Interaction of Bile Acids, Phospholipids, Cholesterol and Triglyceride with Dietary Fibers in the Small Intestine of Rats[1]

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ABSTRACT Certain dietary fibers have been reported to lower plasma cholesterol by binding bile acids and reducing their recycling through the enterohepatic circulation. In addition, certain fibers may delay the digestion and absorption of fat. In the present study, the interaction of bile acids with guar gum (GG), konjac mannan (KM) and chitosan (CH) was determined. Rats were fed during a 20-min period a test meal containing either 5% cellulose (CE), GG, KM or CH and also containing ^{14}C-labeled triolein and ^{3}H-labeled cholesterol. The group fed CE served as control, since CE does not bind bile acids or phospholipids in vivo. Two hours after presentation of the test meal, rats were killed and the stomach and small and large intestine removed. All four groups ate the same amount of the test meal, about 1.9 g. The aqueous phase of the small intestinal contents was separated by ultracentrifugation, and the amount (μmol) of bile acids and phospholipids in the total intestinal contents and in the aqueous phase was estimated. The ratio of bile acids in the aqueous phase to that in total intestinal contents was significantly higher in the GG and KM groups and significantly lower in the CH group than that in the CE group, demonstrating that the bile acids are bound or trapped by each of these fiber sources. Only CH appeared to bind phospholipids, reducing the proportion in the aqueous phase compared to that in the CE group. Of the ^{14}C and ^{3}H emptied from the stomach, a greater percentage remained in the small intestine of the GG and KM groups than in that of the CE group, indicating that absorption of both cholesterol and triglyceride from the small intestine was delayed due to feeding GG or KM. *J. Nutr.* 119: 1100–1106, 1989.

INDEXING KEY WORDS:

• *bile acids* • *cellulose* • *konjac mannan*
• *chitosan* • *guar gum* • *lipid* • *cholesterol*
• *triglycerides* • *rat* • *fiber* • *small intestine*

Numerous in vitro studies have demonstrated that some, but not all, sources of dietary fiber bind bile acids (1–3), leading to the hypothesis that bile acid binding is a mechanism whereby certain sources of dietary fiber lower plasma cholesterol. In human studies, consumption of certain sources of dietary fiber leads to an increase in the fecal excretion of bile acids (4). However, a review of these studies has indicated that the response is not consistent, nor is it likely to be sufficient to completely explain the hypocholesterolemic response to sources of dietary fiber (4, 5). For example, cellulose does not bind bile acids in vitro and in vivo, nor does it lower plasma cholesterol, yet it has been reported to increase fecal bile acid excretion (4). On the other hand, pectin, guar gum and oat bran, which are hypocholesterolemic, have been reported to increase fecal bile acid excretion, while beans reportedly lower plasma cholesterol, yet decrease bile acid excretion (4).

In a previous report, bile acid and phospholipid binding was examined under physiological conditions by determining binding in the small intestinal contents and relating the binding to the amount of lipid solubilized and available for absorption (6). In this earlier study it was shown that cellulose does not bind either bile acids or phospholipid or reduce the amount of lipid solubilized in the aqueous phase of the small intestinal contents. In the present study the ability of guar gum and konjac mannan, two viscous, soluble polysaccharides, and chitosan, an aminopolysaccharide with in vitro bile acid binding capacity, to interact with bile acids and phospholipids and to slow the disappearance of both cholesterol and triglyceride were compared to that of cellulose. The results indicate that the physical properties of the sources of dietary fiber within the intestinal contents will have an important effect on the response in terms of availability of bile acids and phospholipids, and lipid disappearance from the gastrointestinal tract.

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MATERIALS AND METHODS

Animals and diet. Male Wistar rats (Simonsen Laboratories, Gilroy, CA) weighing 140–160 g were housed individually in stainless steel wire-bottomed cages and given water and a purified diet ad libitum for 10 d. The composition of the diet (7) was (%): cellulose (CE) (Solka Floc; Brown Co., Berlin, NH), 5; casein, 18; glucose, 63; corn oil, 10; mineral mixture, 6; vitamin mixture, 3; and butylated hydroxytoluene (BHT), 0.02. A 12-h dark-light, reversed light cycle was used, with lights off at 0800 h. Food was only available during the dark cycle. The test diets, which were fed on the day of the experiment, were prepared by the addition of 5% dietary fiber to a basal diet mixture of the following composition (%): casein, 18; glucose, 43; corn oil, 30; mineral mixture, 6; vitamin mixture, 3; and butylated hydroxytoluene (BHT), 0.05. The added dietary fibers in the test diets were: guar gum (GG; Sigma Chemical, St. Louis, MO); konjac mannan (KM, a glucomannan; (a gift from Shimizu Chemical Industries, Mihara, Hiroshima, Japan); cellulose (CE, Solka Floc); or chitosan (CH; a gift from Kyowa Yushi, Co., Funabashi, Chiba, Japan). Each diet also contained 2.91 μCi of [1,2(*n*)-^{3}H]cholesterol (1 Ci/mmol) and 2.15 μCi of [*carboxyl*-1-^{14}C]triolein (55 mCi/mmol), both from Research Products International, Elk Grove Village, IL. These diets contained a high level of dietary fat to ensure that sufficient fat was present in the small intestine 2 h after the meal.

Experimental design. On the afternoon of the 10th d, food cups were removed and the rats were divided into four groups. Between 0900 and 1100 h of the following morning, the rats were given a 2-g meal of one of the test diets for 20 min. Two hours after the initial presentation of the meal, the rats were killed by heart puncture under ether anesthesia, and then the stomach, the small and large intestine and the liver were removed. The liver and large intestine were frozen immediately in liquid nitrogen and stored at −20°C. The small intestine was clamped at the pylorus and the ileal-cecal junction, removed, stripped of mesentery and fat, and chilled. The small intestine was quartered to facilitate handling, and the contents of each section emptied into a calibrated tube by finger stripping. Finger stripping was used to obtain intestinal contents because it avoids dilution of the contents, allowing determination of aqueous phase volume and radioactivity, bile acid and phospholipid concentration in the intestinal contents and aqueous phase. To inhibit pancreatic lipase, 50 μl of 1 M bromophenylboromic acid (Aldrich Chemical, Milwaukee, WI) in methanol was added to the contents after emptying the first section. The contents in the tube were thoroughly mixed with a spatula after the contents of each section were added. Subsequently, the tube was placed in a 70°C water bath for 10 min immediately after collection of the contents was completed. The tube was then cooled to room temperature, centrifuged at 50 × *g* for 1 min at 23°C to pack the contents and remove air bubbles, and the total intestinal contents volume was determined.

An aliquot (~ 0.8 ml) of the small intestinal contents was centrifuged at 100,000 × *g* for 3 h at 30°C. Three distinct phases formed upon ultracentrifugation: a pellet containing undigested diet and cellular debris; an infranatant, which will be referred to as the aqueous phase; and a thin oil layer on top (8). The aqueous phase proportion of the tube contents was estimated by measuring the height (mm) within the tube of the total contents and of the aqueous phase, and calculating the aqueous phase volume of the total contents. The aqueous phase was collected as completely as possible after the oil layer was removed by tube slicing. The remainder of the uncentrifuged total intestinal contents was homogenized. The homogenized, total intestinal contents and the aqueous phase portion obtained after ultracentrifugation were stored at −20°C under N_2 until assayed for radioactivity, bile acids and phospholipids.

The intestinal tissue, stomach contents and large intestine were collected into tared vessels and immediately lyophilized. The lyophilized tissue samples were ground and mixed in a mortar and pestle and then stored in the desiccator under anhydrous $CaSO_4$ until analyzed.

Determination of radioactivity in the lyophilized tissue samples. Duplicate samples of 50 mg of stomach contents, intestinal tissue and liver, 25 mg of large intestine, 10 μl of the intestinal contents, and 10 μl of the aqueous-phase portion were placed in liquid scintillation vials, to which 200 μl of distilled water and 1 ml of tissue solubilizer (TS-1, Research Products International) were added. Vials were placed in an oven for 2 h at 50°C. One or two drops of glacial acetic acid and 14 ml of scintillation cocktail (Econofluor, New England Nuclear, Wilmington, DE) were added to each vial and radioactivity determined by liquid scintillation spectrometry, using an external standard for quench correction (Packard Tri-Carb model 2610, Downers Grove, IL).

Assay of bile acids and phospholipids in the small intestinal contents and aqueous phase. The extraction of bile acids from the intestinal contents and the partial purification of extracted intestinal contents were completed as published previously (6). Total bile acids in the partially purified intestinal contents extract and unextracted aqueous phase were determined enzymatically by use of α-hydroxysteroid dehydrogenase according to the method of Sheltawy and Losowsky (9).

Phospholipids were extracted by the method of Folch, Lees and Stanley (10) and assayed according to Bartlett (11). Lipids in intestinal contents (20 μl) and aqueous phase (20 μl) were extracted according to the method of Folch, Lees and Stanley (10).

Statistics. Differences between treatments were determined by analysis of variance, and least significant difference was used to determine differences between

means. When data reported as percentage were less than 20%, the arcsin transformation was used to compare differences between means (12). The package Statview (13) was used for statistical analysis.

RESULTS

Before receiving the test meal, all rats were adapted to the same purified diet, and there were no differences in body weight among the experimental groups. Intake of test meal, stomach contents dry weight, volume of intestinal contents and volume of the aqueous phase are shown in **Table 1**. All the experimental groups consumed a similar quantity of the test meal. The dry weight of the stomach contents was significantly higher in the GG group than in the CE and KM groups. Stomach contents weight tended to be higher in the CH group than in the CE and KM groups, but the difference was not significant. The volume of the total intestinal contents and the aqueous phase was significantly higher in the KM group than in the other three groups. In the GG group, the volume of the aqueous phase was significantly higher than that of the CE and CH groups. The volume of the total intestinal contents was significantly correlated with the volume of the aqueous phase ($r = 0.885$, $P < 0.0001$, $d_f = 36$).

The amount of bile acids and phospholipids in the intestinal contents and their concentration in the aqueous phase are shown in **Table 2**. The total amount of bile acids in the KM group was significantly higher than in the CE and GG groups, and the amount in the CH group was significantly higher than in the GG group. The total amount of phospholipids in the KM group was significantly higher than in the CE, GG and CH groups. In the aqueous phase of the intestinal contents, the concentration of bile acids was significantly lower in the CH group than in the KM and GG groups. Phospholipid concentration in the aqueous phase was significantly higher in the CE group than in the other three dietary groups and significantly higher in the KM group than in the GG and CH groups. The distribution of bile acids and phospholipids between the aqueous phase and total intestinal contents is illustrated in **Figure 1**. The ratio of bile acids in the aqueous phase to total intestinal contents in the GG, KM, and CH groups differs significantly from the ratio in the CE group (Fig. 1*A*). The ratio in the GG and KM groups was significantly higher than in the CE group, whereas in the CH group, it was significantly lower. In both the GG and CH groups, the ratio of phospholipids in the aqueous phase to total intestinal contents was significantly lower than the ratio in the KM and CE groups, and the ratio was significantly lower in the CH group than in the GG group (Fig. 1*B*). Estimation of the amount of bile acids in the aqueous phase from the concentration of bile acids (Table 2) and volume (Table 1) of the aqueous phase is in agreement with the pattern of distribution shown by the ratios in Figure 1: the GG and KM had higher amounts of bile acids in the aqueous phase than did the CE and CH groups.

The activities of ^{3}H from cholesterol and ^{14}C from triolein in the stomach are shown in **Table 3**. In the KM group, the ^{3}H activity was lower than in the GG and CH groups. The ^{14}C activity was lower in the KM group than in the CH and CE groups. Both ^{14}C and ^{3}H activities were significantly correlated with the weight of the stomach contents. Since food intake did not differ among the groups, the differences in activity between groups suggest a difference in gastric emptying. The activity emptied from the stomach was calculated by subtracting the amount in the stomach from the intake of ^{14}C or ^{3}H; the values are shown in Table 3. The ^{3}H activity emptied from the stomach was significantly lower in the GG and CH groups than in the CE and KM groups. The ^{14}C activity emptied was significantly different among all groups, with GG the lowest, followed by KM, then CH, and finally CE. The ^{14}C activity emptied was significantly correlated with the ^{3}H activity emptied ($r = 0.68$, $d_f = 36$, $P < 0.0001$).

The distribution of ^{14}C and ^{3}H in the small intestine was expressed as a percentage of the label that was emptied from the stomach (**Table 4**). More of the ^{3}H label than the ^{14}C label remained in the small intestine, which supports the observation by other researchers that cholesterol is absorbed less efficiently than triacylglycerides. The KM group had a significantly higher percentage of ^{3}H from cholesterol remaining in the to-

TABLE 1

***Test meal intake, stomach content weight, intestinal content volume and aqueous phase volume*[1]**

Diet	Test meal	Stomach contents	Intestinal contents	Aqueous phase
	g	*g dry wt*	*ml*	
Cellulose	1.90 ± 0.05[a]	0.76 ± 0.08[a]	1.70 ± 0.08[a]	0.90 ± 0.04[a]
Guar gum	1.84 ± 0.06[a]	0.98 ± 0.08[b]	1.93 ± 0.14[a]	1.36 ± 0.12[b]
Konjac mannan	1.93 ± 0.05[a]	0.75 ± 0.05[a]	2.31 ± 0.11[b]	1.65 ± 0.10[c]
Chitosan	1.86 ± 0.04[a]	0.90 ± 0.05[ab]	1.68 ± 0.06[a]	0.83 ± 0.04[a]

[1]Values are means ± SEM for $n = 9$ rats for the cellulose, guar gum, and chitosan groups and $n = 10$ rats for the konjac mannan group. Values not sharing a common superscript letter are significantly different ($P < 0.05$).

TABLE 2

***Total amount of bile acid and phospholipid in the small intestinal contents and their concentration in the aqueous phase*[1]**

Diet	Intestinal contents		Aqueous phase	
	Bile acids	Phospholipids	Bile acid	Phospholipid
	μmol		*mM*	
Cellulose	33.89 ± 0.95[ab]	24.98 ± 0.85[a]	13.3 ± 0.6[ab]	5.28 ± 0.38[c]
Guar gum	30.34 ± 1.15[a]	23.73 ± 1.22[a]	15.0 ± 1.8[bc]	2.75 ± 0.33[a]
Konjac mannan	37.94 ± 1.71[c]	29.91 ± 1.19[b]	18.0 ± 0.6[c]	3.61 ± 0.19[b]
Chitosan	35.56 ± 0.89[bc]	25.80 ± 0.77[a]	9.9 ± 0.4[a]	2.66 ± 0.17[a]

[1]Values are means ± SEM for $n = 9$ rats for the cellulose, guar gum, and chitosan groups and $n = 10$ rats for the konjac mannan group. Values not sharing a common superscript letter are significantly different ($P < 0.05$).

tal small intestinal contents than did the other dietary groups, and the GG group had a higher percentage of ^3H in the total intestinal contents than did the CE group. Within the aqueous phase of the intestinal contents, the KM group had a higher percentage of ^3H than did the other dietary groups, and the CH group had a significantly lower percentage than did the GG group. Most of the ^3H in the small intestine was in the aqueous phase, as indicated by the ratio of ^3H in the aqueous phase to the total intestinal contents. However, the ratio differed among all the groups; the CH group had the lowest ratio, followed by the GG, then KM, and CE groups. In the small intestinal tissue, the percentage of ^3H emptied from the stomach was significantly lower in the KM group than in the other diet groups, and significantly lower in the GG group than in the CE group. The distribution of ^3H in the large intestine was also affected by diet. The percentage in the KM group was substantially lower than in the other dietary treatments, and the GG group had a higher percentage than did the CE and CH groups.

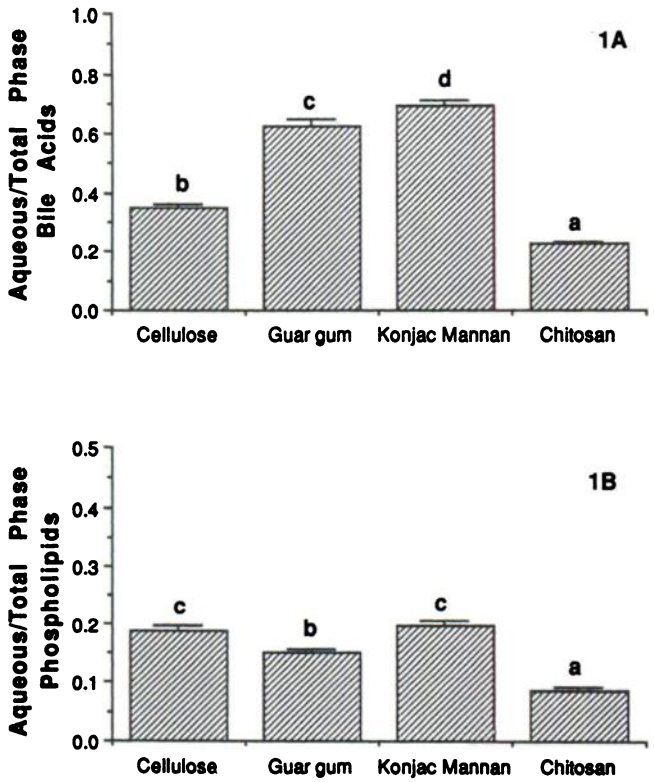

FIGURE 1 The ratio of ***A***) bile acids and ***B***) phospholipids in the aqueous phase of the small intestine contents to the total intestine contents. Bars without common superscript letters differ significantly ($P < 0.05$); $n = 9$ rats for the cellulose, guar gum, and chitosan groups and $n = 10$ rats for the konjac mannan group.

The KM group had a significantly higher percentage of ^{14}C from triolein remaining in the total small intestinal contents than did the other dietary groups, and the GG group had a significantly higher percentage remaining than did the CE and CH groups. As with the ^3H, most of the ^{14}C was in the aqueous phase, as indicated by the aqueous phase/total intestinal contents ratio, and the pattern of differences among the dietary treatments found in the aqueous phase was the same as the differences in total intestinal contents. The KM group had the lowest percentage of ^{14}C in the small intestinal tissue and large intestine, and the GG group had significantly less ^{14}C in the small intestinal tissue than did the CE and CH groups and significantly less in the large intestine than did the CH group. In the aqueous phase of the small intestinal contents, the activity from ^{14}C-triolein was significantly correlated with the activity from ^3H-cholesterol ($r = 0.868$, $P < 0.0001$, $d_f = 36$).

DISCUSSION

The four sources of fiber selected for this study have unique chemical and physical properties. Cellulose, which is considered an insoluble fiber source, has been shown previously to not bind bile acids or phospholipids in vivo (6) or in vitro (1). Because it does not bind bile acids or phospholipids, cellulose is a useful control to compare the in vivo binding capacity of the other three fiber sources fed in this study. Guar gum and konjac mannan are both sources of viscous polysaccharides; however, KM has a higher viscosity than GG (14). GG has been shown to bind or entrap bile acids in the intestinal contents (6). Chitosan is partially sol-

TABLE 3

Stomach content of ^{3}H from cholesterol and ^{14}C from triolein and the amount of isotope emptied from the stomach[1]

Diet	^{3}H		^{14}C	
	Stomach	Emptied[2]	Stomach	Emptied[2]
		dpm × 10^{6}		
Cellulose	2.27 ± 0.24^{ab}	3.64 ± 0.16^{b}	1.84 ± 0.20^{bc}	2.93 ± 0.13^{d}
Guar gum	2.76 ± 0.22^{b}	2.71 ± 0.17^{a}	1.44 ± 0.12^{ab}	1.55 ± 0.08^{a}
Konjac mannan	2.04 ± 0.15^{a}	3.55 ± 0.18^{b}	1.12 ± 0.07^{a}	1.91 ± 0.09^{b}
Chitosan	2.72 ± 0.17^{b}	2.86 ± 0.12^{a}	2.18 ± 0.14^{c}	2.33 ± 0.10^{c}

[1]Values are means ± SEM for n = 9 rats for the cellulose, guar gum, and chitosan groups and n = 10 rats for the konjac mannan group. Values not sharing a common superscript letter are significantly different ($P < 0.05$).

[2]Amount emptied was calculated as the difference between intake and the amount in the stomach.

uble in weak acid solutions but at the pH of the small intestine is insoluble (15). Chitosan has an ion exchange capacity and has a strong bile acid binding capacity in vitro (15).

Based on the weight of the stomach contents and the amount of ^{14}C and ^{3}H in the stomach, GG appeared to delay stomach emptying compared to CE. Other studies have indicated that GG can slow the rate of gastric emptying due to its viscosity. KM, which is also a viscous polysaccharide, however, did not appear to delay gastric emptying compared to CE. This difference in response may be because KM has a slower swelling rate than GG.

Within the small intestinal contents, the water-holding capacity of both GG and KM was evident in the higher volume of the intestinal aqueous phase in these groups than in the CE group. The total intestinal volume was also higher in the KM group, reflecting the high water-holding capacity of this substance. CH is likely to be insoluble at the pH of the small intestine, and the CH group had similar total intestinal contents volume and aqueous phase volume as had the CE group.

The results of this study indicate that GG, KM and CH bind or sequester bile acids, and that CH binds phospholipids in the intestinal contents compared to CE. The binding or entrapment of bile acids and phospholipids was demonstrated by the change from the CE group in the ratio of aqueous phase to total intestinal contents bile acids and phospholipids (Fig. 1). In a previous study (6), it was shown that cellulose does not bind bile acids or phospholipids in the intestinal contents, and GG was shown to bind or sequester bile acids in the intestinal contents. Kiriyama, Enishi and Yura (16) reported that KM did not bind bile acids in vitro but did inhibit bile acid uptake in the ileum. The ability to reduce bile acid reabsorption by entrapment with the KM in the aqueous phase would account for the significantly greater amount of bile acids in the intestinal contents and the greater concentration of bile acids of the KM group compared to the CE group observed in the present study. The bile acid binding capacity of CH has been demonstrated previously in vitro (17). Differences in the concentration of phospholipids in the aqueous phase among groups (Table 2) also occurred.

TABLE 4

Distribution in the large and small intestine of ^{3}H from cholesterol and ^{14}C from triolein, which were emptied from the stomach[1]

Diet	Small intestinal contents	Aqueous phase	AP/TP[2]	Small intestinal tissue	Large intestinal tissue
	%			%	
^{3}H-Cholesterol					
Cellulose	33.2 ± 2.6^{a}	30.5 ± 2.3^{ab}	0.921 ± 0.006^{d}	57.2 ± 2.8^{c}	0.16 ± 0.03^{b}
Guar gum	42.9 ± 4.8^{b}	37.5 ± 4.3^{b}	0.873 ± 0.006^{b}	45.1 ± 3.2^{b}	0.34 ± 0.04^{c}
Konjac mannan	58.3 ± 1.0^{c}	52.9 ± 0.9^{c}	0.907 ± 0.002^{c}	26.7 ± 1.2^{a}	0.05 ± 0.01^{a}
Chitosan	37.6 ± 2.7^{ab}	29.7 ± 2.1^{a}	0.790 ± 0.003^{a}	51.3 ± 2.8^{bc}	0.23 ± 0.04^{b}
^{14}C-Triolein					
Cellulose	6.88 ± 0.38^{a}	6.25 ± 0.34^{a}	0.909 ± 0.011^{b}	19.69 ± 0.89^{c}	0.16 ± 0.03^{bc}
Guar gum	9.11 ± 0.96^{b}	8.29 ± 0.95^{b}	0.904 ± 0.017^{b}	11.36 ± 0.70^{b}	0.12 ± 0.02^{b}
Konjac mannan	11.98 ± 0.22^{c}	10.93 ± 0.22^{c}	0.914 ± 0.011^{b}	0.99 ± 0.29^{a}	0.03 ± 0.003^{a}
Chitosan	6.99 ± 0.46^{a}	5.84 ± 0.39^{a}	0.834 ± 0.012^{a}	17.67 ± 0.95^{c}	0.24 ± 0.07^{c}

[1]Values are means ± SEM for n = 9 rats for the cellulose, guar gum, and chitosan groups and n = 10 rats for the konjac mannan group. Values not sharing a common superscript letter are significantly different ($P < 0.05$).

[2]Ratio of isotope in the aqueous phase to total small intestinal contents.

In the CH group, the concentration was lower than in the CE group, most likely because of binding the phospholipids, whereas in the GG and KM groups, the concentration was lower than in the CE group, because the phospholipids were not bound and the volume of the aqueous phase was greater. The binding of bile acids, reducing their reabsorption, has been proposed as a mechanism to lower plasma cholesterol. The three fibers that bound bile acids, GG, KM and CH, have all been reported to lower plasma cholesterol in human feeding studies or animal models (4, 14, 18–21).

The other objective of this study was to determine whether the interaction of these fibers in the gastrointestinal tract would delay the disappearance of lipid, which was part of a meal. To examine this question, ^{3}H-labeled cholesterol and ^{14}C-labeled triolein were incorporated into the meal. In the GG and KM groups, compared to the CE group, a greater percentage of the ^{3}H and ^{14}C emptied from the stomach still remained in the small intestine. Both of these groups also had the lowest percentage of the isotope in the intestinal mucosa, suggesting that uptake from the intestinal contents had been slower due to the presence of KM or GG. Viscous, gel-forming fiber sources, such as pectin, guar gum and konjac mannan, increase the unstirred layer in the intestine and slow glucose diffusion, decreasing glucose absorption (22, 23). The diffusion rate of solute molecules depends on their molecular weight and particle size. It is likely that the diffusion of micelles in the intestinal lumen will be affected by konjac mannan and guar gum, since micelles are larger particles than glucose or other monomer nutrients. Konjac mannan has been shown to interfere with micelle diffusion in vitro (24). Within the intestinal contents, another factor affecting diffusion rate will be the availability of free (unbound) water in the aqueous phase. Because of their water-holding capacity, konjac mannan and guar gum are likely to decrease the availability of unbound water in the aqueous phase of the intestinal contents. In general the konjac mannan had a greater effect on the disappearance of ^{3}H and ^{14}C than did guar gum, which is probably related to konjac mannan's greater gel-forming capacity. In a 0.3% solution, konjac mannan has about five times the viscosity of a guar gum solution (14). From these studies it is apparent that the change in the physical properties of the small intestinal contents due to different fiber sources is important in understanding their effects on the disap- from the intestinal contents.

Compared to cellulose, chitosan bound both bile acids and phospholipids; however, the amount of ^{3}H and ^{14}C remaining in the intestinal contents and in the intestinal tissue did not differ between the two groups, suggesting that bile acid and phospholipid availability was adequate for micelle formation and lipid absorption. Since the chitosan is not soluble at the pH of the small intestinal contents, it would not be present in the aqueous phase to retard micelle diffusion and slow lipid absorption. Furda (15) suggested that precipitation of chitosan at the pH of the small intestine could entrap micelles in the nonsoluble portion of the intestinal contents. Our results support this suggestion, since the ratio of aqueous phase to total intestinal contents was lower than the cellulose group for bile acids, phospholipids, ^{3}H and ^{14}C, which would all be a part of the micelle structure. Interestingly, entrapment of some micelles did not lead to an overall reduction in the disappearance of lipid from the small intestine at 2 h nor has chitosan been reported to increase bile acid excretion (15).

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