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A Comparison of the Lipid-Lowering and Intestinal Morphological Effects of Cholestyramine, Chitosan, and Oat Gum in Rats (42773)

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Abstract. Cholestyramine, chitosan, and oat gum are lipid-lowering compounds. Cholestyramine use in humans may contribute to colonic adenocarcinoma; chitosan and oat gum are being studied in the rat to determine their potential for human use. To compare these compounds, we fed three groups of 10 male Sprague–Dawley rats one of the substances at 5% of diet with 1% cholesterol and 0.2% cholic acid; two other groups were fed cellulose with and without 1% cholesterol and 0.2% cholic acid. All groups had similar food intake and weight gains. Cholesterol feeding increased total liver lipids almost 3-fold and liver cholesterol concentration almost 10-fold. Cholestyramine, oat gum, and chitosan all significantly lowered liver cholesterol with cholestyramine feeding yielding levels identical to the noncholesterol-fed basal group. Chitosan and oat gum lowered liver cholesterol moderately. Cholestyramine and chitosan both significantly lowered serum cholesterol compared to the cellulose group. Oat gum was less effective. Hemoglobin and serum iron were similar in all groups except the oat gum group, which had decreased serum iron. Histological examination of small and large bowel with morphometry revealed statistically significant increases in both proximal and distal small bowel and distal large bowel mucosal thickness in the cholestyramine-fed group. No changes were noted in the proximal large bowel. Neither chitosan nor oat gum produced mucosal change other than an increase in the distal small bowel with the oat gum diet. Chitosan may have lipid-lowering effects similar to those of cholestyramine without the deleterious changes in intestinal mucosa.

Cholestyramine effectively lowers lipid levels in humans. Its potent bile acid binding capacity results in decreased intestinal cholesterol absorption and increased fecal excretion of bile acids (1). Unfortunately, cholestyramine is possibly linked to colon cancer in humans (2–5) and rat models (6). Though mechanisms of cancer induction remain unclear, recent histological studies indicate that cellular disruption in the rat colon occurs with cholestyramine feeding.(7).

Chitosan and oat gum lower lipid levels in rats (8–10). Several researchers are examining chitosan, an aminopolysaccharide extracted from shellfish chitin, for its potential hypocholesterolemic effects in rats. Preparation by deacetylation of insoluble chitin produces acid soluble chitosan which has a viscosity similar to that of pectin or guar (2), substances which are also known to lower lipid levels in rats (10). Chitosan and cholestyramine are equally effective in lowering rat serum and liver cholesterol levels (11).

Oat gum may be extracted from oat flour or bran (12, 13). The major component is a (1-3) (1-4)-β-D-glucan (β-glucan) found in the oat endosperm cell walls and concentrated in the outer (bran) layers (14). Oat gum, like pectin and guar gum, is water soluble, and aqueous solutions have viscosity characteristics similar to those of guar gum (15). Both pectin and guar gum are lipid lowering agents (10). Studies show that oat bran has high potency in lowering rat cholesterol levels in liver and plasma, while increasing HDL-cholesterol concentrations, an effect which might be attributed to the β-glucan component of the bran (16). Since chitosan and oat gum show potential for controlling hypercholesterolemia in humans, we examined their lipid-lowering effects relative to cholestyramine in a rat model. We also examined the apparent safety of each substance by feeding it to rats at 5% of diet and measuring serum iron and hemoglobin, as well as examining the effects of each compound on

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intestinal mucosa by light microscopy with morphometry.

Materials and Methods. *Diets.* Dr. Ivan Furda, Minneapolis, Minnesota, provided the chitosan. Analysis provided by Dr. Furda revealed 70.03% deacetylation, ash (0.20%), moisture (13.60%), and mol wt 500–1000 kDa. Oat gum was prepared at the POS Pilot Plant, Saskatoon, Saskatchewan, by Dr. Peter Wood. Enzymic analysis (17) of the oat gum showed 78% water-soluble β-glucan, pentosan (2.9%), starch (7.9%), protein (7.2%), and ash (1.6%). Cholestyramine (Questran) was obtained from Mead Johnson. Other ingredients such as sucrose, etc., are widely available. ICN Nutritional Biochemicals, Cleveland, Ohio, prepared the diets. Table I gives the composition of the diets.

Animals. Fifty male Sprague–Dawley rats, 10 weeks old, were divided into 10 blocks each consisting of five rats. The five rats in each block were similar in their initial body weight. Then, the rats from each weight stratification group were randomly assigned to each of the five different fiber-containing diets to provide five dietary groups of 10 rats each (Table I). This achieved random selection of rats for dietary groups while maintaining similar initial weights for all rats in the study. They were housed individually in wire cages in an air-conditioned room with a 12-h light/dark cycle. After rats were received from the supplier they were fed standard Purina lab chow for at least 7 days for acclimitization, then were stratified into the five dietary groups and fed the test diets *ad libitum* for 21 days. At the end of the experimental period, rats were anesthetized with pentobarbital (0.1 mg/100 g body wt) and exsanguinated by cardiac puncture. Livers were removed, weighed, and frozen immediately on dry ice for later analysis. In addition, segments of jejunum, ileum, and ascending and descending colon were removed, opened, pinned flat, washed at 4°C with phosphate-buffered saline solution, and immersed immediately in 10% formaldehyde prior to preparation for histologic study.

Analysis. Lipid extraction was by modification of the Folch procedure (18). The separated chloroform layer was used for gravimetric determination of total liver lipids. $FeCl_3$ and H_2SO_4 were used to colorimetrically analyze liver and serum cholesterol concentrations (19). Liver and serum triglyc-

TABLE I. COMPOSITION OF DIETS

	Basal	Cellulose[a]	Oat gum[b]	Chitosan[c]	Cholestyramine[d]
			G/100 G		
Carbohydrate, total	68.7	67.5	67.5	67.5	67.5
Sucrose	51.5	51.5	51.5	51.5	51.5
Starch	17.2	16	16	16	16
Protein (casein)	15	15	15	15	15
DL-Methionine	0.3	0.3	0.3	0.3	0.3
Fat (cotton seed oil)	6	6	6	6	6
Cholesterol		1	1	1	1
Cholic acid		0.2	0.2	0.2	0.2
Vitamin mixture[e]	1	1	1	1	1
Salt mixture[f]	4	4	4	4	4
Cellulose	5	5			
Oat gum			5		
Chitosan				5	
Cholestyramine					5

[a] Alphacel, ICN Nutritional Biochemicals, Cleveland, OH.
[b] Prepared at the POS Pilot Plant, Saskatoon, Saskatchewan.
[c] Dr. Ivan Furda, Minneapolis, MN, provided the chitosan.
[d] Mead Johnson Corp., Pharmaceutical Division, Evansville, IN, provided the cholestyramine.
[e] Vitamin diet fortification mixture, ICN Nutritional Biochemicals, Cleveland, OH.
[f] USP XVII salt mixture (ICN Nutritional Biochemicals, Cleveland, OH) contains 0.54% iron and is supplemented with 0.081% zinc sulfate.

erides were determined colorimetrically by the Dade method (20). Serum HDL-cholesterol was precipitated with sodium phosphotungstate–magnesium (21) and measured colorimetrically. Gibson (22) reported that precipitation methods employing phosphotungstate–magnesium will precipitate some apo E-containing HDL which, in some species, might include a fairly large percentage of the HDL present. Thus this method may, in some circumstances, cause an underestimation of HDL levels. In this study, however, this underestimation would not prevent the comparison of differences among groups. Data were analyzed using one-way analysis of variance (23) and the Student *t* test. Hemoglobin levels were determined by the standard cyanmethemoglobin method. Serum iron levels were determined on the DuPont ACA using an adaption of the Megraw–Bouda modification of the bathophenanthroline procedure originally described by Goodwin *et al.* (24–26).

Histologic examination. A cross-section of the mid portion of each harvested bowel segment was processed in a Fisher Histomatic tissue processor (Model 266 MP). The processed portions of bowel were then oriented so that the plane of sectioning was perpendicular to the lumen and embedded in paraffin. Five-micron sections were stained with hematoxylin and eosin and reviewed for appropriate orientation for morphometric measurements. Those sections with improper orientation were reembedded or resubmitted from the remaining gross tissue.

Morphometry was done with an American Optical microscope and optical micrometer which had been calibrated against an American Optical micrometer disk with a 5-mm scale divided into 50 units. Two separated sections of each bowel segment were measured five times to obtain mucosal height. Measurements were taken from the basement membrane at the base of the villus or gland to the highest point of the glyocalex of the same villus or gland. Only glands or villi which were contained entirely within the plane of sectioning were utilized for measurement. The five greatest measurements obtained for each section were combined and the mean of the 10 measurements was assigned as the mucosal height for that particular segment of bowel. All measurements were taken without knowledge of the test group to which that particular animal belonged. Test groups were revealed only at the time of statistical analysis.

Results. *Food intake and weights.* All groups had similar initial and final weights with no differences noted among any of the diet groups (Table II). Rats fed cholestyramine had significantly higher food intake than rats fed basal, cellulose, or oat gum diets. Rats fed chitosan had significantly greater intake than rats fed oat gum.

Serum lipids. Chitosan and cholestyramine diets lowered total serum cholesterol significantly as compared to the cellulose diet. Oat gum lowered serum cholesterol to a lesser although significant degree. Adding cholesterol to the diets lowered serum HDL-cholesterol. Feeding cholestyramine resulted in an elevated HDL-cholesterol concentration almost equal to that of basal levels. Compared to cellulose, chitosan and oat gum slightly raised HDL-cholesterol concentration. None of the three substances altered serum triglycerides.

Liver weight. Cholesterol feeding increased liver weight significantly as compared to basal levels. All three test substances decreased liver weights. Cholestyramine-fed rats had values slightly lower than basal values, while chitosan- and oat gum-fed rats had values between those for basal and cellulose groups.

Liver lipids. Cholesterol feeding increased total liver lipids almost 3-fold. Cholestyramine lowered lipids the most, and oat gum, the least. Cholesterol feeding increased liver cholesterol almost 10-fold. As compared to cellulose, oat gum, chitosan, and cholestyramine all significantly lowered liver cholesterol. With cholestyramine feeding, values were identical to those for the basal group, while chitosan and oat gum had intermediate effects. Liver triglycerides followed a virtually identical pattern.

Hemoglobin and serum iron. Oat gum and chitosan did not alter hemoglobin concentrations. Cholestyramine-fed rats had significantly higher hemoglobin levels than any of the other groups. Feeding oat gum produced a lower serum iron concentration as compared to basal levels. Cholestyramine raised

TABLE II. EFFECTS OF OAT GUM, CHOLESTYRAMINE, AND CHITOSAN ON BODY WEIGHT, FOOD INTAKE, PLASMA, AND LIVER LIPIDS, HEMOGLOBIN, AND IRON[a]

	Basal	Cellulose	Oat gum	Chitosan	Cholestyramine
Initial body wt (g)	219 ± 1.6	218 ± 1	220 ± 1	220 ± 1.2	221 ± 1.3
Final body wt (g)	294 ± 5.7	298 ± 5.9	292 ± 7	300 ± 5	293 ± 3.3
Food intake (g/day)	17.6 ± 0.6*†	18.4 ± 0.7*†	16.7 ± 0.7†	18.7 ± 0.5*‡	20.3 ± 0.7‡
Relative liver size (g liver/100 g body wt)	3.89 ± 0.1*	5.08 ± 0.11†	4.63 ± 0.11‡	4.5 ± 0.13‡	3.64 ± 0.08§
Plasma total cholesterol (mg/100 ml)	88 ± 3*	151 ± 11†	117 ± 7‡	99 ± 5*§	99 ± 4§
Plasma HDL cholesterol[b] (mg/100 ml)	66 ± 3*	47 ± 4†	56 ± 5‡§	55 ± 4†‡	64 ± 5*§
Plasma triglyceride (mg/100 ml)	89.4 ± 8	84.8 ± 8.8	87.6 ± 6.6	92.8 ± 5.5	84.5 ± 7.2
Liver total lipid (mg/g wet liver)	43 ± 0.5*	122 ± 4†	76 ± 4‡	61 ± 3§	44 ± 0.9*
Liver triglyceride (mg/g wet liver)	5.5 ± 0.2*	25.1 ± 1.6†	15 ± 1.1‡	11.2 ± 0.8§	5.7 ± 0.3*
Liver cholesterol (mg/g wet liver)	3.8 ± 0.1*	37 ± 1.9†	16.9 ± 1.8‡	11.3 ± 1.3§	3.8 ± 0.1*
Hemoglobin (mg/100 ml)	14.2 ± 0.2*	13.9 ± 0.3*	14 ± 0.2*	14.2 ± 0.2*	15.4 ± 0.1†
Total serum iron	170 ± 7*†	155 ± 7*†‡	147 ± 4‡	167 ± 6*§	181 ± 9†§

[a] Means ± SEM for 10 rats. Means in the same row with different superscript symbols differ significantly ($P < 0.05$).
[b] High-density lipoprotein.

iron concentrations above cellulose and oat gum levels. Feeding cholesterol produced a slight decrease from basal levels. Of the three test substances, chitosan showed the least change from basal levels in total serum iron.

Microscopy. The mean mucosal height of all rats in each test group at each anatomical location is given in Table III. Analysis of variance show the mean heights of the groups to be significantly different in the proximal ($P < 0.01$) and distal small bowel ($P < 0.001$) and the distal large bowel ($P < 0.001$). The means in the proximal colon did not differ significantly. Student t test comparison of individual means shows that the mean mucosal height of the cholestyramine-fed rats was significantly greater than the cellulose group for all areas of bowel except the proximal colon. The differences in mean mucosal height between the cholestyramine and cellulose group were greatest in the proximal small bowel. The oat gum group showed a slight but insignificant decreased mucosal height in the proximal small bowel and distal colon. The mean mucosal height for the chitosan-fed group showed no statistical difference from the cellulose group in any area of bowel.

No evidence of inflammatory change, mucosal ulceration, invasive carcinoma, mucosal dysplasia, or infectious agents was found in any histologic section taken. No tumors, polyps, or other mucosal lesions were seen upon gross examination.

Discussion. Oat gum, cholestyramine, and chitosan effectively reduced serum lipids.

TABLE III. MUCOSAL HEIGHTS OF RAT BOWEL FOR EACH TEST GROUP AND AT EACH ANATOMIC LOCATION[a]

	Proximal small bowel	Distal small bowel	Proximal colon	Distal colon
Basal	58.7 ± 1.54*†	40.12 ± 1.74*	21.4 ± 0.82	23.0 ± 0.88*
Cellulose	56.3 ± 1.40*	29.6 ± 1.13†	22.0 ± 0.95	23.1 ± 1.14*†
Oat gum	55.4 ± 1.73*	34.7 ± 2.02*‡	22.8 ± 0.96	20.78 ± 0.47†
Chitosan	59.00 ± 1.05*	30.89 ± 1.18†‡	21.4 ± 0.58	24.1 ± 0.87*‡
Cholestyramine	63.4 ± 1.65†	34.44 ± 1.30‡	19.6 ± 0.63	27.7 ± 1.47‡

[a] Values represent mean mucosal height in hundredths of a millimeter ± SEM for 10 rats. Means in the same column with different superscript symbols differ significantly ($P < 0.05$).

Cholestyramine lowered serum cholesterol 34% below cellulose levels, consistent with previous rat studies (8, 9, 27, 28).

Chitosan has similar cholesterol lowering effects in laboratory rats (8, 9, 27, 28). In our study, a 5% chitosan diet also reduced serum cholesterol 34%, confirming Sugano's earlier report (9). Other researchers note chitosan surpasses or equals the cholesterol lowering effects of cholestyramine or plant fibers such as pectin (11, 27, 28). Sugano (8, 9) also reports that chitosan can cause growth reduction or increased food intake, but 5% chitosan in our studies produced neither of these effects.

Both cholestyramine and chitosan reduced serum cholesterol more than did oat gum. Oat gum at 5% of diet reduced serum cholesterol 23%. A previous oat gum study in rats showed a 41% reduction in serum cholesterol when fed at 10% of diet (10). Studies indicate that water-soluble plant fibers in combination with low cholesterol diets are therapeutically useful in controlling hypercholesterolemia in humans (29).

In human studies atherosclerosis correlates positively with low-density lipoprotein (LDL)-cholesterol and inversely with HDL-cholesterol. Evidence suggests that increased HDL-cholesterol may protect from coronary heart disease (30). Thus, any study of cholesterol lowering diets, even in animals, should also evaluate the effects on HDL-cholesterol as well as total cholesterol. Cholesterol feeding lowers HDL-cholesterol in humans and experimental animals (16, 31, 32). In this study we saw a 29% decrease in HDL-cholesterol with cholesterol feeding. As compared to cellulose, cholestyramine, chitosan, and oat gum elevated HDL-cholesterol levels by 36, 17, and 19%, respectively.

Cholestyramine, chitosan, and oat gum did not affect serum triglycerides in these studies. Most previous studies on similar substances indicate no alterations in serum triglycerides. In a rat study (10), oat gum lowered serum triglycerides. However, in two clinical studies (29, 33) with oat bran, both a lowering of serum triglycerides and no effect were observed. We need to further examine possible effects of oat gum on serum triglycerides.

As Kritchevsky notes (34), most studies use dietary cholesterol ranging from 0.25 to 1.0%, and some add bile acids to enhance the cholesterolemia, as in this and previous experiments (10, 16, 35). Using this model, liver lipid changes followed the pattern of serum cholesterol. Cholesterol feeding significantly raised liver total lipids, liver triglyceride, liver cholesterol, and liver weight. Cholestyramine produced the greatest reductions in liver lipids. Liver total lipids decreased 64% with cholestyramine and 50% with chitosan. Oat gum was the least potent, with a reduction in total liver lipids of 38% from control cellulose levels. Results of liver cholesterol, liver triglyceride, and liver weight were comparable, with cholestyramine being the most potent and oat gum the least potent.

Our results show that oat gum reduces total serum iron 5% below cellulose values and 14% below basal levels. This reduction may be due to iron binding by oat gum, decreasing its availability for absorption. Previous human and *in vitro* studies show that wheat bran decreases iron absorption (36–38). In rats, the fiber portion of the fiber–iron complex is not degraded in the intestine so the iron remains bound throughout its transit and becomes unavailable (39).

Alternatively, lower serum iron levels may result from the ability of oat gum to slow migration of rat epithelial crypt cells (40). The columnar epithelial cells control iron absorption by sequestering iron. These cells then accumulate excess dietary iron and continually block iron absorption. In contrast, cholestyramine increases migratory rates. Our results show an increase in serum iron 17% over control cellulose levels with cholestyramine feeding. Cholestyramine also raised hemoglobin levels significantly and produced an enteric mucosal hypertrophy.

Gordon reports chitosan at 10 and 20% of diet decreases rat hemoglobin levels (39). He postulates that chitosan may reduce hemoglobin levels by producing an inflammatory state in the small intestine. The adverse effect of chitosan on growth and hemoglobin levels in the rat were also seen in a second study by Gordon *et al.* (41).

We saw no inflammatory changes in any of the histologic sections taken in this study.

Our findings of normal hemoglobin and iron along with reduced lipid levels suggest that chitosan is effective in rats with relatively minimal side effects when given at 5% of diet.

Cholestyramine is currently used successfully to treat hypercholesterolemia in humans (42). Although very effective, its use has been questioned because of reports of a possible link to colon cancer in humans (2–5) and rat experimental models (6). Cholestyramine sequesters bile salts in rats (43) and alters intestinal morphology in humans (2). Long-term cholestyramine feeding in rats alters cell repair and renewal rates due to provocation by the bile salts (6). Furthermore, two studies by Nigro *et al.* showed a significant increase in rat intestinal tumor induction by various agents when cholestyramine was added to the diet. The increase tumor induction was seen primarily in the large intestine (6, 44).

Certain dietary fibers may help protect against colon cancer in rats (7, 40), but substances such as pectin and alfalfa, which bind bile salts, produce abnormal intestinal mucosal ultrastructure (6). Oat bran decreases crypt cell replication and alters cell morphology in rats (40), and binds bile acids in humans (29), but has not yet been linked to colonic cancer. Likewise chitosan has yet to be linked with tumor-promoting effects in rats or colon cancer in humans. Different mechanisms of actions of chitosan and cholestyramine (2) may account for the differential mucosal effects seen in this study. Chitosan has been reported to bind lipid droplets *in toto* unlike cholestyramine, which is selective for bile salts. If bile salts play an important tumor-promoting role in colon malignancies, then the different effects of chitosan and cholestyramine on intestinal lipids may explain the differential intestinal mucosal effects seen in this study.

Our data confirm the alteration of intestinal histology by cholestyramine. Mucosal height was significantly greater in the cholestyramine-fed group for jejunum, ileum, and distal colon. Only the proximal colon failed to show an increase in mucosal height. This is consistent with a previous report of altered cell renewal rates in cholestyramine-fed rats (6). The differential effect on proximal and distal colonic mucosa is also consistent with previous reports showing tumor promotion by cholestyramine to be significantly greater in the distal than in the proximal colon (6, 44). The consistency of these findings suggests that 3 weeks of cholestyramine feeding of rats is adequate to demonstrate the mucosal stimulating effects of cholestyramine. This also suggests that the absence of significant hyperplastic responses in the chitosan-fed group is meaningful. No areas of mucosal atypia or discrete hyperplastic lesions were seen in any of the test groups, though these changes may require exposure times greater than 3 weeks or the presence of initiating factors. The chitosan group showed no significant differences in mucosa from those of the control group.

In summary, this study suggests that chitosan at 5% of diet in rats does not reduce serum iron or hemoglobin, and has lipid lowering effects equivalent to those of cholestyramine without producing similar deleterious effects upon intestinal mucosa. Thus, chitosan may show promise as an effective lipid lowering agent in humans, without the associated increase in colon cancer suspected in patients receiving cholestyramine.

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