed at dietary levels of 10% and 20%. However, such high
levels of intake are atypical. At dietary levels below 5%,
neither compound appears to compromise element absorption.

Acknowledgments

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Citrus Pectic Polysaccharides—Their in Vitro Interaction with Low Density Serum Lipoproteins

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Dietary fiber," the skeletal remains of plant cells that
are resistant to digestion by enzymes of man" (1) include at
least five different known components: lignin, cellulose,
hemicellulose, gums and pectins. Lignin is a non-carbohydrate
component and appears to have no known role in human nutri-
tion. Cellulose and hemicellulose affect large bowel function
and cause an increase in stool weight and decrease in transit
time. Gums and pectins can also increase intestinal transit
time, adsorb fecal water, influence glucose and lipid metabol-
ism, mineral absorption and, in addition, may possibly play a
role in colon cancer.

With the exception of cellulose, a polymer of glucose, the
chemistry of hemicelluloses, gums and pectins is extremely
complex (2). Pectin, a heterogeneous mixture of a number of
complex pectic polysaccharides, appears early during plant cell
wall biogenesis. A series of complex biochemical steps result
in the formation of cell plate followed first by its growth in
area (primary cell wall) then in thickness (secondary cell
wall). Exclusive of randomly oriented cellulose fibrils, pri-
mary cell wall is composed mainly of pectic polysaccharides
(3). These pectic polysaccharides are rich in D-galacturonic
acid, D-galactose and L-arabinose residues. With growth in
thickness, there occurs a replacement of pectic polysaccharide
deposition with polysaccharides rich in D-glucuronic acid, D-
xylose and D-glucose residues. These polysaccharides which add
to the growth in thickness of the plant cell wall are
classified as alkali-soluble hemicelluloses. Previous studies
(4) the chemistry of pectin suggested the presence of three
major polysaccharides in pectin, namely 1) a glacturonan poly-
mer containing 1,4 linked α-D-galacturonic acid residues, 2) a
galactan polymer containing chains of 1,4 linked α-D-galacto-
pyranose residues and 3) and arabinan a branched polymer con-
taining 1,5 and 1,3 linked L-arabinofuranose residues. Com-
paratively recent studies (2-3,5-6), however, suggest that the
chemistry of pectin is much more complex than previously

believed. Presence of covalantly-linked neutral sugars especi-
ally galactose, rhamnose and arabinose to polymers of galactur-
onic acid residues was demonstrated. Also, demonstrated was
the presence of L-fucose, D-apiose and D-xylose covalently
linked to three major polysaccharides, i.e., galacturonans,
arabinans and galactans as mentioned earlier. The demonstrated
covalently-linked amino acids such as hydroxy proline within
the structural framework of various pectic polysaccharides
added further complexity to the chemistry of pectin.

     Cholesterol Lowering Influence of Pectin: Several
investigators (7-9) have clearly suggested that pectin when
supplemented in the diet of laboratory animals and/or human
volunteers causes significant reduction in serum cholesterol
levels. This, indeed, is an important observation, as it has
been shown that the etiology of atherosclerosis and coronary
artery disease is associated with elevated serum cholesterol
levels, and the observed reduction of serum cholesterol level
by pectin holds promise for the treatment and/or prevention of
these diseases. It is relevant here to point out that dietary
pectin has shown to retard induced avian atherosclerosis (10).

     Pectin Bile Salt Interaction. The biochemical basis by
which pectin causes lowering of serum cholesterol level remains
elusive. It has been suggested that dietary fibers in general
cause lowering of cholesterol levels by binding with bile salts
and consequently causing a break in the enterohepatic circula-
tion of bile acids and increasing cholesterol turnover (11-
13). This explanation is similar to one where cholestyramine,
an ion exchange resin (Dowex 1x2 Cl⁻), used as a drug (Cuemid,
Questran, Quantalan) to affect the lowering of serum choles-
terol levels when given to patients with hypercholesterolemia.
Critical evaluation of data suggesting the binding of bile
salts to dietary fiber reveals that the observed in vitro affi-
nity of various dietary fibers to bile acids is much lower when
compared with binding of bile acids with cholestyramine. The
in vitro affinity of cholestyramine for acids is 1.0 mg bile
acid/mg of resin estimated at pH 6.4 in 0.9% saline (14). On
the other hand, the in vitro binding of bile acids to fibers
such as wheat straw, sugar cane pulp, bran, oat, etc., ranges
from 0.007 mg to 0.024 mg of bile acid per mg of fiber in nor-
mal saline. These values amount to 41-142 times less than the
affinity of bile acids to cholestyramine. Although alfalfa,
guar gum and pectin, unlike the various fibers mentioned above,
showed better affinity for bile acids, this affinity is about 5
times less than the affinity of bile acids to cholestyramine.
For a physicochemical phenomenon to be of biological signifi-
cance, the reactants mush exhibit a stoichiometric
relationship. In the studies mentioned above (12,13), although
increasing the amount of various fibers in the incubation

mixture did cause an increase in the amount of bile acid bound
to the various fibers studied, this observed increase was not
stoichiometric. Furthermore, no binding of bile acids to puri-
fied dietary fibers was observed by these investigators. It is
of interest to mention the results of a recent study (15) which
failed to demonstrate the in vitro binding and/or interactin of
pectin with bile acids when examined by utilizing NMR spectro-
scopy. The observations made by these investigators are simi-
lar to those where interaction of bile acids and dietary fibers
from a variety of sources was studied in vitro by utilizing
labelled bile acids, equilibrium dialysis and/or centrifugation
techniques (16). Increased excretion of bile salts has also
been presented as an evidence for the binding of bile acid to
dietary fibers consequently resulting in hypocholesterolemia
(17,18). However, in a recent study (19) where fecal bile acid
excretion was studied in response to intake of a variety of
fibers, no significant alterations in the fecal bile acid con-
tent was observed. It is evident from the above that our
current understanding of the biochemical basis by which dietary
fiber causes lowering of cholesteol levels is far from com-
plete, and therefore it needs to be researched and re-
evaluated.

     Pectin Lipoprotein Interaction. Cholesterol in serum is
not found free but is carried by a number of lipoproteins found
in serum. Low density lipoprotein (LDL) is the major carrier
of circulating cholesterol. Elevated levels of LDL in the
serum contribute significantly to the risk of coronary heart
disease, and development of atherosclerosis. Atherosclerotic
lesions are characterized by intimal proliferation of smooth
muscle cells accompanied by accumulation of large amounts of
connective tissue components such as collagen, elastin and
glycosaminoglycans. In atherosclerosis, presence of large
amounts of LDL and glycosaminoglycan complexes in plaques have
been demonstrated (20,21). Glycosaminoglycans, like pectin,
are polyanionic complex carbohydrates found in the connective
tissue of animals.
     In view of the fact that pectin lowers serum cholesterol
levels and cholesterol is carried by lipoproteins in the serum,
we investigated the interaction of pectin obtained from grape-
fruit albedo with various human serum lipoprotein fractions in
vitro (22). It was observed that pectin interacted specifical-
ly with LDL, while no interaction was observed between pectin
and high density or very low density lipoproteins. The biolog-
ical significance of this observed in vitro interaction of
pectin with LDL remain unclear, primarily because of lack of
evidence suggesting entry of pectin or some component of it
into the blood streatm. Therefore, unless such entry and/or
absorption of pectin or one of its components is demonstrated,
the biological implication of this observed interaction

resulting in the formation of pectin-LDL complexes and the
metabolic fate of such complexes if found remains hypothetical
in nature.

Our ongoing studies aimed to gain an insight into the bio-
chemical basis of this observed interaction between LDL and
pectin have revealed that selective modification of basic amino
acid residues such as histidine, arginine and lysine, found in
LDL caused a significant loss of interaction between pectin and
LDL. These results suggest that various basic amino acids are
involved in the observed interaction between pectin and LDL (23).

Results from our recent experiments designed to evaluate
the comparative effectiveness of cholystyramine and pectin in
lowering serum cholesterol levels in rats (24), clearly suggest
that pectin is comparatively as effective as cholestyramine in
lowering serum cholesterol levels and this observed lowering of
serum cholesterol by pectin is mediated through an exclusive
lowering of cholesterol associated with LDL. Selective lower-
ing of LDL by pectin (25) and by other dietary fibers such as
oat bran (26) has also been reported by other investigators.
Based on these observations, it could be concluded that the
mediation of serum cholesterol lowering effect of pectin is
accomplished by lowering of cholesterol associated with serum
low density lipoproteins by mechanisms which remain elusive.
Furthermore, experimental attempts to elucidate the possible
mechanism by which pectin causes lowering of cholesterol level
must take into account the heterogeneous nature of pectin, for
a unique pectic polysaccharide found in pectin may be solely
responsible for the observed hypocholesterolemic activity of
pectin.

Selective Interaction of various Pectic Polysaccharides
with Low Density Lipoproteins. As mentioned earlier, in vitro
interaction between unfractionated pectin and LDL results in
the formation of insoluble complexes (22). Further insight
into this observed interaction was gained by examining this
interaction between isolated pectic polysaccharides found in
pectin and human LDL. Various pectin polysaccharides found in
citrus pectic were resolved by employing DE-52 cellulose ion
exchange column chromatography techniques (27). Four chemical-
ly distinct pectic polysaccharides were resolved following
sequential elution of column bound pectin with 0.025, 0.1, 0.25
and 0.5 M sodium phosphate buffer, pH 6.0, and composed of 13%
(Fraction I), 5% (Fraction II), 62% (Fraction III), and 20%
(Fraction IV), respectively, of the total pectin subjected to
ion exchange chromatography. The galacturonic acid contents of
these individual pectic polysaccharide fractions were not stri-
kingly different and ranged between 70-80% of the total car-
bohydrate contents. However, the neutral sugar composition of
these pectic polysaccharides was found to be different. A pro-
gressive decrease in the degree of methylation of these pectic

polysaccharides was also observed in that pectic polysaccharide eluted with 0.1 M salt (Fraction II) was highest in its methoxy content (14.85%), whereas pectic polysaccharide eluted with 0.5 M salt (Fraction IV) was lowest in its methoxy content (4.42%). The methoxy content of Fraction I, the fraction eluted with 0.025 M salt, and not retained by ion exchange resin was found to be similar to unfractionated pectin (8.9%). Examination of neutral sugar composition of various pectic polysaccharides revealed that Fraction I and Fraction IV were rich in rhamnose content, when compared with other pectic poly-saccharides (28).

Examination of interaction between these pectic polysac-charide fractions and LDL in vitro by employing experimental techniques described earlier (22), revealed the selective na-ture of this interaction. Pectic polysaccharides I and IV, i.e., polysaccharides rich in rhamnose content were the only polysaccharides which interacted specifically with human LDL, causing their precipitation in vitro. No or little interaction between LDL and pectic polysaccharide Fractions II and III was observed. These observations suggest that pectic polysaccha-rides rich in rhamnose sugar residues, specifically interact exclusively with human LDL, and this interaction appears to be independent of the methoxy content of the polysaccharides.

In summary, studies demonstrating in vitro interaction between a variety of dietary fiber and bile acids (12,13) may be of importance in part in explaining the biochemical basis by which ingestin of dietary fiber causes lowering of serum cho-lesterol levels. However, in view of results obtained from comparatively recent in vitro (15,16) and/or in vitro (19) studies indicating lack of interaction between dietary fiber and bile salt, the existance of a much more complex mechanism by which dietary fiber causes lowering of serum cholesterol level could not be overruled. The finding of specific in vitro electrostatic interaction between pectin and human low density lipoprotein (22) adds further complexity to the fiber and serum cholesterol connection. Further complexity is added to this connection by observations suggesting heterogeneous nature of dietary pectin, and selective interaction of rhamnose rich pectic polysaccharides with human low density lipoprotein. It is, therefore, safe to conclude that, only through studies aimed towards defining the psysicochemical nature, metabolism and cholesterol-lowering capability of well defined individual polysaccharides found in dietary fiber, can an insight into the elusive biochemical basis by which dietary fiber causes lower-ing of serum cholesterol be gained.

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Washed Orange Pulp: Characterization and Properties

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Hamlin and Valencia washed orange pulp from
several crop years were dried and analyzed.
The range in values of total pectin, neutral
detergent fiber and crude fiber were
26.0-45.6%, 15.8-31.0% and 9.9-20.6% respec-
tively. In addition, detailed nutrient and
dietary fiber analyses were obtained for
selected samples. Functional properties
such as flavor muting, chromatophore
formation and oil holding properties were
noted. Food processing modification yields
a novel form of the hydrated pulp with
extended functional properties.

Interest in dietary fiber has refocused attention on a
known but underutilized fiber source: <u>washed orange pulp</u>.
Its precursor, orange pulp, is that tissue produced upon
expression of orange juice from the juice vesicles. With
further extraction(s) of residual soluble sugars from the
pulp, an osmotic re-equilibrium occurs to yield the <u>washed</u>
orange pulp. This washed orange pulp is obtained as a
hydrated complex of vesicular carbohydrates at 4-10% solids.
As a byproduct of the juice concentrate industry, an estimated
500,000 to 1,000,000 tons of wet pulp are produced each crop
year. Unlike suggested more exotic sources of dietary fiber,
orange pulp is already an established, commonly consumed food
component in the American diet. The functional and nutrient
properties of washed orange pulp are intimately related to a
series of factors. The foremost of these is the orange species
but also significant are climatic and crop year variants,
harvest maturity, expression and extraction processing
variables, uncontrolled enzymatic and microbial activity as
well as method of drying. Because of variations in the
material, application and development as a fiber has been
limited.

Methods and Materials

     Valencia and Hamlin washed orange pulp were obtained
from central Florida cooperatives in the 1978, 1979 and 1980
crop years. The pulp was steam injected to give a minimum
temperature of 95°C, then packed and frozen. Samples were
thawed immediately before experimental use. Samples of drum
dried, freeze dried and spray dried washed orange pulp were
prepared in the pilot plant facilities of the General Mills
James Ford Bell Technical Center as needed. Compositional and
nutrient analyses were performed by Medallion Laboratories
according to standard AOAC and AACC procedures. Sugar
analyses were determined by HPLC according to DeVries
et al. (1). Water holding (and oil holding) capacities
were determined according to Porzio and Baumann (2). In
this procedure, a weighed sample, approximately 1 gram and
of uniform particle size, was suspended in an excess of liquid,
stirred at ambient temperature for 30 minutes and transferred
to a vacuum filtration apparatus. Excess liquid was removed
by filtration at 60-100 mm Hg pressure for 3 minutes and the
weight gain of liquid reported as water (or oil) holding
capacity in grams per gram of sample.
     Total pectin is the sum of soluble pectin and protopectin.
Soluble pectin was determined by the following procedure:
macerated tissue was extracted at 80-90°C in distilled water
for 1 hour, the supernatant separated by centrifugation and
an additional extraction-centrifugation step repeated. The
combined supernatants were brought to 0.5 $\underline{N}$ NaOH, saponified
for 4 hours at 26°C, neutralized with acetic acid to pH 5 and
precipitated as the calcium pectate at a final calcium
concentration of 0.3% (w/v) by addition of $CaCl_2$. The heat
flocculated precipitate was filtered on a glass wool support
in a 50 ml fritted crucible, rinsed free of calcium salts
with distilled water, then washed with acetone to remove
remaining lipids and moisture. The precipitate was dried at
70°C in a vacuum oven overnight and reported as calcium
pectate. Protopectin was determined on recovered fiber
solids from the soluble pectin determination. Fiber solids
were hydrolyzed in 0.15 $\underline{N}$ HCl for 1 hour at 80°C. The sample
was centrifuged and supernatant retained, tissue rewashed
with distilled water and centrifuged. The combined super-
natants were neutralized to pH 7, saponified as described
above and calcium pectate determined. The acid extracted
solids fraction was adjusted to 0.05 $\underline{N}$ NaOH and saponified
for 4 hours at 26°C. After the pH was brought to 5 with
acetic acid, the sample was centrifuged to yield neutralized
supernatant which was then precipitated at 0.3% calcium
chloride. The two precipitates from the acid and base
extractions were combined, rinsed with distilled water,
dried, and reported as protopectin.

Solvent extractions were performed by treatment of
100-150 grams of 93% moisture washed orange pulp in a Soxhlet
apparatus charged with 600 ml of acetone or isopropanol. The
extraction was continued 3-6 hours by which time no additional
yellow pigment could be observed in the extracting solvent.
The tissue mass was dried at ambient temperature until no
detectable residual solvent was evident. Aqueous extractions
of the 93% moisture washed orange pulp were performed with
0.4 $\underline{M}$ sodium citrate, 0.2 $\underline{M}$ EDTA, 0.1% H_3PO_4, 0.5% Na_2CO_3 and
5% $\overline{Na}OH$ solutions in respective experiments. In each case
200 ml of extracting solution was added to 100 grams of pulp
and stirred for three hours of 25°C. After filtration the
extracted tissue was washed three times with 200-250 ml of
50°C distilled water and filtered. Those samples for OHC or
WHC were then freeze dried. Samples for microscopic examina-
tion were kept in the hydrated state.
     Soluble and insoluble dietary fiber analyses were
performed according to Furda (3).
     Phenolic(s) were analyzed colorimetrically utilizing
reagent 2,6-dibromo-N-chlorobenzo-quinoneimine. Catechin was
the reference standard and results are reported as mg "catechin
equivalent" per gram sample.

Results and Discussion

Washed orange pulp, obtained from FMC juice finishers is
a relatively homogeneous fraction of the juice vesicle (4).
This material also contains chromoplast lipids and
cytoplasmic materials such as proteins, bioflavanoids,
soluble carbohydrates, limonoids and hydrolytic enzymes (5).
The processor can control the uniformity of the material but
care is necessary to prevent indigenous pectinesterases from
rapidly altering the character of the tissue. Microbial
activity can also lead to undesirable changes. Therefore
steam injection to pasteurize and stabilize the material is
extremely desirable. Washed orange pulp has a membranous
structure which is shown in Figure 1A. A view of the surface
is presented in Figure 1B where the fibrillar organization of
the cell wall pectins is evident at the higher magnification.
Figure 1C shows an SEM photomicrograph of an identical sample
of washed orange pulp.
     Hamlin and Valencia washed orange pulps were obtained
and analyzed and the results are presented in Table I.
Because of low solids content of wet pulp, analyses were
performed on the drum dried product and the reported moisture
level reflects the processing conditions of drying. The
change in tissue morphology upon drum drying is shown in SEM
photograph Figure 1D where the surface results from contact
of the pulp and its soluble solids with the hot drier surface.
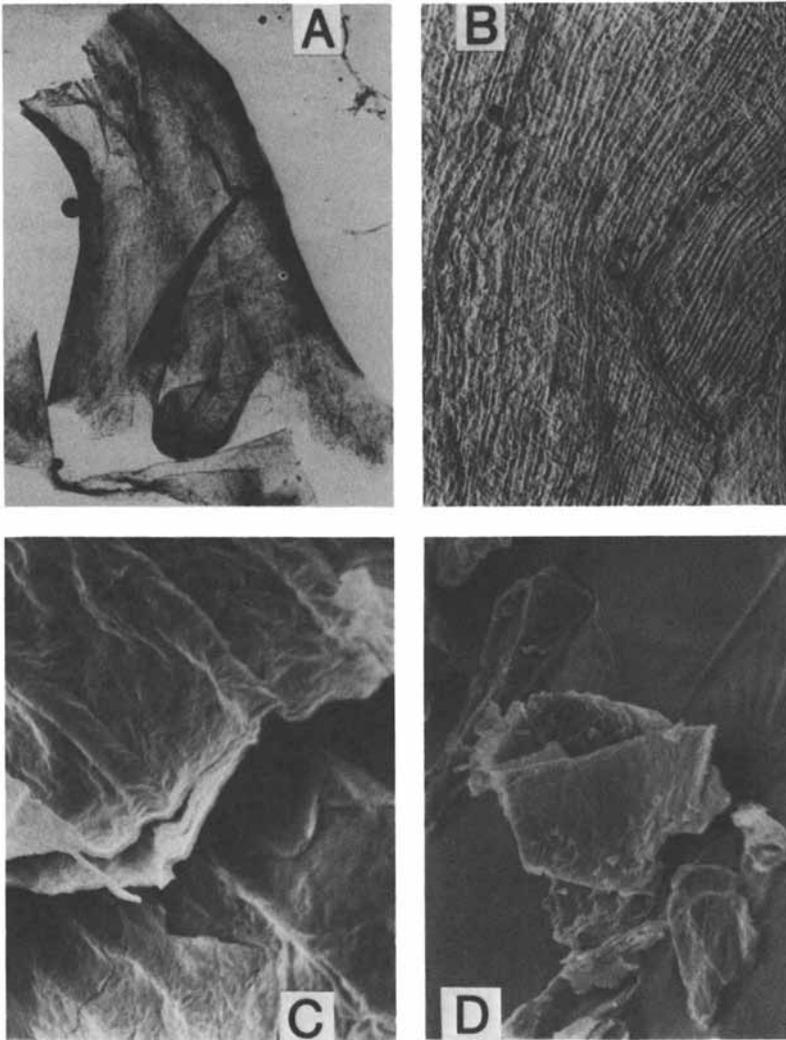
Figure 1.   Washed orange pulp (juice vesicles). Key: a, whole juice vesicle, 13✕; b, enlarged view of vesicle surface showing pectin ridges, 140✕; c, scanning electron microscopy (SEM) of untreated juice vesicle, 200✕; and d, scanning electron microscopy (SEM) of drum-dried washed orange pulp, 220✕.

TABLE I

COMPONENT COMPOSITION OF WASHED ORANGE PULP (a,b)

| | ORANGE VARIETY | | | |
| | (YEAR) | | | |
	HAMLIN (1978-79)	HAMLIN (1977-78)	VALENCIA (1978-79)	VALENCIA (1979-80)
MOISTURE	2.89	3.00	9.67	7.44
PROTEIN (N*6.25)	8.89	9.63	7.82	7.47
SUGARS, TOTAL	35.51	11.11	18.91	8.59
SUGARS, REDUCING	18.10	7.92	9.83	5.30
FAT	0.91	0.47	0.84	0.73
ASH	3.27	1.85	2.08	3.11
NDF	15.80	26.08	22.94	31.02
ADF	13.76	-	-	-
CRUDE FIBER	9.92	16.88	15.40	20.55
PECTIN, TOTAL	26.00	38.84	34.39	45.63
PECTIN, PROTO-	17.89	26.31	28.29	36.46
PECTIN, SOLUBLE	8.11	12.53	6.10	9.17

(a) Grams/100 grams.
(b) Samples were drum dried and milled before
    analysis; NDF, neutral detergent fiber; ADF,
    acid detergent fiber

    Analyzed sugar values mainly reflect the efficency of an
individual processor in removing the soluble juice sugars.
The 8.6% sugar value probably represents the lowest practical
limits of commercial processing efficency and economics. In
Table II the component compositions of Table I have been
recalculated to a moisture and sugar free basis. Agreement
of pectin, protein and neutral detergent fiber values now
reflect inherent insoluble cell component composition. A
more accepted method in the analysis of fiber components for
the sugar rich washed orange pulp utilizes preparation of
alcohol insoluble solids as reported by Ting (6). In our
case the drum dried pulp is more representative of a food
fiber ingredient and so analyzed. Dietary fiber composition
of washed orange pulp by the method of Furda (3) yields both
soluble and insoluble dietary component. These values are
found in Table III along with selected reference fiber
materials. A more complete nutrient analysis of drum dried
washed orange pulp is presented in Table IV along with a
reference fiber-wheat bran. The significant difference
between these two fibers is noted in the large component of
starch and low pectin in bran as compared to a much greater
equivalent of pectin of the pulp. Two heat sensitive
vitamins, A & C, were determined additionally on a freeze
dried washed orange pulp sample. The expected larger values
reflect original levels in the tissue before drum drying.
    Pectin has become a subject of increasing interest with
elucidation of the role of dietary fiber in cholesterol
reduction (7). As noted, washed orange pulp is a rich source
of pectin(s). We have reported pectin as soluble pectin as
well as protopectin, i.e. large molecular weight, methoxylated
insoluble pectin. In our procedure protopectin values may
include some hemicelluose material as a function of the
analysis. Distinction between the soluble and protopectin
can be seen with microscopic examination of extracted tissue.
Figure 2A shows a washed orange pulp juice vesicle after mild
extraction in dilute sodium carbonate solution at ambient
temperature. Degredation of the pectinaceous fibril surface
is evident. There is retention of the inherent tissue
organization even while segments of soluble pectin are
removed. Upon more rigorous extraction with 5% NaOH, complete
loss of pectinaceous material results leaving the inner
lamella composed of relatively pure cellulose (Figure 2B).
    The property of dried citrus pulp to be "wet" by oils
has been described by Kesterson and Braddock (8). Washed
orange pulp solids normally contain 0.5-1.5% ether extractible
lipids. Also present is cuticular wax which gives lipophilic
character to the pulp solids (9). Both the water holding
capacity (WHC) which encompasses sorption and hydration
phenomena, and oil holding capacity (OHC) which can be
described in terms of adsorption and capillary entrapment of

TABLE II

COMPONENT COMPOSITION OF WASHED ORANGE PULP

ADJUSTED TO MOISTURE AND SUGAR-FREE BASIS (a)

| | ORANGE VARIETY | | | |
| | (YEAR) | | | |
	HAMLIN (1978-79)	HAMLIN (1977-78)	VALENCIA (1978-79)	VALENCIA (1979-80)
PROTEIN	14.43	11.21	10.95	8.90
FAT	1.48	0.55	1.16	0.86
ASH	5.33	2.15	2.91	3.70
NDF	25.77	30.38	32.08	36.92
CRUDE FIBER	16.15	19.65	21.56	24.41
PECTIN, TOTAL	42.41	45.22	48.15	54.34
PECTIN, PROTO-	29.18	30.63	39.61	43.42
PECTIN, SOLUBLE	13.23	14.59	8.54	10.92

(a) Calculated from data in Table I; reported as
    grams/100 grams

TABLE III

COMPOSITIONAL AND NUTRIENT ANALYSIS OF DRUM DRIED
VALENCIA WASHED ORANGE PULP AND AACC REFERENCE
WHEAT BRAN (a,b)

COMPONENT	WASHED ORANGE PULP	WHEAT BRAN	UNITS
PROTEIN (N* 6.25)	8.61	15.96	G/100 G
FAT	1.12 (c)	5.83 (d)	"
ASH	3.70	5.71	"
SUGARS, TOTAL	16.81	7.86 (e)	"
SUGAR, FRUCTOSE	4.15	-	"
SUGAR, GLUCOSE	3.59	-	"
CRUDE FIBER	18.97	9.94	"
NEUTRAL DETERGENT FIBER	32.89	44.87	"
PECTIN, TOTAL	48.47	3.30	"
PECTIN, PROTO-	36.34	-	"
PECTIN, SOLUBLE	12.13	-	"
STARCH	(f)	19.4	"
VITAMIN A	285. [796,g]	-	I.U./100 G
VITAMIN B1	0.45	0.87	Mg /100 G
VITAMIN B2	0.16	0.44	"
VITAMIN B6	0.23	0.65	"
VITAMIN C	22.30 [88.8,g]	-	"
NIACIN	1.71	23.3	"
CALCIUM	528.5	1300.	"
MAGNESIUM	123.6	480.	"
SODIUM	65.8	112.	"
POTASSIUM	466.2	1540.	"
IRON	0.3	0.14	"
ALUMINUM	0.3	< 0.01	"

(a) Values reported on a moisture free basis; (b) AACC
wheat bran standard, R07-3691; (c) ether extractable;
(d) by acid hydrolysis; (e) as invert sugars; (f) not
normally analyzed; (g) freeze dried sample.

TABLE IV

FIBER ANALYSIS OF EEC/IARC REFERENCE SAMPLES
AND HAMLIN WASHED ORANGE PULP, % DRY MATTER [a,b]

MATERIAL	H_2O	FAT	NDF	TDF	IDF	SDF TOTAL	% PECTINS	% N.P.[b]	TOTAL PECTINS IN SAMPLE
WHEAT BRAN	9.0	2.8	42.1	43.0	37.0	11.0	26.0	74.0	2.9
APPLE PULP	8.5	0.3	8.7	12.7	9.1	3.6	85.8	14.2	3.1
POTATO POWDER	9.2	-	2.9	10.5	6.0	4.5	57.6	42.4	2.6
CITRUS PECTIN	8.7	0.1	0.3	92.5	0.3	92.5	92.1	7.9	84.9
WASHED ORANGE PULP[c]	4.5	1.2	32.9	46.5	21.9	24.6	8.1	91.9	26.6

a Reprint from reference 3, pg. 169, by courtesy of Marciel Dekker, Inc.
b N. P. - neutral polysaccharides; IDF - insoluble dietary fiber; SDF - soluble dietary fiber.
c Hamlin
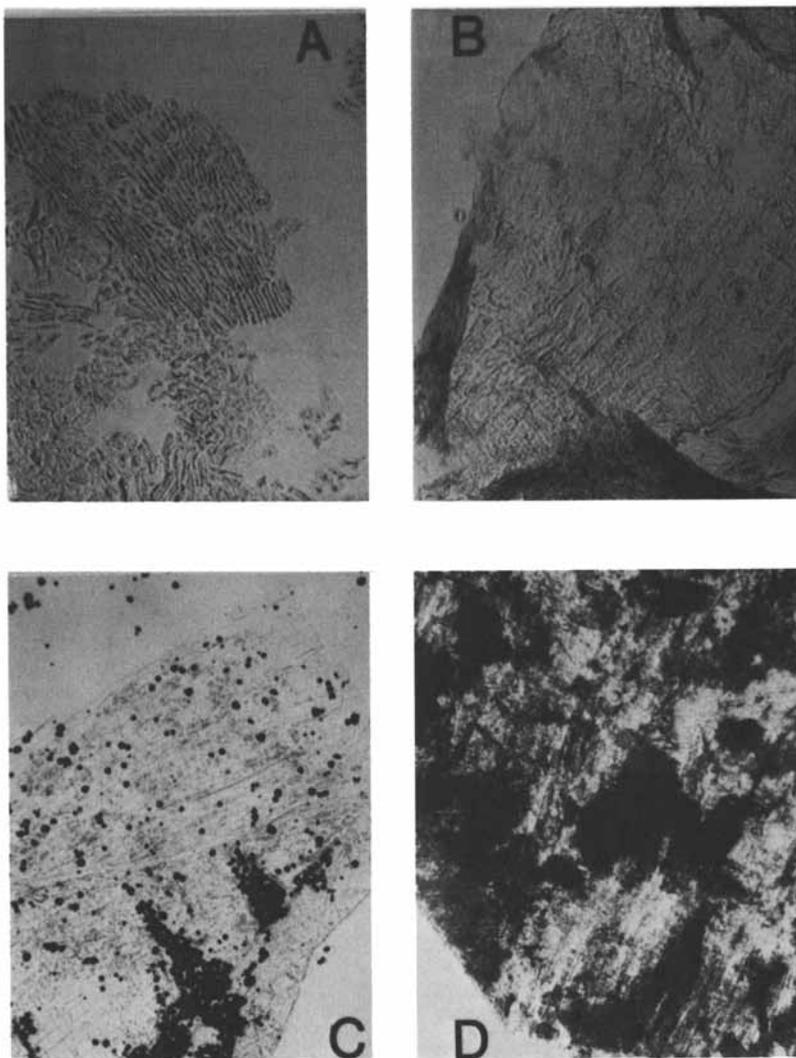
Figure 2. Structural characterization of juice vesicles. Key: a, extracted juice vesicle showing partially removed pectin, 100×; b, cellulose inner lamella of juice vesicle following 5% NaOH extraction of A, 110×; c, starting material in processed base with iodine stained starch granules, 82×; and d, finished processed base with starch bound to juice vesicle, 82×.

the oil were determined for different dried and extracted
samples (Table V). Density of the fiber particulates was
found to have a reciprocal relationship with OHC. Solvent
extracted samples contain less than 0.2 % lipids yet exhibited
increases in OHC. The extraction is a low temperature drying
and yields fiber without major changes in structure. This
could explain in part the increase in OHC. However, freeze
dried samples of approximate equal density (without extraction)
do not show an equivalent oil holding capacity. Tissue bound
lipoproteins may remain after extraction to impart surface
lipophilicity.

Samples extracted with calcium complexing agents such as
EDTA or sodium citrate show extensive loss of soluble pectin.
This loss is reflected in the density of the dried fiber
solids. In Table V the increase in OHC and WHC of extracted
samples are evident as well as changes in density. The loss
of pectin gives a porous matrix upon drying so the method
used for OHC reflects an increase of entrained oil.

Citrus pulp, like other plant tissue, are rich in
bioflavanoids and phenolics. These compounds are found as the
substituted flavanones, 3-hydroxyflavanones, flavones,
3-hydroxyflavones, and their glycosidic derivatives as well
as the simpler phenolics (10). Colorimetric analysis of
washed orange pulp yields 19.9 ± 0.3 mg 'catechin equivalents'/
gram of pulp. This level is sufficient for two specific
reactions to occur: formation of green chromatophores in the
presence of $Fe(+3)$ at neutral pH and the production of a
'flavor muting' complex. This latter property is a mani-
festation of a reaction noted in a patented process for
production of a "material to remove odor from air" derived
from citrus tissue (11). Plant phenolics react with polyols
catalyzed by light or radical initiators. This reaction was
verified to occur under food processing conditions. A model
system reacted fresh washed orange pulp, starch, and sugars
in presence of glycerol and flavor concentrates. Samples
were prepared and dried, then tested organoleptically. In
each case where glycerol and pulp were present together, a
significant lowering of flavor intentsity was observed in the
model product.

Utilization of washed orange pulp in food processing and
product applications was explored. In one specific case, the
hydrated pulp was combined with ungelatinized starch, edible
acids, nutritive sweeteners and additional water. The
mixture was heated and the system brought to 48-53% solids
from the 30-40% solids starting level. This process leading
to an ingredient is the basis of U.S. patent 4,232,049 (12).
The processing of the pulp initiates multiple reactions.
They include hydrolysis of sucrose, hydration and gelatiniza-
tion of starch, conversion of protopectin to the soluble

TABLE V

OIL AND WATER HOLDING CAPACITIES
OF WASHED ORANGE PULP

SAMPLE TREATMENT (a)	DENSITY (G/CC)	WHC (G/G)	OHC (G/G)
DRUM DRIED	0.22	3.0	0.7
FREEZE DRIED	0.11	3.1	1.3
SPRAY DRIED	0.33	5.2	0.2
ISOPROPANOL EXTRACTED	0.09	4.8	2.2
ACETONE EXTRACTED	0.13	5.5	1.3
0.2 $\underline{M}$ EDTA EXTRACTED	0.02	6.7	11.6
0.4 $\underline{M}$ CITRATE EXTRACTED	0.03	13.3	8.4
0.1% H_3PO_4 EXTRACTED	0.02	9.6	4.5

(a) solvent extracted samples were air dried overnight;
aqueous extracted samples were freeze dried

pectin, sucrose-pectin gel interactions, volatilization of
citrus oils and microbial inactivation. In addition, it was
observed that the hydrated, gelatinized starch interacts and
is deposited on the vesicle surface of the washed orange
pulp. Figure 2C shows the unheated starting mixture where
the starch granules are stained with iodide. Figure 2D shows
the same sample at the conclusion of the heating process.
Starch associates with the vesicle and can be compared with
the equivalent process of "sizing" reported in paper manu-
facturing. The washed orange pulp was then utilized in a
series of food products including frostings (12) and a
nondairy ice cream analogue (13).

Conclusion

     Washed orange pulp was evaluated as an unconventional
source of dietary fiber. The pulp is an excellent source of
soluble and insoluble dietary fiber. Dried samples generally
contain 25-45% pectins and 9-20% crude fiber. Several prop-
erties of the pulp are a function of its cellular components
and must be considered in utilization as a food ingredient.
Additional functional properties can be obtained by process-
ing the hydrated pulp and have extended the potential use
of this byproduct of the juice concentrate industry.

Acknowledgment

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Dietary Fiber from Citrus Wastes: Characterization

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Nearly 5 million metric tons of citrus peel and pulp waste materials are produced annually in the manufacturing of concentrated citrus juices in Florida. Although these materials are currently processed into cattle feed, they are rich in polysaccharides and can be considered as a source of dietary fiber. Alcohol-insoluble solids (AIS) of 3 different fractions of the processing residues discharged from commercial juice manufacturing equipment were analyzed for pectin, easily hydrolyzable polysaccharides, and cellulose. Two varieties of orange and one of grapefruit that are the predominant citrus fruit used in juice production were included in this study.

Between 50-60% of the AIS of all samples was removed by a 0.05 M NaOH treatment and about 10-12% by a subsequent acid hydrolysis of the residue. The "cellulose" fraction remaining was nearly completely dissolved in 12 M H_2SO_4. Protein and ash comprised about 10% of the AIS and were found mostly in the dilute alkali extract. The neutral sugars were determined by HPLC and GLC of their silylated derivatives of the hydrolysate after removal of acid. Arabinose and galactose were found in the dilute alkali extract. Xylose, arabinose, galactose and glucose were all present in the easily hydrolyzable fraction. Glucose was the main sugar component in the fraction dissolved in the 12 M H_2SO_4. Carbazol colorimetric method was used to determine the uronic acids in the fractions.

Citrus juice processing is one of the more important food
industries in the world. Annual production of frozen
concentrated orange juice in the United States and Brazil, the
two leading citrus concentrate producing countries, utilize
nearly 15 million tons of citrus fruit with an enormous
quantitity of processing residue. Only about 45-50% of
grapefruit and 50-55% of oranges are recovered as juice (1).
The residue from the fruit after the juice is removed by
mechanical extractors consisted of the peel, the central core
and the membrane, and the coarse pulp which is removed by a
screen-type finisher. Seeds are included in the residue when
seedy varieties of citrus are processed. A distribution of
component parts or oranges and grapefruit is shown in Table I.

Table I. Average distribution of component
parts of citrus fruits.

Fruit and variety	Year	Peel (Flavedo & albedo)	Membrane, core & seeds (Rag)	Juice sacs (pulp)	Juice
'Pineapple'	1960-61	19.9	17.5	23.6	39.0
orange	1961-62	22.7	17.7	21.9	37.7
'Valencia'	1959-60	19.2	9.8	19.9	51.1
orange	1960-61	20.8	12.8	23.7	42.7
'Marsh'	1961-62	27.2	13.4	21.5	37.9
grapefruit	1962-63	29.2	14.7	19.1	37.0

Rouse et al. (2, 3, 4).

Most of the citrus residues are now manufactured into
animal feed. The production of this product is over a million
tons annually (1). Although the processing of these residues
to such by-products gives a fair return to the producers, more
sophisticated products can be obtained from them which are
suitable as human food.

Between 45-75% of the total solids in the citrus peel and
membrane has been found to be insoluble in alcohol (Table II),
and the main portion of this alcohol-insoluble material is
complex carbohydrate (5). The alcohol-insoluble solids (AIS)
of the coarse pulp is also largely complex carbohydrate, and
it also contains proteins and inorganic salts (6). Of the
complex carbohydrates, pectin is by far the most important
fraction. Rouse et al. (7) divided the pectic substances into
water soluble, oxalate soluble, and sodium hydroxide soluble
fractions in their study. Eaks and Sinclair (8) found a
difference in the composition of the hemicellulose-cellulose
fraction between the mature and immature 'Valencia' orange
peel AIS.

Table II. Proximate composition of citrus waste material.

Fruit and variety	Component	Water	Total solids	Alcohol insoluble solids (AIS)	Total protein as % AIS	Ash as % AIS
			(g/100 g)			
'Pineapple' orange	Peel	77.1	22.9	13.0	5.2	2.7
	Rag	81.2	18.8	10.2	8.1	2.0
	Pulp	89.5	10.5	3.6	11.5	1.6
'Valencia' orange	Peel	76.6	23.4	15.2	5.6	2.9
	Rag	81.6	18.4	10.4	9.7	1.8
	Pulp	84.2	15.8	6.4	9.6	1.5
'Marsh' grapefruit	Peel	83.4	16.6	8.5	6.2	2.3
	Rag	82.0	18.0	9.3	6.8	1.8
	Pulp	90.0	10.0	2.5	14.0	1.5

The water-soluble pectins include the more highly
methoxylated pectic acid, while the oxalate-soluble pectic
compounds are the calcium pectates of the middle lamellas of
the cell wall. Sodium hydroxide easily hydrolyzes both the
methoxylated pectates and the calcium pectate. Thus,
extraction with dilute alkali of the AIS would include all
these pectic fractions.

The hydrophilic nature of these carbohydrate constituents
could be an important factor in considering them as a source
of dietary fibers. Ferguson and Fox (9) studied the
possibilities of manufacturing a high fiber food product from
citrus waste. Belshaw (10) suggested the use of a flour
manufactured from the peels of oranges and grapefruit in bread
to increase the fiber content. Such flour can also
incorporate some desirable flavor. Recently, Braddock and
Graumlich (11) analyzed the insoluble carbohydrates of
different fruit parts of orange and grapefruit and discussed
the possible utilization of citrus peel as a source of dietary
fiber.

The purpose of this work is to study the composition of
the residues from citrus processing and to devise a method for
separating and estimating the nature and quantities of various
fractions of the complex carbohydrates from such materials.

Analyzing Citrus Residue

Processing residues. The processing residues used in
this study were collected from an FMC Model 091 in-line juice
extractor and from an FMC Model 035 juice finisher (FMC
Corporation, Citrus Machinery Division, Lakeland, FL). The
juice extractor discharges the peel partially shredded, while
the central core, section membrane and the entire inner part
of the fruit are pressed in the central extraction tube.
After the juice is expressed and passed through a prefinisher
in the central extraction tube, the residue discharged is
collectively called the rag. The prefinished juice then
passes through a fine screen (.020 in.) in the finisher which
removes the pulp. The pressure applied to the finisher which
is adjustable can determine the amount of liquid retained by
the pulp. Two cultivars of orange, 'Pineapple' and 'Valencia'
and one of grapefruit, 'Marsh' were used in this study as they
are the important cultivars in juice manufacturing.

Sample preparation. Approximately 2 kg of each residue
component was obtained from each cultivar. The peel and rag
were separately passed through a hand-operated grinder. After
thorough mixing, duplicate 100 g aliquots of each of these
components were weighed out and placed in separate quart Mason
jars each containing about 400 ml of 95% ethanol. The pulp

samples were treated in the same manner but without prior grinding. These alcohol-preserved samples were used in the preparation of AIS. Total solids was determined by drying the samples in a vacuum oven at 75°C for 16 hours followed by 2 hours at 100°C in a regular forced-draft drying oven.

Preparation of alcohol-insoluble solids. The sample preserved in alcohol was placed in a Waring blender and macerated at high speed for 10 minutes and filtered through a Whatman No. 1 filter paper in a Buchner funnel under vacuum from a water aspirator to remove the alcohol solubles which were mostly sugars and organic acids. The residue on the filter was returned to the Waring blender with another 400 ml of 80% ethanol. It was again ground for 10 minutes and filtered. This operation was repeated, and the residue finally extracted with 200 ml of acetone. The white residue was air dried and finally dried in an oven at 75°C overnight. The weight was considered as percent AIS. Each sample was ground in a Wiley mill to pass a 40 mesh screen and stored in tightly stoppered bottles until analyzed.

Total nitrogen of the AIS was determined by a semi-micro Kjeldahl procedure (6) on a 0.50 g sample and the protein content calculated using the factor 6.25. The ash content was determined on a 0.5 g sample of the AIS ignited in a muffle furnace at 550°C for 16 hours.

Extraction of pectic substance fraction. A one-half gram of the AIS was placed in a 100 ml volumetric flask, moistened with about 1 ml of 95% ethanol and made to volume with 0.05 NaOH. The contents were allowed to stand at room temperature for 2 hours with occasional shaking. The mixture was poured onto a filtering cloth (Mira-Cloth, American Can Co.) supported on a Buchner funnel. Fifty ml of the filtrate was transferred into a 100 ml volumetric flask and acidified with 2.5 ml of conc. acetic acid. The content was made to volume and aliquots were used in the analysis of pectin and the accompanied monosaccharides. The residue was washed with a copious amount of water followed by 95% ethanol. It was rinsed from the filter cloth with a stream of acetone into a beaker and the mixture filtered through a tared sintered glass funnel. After air-dried, it was dried in an oven at 75°C and weighed. The difference in weight was considered as that removed by the dilute alkali. A flow diagram describing the fractionation, hydrolysis and subsequent analysis of the fractions are shown in Figure 1.

Uronic acid analysis. Carbazol colorimetric method (7) was used for the determination of total pectins in the dilute alkali extract of the alcohol-insoluble solids. The solution has to be diluted in order for the concentration to fall within the range of determination by this method.
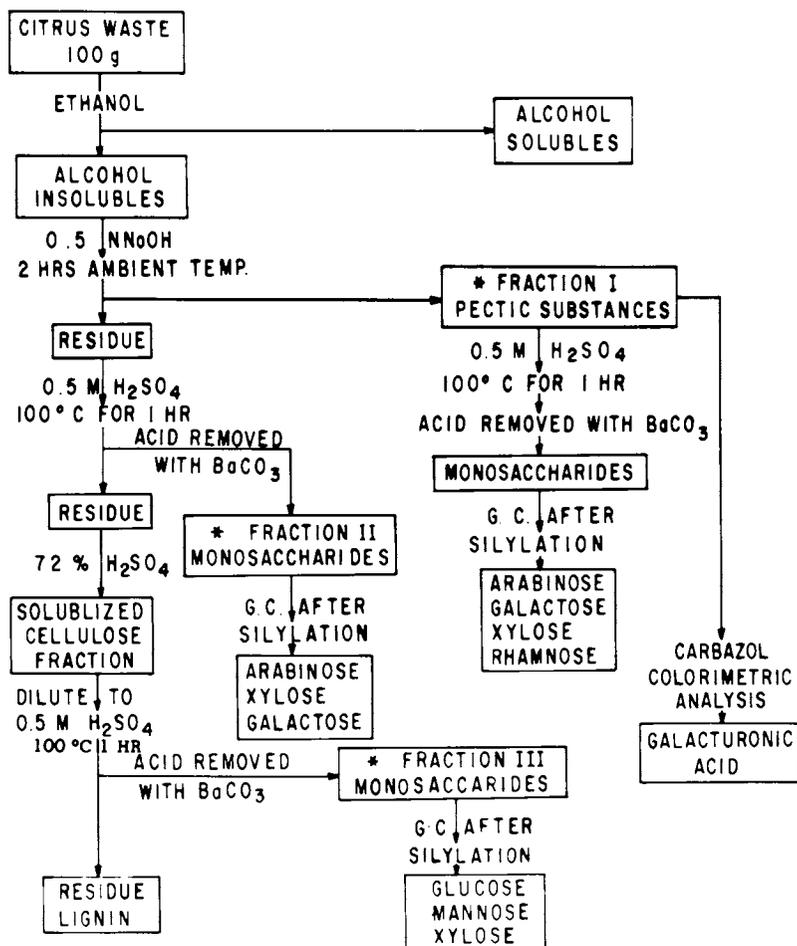
Figure 1. Preparation, fractionation, hydrolysis, and analysis of citrus processing waste.

Hydrolysis of the dilute alkali extract. Five ml of the
acidified extract was combined with an equal volume of 1 M
H_2SO_4 in a 15 x 125 mm screw capped culture tube. The mixture
was heated for 1 hour in a boiling water bath. After cooling,
0.8 g of $BaCO_3$ was added, and the tube was sonicated for 10
minutes and allowed to stand overnight. After centrifugation,
5 ml of the neutralized supernatant fluid was used for gas
chromatographic analysis.

Easily hydrolyzable polysaccharides. The residue after
the dilute alkali extract was hydrolyzed with 10 ml of 1 M
H_2SO_4 in a 15 x 125 mm screw-capped culture tube in a boiling
water bath for 1 hour. The hydrolysate was decanted, after
centrifuging, into a 125 ml Erlenmeyer flask, and the residue
in the tube washed with 5 ml of water. The washing after
centrifuging was combined with the hydrolysate. The residue
was washed once with ethanol followed by acetone. Each time
it was centrifuged and the washings discarded. The residue
was dried, weighed and considered as the "cellulose" fraction.
The hydrolysate was neutralized with 1.5 g $BaCO_3$, filtered to
remove $BaSO_4$ and evaporated to dryness. Twenty-five ml of
water was added to dissolve the solids and 1 ml aliquots of
the solution used for chromatograpic analysis.

Hydrolysis of the "cellulose" fraction. The residue from
the dilute acid extraction was suspended in 1 ml of 72% H_2SO_4
and allowed to stand at room temperature for 2 hours. The
contents were transferred into a 25 x 400 mm test tube with 50
ml of water. The tubes are placed in a boiling water bath for
1 hour. After cooling the large portion of the clear
hydrolysate was transferred over to a 100 ml volumetric flask
and the remainder was transferred into a 50 ml centrifuge
tube. After centrifuging, the supernatant fluid was combined
with the previous portion in the volumetric flask. The
residue was washed once in the centrifuge tube and the
washings combined with the hydrolysate. The dark precipitate
was washed with acetone, air dried and, finally, oven-dried
and weighed. The hydrolysate was made to volume and a 1-ml
aliquot was used for gas chromatography.

Gas chromatographic and HPLC analysis of monosaccharide.
The sugars in each fraction were analyzed as their silylated
ether (12) by gas chromatography. The aliquot of the
hydrolysate was first evaporated to dryness and treated with 1
ml of Trisil (Pierce Chemical Company, Rockford, IL). A
Hewlett Packard Model 5730 gas chromatograph with a 1/4 inch
glass column packed with 3% SE 30 on Chromosorb (Supelco Co.,
Bellefonte, PA) was used. Temperature was programmed from 100

to 220°C at 4° per minute. Both injection and detection
temperatures were set at 300°C. Injections were made with the
aid of a Hewlett Packard Model 7672A autosampler, and the
concentrations of each sugar determined on a Hewlett Packard
Model 3385A calculating intergrator using inositol as the
internal standard and appropriate standards.
     The sugars were also analyzed by HPLC using a Waters HPLC
system with a carbohydrate column. The monomers can be
separated but the concentrations of the minor constituents in
the mixtures were too low for the refractive index (RI)
detector.

Composition of Citrus Processing Residue

     The proximate composition of these citrus processing
residue components are shown in Table II. The peel contained
about 10-15% AIS on fresh weight basis or about 50 to 65% of
the dry weight. Grapefruit had lower AIS and total solids
than the 2 varieties of oranges. 'Valencia' oranges had
higher alcohol-insoluble solids in the pulp than either the
'Pineapple' orange or the grapefruit. The AIS of the rags
showed the least differences among the cultivars.
     Protein is higher in the pulp and rag than in the peel.
The peel had between 5 and 6% protein, whereas the pulp had
more than twice that value. The ash content is slightly
higher in the peel.
     The dilute alkali removed 46 to 53% of the AIS of the
peel, 50 to 60% of that of the rag and 62 and 64% of that of
the pulp of 'Pineapple' orange and 'Marsh' grapefruit. In the
pulp of 'Valencia' oranges, which has much higher AIS content,
only 46% was removed (Table III).
     The dilute acid hydrolysis of the residue following the
first extraction removed about 25% of the AIS of orange peel
but only 17% of that of grapefruit peel. The rag and pulp
components of the 'Valencia' oranges, which had less amounts
removed by the dilute alkali extract, were much higher in this
fraction than those of either the 'Pineapple' orange or the
'Marsh' grapefruit. The "cellulose" fraction of the peel was
about 28% in all three kinds of fruit but only about 15 to 20%
in their pulp components, with the rags occupying the values
in between.
     The use of a 0.05 M NaOH to extract the AIS of citrus
tissues removes all the pectins (7). In all three types of
citrus waste components these hydrolysates contained mostly
uronic acids, but arabinose, galactose and small amounts of
xylose were also present (Table IV). Arabinose is generally 2
to 3 times higher than galactose. In some samples, especially
in the peel of 'Pineapple' orange, considerable amounts of
rhamnose were found. Only a small amount of rhamnose was
found in the 'Valencia' peel sample and none in the grapefruit

Table III. Percent distribution in fractionation of AIS of citrus waste components

Fruit and variety	Waste component	0.05N NaOH Extract (pectin containing)	1 M H_2SO_4 Hydrolysate	Cellulosic fraction	Residue after 72% H_2SO_4 solubilization	Total
'Pineapple' orange	peel	46.0	24.0	28.8	1.0	99.8
	rag	60.0	18.0	22.6	0.7	101.3
	pulp	64.0	14.0	16.0	3.3	97.3
'Valencia' orange	peel	49.0	24.0	27.4	1.2	101.6
	rag	49.0	25.0	24.1	1.4	99.5
	pulp	46.0	27.0	20.6	3.4	97.0
'Marsh' grapefruit	peel	53.0	17.0	28.5	1.6	100.1
	rag	55.0	17.0	26.0	1.3	99.3
	pulp	62.0	17.0	15.1	4.3	98.4

Table IV. Neutral sugars and uronic acid in the 0.05 N NaOH extract of the AIS of citrus processing waste.

Fruit and variety	Type of residue	Carbohydrate monomers (mg/g AIS)					Uronic acids	Total	% of Extracted material
		Arab.	Galac.	Rham.	Xyl.	Glu.			
'Pineapple' orange	peel	21.3	8.2	30.0	5.6	—	200.0	265.1	57.6
	rag	45.6	11.5	—	3.4	23.0	324.4	407.9	68.0
	pulp	74.8	30.8	—	6.3	—	320.6	432.5	67.6
'Valencia' orange	peel	30.0	7.9	3.2	3.0	4.6	236.4	285.1	58.2
	rag	46.2	12.8	—	8.3	8.5	262.4	338.2	69.0
	pulp	59.0	20.1	—	5.3	—	192.4	276.5	60.1
'Marsh' grapefruit	peel	17.0	6.0	—	—	—	280.4	303.0	57.2
	rag	20.3	11.3	—	1.8	1.0	356.0	390.4	71.0
	pulp	32.4	15.2	—	3.4	—	287.6	338.6	54.6

peel sample. Xylose was found in all samples except in that
of the grapefruit. Glucose was found in all rag samples, but
in that of grapefruit the amount was smaller than those in the
corresponding components of the oranges.

The uronic acid content of the total monomers amounted to
70% in the pulp of 'Valencia' orange to 92% in that of
grapefruit peel. Grapefruit peel is known to produce better
pectin than orange peel (13) and the ratio of its uronic acid
to the sugar monomers is much higher. The sum of all monomers
of all components only accounted for 55 to 70% of the total
amount extracted. These values did not vary much from those
reported earlier (5). Some of these differences probably were
due to protein and minerals. The incomplete hydrolysis could
be another explanation of the low recovery.

Arabinose, galactose, xylose and glucose were also found
in the dilute acid hydrolysate of the residue following the
dilute alkali extraction (Table V). This easily hydrolyzable
fraction contained most of the hemicellulose. In grapefruit
peel, the arabinose and galactose were lower compared to the
similar components of the two oranges, but the xylose was
slightly higher. The rag and pulp of the 'Valencia' orange
were higher in arabinose and galactose than the other two
varieties. Overlapping peaks of glucose and mannose were not
easily separated, but they comprise less than 10% of the total
sugars accounted for.

Glucose is the major monomer in the 72% H_2SO_4 soluble
polysaccharide and comprises about 80 to 85% of the total
monomers accounted for (Table VI). Only a trace of arabinose
was detected in some peel samples but xylose was found to be
in measurable quantities. Several peaks on the chromatograms
were found in the various components. They were not
identified positively. Peaks with retention time similar to
some anomers of mannose not masked by glucose were found.
Mannose has been previously noted by paper chromatography from
citrus peel (5).

Conclusion

With more than 4 million tons of processing waste each
year from the citrus juice manufacturing industry in Florida,
production of food grade products with high dietary fiber
content can be profitable. Many food uses of the pulp
component have already been proposed (9, 14). Studies of
chemical composition of these waste components gave a better
understanding of the nature of these materials and might lead
to their better usage.

The fractionation scheme using dilute alkali, dilute acid
and 12 M sulfuric acid divides the polysaccharides into the
three definite parts. All of these waste components of both
oranges and grapefruit vary only slightly in their

Table V. Carbohydrate monomers in the easily hydrolyzable fraction of the alcohol insoluble solids of citrus processing residue.

Fruit and variety	Type of residue	Carbohydrate monomers (mg/g AIS)					% of Extracted material
		Arab.	Xyl.	Gal.	Others	Total	
'Pineapple' orange	peel	67.0	16.8	40.4	13.6	137.8	57.4
	rag	56.4	18.0	41.6	8.6	124.4	69.1
	pulp	38.0	12.2	31.2	8.8	90.2	64.4
'Valencia' orange	peel	78.8	20.0	73.6	22.2	94.8	77.0
	rag	71.6	16.6	51.6	19.6	159.4	63.8
	pulp	66.6	11.0	48.8	20.0	146.4	69.7
'Marsh' grapefruit	peel	46.0	30.8	29.6	18.6	125.4	73.8
	rag	34.8	21.6	19.8	5.2	82.0	48.2
	pulp	31.4	16.0	20.8	6.6	75.4	44.4

Table VI. Monosaccharide in the "cellulose" fraction of the AIS of citrus processing wastes.

Fruit and variety	Type of residue	Carbohydrate monomers (mg/g AIS)					% of Extracted material
		Arab.	Xyl.	Gluc.	Others	Total	
'Pineapple' orange	peel	trace	10.5	106.0	17.0	134.5	46.5
	rag	--	6.0	146.0	18.5	170.5	75.4
	pulp	--	4.0	113.5	17.5	135.0	84.4
'Valencia' orange	peel	trace	6.0	136.5	20.0	163.8	59.4
	rag	--	4.0	120.0	15.0	139.0	57.6
	pulp	trace	3.0	111.5	21.5	137.0	66.5
'Marsh' grapefruit	peel	--	9.5	149.5	24.5	183.5	64.4
	rag	--	7.0	141.0	16.0	164.0	63.0
	pulp	--	5.0	106.0	24.0	135.0	89.4

polysaccharide patterns. The AIS of the peel contained more
cellulose than the rag or pulp. The dilute alkali extracts of
all components contained mostly polyuronides and the dilute
acid extracts the hemicellulose. The AIS, especially that of
the pulp, contained some substances that are not solublized by
the strong H_2SO_4. Lignin is usually considered to be in that
fraction (5). The juice vesicle membrane which composes the
main portion of the pulp AIS has been reported to contain
cuticle wax, a lipoic compound (15), and lipids are also found
in the juice sac contents (16). These compounds which are not
removed by alcohol could be in the AIS of these materials.

    Rhamnose was found in the dilute alkali fractions of the
peel. The galacturonic acid is by far the major constituents
in this extract of all three components. Arabinose is two or
three times that of galactose and some xylose was also found.

    More xylose was found in the dilute acid hydrolysate than
in the other two fractions. Only about 55 to 70% of the AIS
extracted by the dilute alkali can be accounted for by the sum
of the sugars and the uronic acid. The recovery of the dilute
acid extract as the monomers was also low, amounting to
between 44 and 79%. There were some peaks on the chromatogram
of this fraction, but the aggregated integrated area was only
around 10% of the total. Peak with retention times close to
glucose and mannose were present.

    Xylose is also found in cellulose containing fractions,
but glucose is the major sugar. Unidentified peaks occupied a
noticeable amount of the total identified sugars on the
chromatograms.

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Cellulose, Xylan, Corn Bran, and Pectin in the Human Digestive Process

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Acid hydrolysis of human fecal samples collected after ingestion of different dietary fibers produced monosaccharides that were related to the fibers ingested. Dietary fibers studied in five normal male subjects were cellulose, xylan, corn bran and pectin. Increases in monosaccharides in recovered hydrolyzed feces from fiber diets compared to those from the fiber-free diet were: galactose for the pectin diet; xylose and glucose for the cellulose diet; xylose and mannose for the xylan diet; and arabinose, xylose, galactose and glucose for the corn bran diet. Comparison of the fecal results to those for the intact food fibers showed that fermentation of fiber components in the colon was different for each fiber.

Dietary fiber is often considered in terms of its physiological effects in man. It has been defined as that portion of plant foods resistant to hydrolysis by the alimentary enzymes of man (1) and as the indigestible material in the diet derived mainly from plant cell walls, including the sum of the polysaccharides and lignin in the diet that are not digested by the endogenous secretions of the lumen of the digestive tract (2). In addition to these physiological definitions a philosophical one has been proposed which states that dietary fiber includes all components of the diet which are imagined to pass through the small intestine substantially unchanged (3). It is also not a single substance, nor is it inert, indigestible material which simply passes through the human gut (4). Thus plant gums and mucilages are not cell wall components but are polysaccharides related in chemical structure and properties to cell wall constituents and not hydrolyzed by the human gastrointestinal enzymes. These materials are often in the diet as emul-

sifiers, viscosity agents and stabilizers and not recognized as dietary fiber per se.

This broad understanding of dietary fiber makes it difficult to describe or characterize chemically and to elucidate its behavior in the gastrointestinal tract. Nevertheless with changing dietary habits and recommendations for food intake for good health and nutrition, it is important that the chemistry of this class of food components be more readily characterized and understood. Since much dietary fiber is of cell wall origin, some of the approaches that have been successfully used to define and explain the chemistry of plant cell walls (5, 6) should be applicable to research on the characterization and function of dietary fiber (7, 8, 9). To this end we have investigated several dietary fibers derived from plant cell walls both before and after complete passage through the human gastrointestinal tract.

Plant cell walls are chemically complex and many of the components we are primarily interested in are basically insoluble. These two facts have hampered structural studies on plant cell walls as well as studies designed to determine what these substances do in the gastrointestinal tract. Thus, for example, in order to study the chemistry of individual components of the cell wall they must be solubilized and purified, a task that is difficult to do without physically or chemically altering the components. In this study we have investigated those portions of several cell wall derived dietary fibers that are susceptible to hydrolysis in trifluoroacetic acid (TFA). Dietary fibers with significantly different compositions of acid hydrolyzable neutral monosaccharides were used in the study. They were a cellulose, a xylan, a corn bran and a pectin. Monosaccharides released by direct acid hydrolysis of the dietary fibers were determined before consumption by human subjects and compared to the monosaccharides released by direct acid hydrolysis of fecal material resulting from ingestion of each fiber and of fecal material from the subjects on a fiber free diet. Those portions of dietary fiber that did not survive the gastrointestinal tract were either digested and absorbed in the small intestine or were likely nutritional substrates for intestinal microflora growth and the production of microflora by-products. These include volatile fatty acids (VFA's) that can be absorbed and utilized by the host (10, 11) and the gases, carbon dioxide, hydrogen and sometimes methane (12, 13).

This study is part of a continuing investigation of the relationship between dietary fiber sources and bioavailability of nutrients. Specific objectives of this research were to investigate chemical differences of various sources of edible fiber and to determine the effects of the human gastrointestinal tract on these fiber sources.

Experimental

      The design for the feeding portion of the experiment is
described by Marthinsen and Fleming (14). Briefly, six normal
healthy male volunteers, 21-35 years old with no histories of
gastrointestinal disorders or food allergies, were selected for
the study. Initially, subjects were provided with a nutrition-
ally adequate blended-food diet on an outpatient basis for two
weeks. Once subjects took up residence in the metabolic unit
they undertook a strict daily regime. The 63-day metabolic
study was divided into seven metabolic periods, each of nine
days duration (Table I). All subjects received the basal,
fiber-free diet during the first metabolic period. The subse-
quent test diet sequence was designed to minimize duplication
of any previous diet effects (Table I). Diets included two
bland formulations providing either 100% or 85% of the daily
energy requirements for the individual. Four diets contained
fiber sources including a cellulose (Alphacel from ICN Phar-
maceuticals, Inc.), a pectin (from Sigma Chem. Co.), a xylan
(from ICN Pharmaceuticals), and a raw corn bran (from Quaker
Oats). The basal formulation provided 0.8g protein/kg body
weight daily from egg albumin (Seymour Foods, Inc.). Fat (but-
terfat and cottonseed oil) provided 30% of the caloric require-
ments and the remainder was provided by cornstarch (CPC Inter-
national), dextromaltose (American Maize Products Co.) and su-
crose in a ratio of 5:5:1. Daily supplements of vitamins,
minerals, and choline were taken.
      During the test, dietary fiber was provided with each
meal. Alphacel, xylan, and pectin were fed at 0.5g/kg body
weight per day. Corn bran was considered to be approximately
80% dietary fiber and was fed at 0.6g/kg body weight. Each
fiber was consumed as a pudding containing other dietary con-
stituents. The remaining dietary constituents were consumed
as a milkshake style drink.
      Feces were collected daily, frozen, compiled into three-
day pools, diluted with water and homogenized using an electric
colloid mill. Homogenates were kept frozen prior to removing
aliquots for analysis. Pooled collections from days 7-9 for
each diet were used for the analyses except for subject #5 on
the xylan diet where the collection from days 4-6 was used
because of the loss of the 7-9 day sample. Dry weights were
obtained by drying aliquots under vacuum at 70°C for 16 hours.
      Hydrolyses were performed following the procedures of Al-
bersheim et al (15) for the characterization of plant polysac-
charides and described by us earlier for the hydrolysis of
dietary fiber samples (16). For these fiber samples 2-6mg
were hydrolyzed in 1ml of 2N TFA at 121°C for 1 hour. Fecal
samples contained other components in addition to the polysac-
charides or polysaccharide residues from the ingested food fi-
ber so that aliquots for the hydrolyses were increased to 10-25mg

Table I. DIETARY SCHEME FOR SUBJECTS

Diet

Metabolic Period	Subject					
	1	2	3	4	5	6
I	Basal	Basal	Basal	Basal	Basal	Basal
II	Cellulose	Xylan	Pectin	Corn bran	Basal	85% Energy
III	Xylan	Basal	85% Energy	Cellulose	Pectin	Corn bran
IV	85% Energy	Cellulose	Xylan	Pectin	Corn bran	Basal
V	Corn bran	Pectin	Cellulose	Basal	85% Energy	Xylan
VI	Pectin	Corn bran	Basal	85% Energy	Xylan	Cellulose
VII	Basal	85% Energy	Corn bran	Xylan	Cellulose	Pectin

(dry weight). TFA was evaporated and the sugars reduced with sodium borohydride. Excess sodium borohydride was destroyed with acetic acid and borate was removed with methanol. The alditols were converted to the corresponding acetates by adding 1ml of a 10:1 acetic anhydride/pyridine solution and heating at 121°C for 2 hours. The acetates were quantitatively separated by injecting 1μl of the reaction mixture onto a 1/8" X 6' stainless steel column packed with Supelco 2330 using 25 ml/min He as carrier gas and a FID detector. The oven was programmed from 200–224°C. The gas chromatograph unit was a Hewlett Packard 5880 Level 3. Mixtures of known sugars were reduced and acetylated to serve as standards. Samples were analyzed in triplicate and the results averaged.

Results And Discussion

Dietary Fiber. Acid strength, temperature, time, and amount of sample per ml of acid solution all influence the amounts of monosaccharides produced on hydrolysis. From the results of preliminary studies conditions were selected for these hydrolyses which appeared to produce close to maximum amounts of each monosaccharide. All of the hydrolyses were conducted following the same procedure. We have previously shown that different dietary fibers are composed of significantly different complements of acid hydrolyzable neutral monsaccharides as determined by direct TFA hydrolysis (16). Not only did different dietary fibers give different results but different lots of the same fiber type gave different results.

TABLE II

COMPOSITION OF DIETARY FIBERS BY TFA
HYDROLYSIS AS PERCENT OF ORIGINAL MATERIAL

Fiber	Rham	Arab	Xyl	Mann	Gal	Glu
Alphacel	0	0	4.6	0	0	6.3
Xylan	0	0.20	25	39	0	13
Pectin	1.0	1.2	0.1	0	2.0	0
Corn bran	0.1	13	24	0	3.4	20

The composition of dietary fibers fed in this study as determined by TFA hydrolysis are shown in Table II. The Alphacel contained 11% hydrolyzable sugars, the xylan 77% and the corn bran 60%. The small amount of xylose recovered from the Alphacel is most likely from hemicellulose present in the original wood

from which the Alphacel was prepared. The xylan was reportedly
from larchwood. The principle recovered sugars from the xylan
were mannose, xylose and glucose in that order. The mannose
to glucose ratio of 3 suggested the presence of a glucomannan,
a hemicellulose found in wood. The free glucose content of the
fiber was less than 0.1% (dry weight basis) as determined by
water extraction and Statzyme analysis (Worthington Diagnostics)
for glucose. Eighty percent of the glucose recovered from the
corn bran was starch as determined by amyloglucosidase hydro-
lysis followed by analysis for glucose. Pectin contained small
amounts of rhamnose, arabinose and galactose as expected.

   Subject Responses. Early in the study subject #4 dropped
out and was not replaced. In the following presentation and
discussion of data results from subject #6 are sometimes quite
different from those of the other subjects although in most
cases directionally consistent. No explanation for this is
offered at this time. Evaluation of the 100% and 85% energy
diets will be made at a later time. Excretion of breath and
flatus gases by the subjects has been reported (14).

   Fecal Hydrolyses. The rhamnose, arabinose, xylose, mannose,
galactose and glucose from TFA hydrolysis of aliquots of the
3-day fecal samples for each subject on each diet sequence are
shown in Figures 1-5. For subject #1 (Figure 1) on the fiber
free diet, small amounts of all of the sugars were produced on
hydrolysis of the fecal sample. Fecal material from this subject
on the Alphacel diet gave more glucose, on the xylan diet more
xylose, on the corn bran diet more arabinose, xylose, galactose
and glucose, and on the pectin diet more rhamnose and galactose.
Similar results were observed for subjects 2, 3, 5, and 6
(Figures 2-5). For every subject the amounts of the sugars
produced on acid hydrolysis of feces were different for each
diet fed and were related to the specific fiber fed. Comparison
of results from the several fiber-free diets run in each sequence
showed directional increases in hydrolyzable monosaccharides in
the feces which appear to be related to immediate diet history.
   The amount of hydrolyzable glucose in feces following the
Alphacel diets was more than could be accounted for if all of
the Alphacel had been excreted, assuming the same amount of
glucose was produced as that from the original Alphacel. Fur-
ther examination of the samples showed that they contained both
free glucose and starch. Free glucose was estimated by analyzing
water extracts for glucose by both the Statzyme procedure (Worth-
ington Diagnostics) and gas chromatography of the alditol ace-
tates. Starch was estimated by treatment of autoclaved fecal
samples with amyloglucosidase for 3 hours at 55°C followed by
the same analyses for glucose. In feces from the Alphacel diets
free glucose accounted for 5-15% and glucose from starch ranged
from 15-25% of the total glucose recovered following TFA hydro-
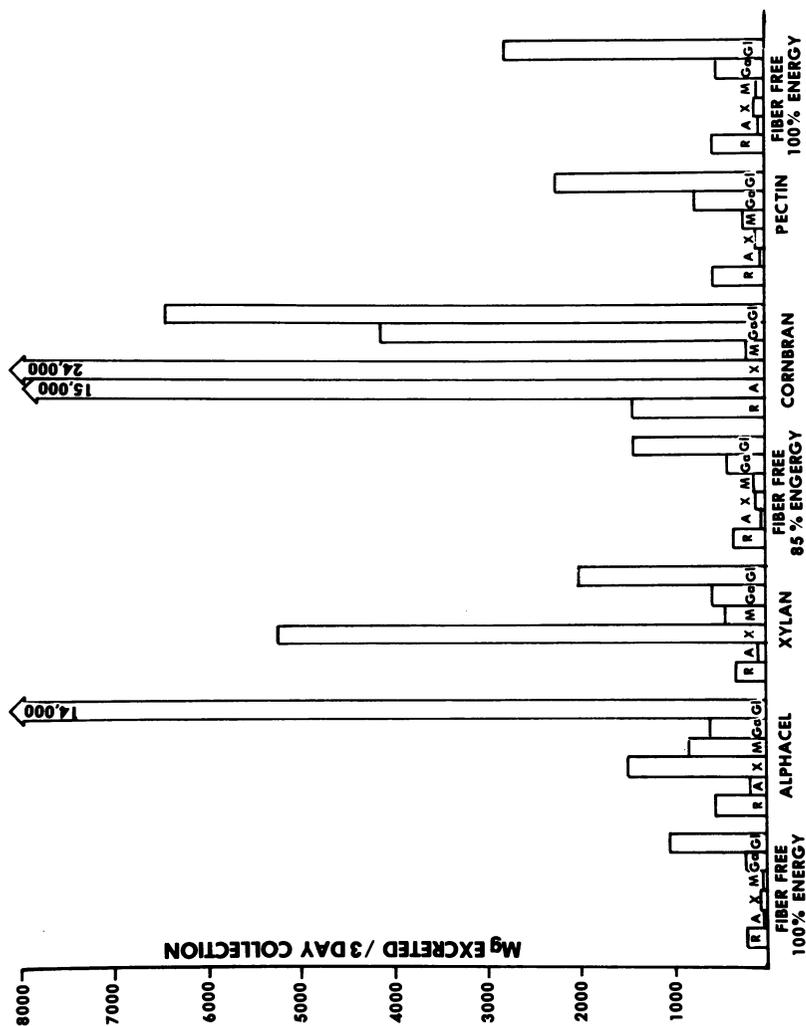
Figure 1. Monosaccharides from the hydrolysis of aliquots of 3-day fecal collections for Subject 1 on the indicated diets. Key: R, rhamnose; A, arabinose; X, xylose; M, mannose; Ga, galactose; and Gl, glucose.
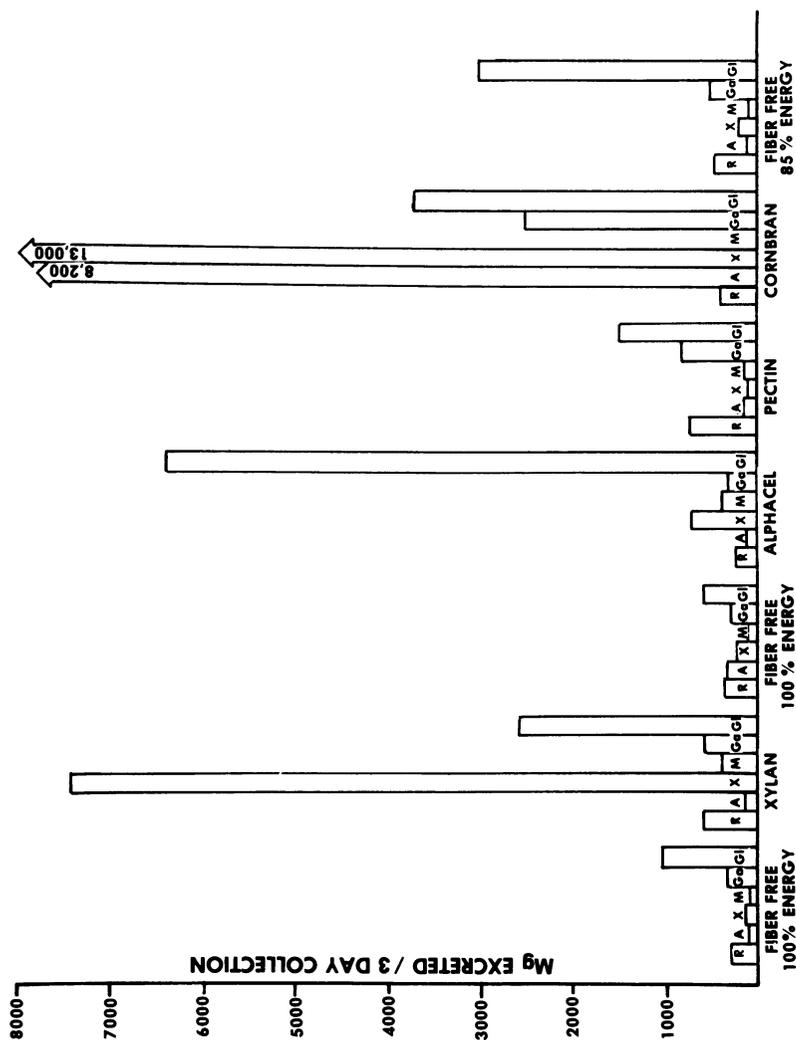
Figure 2. Monosaccharides from the hydrolysis of aliquots of 3-day fecal collections for Subject 2 on the indicated diets. Key is the same as in Figure 1.
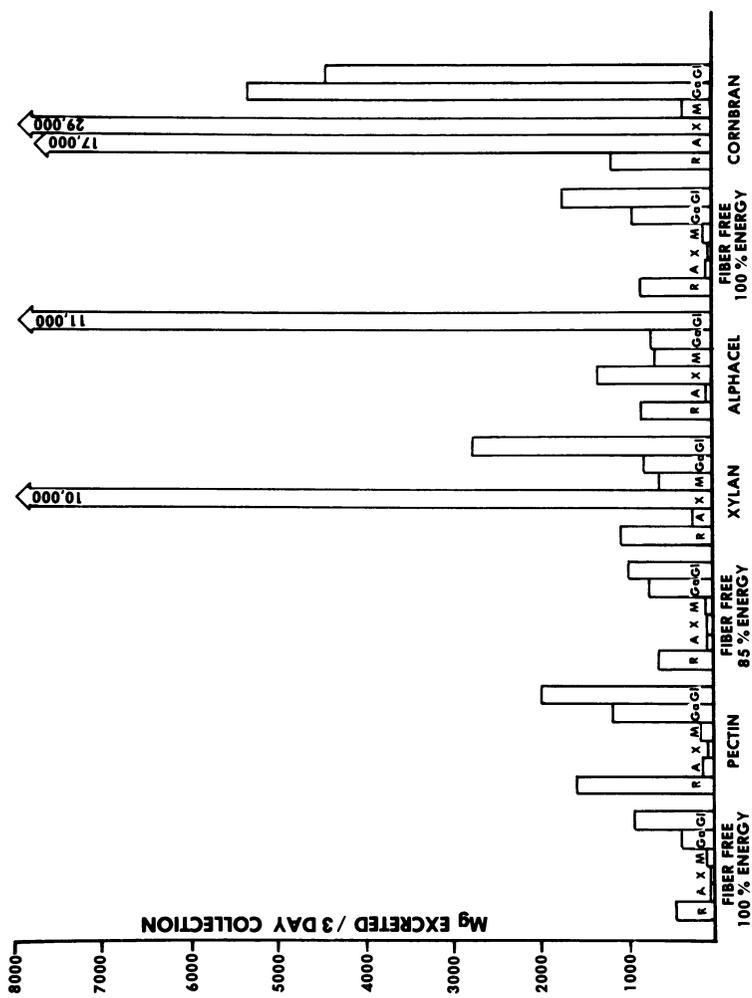
Figure 3. Monosaccharides from the hydrolysis of aliquots of 3-day fecal collections for Subject 3 on the indicated diets. Key is the same as in Figure 1.
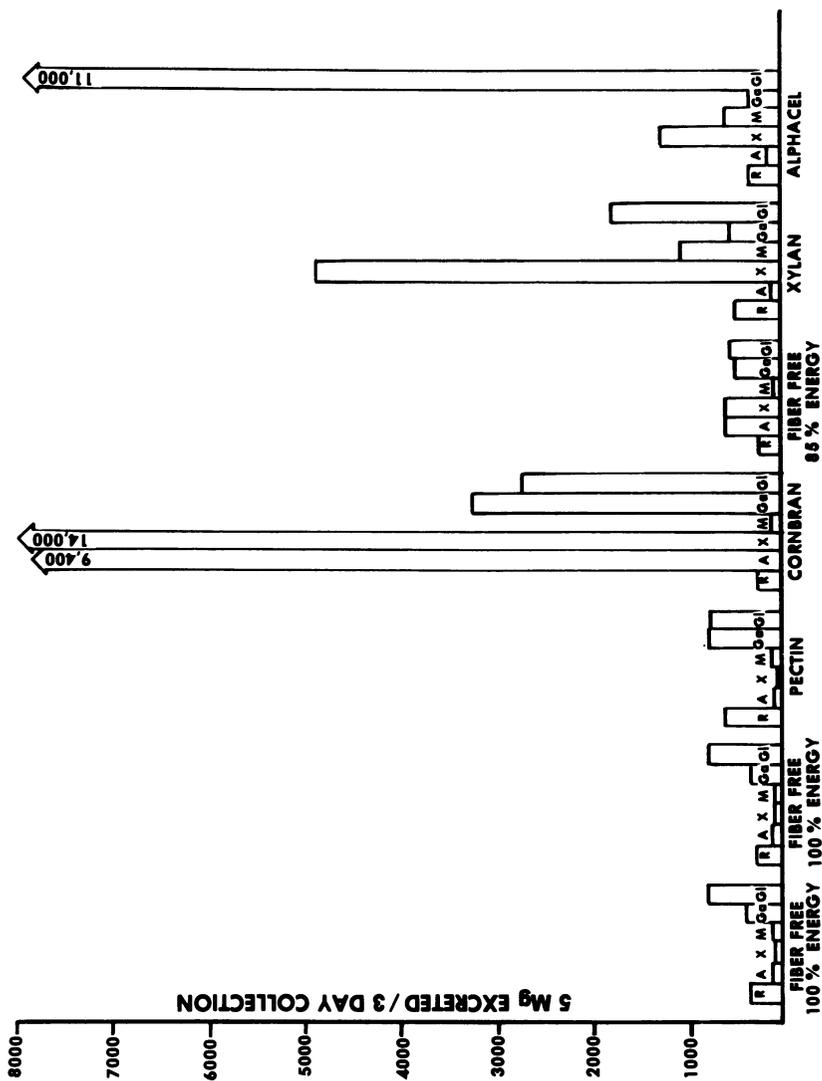
Figure 4.   Monosaccharides from the hydrolysis of aliquots of 3-day fecal collections for Subject 5 on the indicated diets. Key is the same as in Figure 1.
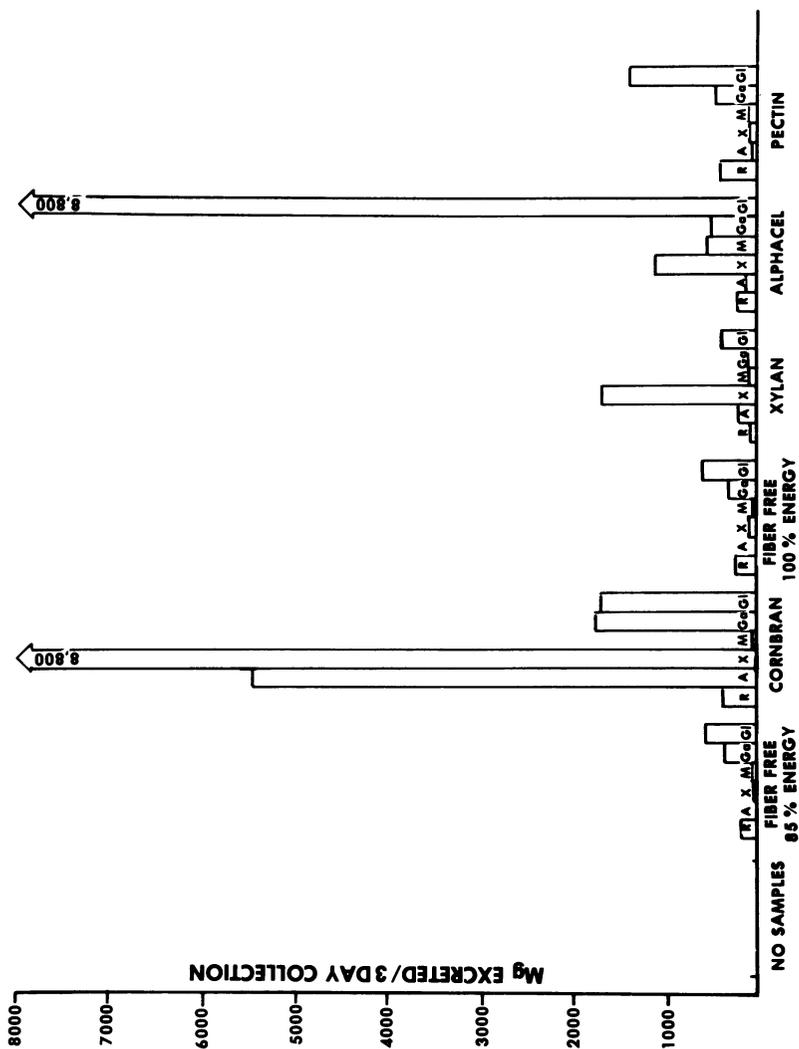
Figure 5. *Monosaccharides from the hydrolysis of aliquots of 3-day fecal collections for Subject 6 on the indicated diets. Key is the same as in Figure 1.*

lysis. This partially accounted for the large amount of glucose recovered from this diet. However, similar amounts of free glucose and starch were also found in fecal samples from the corn bran diet while smaller amounts were found in the fecal samples from the other diets including the fiber-free diets. While difficulties in these analyses prevented better quantitation, the results suggest that both Alphacel and corn bran may have inhibited starch digestion. This may be partially due to bulking effect and less effective substrate/enzyme interaction.

Small amounts of glucose were produced from cellulose preparations by hydrolysis with TFA as already noted (Table II) and reported by others (19). The amount produced probably depends on the physical form of the cellulose since different amounts were obtained from different preparations. Subtracting the amounts of free glucose and that derived from starch from the total amount obtained by direct TFA-hydrolysis of feces from some of the Alphacel diets still leaves more glucose than can be accounted for from unchanged cellulose itself. This suggests that passage through the gastrointestinal tract may have produced changes that made cellulose in recovered feces more susceptible to TFA hydrolysis than that which was fed.

Xylan and Corn Bran. The xylan and corn bran fibers were mainly hemicellulose and contained more complex mixtures of hydrolyzable monosaccharides than the other fiber sources. It was of some interest to consider the results from the hydrolysis of fecal samples from these diets in an alternate manner. The data were replotted to show monosaccharides recovered as percent of the amount fed for each subject. (Figures 6 and 7). For comparison the composition of the original fiber is shown on the extreme left as percent dry weight of the fiber.

As already noted the composition of the xylan was interesting because of its high mannose and glucose content, suggesting the presence of glucomannan in the xylan. The feces examined for hydrolyzable polysaccharides showed that the mannose (or mannan) was highly fermentable since only 0-3% of it survived to be excreted. Similarly less than 10% of the glucose originally present in the xylan ingested was recoverable by hydrolysis of fecal samples. Over 70% of the xylose in the xylan also disappeared. Thus, the original xylan may be composed of at least two readily fermentable polysaccharides, a xylan (β1-4 linked xylose units) and a glucomannan (β1-4 linked mannose and glucose in the ratio 3:1). Of the two polysaccharides the glucomannan was more readily fermentable since less of it survived the digestive process.

Corn bran has a large xylose to arabinose ratio of 1.8. Recovery of these sugars in the feces was the highest of any of the fibers fed. The xylose to arabinose ratio was also close to that in the bran fed, ranging from 1.5-1.7. Galactose, while in quite small amounts, survived in roughly the same
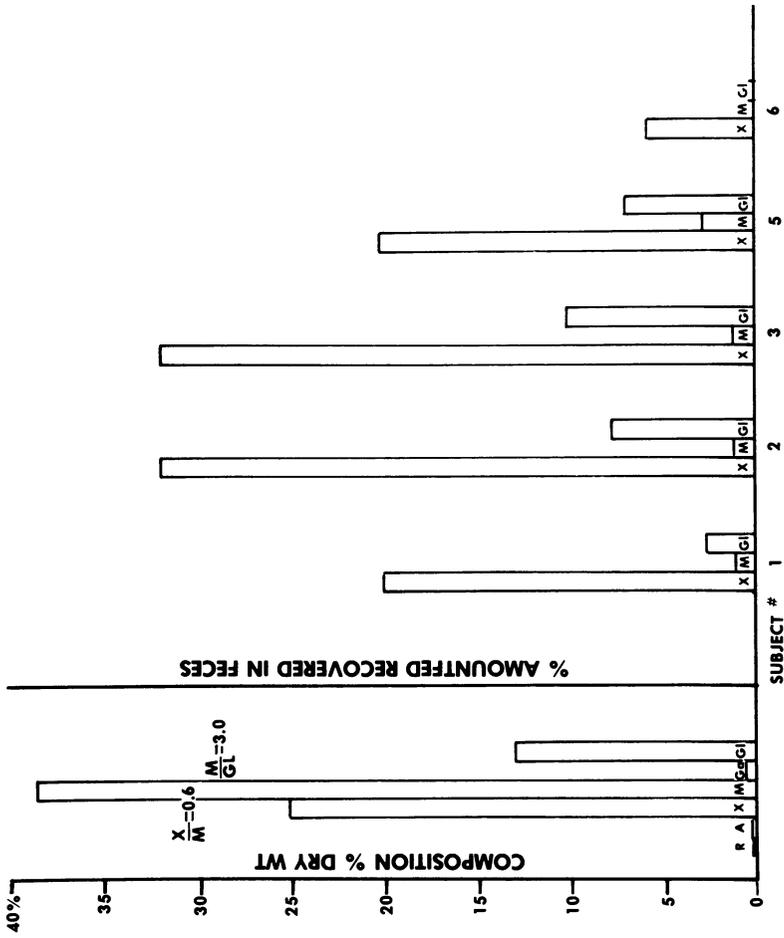
Figure 6.   Recovery of monosaccharides from xylan diets from each subject as percent of amount fed. Composition of fiber fed is shown at left.
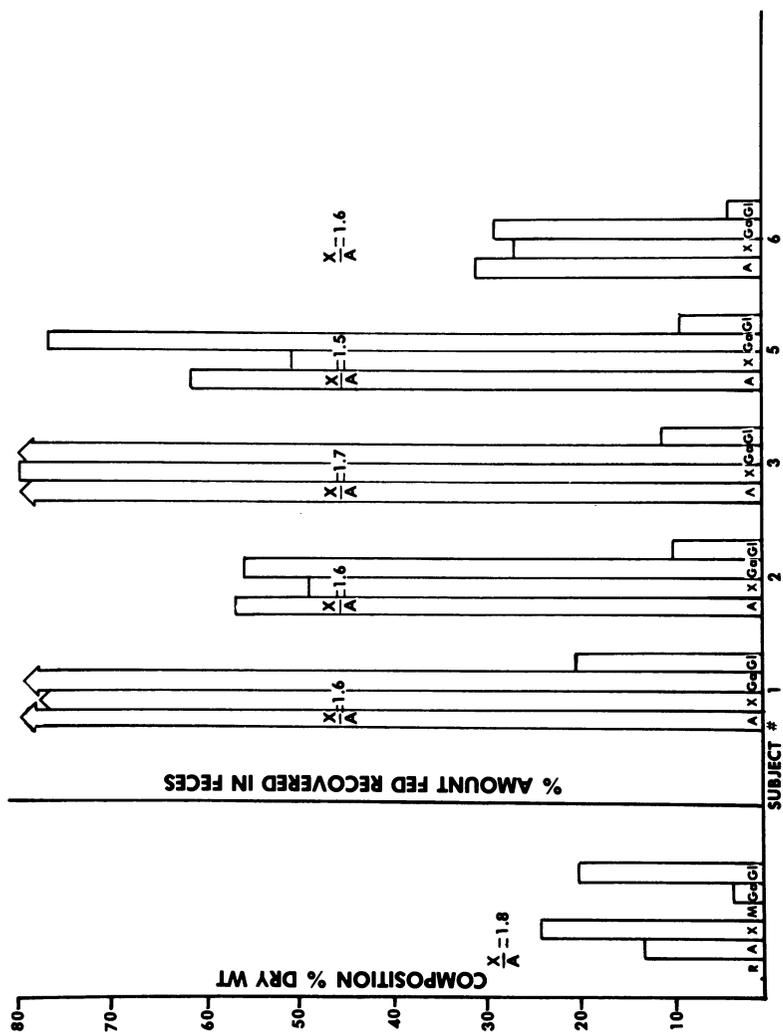
Figure 7. Recovery of monosaccharides from corn bran diets from each subject as percent of amount fed. Composition of fiber fed is shown at left.

proportions. The glucose value of 20% for the corn bran in-
cludes 16% starch and, by difference, 4% of the glucose is
probably tied up in hemicellulose. Then a calculated value of
hydrolyzable glucose in the feces based only on glucose in
hemicellulose in the original fiber would be 4X that shown in
Figure 7.

Comparison of these two sources of hemicellulose in the
diets and their recoveries in the feces indicated that their
behavior in the gastrointestinal tract may depend both on their
composition and particle size. The xylan was a finely divided
preparation while the corn bran was composed of much larger
particles. Very little of the xylan was recovered in the feces
compared to that recovered from the corn bran.

Fiber Input: Fecal Output. Fecal output on a fiber-free
diet may include bacterial detritus, bile salts, mucus, intes-
tinal wall cell debris, and perhaps fiber type material in the
"fiber-free" diet that is as yet not recognized as such (e.g.
material added as emulsifiers, etc). Fecal output from a fiber
supplemented diet minus that from the fiber-free output should
indicate the extent of the fiber itself that was excreted plus
the increase in bacterial detritus, mucus and other material
perhaps rendered less digestible due to the presence of the
fiber. In Figure 8 the amount of each fiber consumed is shown
compared to a corrected average (of the five subjects) total
3-day dry weight of fecal output and amounts of TFA hydrolyzable
sugars present. The correction was made by subtracting the
average fecal output and hydrolyzable sugars (shown in the ex-
treme right in Figure 8) obtained from the fiber-free diets from
the values obtained on the fiber diets.

Average fecal outputs for all of the fiber diets are larger
than those from fiber-free diets. Most of the TFA-hydrolyzable
sugars from the pectin and xylan fibers are not recovered in
the feces (9, 17, 18). Both the Alphacel and corn bran diets
produced large increases in fecal dry weights. For corn bran
relatively similar proportions of xylose, arabinose and galactose
found in the bran were found in the feces. The large bran parti-
cles and the chemical form of the polysaccharides probably limits
the fermentability of these hemicelluloses (20, 21). It was pos-
sible to visually identify corn bran particles in fecal material.

Statistical Analysis. Means and 95% confidence intervals
were calculated for the recovered monosaccharides (Table III).
Data was treated by analysis of variance fitting diets and sub-
jects. In some cases transformations were needed to stabilize
the variances between diets. Significant differences in the
composition of feces as a result of different diets are clearly
indicated and are consistant with the previous discussion.
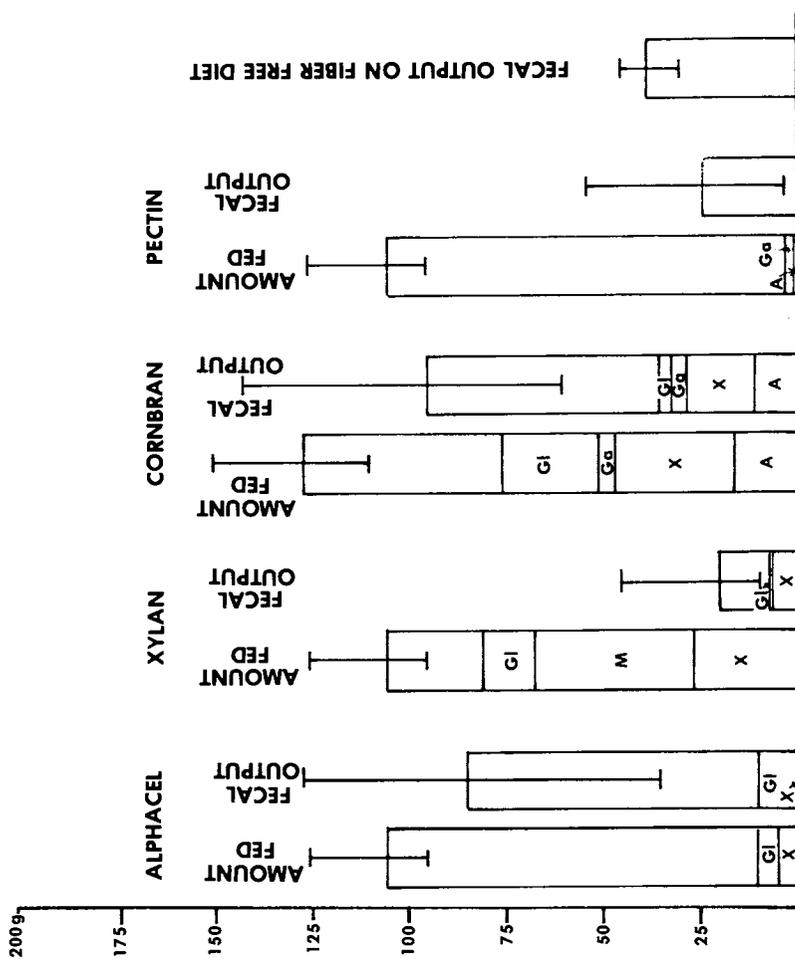
Figure 8. Comparison of fiber input to fecal output (dry weight in grams) corrected for fecal output from fiber-free diet shown at extreme right. Average of five subjects for 3-day total fecal output.

Table III.   FECAL HYDROLYSIS RESULTS: MEANS AND 95% CONFIDENCE INTERVALS*

DIET	RHAMNOSE Mean	95% C.I	ARABINOSE Mean	95% C.I.	XYLOSE Mean	95% C.I.	MANNOSE Mean	95% C.I.	GALACTOSE Mean	95% C.I.	GLUCOSE Mean	95% C.I.
Fiber Free	0.41	0.18 0.64	0.08	0.03 0.13	0.09	0.07 0.13	0.07	0.05 0.11	0.44	0.31 0.57	1.17	0.79 1.67
Pectin	0.77	0.54 1.00	0.09	0.04 0.14	0.06	0.05 0.09	0.13	0.08 0.19	0.80	0.67 0.93	1.50	1.04 2.10
Alphacel	0.43	0.20 0.66	0.12	0.07 0.17	1.13	0.81 1.57	0.61	0.40 0.92	0.49	0.36 0.62	10.1	8.06 12.5
Xylan	0.43	0.20 0.66	0.11	0.07 0.16	4.11	2.97 5.68	0.32	0.21 0.49	0.45	0.32 0.58	1.36	0.94 1.92
Corn bran	0.72	0.49 0.95	10.9	5.1 16.7	16.2	11.7 22.4	0.15	0.10 0.23	3.41	1.61 5.20	3.58	2.67 4.70

*For Grams Excreted/3 Day Pooled Collection; N = 5.

Summary And Conclusions

Marthinsen and Fleming (14) reported that methane excretion was greater while consuming the xylan and pectin diets than while consuming the other diets. Ingestion of these two fibers also caused higher flatus volume and hydrogen and carbon dioxide excretion. The Alphacel and corn bran diets resulted in breath and flatus gas excretion at levels equivalent to the fiber-free diets. These observations are consistent with the high recovery of fiber from the Alphacel and corn bran and the implied fermentation and hence low recovery of the xylan and the pectin.

The results from this work show that dietary fiber sources produce different patterns of monosaccharides on direct TFA hydrolysis. The results also show that it is possible to obtain significant information on the fate of dietary fiber sources by direct TFA hydrolysis of aliquots of recovered fecal material. Different dietary fiber sources give different amounts and patterns of monosaccharides that were relatable to the fiber sources ingested.

Additional experiments are needed to relate not only the monosaccharide composition of the fibers fed to fecal output but also the structures of those fibers and how they may have been modified in the digestive tract even if not fermented. The effect of fiber particle size and pretreatment should be studied. Free sugar, starch, cellulose and uronic acid measurements should be made in order to obtain a more complete picture of what survives and what is metabolized. The effect of dietary fibers on the digestion and utilization of other polysaccharides and other food components should be studied. This information, together with fermentation data, including gas and VFA production, will provide a better understanding of the role and value of different dietary fibers and their effects on nutrient bioavailability.

Acknowledgment

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Properties of Wood Lignin

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            To further the understanding of lignin as a
       component of the dietary fiber, studies of
       three aspects of the properties of enzymati-
       cally isolated wood lignin are discussed
       here: (1) the effect of cations and com-
       plexing ligands on adsorption of nitrosamines
       on lignin; (2) the binding of heavy metals on
       lignin; and (3) the adsorption of bile acids
       on lignin, wood and cellulose.

       Lignin is a highly branched polymer composed of
phenylpropane units that are believed to be chemically
bound to the carbohydrates in wood. Either degradative
processes, such as those used in producing kraft or
Klason lignins, or extraction with organic solvents,
such as those used in producing Brauns lignin, are used
to isolate lignins from wood. Lignins isolated by these
methods are unlikely to be useful for elucidating the
role of lignin in dietary fiber because they are either
chemically or structurally different from naturally
occurring lignin (1). We have concentrated our efforts
on lignins obtained from wood by enzymatic digestion (2).
Lignins prepared by the enzymatic removal of the carbo-
hydrates of plant materials bear a closer resemblance
to the substances found in the digestive tract. To
further the understanding of lignin as a component of
dietary fiber, studies of three aspects of the proper-
ties of wood lignin are discussed here: (1) the effect
of cations and complexing ligands on adsorption of
nitrosamines on lignin; (2) the binding of heavy metals
on lignin; and (3) the adsorption of bile acids on
lignin, wood and cellulose.

Effect of Cations and Complexing Ligands on the Adsorption of Nitrosamines on Lignin

The marked differences in the adsorption of N-nitrosodiethylamine on different lignin preparations enabled us (3) to correlate the adsorption of this nitrosamine with chemical studies of the various lignins. The adsorption of N-nitrosodiethylamine was also used as a diagnostic tool in defining optimum conditions for preparing the enzyme lignin. Two factors proved to be of particular importance: mechanical pre-treatment of wood and the conditions of the enzymatic digestion process (4). The effect of these factors on the metal content in wood and lignin is discussed here.

The mechanical comminution process can introduce heavy metals, particularly iron, into the samples. Analysis by atomic absorption has shown (Table I) that the calcium, magnesium, and zinc content was not affected by the comminution method. Samples of wood pulverized by a Hurricane Pulverizer (a hammer mill), however, had an iron content one order of magnitude higher than those treated in a Wiley Mill (a rotating steel cutter). In both cases the samples were further ball-milled with ceramic balls. The enzymatic lignin prepared from the pulverized and ball-milled wood showed a high iron content (Table I). The content of metal ions in the wood samples was reduced by deionization by extraction with 1 $\underline{M}$ HCl and 1 $\underline{M}$ NaCl and subsequent dialysis. Similar treatment of the lignins showed a decrease in calcium, magnesium, and zinc content, with a relatively low degree of removal of iron. This indicates that lignin binds iron more strongly when isolated from the wood polysaccharides.

Another source of the metal ion content of lignin is from the enzyme solutions. Two types of enzymatic preparations were used: a mixture of the cellulases produced by cultures of Schizophyllum commune and Trichoderma reesei (5); and a commercial preparation (Cellucast, Novo Industry, Denmark) which is a concentrate of the cellulases produced by Trichoderma viride. Both types of enzyme preparation contain metal ions derived from the culture media.

N-nitrosodiethylamine is strongly adsorbed onto kraft lignin (6), Klason lignin (7), and onto freshly prepared or stored samples of enzyme lignin with low residual sugar prepared from Wiley-milled wood. By contrast all of the enzymatic lignin samples prepared from Hurricane-pulverized wood were poor adsorbers of nitrosamine. We believe that iron, introduced by the Hurricane pulverizer, forms a complex with lignin that alters its adsorptive properties. In one enzyme lignin sample freshly prepared from Hurricane-pulverized wood, the adsorption of nitrosamines was enhanced by washing

Table I

Minerals in Aspen Wood and Lignins
(mg/kg) [a]

	Ca	Mg	Fe	Zn
Wood, Hurricane- pulverized, ball-milled	1600	220	350	50
Wood, same, deionized[b]	40	3	90	6
Wood, Wiley-milled ball-milled	1400	170	30	50
Lignin, enzymatic[c]	1200	75	370	8
Lignin, same, deionized[b]	90	9	280	7

[a]Determined by atomic absorption.

[b]Extracted with 1 M HCl and 1 M NaCl, dialyzed against
distilled water.

[c]From Hurricane-pulverized, ball-milled wood.

the lignin with a 1 molar solution of calcium, sodium
or lithium chloride (Figure 1). The adsorption proper-
ties of lignin increase with increasing ionic radius of
the cation. This indicates that probably an ionic
strength effect is involved, affecting the composition
of the layer of solvent (water) at the surface of
lignin. However, when the sample of lignin was stored
in a semi-wet state at 4°C for a month, this enhance-
ment of nitrosamine adsorption by contact with concen-
trated salt solutions was lost.

     Another sample of enzymatic lignin freshly pre-
pared from Hurricane-pulverized wood was suspended in
0.1 $\underline{M}$ EDTA and stored at 4°C for one week. This treat-
ment also enhanced the adsorption of nitrosamine
(Figure 2). It therefore appears that ion exchange of
heavy metals, notably iron, by complex formation with
EDTA enhances the adsorption of nitrosamine on enzy-
matic lignin. Systematic control of the heavy metal
ions in enzymatically prepared lignins is a necessary
feature of future studies.

Binding of Heavy Metals on Lignin

     Adsorption of copper(II) and zinc(II) ions from
unbuffered aqueous suspensions of lignin was followed
by polarographic determination of the concentration of
those ions remaining in solution, using varying con-
centrations of Cu^{2+} and Zn^{2+} ions equilibrated with
lignin preparation for 20-24 h. The amount of the
metal ion adsorbed by one gram of lignin (Figures 3, 4)
was similar for both metals. The binding increases in
the sequence: kraft lignin < enzymatic lignin < brown
rot wood, for both copper and zinc ions, and the shapes
of the adsorption isotherms were similar. The presence
of two limiting regions, most clearly demonstrated for
the adsorption of zinc ions on brown rot wood, indicates
at least two different binding centers. The sequence
of the binding ability of lignin for metal ions differs
from that found for binding nitrosamine and indicates
that binding sites for copper and zinc are distinct
from those that interact with nitrosamine. Enzymatic
lignins may contain residual cellulose and some amount
of adsorbed proteins and/or peptides that adsorb copper
and zinc. Elemental nitrogen and primary amino groups
have been detected in our lignin preparations, but a
systematic study of the effect of these residues on
Cu^{2+} and Zn^{2+} binding is not yet available.
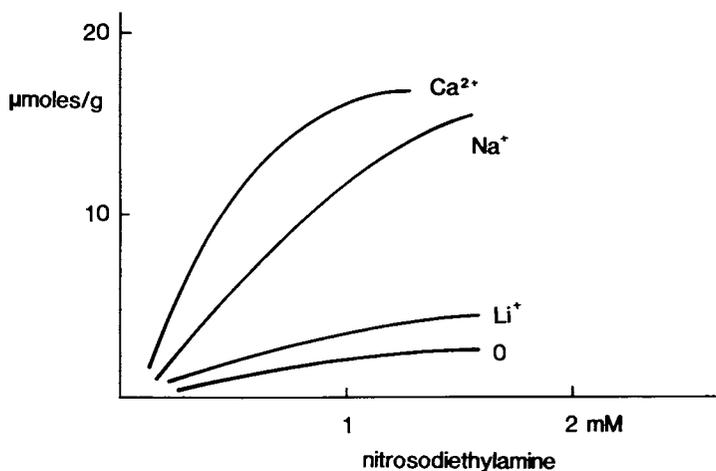
Figure 1.   Effect of high concentrations of solutions of strong electrolytes on the adsorption of N-nitrosodiethylamine on lignins. Dependence of the number of μmoles of the nitrosamine adsorbed on equilibrium concentration of free, unbound N-nitrosodiethylamine.

Lignin was produced by digestion of Hurricane-pulverized, ball-milled aspen wood with an 80:20 mixture of enzyme-containing supernatants from Schizophyllum commune and Trichoderma reesei cultures (final sugar content 4%). Samples were exposed for 30 min to 1 M CaCl₂ (Ca²⁺), NaCl (Na⁺); LiCl (Li⁺), or water (0). Suspension was centrifuged, supernatant discarded, lignin suspended in solutions containing varying concentrations of N-nitrosodiethylamine, equilibrated for 24 h, and remaining concentration of the nitrosamine determined in the supernatant.
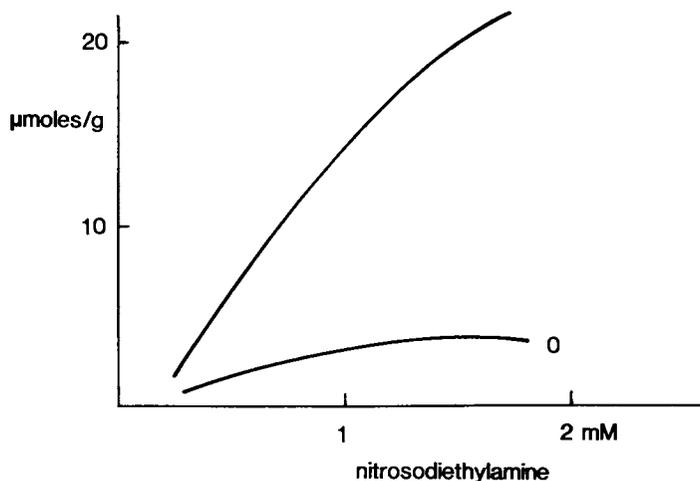
Figure 2.   Effect of treatment with EDTA on the ability of lignin to adsorb N-nitrosodiethylamine. Dependence of the number of μmoles of the nitrosamine adsorbed on equilibrium concentration of free N-nitrosodiethylamine.

Lignin was produced as in Figure 1 with a final sugar content of 16%. The lignin was suspended in 0.1 M EDTA for 1 week. The suspension was then treated as in Figure 1.
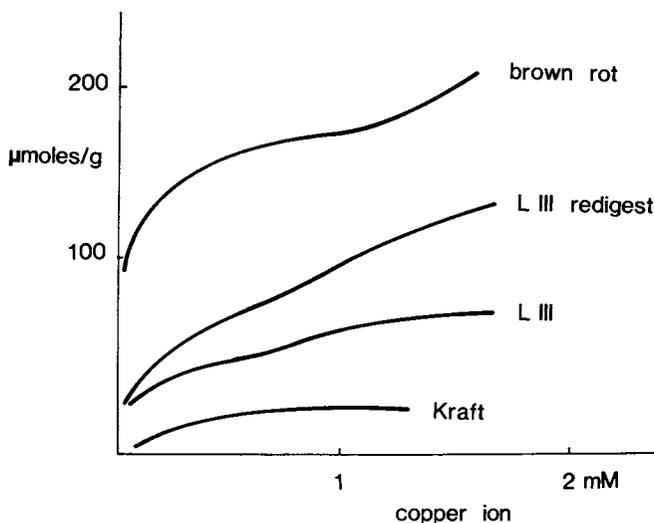
Figure 3.   Adsorption of cupric ions on lignins of various origin and similar mate-
rials. Dependence of the number of μmoles of Cu²⁺ ions adsorbed on equilibrium
concentration of the free Cu²⁺ ions.

"Brown rot" refers to birch wood decayed by Polyporus Betulinum and digested with
Celluclast (without milling pretreatment). One sample of lignin (L III redigest) was produced
from Hurricane-pulverized, ball-milled aspen wood and digested with both Celluclast and
the Schizophyllum and Trichoderma enzyme mixture (final sugar content 12%). Another
sample of Lignin (L III) was produced from Hurricane-pulverized, ball-milled aspen wood
and then digested with the Schizophyllum and Trichoderma mixture (final sugar content 4%);
Kraft lignin was purchased from Westvaco. Samples of lignin were suspended in solutions
containing varying concentrations of copper sulfate, equilibrated for 24 h, and remaining
concentration determined in the supernatant.
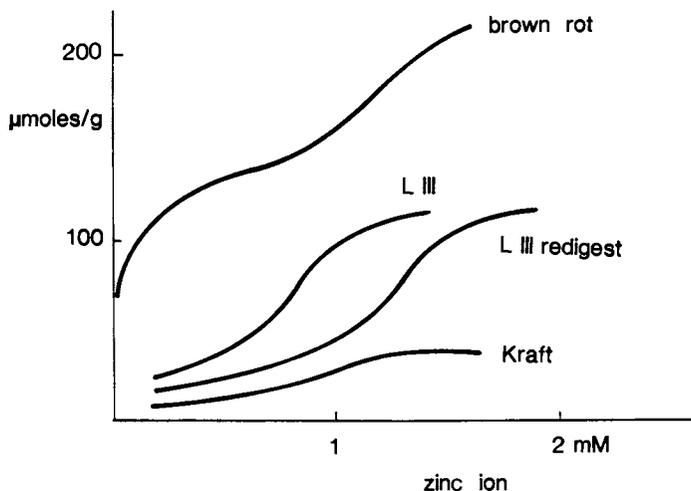
Figure 4.   Adsorption of zinc ion on various lignins and similar materials. The
same samples of lignin as in Figure 3 were equilibrated with a zinc sulfate solution.

The Adsorption of Bile Acids on Lignins

The adsorption of the bile acids from aqueous solutions of their salts was followed by measuring the concentration of the unadsorbed acid in the supernatant, using the measurement of the absorption spectra of polyenylic carbocations formed from bile acids in 72% sulfuric acid (8). For a given bile acid, e.g., cholic acid, much smaller differences in adsorption to a variety of lignins and wood related materials was found, as compared to adsorption of nitrosamines. Bile acids are thus less suitable for discriminating adsorptive properties of lignin preparations. Nevertheless, comparing the adsorptive capacities for a given mass of adsorbent follows a similar sequence for the adsorption of bile acids as was observed for nitrosamines. In both cases, cellulose and wood powders are very weak adsorbers. All of the lignins, both kraft and enzymatic, showed significantly greater absorptivity than wood (Figure 5). The sequence of adsorption properties of cholic, deoxycholic and taurocholic acids onto lignin (Figure 6) follows the same pattern as reported by Eastwood (9) and Kay (10), but it is doubtful if these differences are significant. The small differences in adsorptivity of these bile acids, as well as those of lithocholic and glycocholic, indicate that the side-chain and the number of the hydroxy groups play a minor role in the adsorptivity, probably due to a flat orientation of the rigid sterol molecule at the lignin surface.

The adsorption of bile salts to lignins is practically complete in about 5 h. When samples of lignin with adsorbed bile acid were separated from the supernatant, suspended in water and shaken for 3 h, 50% of the bile acid was desorbed.

Experimental

Trembling aspen wood (*Populus tremuloides* Michx.) was reduced to a powder in a Hurricane Pulverizer. Samples were prepared from extracted pulverized wood by ball-milling for 10 days in a rotating ceramic mill equipped with an air-conditioning unit which prevented the temperature of the contents of the mill from rising above ambient. Digestion of the samples with cellulase was done with a mixture of *Schizophyllum commune* and *Trichoderma reesei* culture filtrates at 30°C for 3 days. One sample (Lignin III redigest) was digested a second time with a commercial enzyme preparation, Celluclast (Novo Industry, Denmark), at 50°C for 2 days. The isolated lignin suspension was stored at 4°C until analysis.
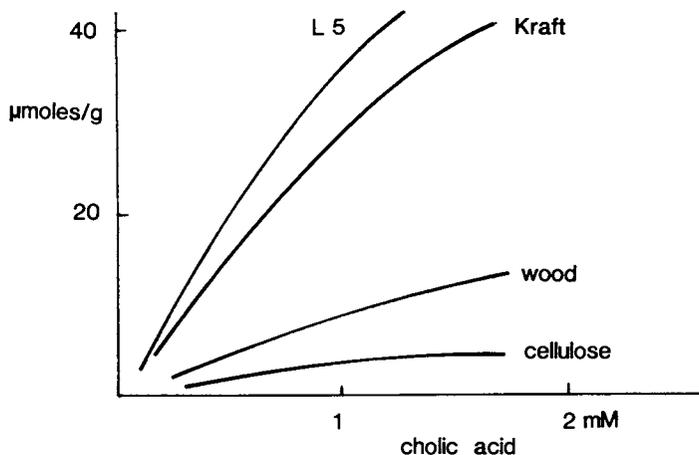
Figure 5.   Adsorption of cholic acid on cellulose, wood and kraft, and enzyme lignins. Dependence of the number of μmoles of cholic acid adsorbed on equilibrium concentration of the free cholic acid.

Lignin (L 5) was produced from Wiley-milled, ball-milled aspen wood and digested with the Schizophyllum *and* Trichoderma *mixture (final sugar content 4%); Kraft lignin was purchased from* Westvaco; *microcrystalline cellulose (Avicel R) was obtained from the FMC Corporation (Newark). Samples of lignin, wood, or cellulose were suspended in unbuffered solutions of sodium cholate, equilibrated for 24 h, and remaining concentration determined in the supernatant.*
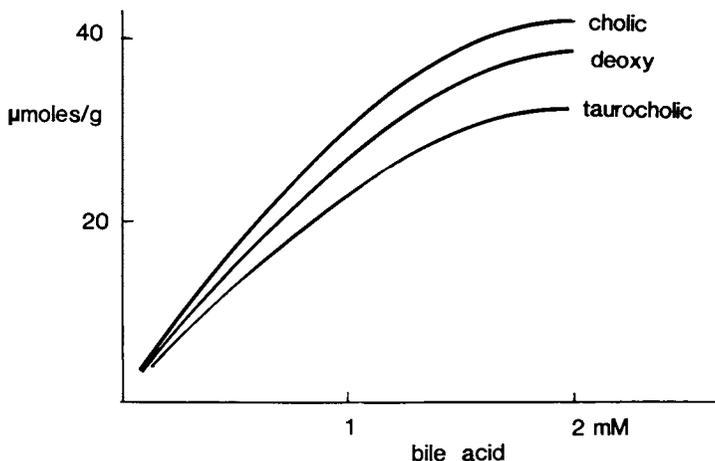
Figure 6.   Adsorption of cholic, deoxycholic, and taurocholic acid on enzyme lignin (L 5).

Lignin L 5 was obtained and treated as in Figure 5, solutions of sodium salts of cholic, deoxycholic, and taurocholic acids were equilibrated with lignin for 24 h, and remaining concentration in the supernatant determined.

One sample (L5) was produced from Wiley-milled,
ball-milled wood which had been digested with the
mixture of enzymes found in S. commune and T. reesei
culture filtrates (5). This lignin sample was dried
under vacuum at ambient temperature and then ground to
a fine powder with a mortar and pestle.

Brown rot wood was prepared from birch wood which
had been decayed by Polyporus Betulinum and digested
with Celluclast without milling pretreatment.

Kraft lignin (lot no. RLX 4290:20B) was obtained
from Westvaco, Charleston Research Center, North
Charleston, SC. Microcrystalline cellulose (Avicel R)
was obtained from FMC, Newark.

In the study of nitrosamine adsorption, lignin
suspensions were stored at 4°C for less than 15 days.
Samples were exposed to 1 M $CaCl_2$, NaCl, LiCl, or water
for 30 min or to 0.1 M EDTA for 1 week. Suspensions
were centrifuged and supernatants were discarded.
Lignin samples were then suspended in unbuffered
solutions containing varying concentrations of N-nitro-
sodiethylamine and equilibrated for 25 h. The remain-
ing concentration of nitrosamine was determined in the
supernatant by polarography (3).

Adsorption of copper(II) and zinc(II) ions was
determined by suspending lignin samples in unbuffered
solutions containing varying concentrations of copper
sulfate or zinc sulfate. After equilibration for
20-24 h, the concentration remaining in the supernatant
was determined by polarography.

Samples of lignin, wood, or cellulose were sus-
pended in phosphate buffer (pH 7.0, $\mu = 0.5$) containing
varying concentrations of sodium cholate, sodium
deoxycholate or sodium taurocholate. The concentration
remaining in the supernatant after equilibration for
20-24 h was determined by measuring the absorption
spectra of polyenylic carbocations formed from bile
acids in 72% sulfuric acid (8).

Adsorption of selenium(IV) ions from unbuffered
solutions of kraft lignin was examined in preliminary
studies. Polarographic determination (11) of
selenium(IV) remaining in solution after 25 h equili-
bration indicated that kraft lignin adsorbs a negli-
gible quantity of selenium. A polarographically
reducible organic compound which was extracted from the
lignin prevented a more detailed analysis of the
adsorption properties of selenium(IV).

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Chemical and Physical Properties of Tobacco Fiber

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Fiber complexes of ten tobacco cultivars as by-
products of the leaf protein extraction process con-
tain levels of neutral and acid detergent fiber com-
parable to that of alfalfa fiber and in excess of
that in wheat bran. Compositional analyses revealed
lesser concentrations of cellulose, lignin, and
lipid but a greater amount of hemicellulose in
tobacco than in alfalfa. In reference to wheat bran,
tobacco fiber has comparable amounts of Na, P, S,
Mn, and Cu but at least 20-fold more Ca and several-
fold more Fe. Among tobacco cultivars, dark green
tobaccos accumulate more starch and protein than
burley and Turkish types. Quantitative variation
of cellulosic components and minerals is also
evident among tobaccos. Average bulk fiber volume
of tobacco cultivars is 14.2 mL/g as compared to
9.5 and 4.5 mL/g for alfalfa fiber and wheat bran,
respectively. The water retention capacity of
tobacco fiber (9 g water/g) is nearly double that
of alfalfa fiber and eight times greater than
wheat bran. Cation exchange capacity ranks the
highest for tobacco, followed by alfalfa and then
wheat bran. Sodium taurocholate binding capacity
is comparable among the three fiber sources al-
though the fiber complex of flue-cured tobaccos
absorbs more than others. The above properties of
tobacco fiber complex suggest its potential as a
source of dietary fiber.

Epidemiological evidence in causative relationships between
fiber-depleted diets and certain characteristically Western
diseases has stimulated research interest in the physiological
and physico-chemical properties of various dietary fibers in the
past decade (1). Dietary fibers are composed of cellulose,
lignin, and noncellulosic polysaccharides, all of which are the

components of plant cell wall. Pure cellulose exerts little
physiological function, whereas lignin and noncellulosic
polysaccharides are capable of binding fatty acids, bile acids,
and carcinogens (2, 3). Substances such as starch, protein,
lipid, phytic acid, and minerals are usually associated with
dietary fiber in forming a dietary fiber complex that alters
physico-chemical properties of the fiber. Among the common
foods of plant origin, breakfast cereals and wholemeal breads
containing wheat bran are high dietary-fiber foods for both
health conscious people and for the treatment and control of
diabetes (4). Enrichment of foods with fiber from cereal hull,
citrus pulp, guar gum and woody tissues is presently in the
experimental stage. Although fibers in the natural foods of
human diet are primarly derived from leafy vegetables, the
purified dietary fibers of leaf tissue origin are not commer-
cially available.

     The recent achievement in extraction of leaf protein from
young tobacco plants has advanced the possibilities for utiliz-
ing tobacco plant materials in human foods (5). Young tobacco
plants grown under high density produced leaf biomass two to
three times greater than stalk weight (6). Fibrous residue
of young tobacco plants from the leaf protein extraction
process can be a potential dietary fiber if the residue, after
removal of plastic pigments, possesses desirable physico-
chemical properties. The present study was therefore under-
taken to evaluate the decolorized tobacco fibrous residues for
chemical and physical properties and to compare them with wheat
bran and alfalfa fibrous residue. Because of a broad genetic
variation in morphology and chemical composition of plants
within Nicotiana tabacum, the present study compared a number
of tobacco types.

Materials and Methods

     A field experiment involving ten tobacco cultivars
representing flue-cured, dark fire-cured, burley, Turkish and
Maryland types was conducted in Lexington, Kentucky, in early
spring 1980. About 110-120 plants per plot (1.5 x 1 m) per
cultivar were established by direct-seeding and cultured accord-
ing to conventional practices in the burley growing region.
When plants reached 50 cm or taller in the middle of June, all
plants within a 0.36 m^2 area were cut about 12 cm from the
ground as one sample with three samples taken per plot. A total
of 30 samples were separately processed to obtain deproteinized
and decolorized fiber complex.

     Deproteinization of fresh tobacco plants was accomplished
by homogenization in a high capacity Waring blender with an
equal weight of ice-cold 2% sodium metabisulfite solution. The
homogenate was strained through three layers of cheese-cloth and
one layer of Miracloth and, subsequently, the green fibrous

residue was washed with a sufficient volume of cold water to
remove residual soluble protein. The deproteinized green
fibrous residue was washed with several volumes of cold 80%
isopropanol until cream colored. In addition, three samples
of fresh alfalfa (before the flower bud stage) which were
deproteinized and decolorized by the same process and wheat
bran from a commercial mill were included for comparison. All
samples were sieved through a 9-mesh screen and oven-dried
(70 C) overnight before chemical and physical characterization.
     All fiber complexes were analyzed for protein, starch,
lipid, cellulose, lignin, and ash concentrations. Protein
quantity was calculated from the difference in free and total
amino acid contents before and after 6N HCl digestion. Ninhy-
drin reaction of amino acids was colorimetrically determined at
570 nm with known quantities of amino acids as standards (7).
Starch was quantitated at 600 nm by the modified iodine stain
method of Gaines and Meudt (8). The weight difference before
and after overnight Soxhlet extraction of fiber complex with
hexane measured lipid content. Cellulose and lignin quantita-
tion on an ash-free basis was performed through gravimetric
methods (9). Ash content was the residual weight of cellulose
and lignin after charring in a muffle furnace at 550 C for 4 hr.
The difference of the above six components from 100% was
considered as apparent hemicellulose which includes hemicellu-
lose,pectic substances, gums, and mucilages, if present. The
same samples were analyzed for neutral and acid detergent fiber
(NDF and ADF) content according to Van Soest's methods (10).
     Mineral elements except phosphorus and molybdenum in the
tobacco fiber were analyzed by use of Varian AA-6 Atomic
Absorption Spectrophotometer. Samples were wet-ashed in nitric
and perchloric acids (9:1, v/v) until completely digested. After
evaporation to dryness, the ashes were solublized in 1N HCl for
analyses. Quantitation of phosphorus was a colorimetric method
using a Technicon Autoanalyzer and the color reaction of Fiske
and Subbarow (11). Molybdenum was quantitated by the method of
Eivazi et al (12) which determines, with good reproducibility,
a quantity as low as 0.02 ppm in plant materials.
     Physical properties measured in the present study included
bulk volume, water retention capacity, and cation exchange
capacity. Bulk volume measurement was the method of Montgomery
and Baugardt (13). whereas water retention capacity was the
amount of water retained by the fiber against a centrifugal
force of 14,000 g for one hr after a 24 hr hydration period at
20 C (14). For determination of cation exchange capacity, the
fiber complex was soaked in 2 N HCl for 24 hr followed by wash-
ing with deionized water. Hydrogen ions were dissociated from
the fiber complex with 2N neutral NaCl solution which was then
titrated against 0.05N NaOH (14).
     Binding of sodium taurocholate by the fiber was the method
of Kritchevsky and Story (15). A 50 or 100 mg sample of fiber

was incubated in 5 ml of HPLC-grade water containing 0.15 M NaCl
and 8 mM (40 μ moles) or 40 mM (200 μ moles) sodium taurocholate
at 37 C for one hr with gentle shaking. For the control, 5 mL
of 0.15 M NaCl solution devoid of sodium taurocholate was added
to the testing sample. Sodium taurocholate in the same NaCl
solution with a concentration series of 40 mM, 20 mM, 10 mM, 5 mM
and 2.5 mM was processed by the same procedure to establish a
calibration curve. After incubation, the sample was filtered
through a 0.2 μm microporous filter and the filtrate was ready
for HPLC analysis. Varian Model 5000 Liquid Chromatograph
equipped with a Vari-Chrom UV-Vis Detector was used. The HPLC
conditions were: Micro Pak MCH-10 column (30 cm X 4 mm I.D.);
eluent, 70% MeOH and 30% 0.15 M NaCl solution, isocratic; flow
rate, 1 mL/min; detection at 210 nm. With a 10 μL injection and
15 min cycle, sodium taurocholate can be detected within 5 min.
Control samples did not show any peaks with retention times
similar to that of sodium taurocholate.

     Nicotine contamination in tobacco fiber was assayed by the
GLC equipped with an alkaline flame ionization detector. The
extraction procedure and GLC conditions have been detailed in a
recent paper (6). The sensitivity of detection is below 1 ppm.

     All data were subjected to variance analyses. Whenever
variation among fiber sources was statistically significant, the
least significant difference (LSD) at the 5% and 1% level of
probability was calculated for statistical inference of the
results.

Results

     Chemical composition of tobacco and alfalfa deproteinized
and decolorized fiber complexes and wheat bran is given in Table
I. Dark green tobaccos including flue-cured (NC 95, NC 2326 and
Coker 139), dark fire-cured (Ky 171 and Ky 151) and Maryland
(MD 609) types had comparable amounts of protein with a mean of
15 g/100 g dry wt. The average value of the three burleys
(Ky 16M, Burley 21 and Ky 14) was 9.46 g/100 g dry wt with
Burley 21 showing significantly less than the other two. Turkish
tobacco Xanthi nc resembled burley in protein content. Alfalfa
fiber had a protein concentration similar to that of dark green
tobacco, whereas wheat bran contained a significantly higher
level among the fibrous samples under comparison. For starch
content, a significant contrast existed between dark green
tobacco and burley or Turkish type with the exception of MD 609
resembling burley in low starch concentration. Incidentally,
Maryland and burley tobaccos are air-cured types. That starch
content was low in alfalfa fiber and reached as high as 30%
weight in wheat bran was not unexpected owing to the nature of
the corresponding plant parts. There was little difference in
lipid concentration among the fibrous residues of tobacco and
alfalfa with a mean of 2.82 g/100 g dry wt. More than three

Table I. Chemical Composition of Tobacco and Alfalfa Fibrous Residues and Wheat Bran.

Fiber source	Protein	Starch	Lipids	Ash	Cellulose	Lignin	Apparent hemicellulose	NDF	ADF
					g/100 g dry wt				
Tobacco									
NC 95	13.54	4.30	2.44	7.14	31.38	5.68	35.52	53.46	41.51
NC 2326	15.48	2.01	3.06	8.11	33.88	4.13	33.33	53.36	41.50
Coker 139	15.61	2.22	2.78	12.55	32.21	8.25	26.08	55.91	47.25
Ky 171	15.88	2.42	2.40	10.75	28.09	4.85	36.62	50.42	41.85
Ky 151	14.27	1.38	3.38	9.31	33.83	5.10	32.75	55.36	42.15
Ky 16M	12.21	0.90	3.55	9.41	32.68	5.38	35.86	56.06	47.89
Burley 21	6.02	0.33	2.67	12.79	32.71	5.77	39.71	53.84	45.15
Ky 14	10.14	0.09	2.48	9.94	30.60	4.55	42.15	51.84	42.07
Xanthi nc	8.41	0.18	3.09	10.79	35.19	6.24	36.27	61.04	46.98
MC 609	15.23	0.88	2.32	14.79	34.00	7.15	25.63	55.74	51.06
Alfalfa	13.12	0.60	2.80	4.20	44.23	9.91	25.05	61.84	50.71
Wheat bran	18.51	30.67	9.54	5.80	10.17	2.94	24.11	47.05	11.56
LSD 0.05	2.44	1.20	0.99	1.07	2.53	1.09	3.62	5.26	2.28
LSD 0.01	3.31	1.63	1.34	1.46	3.44	1.48	4.93	7.15	3.10

times this amount was found in wheat bran. Ash content varied
widely among tobacco fibrous samples which had an average
quantity of 10.56 g/100 g dry wt. The ash content in alfalfa
fiber and wheat bran was about one-half or less than that in
tobacco fiber.

On the same weight basis, wheat bran had only 1/3 to 1/4
the cellulose quantity present in tobacco and alfalfa fibers.
Alfalfa fiber was also rich in lignin with a quantity three
times greater than that in wheat bran. Lignin quantity in
tobacco fiber was in-between those of alfalfa fiber and wheat
bran; however, significant differences existed within and between
tobacco types. Such a range of variation was also evident for
the quantity of apparent hemicellulose in tobacco samples.
Apparent hemicellulose content in alfalfa fiber and wheat bran
was indifferent to the tobacco cultivars being on the low concen-
tration end of the variation. Quantitative variation of NDF and
ADF was present among tobacco cultivars but they lacked the same
pattern of variation. The tobacco cultivar means for NDF and ADF
quantity were 54.70 and 44.74 g/100 g dry wt, respectively.
These values were significantly higher than those of wheat bran.
Alfalfa fiber had the highest amounts of both detergent fibers
although the amounts were not statistically different from those
of the tobacco cultivars ranked high in detergent fiber content.

It has been shown that fiber-rich diets may impair the
utilization of a number of essential minerals including calcium,
iron, zinc, copper, and phosphorus (16). This can be corrected
by dietary intake of minerals from a variety of foods, on the
one hand, and the endogenous source of essential minerals in the
dietary fiber, on the other. Eleven mineral elements were
therefore quantitated in tobacco and alfalfa fibers and wheat
bran (Table II). In reference to wheat bran, tobacco fiber
contains comparable or slightly higher levels of sodium, phos-
phorus, sulfur, manganese and copper but at least 20-fold more
calcium and several-fold more iron. Iron concentration varied
nearly five-fold among tobaccos. Wheat bran was rich in
potassium, magnesium, and molybdenum. Alfalfa fiber showed a
close resemblance to tobacco in mineral content except for
calcium, magnesium, and copper, which were significantly less.

Rasper (17) studied physical characteristics of dietary
cereal fiber including those from wheat bran and reported that
bran fiber had bulk volume around 3 to 5 mL/g depending upon
preparation method. The wheat bran sample in the present
experiment gave the bulk volume of 4.53 mL/g which is in good
agreement with the values cited in literature. The bulk volume
of alfalfa and tobacco fibers doubled and tripled the wheat bran
value (Table III). Among tobaccos, Xanthi nc gave the largest
bulk volume and NC 2326 the least. The variation seemed to be
significant for flue-cured tobaccos but not for burleys. The
average water retention capacity of tobacco fiber was 9 g water/g
with a range of 7.40 to 10.86. Therefore, tobacco fiber held

Table II. Quantities of Mineral Elements in Tobacco and Alfalfa Fibrous Residues and Wheat Bran.

Fiber source	Na	K	Ca	Mg	P	S	Fe	Mn	Zn	Cu	Mo
	%						ppm				
Tobacco											
NC 95	0.49	0.28	1.98	0.12	0.18	0.22	542	115	58	22	0.43
NC 2326	0.39	0.24	2.03	0.16	0.20	0.30	743	104	54	21	0.85
Coker 139	0.44	0.90	2.53	0.20	0.22	0.43	1510	212	40	43	0.91
Ky 171	0.45	0.49	2.71	0.18	0.26	0.30	701	103	30	21	0.65
Ky 151	0.42	0.38	2.68	0.23	0.27	0.41	659	92	56	29	0.54
Ky 16M	0.53	0.69	2.68	0.19	0.31	0.26	293	99	37	12	0.23
Burley 21	0.64	0.71	2.95	0.22	0.40	0.27	880	111	51	21	0.72
Ky 14	0.48	0.51	3.08	0.32	0.38	0.34	738	99	116	38	0.49
Xanthi nc	0.51	0.55	2.92	0.27	0.35	0.31	861	118	59	32	0.57
MD 609	0.52	0.96	3.12	0.25	0.32	0.36	1407	171	78	32	0.75
Alfalfa	0.35	0.11	1.22	0.16	0.13	0.14	357	21	36	7	0.50
Wheat bran	0.35	1.46	0.11	0.54	0.23	0.20	175	114	184	20	1.25
LSD 0.05	NS	0.29	0.45	0.06	0.06	0.09	407	34	46	2	0.49
LSD 0.01	NS	0.39	0.61	0.08	0.08	0.13	553	46	65	3	0.67

Table III. Physical Properties of Tobacco and Alfalfa
Fibrous Residues and Wheat Bran.

Fiber source	Bulk volume (mL/9)	Water retention capacity (g water/g fiber)	Cation exchange capacity (meq NaOH/g) X 10^{-2}
Tobacco			
NC 95	14.90	9.57	21.83
NC 2326	11.33	8.40	17.50
Coker 139	14.77	10.40	21.50
Ky 171	14.87	7.40	20.00
Ky 151	14.00	10.86	19.00
Ky 16 M	13.80	9.18	20.67
Burley 21	13.97	8.47	21.50
Ky 14	14.80	8.55	18.50
Xanthi nc	16.33	8.02	15.67
MD 609	13.23	9.13	19.67
Alfalfa	9.47	4.92	15.33
Wheat bran	4.53	1.18	6.17
LSD 0.05	0.93	0.89	2.47
LSD 0:01	1.25	1.21	5.43

twice the volume of water than its alfalfa counterpart and six-
fold or more volume than wheat bran. The ranking of cation
exchange capacity among the fiber sources followed the same
order of the above physical properties with tobacco fiber being
the highest and wheat bran the least. The average value of
cation exchange capacity for tobacco cultivars was 19.58 X 10^{-2}
meq NaOH/g. The difference between tobacco fiber and wheat bran
was about three-fold. The cation exchange capacity of wheat
bran was one-half the value of the alfalfa sample.

     Results of sodium taurocholate binding from two experiments
employing different combinations of sodium taurocholate and
fibrous material was given in Table IV. There was a significant
difference among tobacco types but not within a given type.
Both experiments revealed that flue-cured tobaccos had higher
sodium taurocholate binding capacity than the other tobacco
types, and the binding amount was in proportion to the quantity
of fiber. By averaging ten tobacco cultivars, the binding
capacity was 2.47 μ moles/50 mg and 6.07 μ moles/100 mg for two
experiments. These values were ranked the highest, followed by
alfalfa; the lowest was wheat bran. The difference between the
latter two was not statistically significant. The fiber of
flue-cured tobacco was significantly higher in binding capacity
than alfalfa fiber and wheat bran in most cases. However, the
high binding capacity of flue-cured tobacco cannot be correlated
with the quantity of any chemical constituents given in Table I.

     Young tobacco plants usually contain about 0.5% of dry wt
as nicotine although nicotine quantity varies widely among
genotypes (18). Nicotine contamination in tobacco fibrous
residue ranged from 180 to 340 ppm with a mean of 260+53 ppm
(data not shown). Samples of Ky 171 fiber which contains 256 ppm
of nicotine were repeatedly washed with cold water (1:10, w/v)
and the washed residues were analyzed for nicotine quantity by
GLC. On the average, each washing removed two-thirds the
nicotine in the residue; and after several washings, nicotine
level approached 1 ppm (Figure 1). This points to the feasibil-
ity of lowering nicotine contamination in the fibrous residue to
a negligible level. The washing process did not alter the
concentration of protein and noncellulosic components.

Discussion

     Purified plant fibers for dietary purposes are combinations
of cellulose, lignin, hemicellulose, gums and pectinaceous sub-
stances that exert chemical and physical actions in the gastro-
intestinal tract. Dietary fiber complexes such as wheat bran
contain not only the fiber components but also other organic
compounds that contribute to nutrition. In comparison to wheat
bran, the deproteinized and decolorized fiber complexes of
tobacco and alfalfa have favorable chemical composition as
dietary fibers. Their low concentrations in starch and lipids

Table IV. Binding In Vitro of Sodium Taurocholate to Tobacco
           and Alfalfa Fibrous Residues and Wheat Bran

Fiber source	Experiment 1 μ moles/50 mg	Experiment 2 μ moles/100 mg
Tobacco		
NC 95	3.65	7.89
NC 2326	3.30	7.13
Coker 139	2.78	7.88
Ky 171	1.89	6.44
Ky 151	1.91	4.78
Ky 16M	2.12	6.56
Burley 21	1.86	4.37
Ky 14	2.54	4.99
Xanthi nc	2.48	5.14
MD 609	2.21	5.56
Alfalfa	2.16	4.93
Wheat bran	2.09	4.84
LSD 0.05	0.88	1.83
LSD 0.01	1.20	2.49
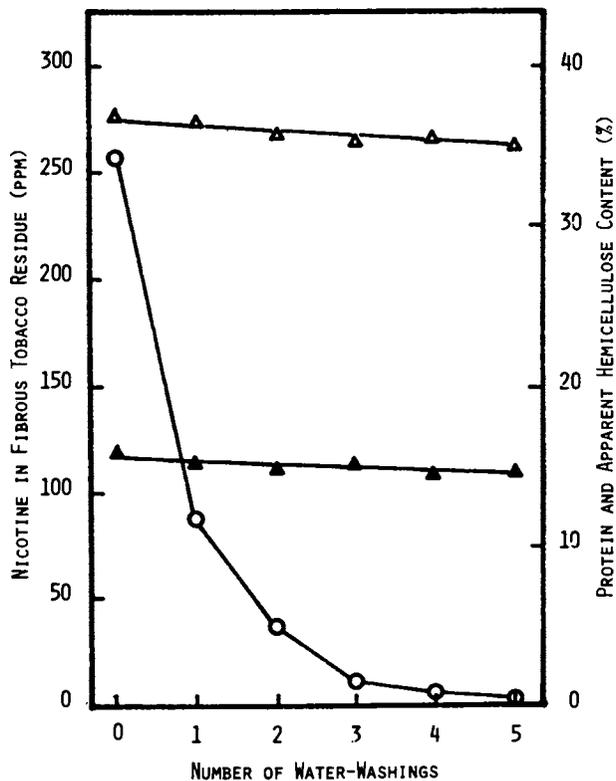
Figure 1. Effects of water-washing on the quantity of nicotine (○), protein (▲), and apparent hemicellulose (△) in the fibrous residue of Ky 171 tobacco.

and high amounts of cellulosic components seem to make them more suitable than wheat bran as low-calorie dietary fibers. This is further supported by the physical properties, especially for tobacco fiber, that a large bulk volume in accompaniment with high water retention and cation exchange capacity would be desirable. In addition, the absorption of bile salt (sodium taurocholate) by fobacco fiber is as effective as, or more so than wheat bran which suggests that tobacco fiber may also have the capability of regulating serum chloresterol levels. Nevertheless, the favorable chemical and physical properties of plant fiber complex measured in vitro still require confirmation from in vivo experimentation since the physiological performance and technological functionality of dietary fiber in fiber-enriched foods are influenced by many factors.

As suggested by Van Soest and Wine (10), the quantitative difference between NDF and ADF gives rise to a good estimation of hemicellulose quantity, and ADF is the sum of cellulose and lignin content. Taking wheat bran in the present study (Table I) as an example, the calculated values for hemicellulose and ADF are 35.49 and 13.12 g/100 g,which are in good agreement with Southgate's findings (19). However, the calculated value of hemicellulose is almost 50% greater than that of apparent hemicellulose. This is not surprising since wheat bran is rich in starch known to contaminate the NDF fraction. On the basis of NDF and ADF quantities, the calculated value of hemicellulose in the alfalfa sample is 11.13 g/100 g, while the average amount for tobacco fiber is 9.96 ± 2.82 g/100 g. The respective values of apparent hemicellulose are 225% and 345% greater. It has been reported that the ADF-reagent sometimes leaves substantial amounts of hemicellulose in the ADF-residue (20). Furthermore, Van Soest's detergent methods do not measure water-soluble components, whereas apparent hemicellulose includes these substances if they are not removed by 80% isopropanol washing. All these factors can contribute to the discrepancy between calculated and apparent hemicellulose values. This led to the suggestion that the pretreatment of fibrous material with lipid extraction and proteolytic and amylolytic enzymes would minimize erroneous results (19). One can also anticipate differences between tobacco and alfalfa and among tobacco cultivars in chemical composition and structure variation of cell wall as well as the nature of complex formation involving cell wall constituents and other substances. It would be highly desirable to compare the sugar constituents of tobacco fiber components with those of conventional dietary fibers.

The high concentration of iron and calcium in tobacco fiber would be desirable from a nutritional viewpoint. The bioavailability of mineral elements in dietary fibers has been discussed in the literature (16). Aside from fiber per se, phytic acid was found to be the major determinant of bivalent metal deficiencies (21). Phytate content is high in wheat bran and other

dietary fibers of seed origin since it is a source of glucuro-
nate precursor for synthesis of cell wall polysaccharides during
germination (22). Among the hundreds of compounds in tobacco
leaf, phytate has never been mentioned in a large volume of
tobacco literatures (18).

In analogy to phytate in wheat bran, tobacco plants
accumulate 2 to 5% of dry weight as oxalic acid, predominately
in the form of calcium salt, in mature leaves (18). Calcium
oxalate is insoluble in water. Young plants of seven burley
cultivars under high density growth had oxalate content in the
range of 0.6 to 1.4% (23). The dietary intake of oxalate poses
a concern for calcium and magnesium availability although it
was documented that oxalate can be formed in vivo from diets
containing ascorbate, glycine, glycolate, and glyoxylate (24).
Oxalate content in tobacco fiber was not determined in the
present study. However, the deproteinization and decolorization
processes would remove soluble oxalate. Calcium oxalate may
remain in the fibrous residue.and will not further deplete
calcium in diets because oxalic acid is already bound to calcium.
Many edible plants are rich in oxalic acid. For example, spin-
ach contains 8 to 10% dry weight as oxalic acid (25). Five cups
of tea per day in the English diet contributes to an intake of
75 mg oxalic acid (26). Oxalate content in tobacco fiber is
probably in the same range as that of certain common foodstuffs.

Another concern of possible adverse effects of tobacco
fiber complex as dietary fiber is nicotine contamination. Nic-
otine is regarded as highly toxic. Gasseline et al (27) re-
ported that the oral LD_{50} of an adult human has been estimated
to be 30 to 60 mg (0.5 to 1.0 mg/kg body wt). They also stated
that tobacco is much less toxic than expected from its nicotine
content because intestinal absorption of nicotine as present in
tobacco is so slow that metabolic inactivation sometimes keeps
pace with absorption and the elimination of nicotine is completed
within 16 hours. About 80 to 90% of the nicotine in human urine
has already been detoxified. Since nicotine is very water-
soluble, the washing of tobacco fibrous residue could lower
nicotine to a negligible level as demonstrated in the present
study. If the water-washed tobacco fiber contains 10 ppm of
nicotine, a 10% incorporation in fiber-enriched formulation
results in 1 ppm of nicotine in the formulated foods. The pre-
sence of nicotine in minute quantities was reported in tomato
and other solanaceous vegetables (28). With the GLC method
employing an alkaline flame ionization detector (6), a broad
array of fresh and preserved foods was analyzed for nicotine
contamination. Nicotine was detected at 2 to 15 ppm (on a dry
wt basis) in tomato, potato, eggplant, and green pepper but not
in alfalfa and wheat bran. Most interesting is the finding of
nicotine in tea, expecially instant tea, at levels of 15-24 ppm.
Nicotine quantity in tobacco can be drastically lowered by
genetic means (29). Varietal difference in oxalate content in

tobacco leaf also indicates possible genetic regulation (23).
If tobacco is grown as a food crop, the concentration of these
undesirable leaf constituents can be lowered by means of breed-
ing.
      The potential of tobacco utilization as a source of soluble
proteins and the deproteinized residue derived from the protein
extraction process as animal feed and safer smoking material has
been mentioned in recent years (5, 6, 30). The present study
suggests that after decolorization tobacco fibrous residue may
be a potential source of dietary fiber. Additional research is
certainly needed to evaluate the nutritional and physiological
significance of tobacco fiber in experimental animals. Some of
these studies are presently being undertaken.

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Products of Wheat Straw Biodegradation by *Cyathus stercoreus*

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The residual biodegradation pro-
ducts of wheat straw lignin
attacked by Cyathus stercoreus
for thirty days were identified.
Low-molecualr-weight nonvolatile
materials constituted less than
5% of the total ^{14}C released
from labeled lignin. In this
portion, syringic acid, vanil-
lic acid, p-hydroxy benzoic acid,
acetic acid, and 2-methoxy-succ-
inic acid were found. The ma-
jority of the water-soluble bio-
degradation products (89%) are
lignin-carbohydrate complexes
with molecular weights greater
than 1000. Analysis of the
gases resulting from lignin
breakdown and metabolism re-
veals primarily CO_2, with
smaller amounts of ethanol,
methanol, and acetone. The
gas phase, flushed out with air
and recovered, constitutes 50%
of the total lignin breakdown
products.

In the field of biomass utilization, separating lignin from cellulose and hemicellulose by biological means has aroused a great deal of interest. Degradation of lignin during the process of separation is not necessarily detrimental if the lignin can be converted to useful chemicals. To fully understand the biological approach to modifying and solubilizing lignin, the biodegradation products of lignin must be identified and quantitated.

Cyathus stercoreus (Schw.) de Toni NRRL 6473 degrades lignin preferentially during a 62-day fermentation of wheat straw, but the biodegradation products have not been characterized (1). Other workers have identified residual phenolics in biodegradation broths of several woods and synthetic or isolated soluble lignins (2). More recent publications have identified functional group changes in degraded lignins rather than specific low molecular weight compounds (3). All essentially identify oxidized species as a result of fungal or bacterial degradation.

Current knowledge in lignin biodegradation supports a mechanism of random oxidation, generating aromatic compounds with various oxygen-containing side groups, aliphatic carboxylic acids from subsequent ring cleavage and ultimately, carbon dioxide (4-7).

The purpose of the present work was to identify and quantitate the residual products after a 30-day fermentation of C. stercoreus on wheat straw. The approach to this problem is limited by a few, significant considerations. Ideally, preliminary extraction of the straw eliminates interfering substances, such as fatty acids and other extractables present in large quantity, which overwhelm minute amounts of biodegradation products and thereby hinder their identification. However, the fungus does not grow well when all soluble nutrients are removed, as will be shown in later sections. Also, sterilization by autoclaving may generate soluble lignins that cannot be attributed to fungal action, and therefore controls must be run. More specifically, reproducibility of fermentation on a solid substrate inoculated with a 1 cm^2 plug of slow-growing C. stercoreus is difficult, especially because this fungus is sensitive to excess water and will not grow in submerged culture, although a high level of moisture is necessary for fungal growth.

After the fermentation is complete, extracted water-soluble degradation products lose much of their water solubility if taken to dryness in air or if heated. Freeze-drying overcomes this problem, but molecular weight distributions should be determined before freeze-drying because polymer chains can be fractured by growing solvent crystals (8).

Materials and Methods

Sample Preparation. Three wheat straw samples were tested. One was a locally obtained, dried and baled wheat straw; the other two were Red River 68 and Butte HRS, grown in an environmental chamber. While monitoring the plant growth and lignin production in the straw as reported by Stone et al. (9), we grew the plants for 38 days (Butte HRS) or 48 days (Red River 68) and cut them for lignin labeling just prior to heading out of the grain.

In order to label the lignin, the roots were cut off and the freshly cut surface was immediately immersed in a 10-ml solution of uniformly labeled ^{14}C-phenylalanine in a growth chamber until the solution was nearly all taken up. The cut surface was then immersed 1-2 cm in distilled water for 3 days, dried at 45° C to constant weight and ground to pass through a 1-mm^2 screen. The ground straw was washed with hexane at 35° C and dried, then soaked in water at 4° C for 16 h, washed, and finally treated with protease at room temperature for 3 h, water washed and freeze-dried. The protease used was Subtilopeptidase-A (Bacterial, Type VII) from Sigma Chemical Company in a pH 7 borate buffer at a concentration of 1 mg/ml. Total ^{14}C content, as decompositions per minute per mg (DPM/mg), was determined by pyrolysis of the straw in an oxygen atmosphere to CO_2 and trapping of the CO_2 in 10 ml of a scintillation cocktail consisting of 1,300 ml toluene, 1,200 ml methanol, 300 ml 2-aminoethanol, 0.0375 g 1,4-[2-(4-methyl-5-phenyl-1,3-oxazolyl) benzene and 30.0 g 2,5-diphenyloxazole.

Unlabeled wheat straw obtained locally was hexane washed, dried, soaked at 4° C in water that was decanted, and then either fermented directly or after further water washing. Also, at the end of the 4° soak the straw was broken up in a Waring Blendor to prevent trapping of water inside the hollow straw stem.

Fermentation was accomplished in air at 25° after inoculation with a 1-cm^2 plug of C. stercoreus that had been grown for 1 week on a potato dextrose agar plate.

Separation of Biodegradation Products. The scheme devised for separation and recovery of biodegradation products is illustrated in Figure 1. The ether phase extract, labeled C in Figure 1, would contain acidic materials such as vanillin, syringealdehyde and similar compounds if they were present. Some polyfunctional acidic compounds remain in the acidified water phase D. An alternate route using ion exchange resin is better suited for separation and identification of polyfunctional acidic compounds, such as hydroxybenzoic acid. These compounds will end up in the water extract labeled E in Figure 1. Unless other soluble compounds mask their presence, these acids can also be identified in the original extracted fermentation broth.
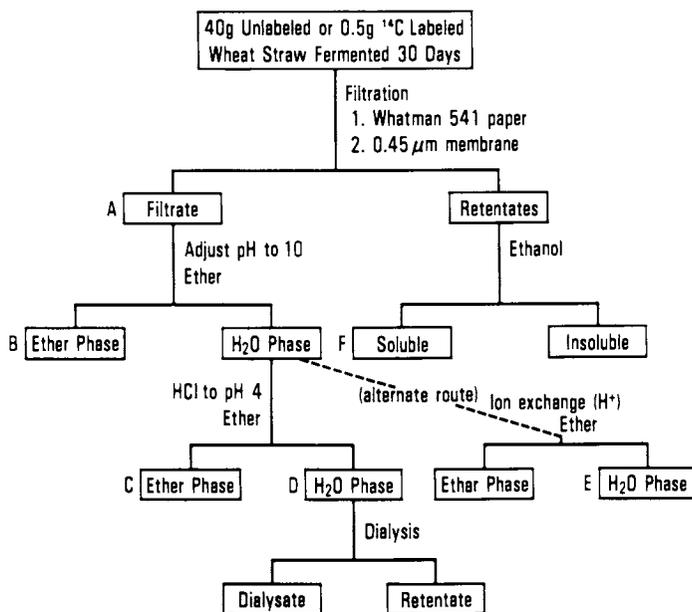
Figure 1.   Separation of biodegraded wheat straw.

Characterization Methods. Labeled materials in soluble
extracts were quantitated by counting 100 μl aliquots of the
various solutions in the same 10 ml scintillation cocktail used
to trap CO_2.

The quantity of ^{14}C in evolved gases was measured by daily
15-min flushes of the sealed fermentations with air into the
same scintillation cocktail. The gas-phase components were
identified by their retention times on a 5 ft X 1/8 inch Porapak Q
column. To trap enough gas for identification, the evolved
gases from 2 days of fermentation were flushed with helium into
a 1 ft X 1/8 inch Porapak Q filled stainless-steel column filled
with Porapak Q and immersed in liquid N_2. The column exit gas
passed through a bubbler containing scintillation fluid. After
the 15-min flush the column was capped on the entrance side and
a new bubbler was attached. The column was then allowed to warm
to room temperature. Trapped CO_2 rapidly eluted as the column
warmed, and when gas evolution stopped the column was inserted
into a Tracor Model 560 gas chromatograph (GC); the entrance
side of the trap column was attached to the injection port and
the exit side to a 4 ft by 1/8 inch Poropak Q column. The
baseline had been established previously but residual CO_2 came
off as helium carrier gas was turned on and the oven was
programmed from 40° to 180° at 5°/min. Samples from the water
phase were tested for volatiles other than CO_2. They were
tested on the same Poropak Q column as gas-phase samples, but
the column was programmed from 100 to 170° at 10°/min and held
at 170° for 20 min.

To ensure that the volatiles other than CO_2 were from
lignin degradation, the same liquid N_2 trapping procedure was
followed. The column was attached at room temperature to a GC
with a thermoconductivity (TC) detector leading to a flowing
scintillation fluid trap for gases as they eluted. Results of
the analysis of volatiles by GC were confirmed by high-pressure
liquid column chromatography (HPLC) and infrared spectra of the
major components.

Less volatile low-molecular-weight biodegradation products,
were characterized by gas chromatography-mass spectroscopy (GC-
MS). Higher molecular weight compounds were characterized by
gel permeation chromatography (GPC), membrane filtration, dialysis
and nuclear magnetic resonance (NMR).

The methylated (CH_2N_2) low-molecular-weight compounds were
analyzed on a Kratos MS-30 equipped with a Perkin Elmer Sigma 3
gas chromatograph and a 3 ft X 1/8 inch OV-1 column. Retention
times of the sample peaks identified by GC-MS were compared to
those of known standards on a 6 ft X 1/8 inch OV-1 column on a
Tracor model 560 GC for confirmation. Lignin content was measured
by the UV method (10).

Control samples of wheat straw oxidized by the standard
nitrobenzene method (11) and separated by the scheme illustrated
in Figure 1 were also tested by the GC-MS method to confirm the
validity of the separation and recovery techniques.

To characterize high–molecular–weight biodegradation products, an Amicon Model 202 Diafiltration Apparatus was used sequentially with XM300, PM10 and UM2 membranes at 10, 17 and 55 psi N_2 pressure, respectively. The samples tested were water–soluble biodegradation products that had never been freeze–dried. Concentrations of the various fractions were determined on aliquots so that GPC curves could be determined on samples that had always been in solution.

High–molecular–weight biodegradation products for NMR were isolated by dialysis and membrane filtration. Dialysis bags of regenerated cellulose were characterized with standard dextrans and raffinose. GPC curves of a dextran–raffinose mixture before and after separation by dialysis indicates a nominal molecular weight of 3,000 or greater was retained for NMR analysis. High–molecular–weight compounds isolated by this method were freeze–dried, and their ^{13}C NMR spectrum determined on a Brüker WH 90 in D_2O. Gel permeation chromatography (GPC) curves were determined with a Waters 244 HPLC on 2 columns of E–linear μ–Bondagel, 7.8 mm X 30 cm each. Water was the solvent at 1 ml/min and ambient temperature. Detection was by refractive index and UV absorbance at 254 mn.

Results and Discussion

Recovery of the gas phase of fermented wheat straw and separation of the residue by the method illustrated in Figure 1 gave a material balance of the various fractions by weight ^{14}C content, as shown in Tables I and II. Although only 4.6% of the original 40 g of straw is extracted as water–soluble material after the fermentation and 9.3% of the radioactive straw by weight, a higher percentage of the original ^{14}C (13.3%) is in the recovered water–soluble material (Table I). Thus there is a higher concentration of lignin degradation products in the fermentation broth than the concentration of lignin in the wheat straw. Autoclaving unfermented straw yields 0.8% of the original radioactivity in the water phase. Careful drying to avoid loss of moderately volatile substances such as vanillin involved freeze–drying the water–soluble material from the 40–g straw fermentations. Additionally, ^{14}C–labeled water solubles were counted by testing aliquots (100 μl) in scintillation fluid, both before and after freeze–drying for 54 h past apparent dryness. Less than 4.3% of the original ^{14}C was lost on freeze–drying and reconstitution, and no ^{14}C was detected in the –70° freeze–drying trap. Some loss of both weight and ^{14}C will occur from dissolved CO_2, methanol and ethanol. From the results in Tables I and II it can also be concluded that the gas phase contains approximately 13% of the original ^{14}C. The total lignin is degraded 27% after 30 days, half in the gas phase and half in the water phase of the fermentation, before either phase is separated for identification. Identification of the components of the gas phase by retention time is illustrated in Figure 2.

A typical flame ionization detection (FID) curve is shown in
Figure 2 (curve 2). Gases and water-phase volatiles identified
by retention time are ethanol, methanol, acetone and acetic
acid. A comparison of counts collected over 10° intervals in
the programmed chromatograph to the retention times of peaks
detected by both FID and TC indicates that all of the gases in
addition to CO_2 have at least part of their origin in the [14]C-
labeled lignin (compare Figure 2, curve 3 to Figure 2, curves 1
and 2). Residual ethanol in the water phase was determined by
HPLC to be 0.02% or 0.033 g in a 30-day, 40-g straw fermentation
with free (filtered) atmospheric gas exchange. CO_2 and water
were evident in the infrared spectra of fermentation gases, with
no evidence of significant amounts of hydrocarbon gases.
     As indicated by weight and ^{14}C analysis (Table II), few if
any low-molecular-weight aldehydic phenols were isolated in
ether phase C. Therefore, the analysis scheme (Figure 1) was
tested by adding 0.05 g vanillin to a 30-day fermented wheat
straw and carrying it through the extraction and freeze-drying
procedure. The added vanillin was easily determined in ether
extract C by GC. Only a trace was found in ethanol-soluble
extract F, but even this trace, 0.1 mg, was detectable, in
contrast to the extracts of biodegraded wheat straw that contained
no aldehydic phenols. The absence of vanillin, syringealdehyde,
and several other phenols in the water-soluble biodegradation
products was confirmed by thin layer chomatography (TLC) in 95/5
benzene/methanol, using UV and radiocarbon detection. Of all
the compounds examined, only carboxylated phenols do not migrate
away from the origin in this solvent.
     Ether extraction procedures have been used also to isolate
aldehydic phenols from alkaline nitrobenzene oxidized lignin to
characterize the lignin in various plants. $Fe(NH_4)_2(SO_4)_2$
washed ether should be used to prevent peroxide oxidation of the
aldehyde groups (12). Using $Fe(NH_4)_2(SO_4)_2$ washed ether
throughout, we demonstrated again that aldehydic phenols were
not present in the ether extracts of biodegraded wheat straw in
which over 2 g of lignin is degraded, but these phenols are
easily found and quantitated when 2 g of wheat straw is oxidized
by the standard nitrobenzene method and the phenols are recovered
as illustrated in Figure 1. Table III lists the compounds found
in nitrobenzene oxidized wheat straw, as well as similar results
for extracts of the biodegradation products. The absence of
low-molecular-weight biodegradation products in the ethanol
extract is confirmed by the observation that the gas
chromatographic curve for the ethanol solubles (silylated) of
fermented wheat straw is identical to the curve for similar
derivatized extracts of unfermented wheat straw carried through
the same extraction procedure.
     To test for polysubstituted phenols in the water soluble
biodegradation broth, the methylated (CH_2N_2) derivative of
freeze-dried extract A (Figure 1) was easily produced and yielded
interpretable mass spectra. Several phenolic standards were

Table I. Quantitation of Biodegradation Products

| | Material Balance by Weight | | |
	Total Sample[a] Basis	Lignin[b] Basis	Total Sample Basis[c] All Soluble Nutrients Removed
Starting material	6.4% H_2O, 2.56 g 15.6% lignin, 6.24 g	100%, 6.24 g	6.4% H_2O, 2.56 g 15.6% lignin, 6.24 g
Hexane extract	0.4%, 0.16 g	-0-	0.4%, 0.16 g
4° Water extract, decanted	5.5%, 2.2 g	4.8%, 0.3 g	
4° Water extract and water wash			5.6%, 2.25 g
Fermented filtered H_2O solubles	4.6%, 1.92 g	7.1%, 0.44 g	2.6%, 1.02 g
0.45 um filtered H_2O solubles			2.3%, 0.939 g
EtOH solubles after fermentation	1.3%, 0.53 g	6.4%, 0.3 ± 0.1 g	
Fermented[e] extracted dry straw	69.5%, 27.8 g	65.2%, 4.07 g	78.8%, 31.52 g
Lost or in gas phase	12.5%, 5.0 g	16.5%, 1.03 g	6.3%, 2.5 g
Trapped gases			

[a] Average of 5 determinations on 40 g wheat straw

[b] Lignin determinations by UV method (10)
[c] Average of 2 determinations.

[d] Decomposition per minute.

[e] Not corrected for fungal mass.

^{14}C Balance DPM^{d}X10^{-6}, Percent			
Sample 1 Butte HR	Sample 2 Butte HR	Sample 3 RR 68	Sample 4 RR 68
38.03	25.04	43.88	35.68
			5.0, 14.0%
5.47, 14.4%	3.33, 13.3%	5.27, 12.1%	4.72, 13.2%
0.13, 0.3%			0.25, 0.7%
25.0, 65.8%	16.9, 67.3%	24.8, 56.5	12.8, 35.8%
6.2%	12.3%	17.6%	-6.4%
5.04, 13.3%	1.27, 5.1%	5.90, 13.5%	20.2, 56.7%

TABLE II.   Separation of Water-Soluble Biodegradation Products

by Ether Extraction (Figure 1)

	Percent of Water-Soluble Products in	
	Ether Extract[a] of Basic H_2O	Ether Extract[b] of Acidic H_2O
By weight	0.41	0.96
By ^{14}C label	0.03	0.4

[a]As illustrated by the extract labeled B in Figure 1.

[b]As illustrated by the extract labeled C in Figure 1.
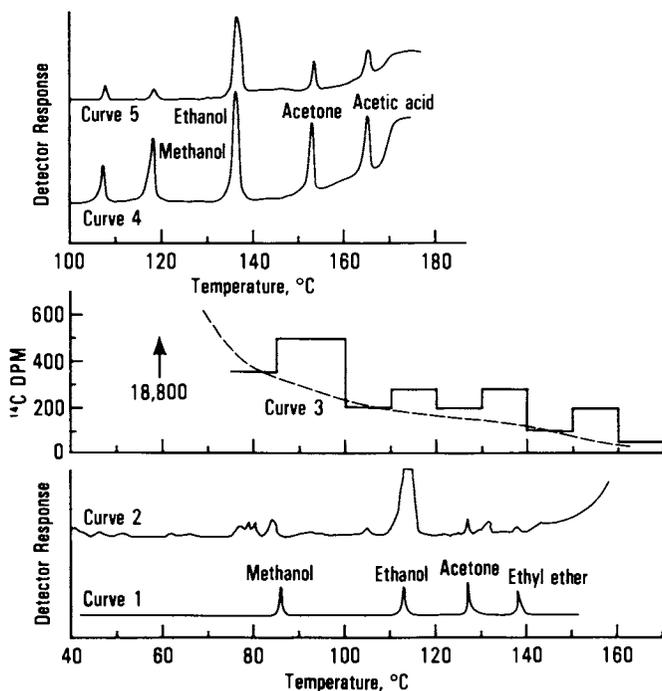
Figure 2.   Gas chromatographic curves for volatiles. Key: Curve 1, standard mix in water of less than 1% each of methanol, ethanol, diethyl ether, and acetone flushed with He, trapped and tested on Poropak Q as were the fermentation gases (FID); Curve 2, gas phase of eight-day fermentation trapped and tested as described in text (FID); Curve 3, collected and counted GC effluents (see text); Curve 4, standard mix of 0.1% each methanol, ethanol, acetone, and acetic acid; and Curve 5, water phase of fermentation.

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methylated for comparison to the unknown. Since methylated
extract A had not been separated into various components before
methylation, the gas chromatograph curve shows many peaks
(Figure 3, curve 1). The smaller peaks in the area of methylated
polyfunctional phenols are therefore difficult to identify from
their mass spectrum alone. A technique called mass chromatography
was used wherein distribution of several major masses that
appear in a standard spectrum is plotted underneath the gas
chromatograph curve as in Figure 3. In this way, coincidence of
several masses that are present in a standard mass spectrum
under a single peak in the gas chromatograph curve identifies
the location of that standard. This, in conjunction with
retention time, confirms the presence of a compound in the
unknown, whereas the absence of such coincidence at a given
retention time confirms the absence of a compound to very low
levels of detectability in the original sample. Using this
technique, methylated vanillic acid, p-hydroxybenzoic acid, 2-
methoxy succinnic acid and syringic acid were found to be present.
The palmitic acid, linoleic acid and triterpenes found earlier
were the dominant materials in the water solubles examined by
this technique, as can be seen in Figure 3. The magnitude of
these three materials prevents putting a larger sample on the
GC-MS instrument to enhance the interpretation of small peaks.
Larger samples possibly could burn out filaments in the mass
detection system. For this reason, pre-extraction of the
triterpenes and fatty acids as much as possible was attempted
with hexane. The fatty acids and triterpenes constitute the
interfering substances referred to in the introduction.

     Since separation of these lower molecular weight compounds
is not easily achieved by ether extraction, they are not
quantitated by the separation scheme of Figure 1. Separation of
higher molecular weight materials for characterization was also
necessary. Both of these goals were achieved by membrane
filtration, dialysis and GPC characterization.

     Membrane filtration results in Table IV gave us considerable
insight into the molecular weight distribution of residual
biodegradation products. We concluded from these results that
few of the residual biodegradation products are present as low-
molecular-weight species. Compounds of less than 1,000 nominal
molecular weight constitute 11% of the water-soluble
biodegradation products, with monomeric aromatics only a portion
of this. GPC curves of the various membrane-separated fractions
confirm that the high-molecular-weight fractions contain the
majority of ultraviolet absorbing species (Figures 4 and 5).

     Figure 6 shows the ^{13}C NMR spectrum of high-molecular-
weight biodegradation products from membrane filtration and
dialysis and the NMR spectrum of a lignin-carbohydrate complex
published by Himmelsbach and Barton (13). The spectrum of the
biodegradation products shows predominantly carbohydrate polymers.
The combination of weight and ^{14}C-label distribution among
sequential membrane filters, the UV absorbance of these species,
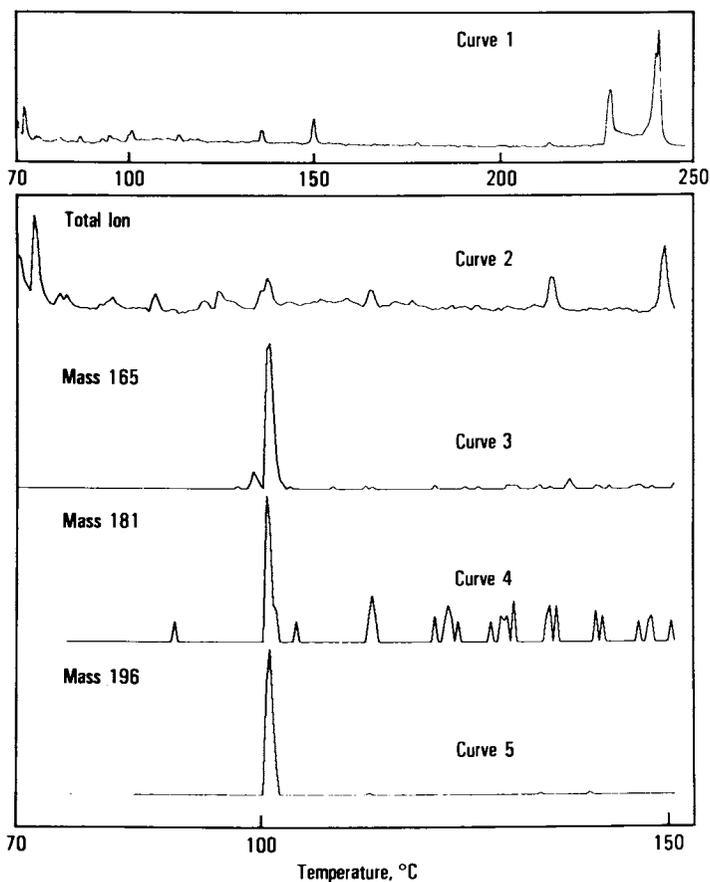
Figure 3. GC-mass spectra curves. Key: Curve 1, total ionization vs. temperature curve for methylated water-soluble biodegradation products; Curve 2, a portion of Curve 1 for searching; Curve 3, plot of mass 165 ions received while Curve 2 was being collected; Curve 4, plot of mass 181 ions received while Curve 2 was being collected; and Curve 5, plot of mass 196 ions received while Curve 2 was being collected.

Table IV. Membrane Filtration of Biodegradation Products

Retained by[a] Membrane	Percent of H_2O Solubles Weight for the 40 g Wheat Straw Fermentation	Percent of H_2O Solubles for the ^{14}C Labeled Lignin Fermentation
X M 300	13.2	22.8
PM 10	10.9	23.9
UM 2	48.8	42.0
Through UM 2	21	11.3
Recovery	93.9	95.5

[a]Nominal molecular weights retained by the XM300 membrane is
>300,000, by the PM10 membrane is 10,000 to 300,000, and
by the UM2 membrane is 1,000 to 10,000.
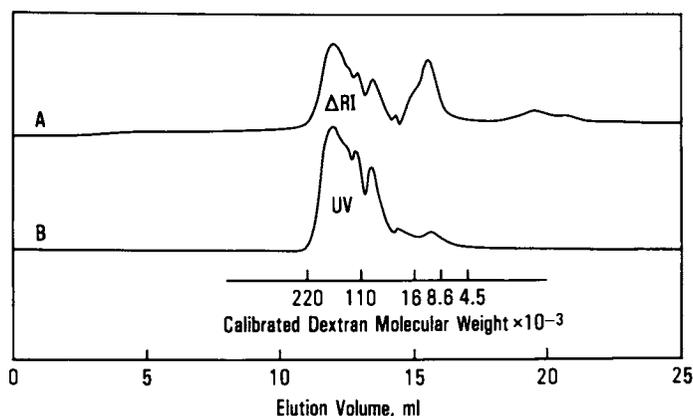
Figure 4.   Gel permeation chromatograms of the total water-soluble biodegradation product. Detection for Chromatogram A is by refractive index change, and Chromatogram B is by UV absorbance at 254 nm.
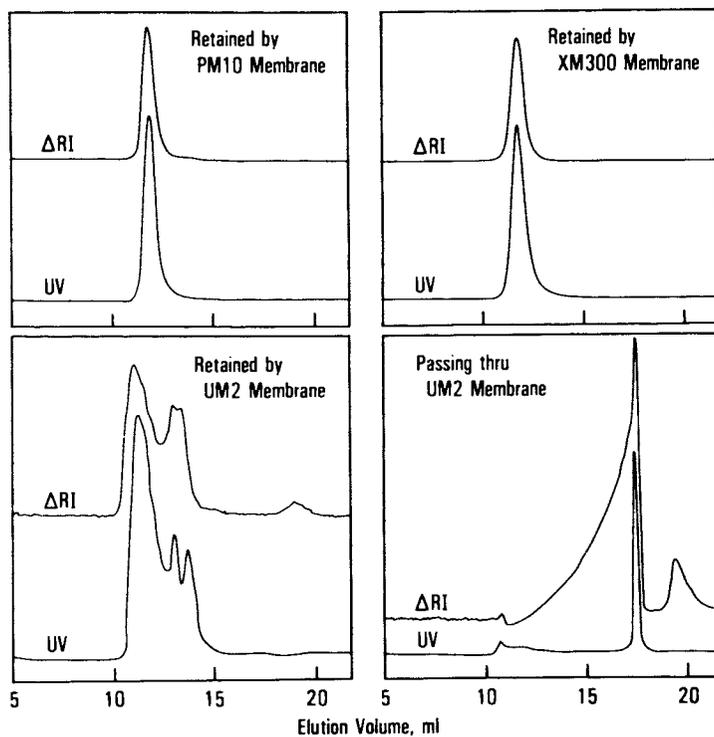
Figure 5. *Gel permeation chromatograms of the membrane filtration fractions using the same conditions as for the unseparated sample described in Figure 4.*
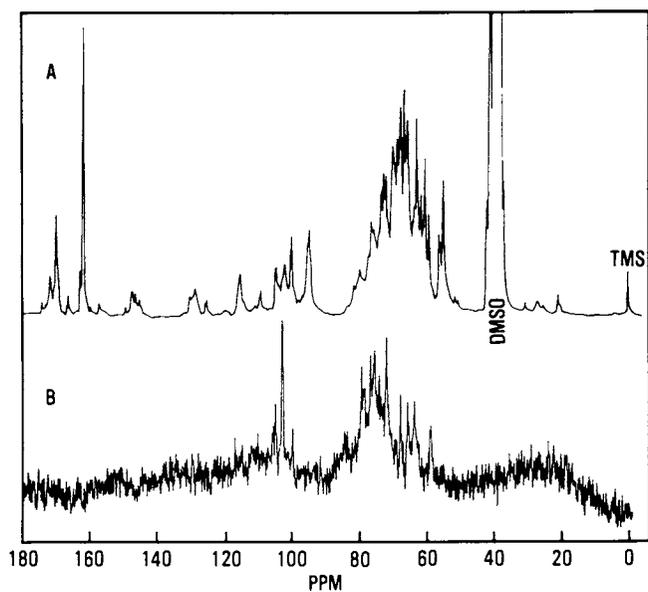
Figure 6. *¹³C-NMR spectra of a lignin-carbohydrate complex A (13), and the high molecular weight water-soluble biodegradation products isolated by dialysis (B).*

and the NMR spectrum in Figure 6 lead to the conclusion that the overwhelming majority of residual biodegradation products are lignin-carbohydrate complexes.

Conclusions

Although some low-molecular-weight oxidized aromatic species can be found in biodegradation products of Cyathus stercoreus on wheat straw, the predominant residual biodegradation species are lignin-carbohydrate complexes. The other major product of degraded lignin is carbon dioxide. Lesser amounts of ethanol, methanol, acetone, acetic acid, 2-methoxy succinnic acid, vanillic acid, syringic acid and p-hydroxy benzoic acid were also found. These findings are consistent with current thinking on mode of attack of fungi on lignocellulosics as described earlier. If random oxidation cleaves high molecular weight water-soluble fractions from lignocellulosics, one would expect that they cannot be metabolized; however, as these are broken down to low-molecular-weight materials by continued oxygenation, these fractions are rapidly metabolized to CO_2. Thus, the residual fermentation broth would contain predominantly indigestible high-molecular-weight species and a few low-molecular-weight species and a significant amount of CO_2 would have been generated. We find such a composition.

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Biphenyl Hydroxylase, Arylhydrocarbon Hydroxylase, and Epoxide Hydrolase

Activities in Intestinal and Liver Microsomes of Rats Fed Selected Types of Dietary Fibers

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"Dietary fiber" has been shown to be protective
against induction of colon cancer by some chemical
carcinogens. One possible mechanism for this pro-
tective action may be altered xenobiotic metabo-
lism. The affects of diets containing different
types of purified fiber on intestinal and liver
microsomal metabolism of biphenyl, benzo(a)pyrene
and epoxides were assessed. Adult, male Wistar
rats were individually fed chemically defined,
nutrient density balanced rations containing
either no dietary fiber, or 15% pectin, Metamucil,
lignin or cellulose for 30 days; a similar group
was fed commercial laboratory chow diet as refer-
ence. The microsomal hydroxylations of biphenyl
and benzo(a)pyrene (BaP) were compared following
incubation with appropriate preparations of liver
and intestinal mucosa. The hydroxybiphenyl
products were determined by fluorimetric HPLC,
hydroxylated BaP was determined by fluorimetry,
and epoxide hydrolase activity was determined
using 3-(p-nitrophenoxy)-1,2-propene oxide as a
substrate. Activities were higher with liver mi-
crosomes than the intestinal microsomal prepar-
ations for each assay. Fiber-fed rats showed sig-
nificantly higher hepatic but not intestinal
epoxide hydrolase levels than did control rats.
Hepatic arylhydrocarbon hydroxylase levels were
higher in synthetic diet-fed rats than lab chow-
fed animals. Variations in intestinal biphenyl
hydroxylase activities were not significant with
respect to fiber type regardless of the specific
metabolite measured. Hepatic biphenyl hydroxylase
activities were significantly higher with syn-
thetic diet-fed rats.

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Some current hypotheses about the etiologies of colon cancer and cholesteremic disease impute a special function to dietary fiber and to some of its components; that function is generally associated with disease prevention. Evidence for the protective function of dietary fiber has been obtained both from epidemiological studies (1-8) and from experiments with animals.

Animal experiments, in particular, have been useful in testing various hypotheses about the relative effectiveness of different kinds of dietary fiber and about possible mechanisms of fiber effects. Thus, studies of bulking action (9-10), of selective secondary bile acid binding (11-12), of increased intestinal transit times (13), of altered bacterial activities and secondary bile acid production (14-15), and selective binding of carcinogens (16-19) have been carried out in laboratory animals. Several related studies have been conducted in humans as well. Among the latter are investigations of the relationship between the bulk fiber content of diets and fecal bile acids or other steroids (4,20-21).

Because of the possible interplay of many factors and processes that might occur among the different chemicals constituting dietary fiber itself and carcinogens or promoters, we have been conducting a systematic study of such interactions using a model experimental design with the rat. Our model consists of feeding purified diets containing different purified fibers, in hydrated form, for a 30 day period to allow for thorough bacterial adaptation(22). At that time each rat was treated per os with a single dose of a xenobiotic chemical selected to exemplify a specific aspect of xenobiotic metabolism. Toxicokinetic analyses were then utilized to quantify the critical parameters, such as absorption and elimination rate constants for the parent compounds, their bacterial metabolites, and metabolites undergoing enterohepatic circulation. By repetitive study with different xenobiotics within the fixed dietary design, a comprehensive understanding of the fiber:animal:carcinogen interactions is being developed (22-24).

In order to fully interpret the toxicokinetic experiments, additional basal data are required from this diet model; it is particulary necessary to establish parameters for the hepatic and intestinal xenobiotic metabolism in rats on the standard fiber diets. This paper presents our findings of the effects of different dietary fiber types on hepatic and intestinal metabolism of reference xenobiotics.

Previous Work Relating to Selection of Substrates

Characteristics of Intestinal vs Hepatic Xenobiotic Metabolism. Intestinal mucosa has been shown both to metabolically activate and to deactivate xenobiotics. The mixed function oxidase (MFO)

of intestinal mucosa responds to inducers and inhibitors differently than hepatic MFO. Intestinal MFO also differs from hepatic MFO in its constituative levels, metabolic patterns, and substrate specificity (25-41).

Biphenyl Hydroxylation. Factors affecting 2-,3-, and 4-biphenyl hydroxylase enzymes are: age, sex, species, nutritional status, genetic background, xenobiotic preexposure, and tissue type. The 2- and 4-hydroxy biphenyl hydroxylases have been demonstrated to be different on the basis of selective induction, conducted both in vivo and in vitro, and on different species specificities(42-51). Toftgard et al.(51) reported that changes in different forms of cytochrome P-450 give rise to altered proportions of biphenyl metabolites. It is now clear that biphenyl 2-hydroxylase activity is conferred only by cytochrome P-448-type induction. Rat hepatic biphenyl 4-hydroxylase is maintained at higher constituative levels than 2-hydroxylase, and is conferred by both cytochromes P-450 and P-448 (52). Wiebkin et al.(53-54) summarized advantages for use of biphenyl as substrate in that its metabolic profile provides an indicator to the toxicity of substrate and products, it highlights further metabolism of primary metabolites, and indicates the relationships among different MFO inducers; for these reasons we chose to use biphenyl as an indicator substrate to assess overall MFO metabolism, as suggested by Billings and McMahon (55).
    Intestinal microsomal metabolism can be monitored via biphenyl hydroxylation as well. Biphenyl hydroxylase assays are more sensitive than some other hydroxylase assays which has permitted measurements of the low constituative enzyme levels in intestinal mucosa and some other extrahepatic tissues (56-59).

Arylhydrocarbon Hydroxylase. Benzo(a)pyrene (BaP) is considered an ideal model compound for monitoring arylhydrocarbon hydroxylase (AAH) MFO levels in rats. Its metabolism has been widely investigated in both rat liver and extrahepatic tissues (40-41,61-70). Arylhydrocarbon hydroxylase has been compared to biphenyl hydroxylase in the intestines of rats (25,28). Intestinal AAH has been shown to be induced up to 30-fold with cytochrome P-448-type inducers, while being insensitive to P-450-type induction. Therefore, we have used intestinal AAH in conjunction with biphenyl hydroxylation, to evaluate the effects of dietary fibers on intestinal metabolism.

Epoxide Hydrolase. Epoxide hydrolase (EH) activity has been studied in numerous tissues with many different substrates (71-72). Hepatic microsomal EH has been shown to be induced by polycyclic aromatic hydrocarbons, but is insensitive to cytochrome P-450 type inducers (73). Epoxide hydrolase has been related to the activation of polycyclic aromatic hydrocarbons into reactive carcinogens (70). Epoxide hydrolase activity has been determined

in the stomach as well as the intestine and other extrahepatic tissues (74-75). Indications of the effects of specific diet fibers on epoxide hydrolase activity could be directly applied to substantiate or modify fiber:carcinogenesis hypotheses.

Methods and Materials

Dietary Fibers. The cellulose used in these experiments was SolkaFlok, KS-1016, a cellulose derived from sulfite processing of wood (birch, beech, and maple) having a particle size of 290 microns and containing 0.15-0.2% lignin and 8-9% hemicellulose (Brown Co.,Berlin, N.H.). The lignin used was Indulin AT, which is a Kraft lignin described as being 99% lignin and 1.0% dry ash, free of simple sugars, insoluble in water and soluble in alkali (Westvaco, North Charleston, S.C.). The lignin was Soxhlet extracted for 30 hours using methylene chloride which was evaporated from the lignin in a fume hood. The extracted, dried lignin was then suspended in deionized water and filtered through a Buchner funnel with Whatman 1 filter paper. This washing process was repeated until the filtrate was light orange in color. The hemicellulose used was Metamucil (Searle Laboratories, Chicago,Ill.), a pharmaceutical preparation of Ispaghula husk from Plantago ovata(Forsk) which has been characterized (76). The pectin used was an NF citrus pectin, finely ground to a powder with particle size of 200 microns (Hercules,Inc., Wilmington,DL).

Diet Preparations. The American Insitute of Nutrition (AIN) synthetic diet formulation was used along with gelatin to form hydrated gelled diets. The ingredient composition in grams/100 grams diet was: dextrose 64.6, casein 16.2, dl-methionine 0.2, AIN salt mixture 3.5, AIN vitamin mixture 1.5, gelatin 6.0, and lard 8.0 for the control diet; fiber diets contained 15% less of each ingredient plus 15g fiber. Dry ingredients were premixed and the hydrated gelled diets were prepared weekly by adding water (190ml/100g) and lard to the appropriate amount of premix, blending for 1 minute, letting the diets gel for 2 hours at 5°C, and reblending the semigelled diets to evenly distribute insoluble ingredients. The prepared diets were stored at 5°C in tubs with plastic film laid directly on the surface of the diets to minimize evaporative loss.

Animal Treatment. Six groups of 11, thirty-day old, male Wistar rats (100-120g), were randomly divided into individual cages. After five days acclimation the groups were fed: Purina Lab Chow ad libitum, control (fiber-free gelled diet) and the gelled diets which contained 15% pectin, Metamucil, lignin, and cellulose, respectively. Every second day each rat was weighed, unused food was weighed and fresh diet placed in feeders secured at the front of each cage. At the end of 28 days, two rats from each group were sacrificed each day for 5 consecutive days.

<u>Enzyme Preparation.</u> Each rat was weighed, killed by cervical dislocation, the liver removed and 2g homogenized in 10 ml 0.05M Tris., 0.15M KCL, pH 7.8 buffer. Intestinal microsomes were prepared according to Stohs et al.(28). The entire small intestine of each rat was removed, washed with buffer, measured for length, split longitudinally with scissors, laid mucosal side up on a glass plate and scraped with a glass slide. The mucosal cells were weighed and homogenized in 10ml buffer with 20% glycerol, 25mg trypsin inhibitor and 2 units heparin. Hepatic and intestinal homogenates were centrifuged at 600g for 2 minutes and then at 13000g for 20 minutes, with the microsomes sedimented at 105,000g for 60 minutes.

<u>Assays.</u> Protein was determined according to Lowry et al(77)). Biphenyl hydroxylase activity was determined with 2 mg microsomal protein, 400 micromoles NADP, 4 micromoles glucose-6-phosphate, 3 micromoles magnesium chloride, and 0.5 units glucose-6-phosphate dehydrogenase in 1 ml total volume. After a 2 minute preincubation, 1 mM biphenyl in 10 microliters of acetone was added to start the 15 minute incubation. The reactions were stopped with 0.5 ml 6N HCL, extracted with isooctane and chromatographed by HPLC on Micropac NH_2-C-10 (Varian) with isooctane:acetonitrile:isoamyl alcohol (100:4:4)(59). Detection of the 2-, 3-, and 4-hydroxybiphenyl products was by fluorescence detection with excitation at 275nm and emission at 38nm. Quantitation of 2-, 3-, and 4-hydroxybiphenyls was accomplished with peak height comparisons to 1.25, 2.50, and 3.75 micrograms of each standard; standards were incubated and extracted with each set of determinations. Epoxide hydrolase activity was determined by the method of Giuliano et al(75) using 3-(p-nitrophenoxy)-1,2-propene oxide as substrate with 0.5mg microsomal protein. The diol was separated using ODS reverse phase HPLC with 40% acetonitrile in water. Detection was accomplished using UV absorbance at 315nm. Arylhydrocarbon hydroxylase activity was determined by the assay procedure of Dehnen et al.(78) on an AmincoBowman spectrofluorometer after incubations with 0.5mg microsomal protein and NADPH generating system. One unit activity is defined as picomoles/mg/minute as based on the fluorescence of 20 ng 3-hydroxybenzo(a)pyrene.

Results and Discussion

Results of body, liver and liver/body weight ratios after 30 days on specified diets are listed in Table I. Each of the treatment groups showed an initial weight loss upon introducing the purified diets. However, all but the lignin-fed animals quickly recovered to steady weight gains throughout the rest of the experiment. Lab Chow-fed rats were significantly heavier than the purified diet-fed rats. Body weights in pectin-and Metamucil-fed rats were significantly (0.05 level) higher than control-,

Table I. Body and Liver Weights and Liver to
Body Weight Ratios of Fiber-Fed Rats.[a]

Diet Type	Body Weight	Liver Weight	Liver Wt./Body Wt.
	g	g	g
Control (No Fiber)	182 ± 12	6.90 ± 1.38	0.037 ± 0.006
Lab Chow	300^b 28	11.14^b 1.63	.037 .004
15% Pectin	220^c 18	7.73 1.19	.034 .003
15% Metamucil	224^c 12	7.83 1.19	.034 .006
15% Lignin	153 15	5.61 0.64	.036 .003
15% Cellulose	172 10	6.10 0.50	.035 .002

[a] Values are means $\pm$ S.D. for groups of 11 rats.

[b] Significantly different from all other groups (P=.05).

[c] Significantly different from the other groups fed purified diets
(P=.05).

lignin- or cellulose-fed animals. Lignin-fed animals could be
considered to be under weight. Lignin- and control-fed animals
ate significantly less purified diet than other treatment groups.
Liver weights in Lab Chow-fed rats were also significantly higher
than in rats fed purified diets. The liver weights of pectin- and
Metamucil-fed rats were slightly higher (not significantly) than
control-, lignin- or cellulose-fed rats. Liver:to:body ratios
were not significantly different in any of the treatment groups.
The influence of specific dietary fibers on small intestine
length, on the amount of mucosal material scraped from the intes-
tine, and on the ratios of grams mucosa/cm or cm/g mucosa is
indicated by the data in Table II, while Table III lists the
intestinal microsomal protein (expressed as total or mg/cm small
intestine). Lab Chow and diets containing pectin or Metamucil
produced intestine lengths that were significantly longer than in
the rats fed the control, lignin, or cellulose diets. These
results support similar findings by deBethizy (23-24) and Roten-
berg and Jakobsen (79). The stable gels formed by Metamucil and
pectin fibers caused significant intestinal tract enlargement,
even though fed in hydrated form. The rats fed Lab Chow and the
pectin diet had the highest mucosal weights, which were signifi-
cantly higher than in control-,lignin-,or cellulose-fed animals.
Lignin-fed animals yielded the least amount of mucosal material,
which coincides with their having the shortest small intestines
and lowest body weights (Table IV). Total intestinal microsomal
protein did not differ significantly (0.05 level) among treatment
groups, although pectin diet and Lab Chow-fed rats had relatively
higher total microsomal protein values. Both liver and intestinal
mucosa microsomal protein values are listed in Table IV. There
were no significant differences between treatment groups for
either tissue.
    Listed in Table V are the values of 2-,3-,4-biphenyl hy-
droxylase activities in liver microsomes of fiber-fed rats.
Values are expressed as nanomoles (2-, 3-,or 4-hydroxybiphe-
nyl)/mg/15 minute incubation; the least detectable amount was
0.05 moles/mg. The hepatic microsomal levels of 4-biphenyl hy-
droxylase were on the order of 40-fold higher than the 2-or 3-
biphenyl hydroxylase activities. This difference is slightly
greater than the higher constituative 4-biphenyl hydroxylase
levels reported by others (46,50,80). The yield of 2-hydroxybi-
phenyl was significantly higher with hepatic microsomes from
lignin-fed animals than with microsomes from any of the other
treatment groups. The slightly higher biphenyl hydroxylase values
for the purified diet-fed rats is notable. Lignin-fed rats also
showed the highest 3-hydroxybiphenyl yields although this effect
was not significant. The levels of hepatic microsomal 4-hydroxy-
biphenyl hydroxylase were significantly lower in Lab Chow-fed
animals than lignin-fed animals. There were no significant dif-
ferences among the purified diet treatment groups, however,
lignin-fed rats again had slightly higher levels of activity.

Table II. Small Intestine Length, Mucosal Weight and their Ratio in Fiber-Fed Rats.[a]

Diet Type	Mucosa	Small Intestine	Mucosal Wt./cm	cm/g Mucosa
	g	cm	g	cm
Control (No Fiber)	2.65 ± 0.47	94 ± 4	0.028 ± 0.005	36.79 ± 6.92
Lab Chow	3.52[b] .42	111[c] 8	.032 .004	31.84 2.88
15% Pectin	3.15[b] .39	108[c] 6	.029 .004	34.70 4.95
15% Metamucil	2.78 .51	108[c] 6	.025 .004	39.86 6.39
15% Lignin	1.98 .35	93 6	.021 .003	48.01 6.74
15% Cellulose	2.30 .35	96 4	.026 .004	41.06 6.48

[a] Means ± S.D. for groups of 11 rats.

[b] Mucosal weight significantly greater (P=.05).

[c] Intestine significantly longer (P=.05).

Table III. Microsomal Protein in Small Intestine
Mucosa of Fiber-Fed Rats.[a]

Type	Total mg	mg/cm Length
Control (No Fiber)	13.77 ± 5.80	0.14 ± 0.06
Lab Chow	14.81 7.88	.13 .07
15% Pectin	15.23 5.45	.14 .05
15% Metamucil	10.50 5.20	.19 .05
15% Lignin	10.09 6.74	.11 .08
15% Cellulose	11.27 6.42	.12 .07

[a] Mean microsomal protein ± S.D. for groups of 11 rats;
differences were not significant (P=.05).

Table IV. Microsomal Protein in Liver and Intestinal
Preparations from Fiber-Fed Rats.[a]

Diet Type	mg Protein/g Liver	mg Protein/g Mucosa
Control (No Fiber)	11.8 $\pm$ 6.0	5.59 $\pm$ 3.48
Lab Chow	14.5 4.6	4.26 2.46
15% Pectin	12.4 5.4	4.97 1.87
15% Metamucil	9.4 3.7	3.71 1.94
15% Lignin	12.1 7.4	5.11 3.83
15% Cellulose	15.1 4.3	4.91 2.30

[a] Values are mean $\pm$ S.D. for groups of 11 rats. Differences among
treatments were not significant (P=.05).

Table V. Biphenyl Hydroxylase Activities in Liver
Microsomes of Fiber-Fed Rats.[a]

Diet Type	Nanomoles/mg/15 min					
	2-OH		3-OH		4-OH	
Control (No Fiber)	0.43	$\pm$ 0.10	0.40	$\pm$ 0.13	15.98	$\pm$ 5.75
Lab Chow	.30[b]	.06	.36[b]	.07	9.77[b]	1.94
15% Pectin	.44	.15	.32	.09	12.46	3.04
15% Metamucil	.41	.08	.29	.07	13.82	4.00
15% Lignin	.81[c]	.35	.62	.18	20.03	5.65
15% Cellulose	.30	.06	.28	.07	11.43	2.57

[a] Values are mean hydroxylase activity $\pm$ SE (N=10) in nanomoles/mg
protein/ 15 min.; min. detectable amount was 0.05 nmole.

[b] Difference from lignin-treated animals significant (P=.05).

[c] Difference from control and all other groups significant (P=.05).

Intestinal microsomal biphenyl hydroxylation for fiber-fed
rats is listed in Table VI. Due to the variability of the data
there were no significant (0.05 level) differences among the
groups of fiber-fed rats. Lignin feeding led to the highest
intestinal 2-biphenyl hydroxylase activities, while all the rats
fed purified diets had higher levels than the Lab Chow group.
Among the groups fed purified diets, the pectin-fed rats had the
lowest 2-biphenylhydroxylase levels. Pectin feeding also gave the
highest intestinal 3-biphenylhydroxylase although that difference
only approached significance at the 0.05 level. Lab Chow-fed
rats had the lowest 3-biphenyl hydroxylase level in intestinal
microsomes.   Intestinal microsomes from fiber-fed rats did not
differ significantly (0.05 level) in 4-biphenyl hydroxylase acti-
vity. Cellulose-fed animals had the lowest level, with the con-
trols and lignin-fed rats showing the highest intestinal microso-
mal 4-biphenylhydroxylase values. Pectin- and Metamucil-fed rats
had relatively low levels of 4-biphenyl hydroxylase activities.
      The results of a preliminary intestinal microsomal biphenyl
hydroxylase trial are listed in Table VII. These rats were on the
same hydrated purified diets containing 15% fiber for 32 days
before the microsomal enzyme determinations. One difference be-
tween these animals and those in the primary experiment reported
herein is that they were started on the diets at 45 days of age
and weighed about 200g at the start. The biphenyl hydroxylation
incubation was done with 5 mg intestinal microsomal protein as
compared to 2 mg protein used in the subsequent incubations. The
biphenyl hydroxylation values were not significantly different
(0.05) in any of the treatment groups although Lab Chow-fed rats
had slightly higher 4-hydroxy biphenylhydroxylase values.
      Benzo(a)pyrene hydroxylase activities in hepatic and intes-
tinal microsomes of fiber-fed rats are presented in Table VIII.
There were no significant differences (0.05 level) among any of
the purified fiber treatment groups in either liver or intestinal
microsomes. Lab Chow-fed rats had the lowest liver microsomal
benzo(a)pyrene hydroxylase (AHH) level. The indication of
enhanced intestinal P-448-dependent hydroxylation of biphenyl in
the pectin-fed rats (seen as elevated 3-hydroxybiphenyl formation
in microsomes, Table VI) was not supported by these preliminary
data.
      Microsomal epoxide hydrolase activities are listed in Table
IX. There was significantly higher (0.05 level) hepatic microso-
mal epoxide hydrolase activity in pectin-,Metamucil-, and
cellulose-treated rats, as compared to control-, lignin- and Lab
Chow-fed animals. There was no significant difference in intesti-
nal microsomal epoxide hydrolase activities among any of the
treatment groups.
      Studies directly probing the interaction of dietary fibers
on foreign compound metabolism are limited. Brown,et al.(81)
reported that rats fed basal chow diets had no liver metabolism
differences compared to rats on diets supplemented with pectin or

Table VI. Biphenyl Hydroxylase Activity in Intestinal
Microsomes of Fiber-Fed Rats.[a]

Diet Type	Nanomoles/mg/15 min					
	2-OH		3-OH		4-OH	
Control (No Fiber)	0.38 ± 0.16		0.79 ± 0.35		1.13 ± 0.74	
Lab Chow	.30	.09	.46	.14	.79	.24
15% Pectin	.19	.05	2.08	.74	.35	.09
15% Metamucil	.50	.01	.88	.13	.92	.45
15% Lignin	.68	.32	1.14	.26	1.32	1.06
15% Cellulose	.47	.07	.46	.13	.22	.08

[a] Values are mean hydroxylase activity ± SE for groups of 7 rats; differences among treatments were not significant ($P=.05$).

Table VII. Small Intestine Parameters and Biphenyl Hydroxylase Activities in intestinal Microsomes of Fiber-Fed Rats.[a]

Fiber Type	Intestine Length (cm) ($\bar{x}$)	Mucosal Weight (g) ($\bar{x}$)	Microsomal Protein (mg/g) ($\bar{x}$)	Biphenyl Hydroxylase[b] 2-OH	3-OH	4-OH
Control (No Fiber)	100 ± 3	2.30 ± 0.45	7.28 ± 0.90	0.95 ± 0.22	0.98 ± 0.09	0.77 ± 0.11
Lab Chow	108 ± 6	2.51 ± 0.70	4.41 ± 1.40	.41 ± .12	.72 ± .09	1.19 ± .56
15% Pectin	115 ± 6	3.78 ± 0.59	3.30[c] ± 1.20	.69 ± .08	.58 ± .14	.23 ± .05
15% Metamucil	119 ± 8	2.55 ± 0.27	6.39 ± 0.64	.83 ± .15	.85 ± .11	.33 ± .07
15% Lignin	101 ± 7	2.56 ± 0.25	3.22 ± 0.50	.97 ± .42	.44 ± .19	.45 ± .07
15% Cellulose	103 ± 5	2.86 ± 0.62	5.73 ± 1.24	.97 ± .21	.73 ± .12	.73 ± .09

[a] Values are means ± S.D. of 3 rats, except hydroxylase values where n=2.

[b] Nanomoles/mg protein in mucosal microsomes/15 min.

[c] Significant difference from control, $P \geq .05$.

Table VIII.   Benzo(a)pyrene Hydroxylase Activities in
Hepatic and Intestinal Mucosa Microsomes
of Fiber-Fed Rats.[a]

| Diet Type | Picomoles/mg/min | |
	Liver	Small Intestine
Control (No Fiber)	216 $\pm$ 87.9	14.5 $\pm$ 1.6
Lab Chow	92[b] 7.1	19.9 6.8
15% Pectin	193 81.1	17.9 4.7
15% Metamucil	263 98.1	18.7 5.3
15% Lignin	255 86.4	19.3 3.4
15% Cellulose	245 98.1	19.6 4.8

[a] Values are means $\pm$ SE for groups of 7. Quantitation was based on the fluorescence of 20ng 3-hydroxy-benzo(a)pyrene and scaled to mg microsomal protein.

[b] Significant difference from controls, $P \geq .05$.

Table IX. Epoxide Hydrolase Activities in
Liver and Intestinal Mucosa
Microsomes of Fiber-Fed Rats.[a]

	Nanomoles/mg/5 min	
Diet Type	Liver	Small Intestine
Control (No Fiber)	38.0 $\pm$ 8.13	14.9 $\pm$ 2.94
Lab Chow	45.0 7.24	13.1 3.28
15% Pectin	64.3[b] 11.81	12.6 3.05
15% Metamucil	62.0[b] 13.32	16.3 3.24
15% Lignin	44.9 4.94	14.3 4.33
15% Cellulose	64.4[b] 22.1	18.6 7.01

[a] Values are means $\pm$ SE for groups of seven scaled to mg microsomal
    protein for 3-(p-nitrophenoxy)-1,2-propene oxide as substrate.

[b] Significantly different from controls, P $\geq$ .05.

wheat bran as judged by antipyrine and acetaminophen metabolism. Chadwick et al.(82), however, demonstrated significant enhancement of lindane metabolism in pectin-fed rats. Proia et al.(83) recently reported that pectin feeding lowered hepatic cytochrome P-450 levels while at the same time increasing intestinal benzo(a)pyrene hydroxylase levels. It remains to be evaluated to what extent the differences observed in drug or other foreign compound in vivo metabolism in rats fed pectin or other purified fibers can be explained by differences in constituative levels of the relevent enzymes in liver and intestinal mucosa. Our data suggest that MFO hydroxylating activities in both tissues are not particularly sensitive to effects of feeding purified fibers at high levels (the diet model used in our work represents extremes in fiber intake). The significant effect on hepatic epoxide hydrolase seen with pectin-, Metamucil-, and cellulose-fed rats (Table IX) may point toward a more important response in relation to chemical carcinogenesis. Studies are in progress in our laboratory to compare GSH-transferase activities and the tissue glutathione status in specific fiber-fed rats. These studies may enhance our appreciation of the contributions of the non-oxidative enzymes in foreign compound metabolism to the relationships between nutritional state and chemical carcinogenesis.

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