# Effects of Certain Dietary Fibers on Apparent Permeability of the Rat Intestine[1]

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ABSTRACT Apparent intestinal permeability was determined indirectly by orally administering a poorly absorbed dye, phenol red, to rats and measuring its recovery in feces and in urine. Increased apparent permeability was recognized by increased dye recovery in urine and by an increased ratio of urinary to fecal dye recovery. Guar gum, pectin, carrageenan type I (80% $\kappa$, 20% $\lambda$), carrageenan type II ($\iota$) and cellulose were each fed at levels of 5 and 15% (wt/wt) of the diet for 31 d to male Fischer 344 rats. The average initial weight of rats was 230 g. Rats fed 15% guar gum gained significantly less weight than most of the other rats ($P < 0.05$). Phenol red recovery was measured at 2 and 4 wk after the beginning of the experiment. At 2 wk urinary recoveries of phenol red were high in rats fed fiber-free and carrageenan type II diets, indicating increased apparent permeability. By 4 wk, adaptation had apparently taken place. Urinary dye recoveries were lower in every diet group, and most fiber-containing diet groups gave significantly lower recoveries than did the fiber-free group. Fecal recovery of phenol red was high in the cellulose, carrageenan I, and 5% carrageenan II groups, intermediate in the 5% pectin and 15% carrageenan II groups, and low in the fiber-free, guar gum and 15% pectin groups at both 2 and 4 wk. The ratio of phenol red recovery from urine to that from feces, another index of apparent intestinal permeability, was higher in the fiber-free diet group than in all the other groups. Rats fed 15% dietary fiber had higher average ratios than those fed the same fiber at 5%. These data are consistent with the hypothesis that intestinal permeability to foreign substances may be altered considerably by diet. J. Nutr. *116*: 223–232, 1986.

INDEXING KEY WORDS dietary fiber • intestine • permeability • rat • adaptation

Epidemiological evidence suggesting that dietary fiber may protect the human colon from cancer (1–6) has been followed by several studies of the effects of dietary fiber on colon carcinogenesis in experimental animals. In many of these studies bran was found to reduce the carcinogenicity or tumorigenicity of dimethylhydrazine (7–11) but in a detailed study of the effects of different diet schedules, feeding bran during carcinogen administration and then feeding a fiber-free diet greatly increased tumor production (12). Pectin increased the tumorigenicity of dimethylhydrazine (13, 14), but decreased that of azoxymethane (11). Carrageenan increased the tumorigenicity of azoxymethane and of the direct-acting colon carcinogen methylnitrosourea (15). Agar increased the tumorigenicity and carcinogenicity of dimethylhydrazine (16). Alfalfa increased the tumorigenicity of azoxymethane and methylnitrosourea (11).

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The mechanisms behind these diverse observations are not clear. Some kinds of dietary fiber may act simply by shortening fecal transit time, reducing the exposure of the gut to toxins or carcinogens in the fecal stream and decreasing the production of these substances by the gut bacteria. Fiber may also act by increasing fecal bulk and volume, thus lowering the concentration, if not the amount of offensive chemicals. Fiber could also increase carcinogenicity by increasing epithelial cell proliferation (12) or mucosal permeability to carcinogens or promoters.

Colonic permeability may be an important factor in the etiology of colon cancer. Chemicals probably need to reach the colonic mucosa before they can induce tumor formation, and they may do so by penetration from the lumen. Therefore agents that increase the permeability of the colon to carcinogens or promoters are likely to increase carcinogenesis. For example, bile acids increase colonic permeability to a number of chemicals (17–20) and enhance colon tumor formation in experimental animals (21–24). Dietary bran counteracts the promoting effect of bile acids (25), perhaps by controlling colonic permeability. Bran was found to prevent the bile salt sodium deoxycholate from increasing cecal and colonic permeability to polyethylene glycol (26).

Colon carcinogenesis is not the only process that may be influenced by intestinal permeability. The toxicities of cyclamates, amaranth and nonionic detergents were reduced by dietary fiber (27–29). Sulfonylurea absorption was reduced by konjac fiber, a rich source of glucomannan (30). In contrast, paracetamol absorption by the rat was increased by dietary pectin (31). While some of these observations may reflect intestinal permeability, other factors such as gastric emptying time and viscosity of intestinal contents may play a role (32).

Some of the barrier properties of the colon are attributable to mucin, which is secreted by goblet cells and is extensively degraded and metabolized by colonic bacteria (33–37). Activities of mucin-degrading enzymes in the intestine are influenced by the diet of the host. Mucinase activity of rat fecal bacteria is high when fiber-free diets are fed and is much lower when diets containing more easily degradable fiber such as pectin or guar gum are fed (38). If mucin degradation reflects mucinase activity and if the secretory activity of the goblet cells fails to compensate for changes in degradation, then changes in mucinase activity may result in changes in intestinal permeability. Therefore dietary fiber may influence colon carcinogenesis by changing mucin degradation and colon permeability. Despite its potential importance, there has been little systematic investigation of the effect of dietary fiber on intestinal permeability.

To study the effects of dietary guar gum, pectin, carrageenan type I ($\kappa$, $\lambda$), carrageenan type II ($\iota$), and cellulose on intestinal permeability, a poorly absorbable dye, phenol red, was administered orally to rats and its appearance in feces and urine was measured. Urinary dye recovery served as a measure of apparent intestinal permeability because phenol red had to cross the alimentary tract mucosa before it could appear in urine.

## MATERIALS AND METHODS

Male Fischer 344 rats (Simonsen Labs, Gilroy, CA) 77 d old and weighing from 218 to 244 g were randomly assigned to 11 experimental diet groups of three animals each. All animals were housed individually, given water and food ad libitum, and kept on a 12-h light:dark cycle; the lights remaining on from 0630 to 1830 h. Ambient temperature was 22 ± 1°C. The composition of the basal diet is shown in table 1. Cellulose (nonnutritive fiber, cellulose-type, Teklad, Madison, WI) and other types of dietary fiber (Sigma Chemical Co., St. Louis, MO) were added at either 5 or 15% of the basal diet (wt/wt). Apparent intestinal permeability was measured after rats were fed their respective diets for 14 d and again after they had been fed for 28 d. Animal weights and food intake were measured. About 6 mo later the experiment was partially replicated with rats weighing 205–231 g randomly assigned to three experimental diet groups of three animals each.

Reagent grade phenol red (Sigma Chemical Co.) was used as permeant. Each of the rats was starved for 18 h and then given 60 mg of phenol red dissolved in 3 ml of water by gastric intubation.

All rats were then placed in metabolic

TABLE 1

*Composition of basal diet*

| Ingredient | Amount |
|---|---|
| | % |
| Casein[1] | 20 |
| Corn oil[2] | 5 |
| Anhydrous glucose (Cerelose)[2] | 70 |
| Vitamin mixture[3] | 1 |
| Mineral mixture[4] | 4 |
| Total | 100 |

[1]Teklad, Madison, WI, about 90% protein. [2]CPC International, Englewood Cliffs, NJ. [3]Vitamin mixture provided in milligrams/100 g of diet: D-biotin, 0.2; cholecalciferol, 0.25 (500,000 IU/g); folic acid, 1.0; thiamin · HCl, 1.5; riboflavin, 1.5; menadione, 1.5; retinyl acetate, 2.0 (500,000 IU/g); pyridoxine · HCl, 1.0; niacin, 5.0; D-Ca-pantothenate, 5.0; all-*rac*-α-tocopheryl acetate, 20 (500 IU/g); choline bitartrate, 100; vitamin B-12, 0.002. [4]Mineral mixture provided in mg/100 g of diet: $CaCO_3$, 782; $CaHPO_4$, 1220; $Na_2HPO_4$, 703; KCl, 790; $MgSO_4$, 464; $MnSO_4 \cdot H_2O$, 16; $CuSO_4$, 2.8; $ZnCO_3$, 1.8; $KIO_3$, 0.1; $FeSO_4 \cdot 7H_2O$, 18.9.

cages where food and water were available. Urine and feces were collected for 48 h, by which time the feces no longer showed any trace of the administered dye.

Unabsorbed dye was measured by the method of Hess and Fitzhugh (39). Feces from each animal were blended with 100 ml of water for about 30 s in a high speed rotary blender (Polytron PT 10/35, Brinkmann Instruments, Westbury, NY), and a 5-ml portion was placed in an Erlenmeyer flask, acidified with 1 ml of concentrated hydrochloric acid and then diluted with 25 ml of water. Ten grams of granular sodium sulfate was added, followed by 20 ml of *t*-butyl alcohol. Then the flask was covered with Parafilm flexible film (American Can Co., Greenwich, CT), and the mixture was agitated vigorously with a vortex mixer. After the two phases separated, the organic (upper) portion containing the dye was removed, and the aqueous portion was reextracted with another 10 ml of *t*-butyl alcohol. Then a 10-ml portion of the combined butanol solutions was incubated at 80°C with 3 mg of chloranil (Sigma Chemical Co.) for 20 min in a closed screw-cap test tube. The solution was cooled, and a 1-ml portion was diluted with 5 ml NaOH. The absorbance of the mixture was measured at 560 nm in a spectrophotometer (Spectronic 100, Bausch and Lomb, Rochester, NY) and was compared to the absorption of standard solutions prepared by extracting mixtures of phenol red and feces from rats fed a stock diet (Rat Chow, Ralston Purina, St. Louis, MO).

Dye that had been absorbed and then excreted in urine was determined by first measuring the total volume of urine collected and then mixing a 1-ml sample with 5 ml 1 *N* NaOH. The absorbance of the mixture was measured at 560 nm, and the concentration of phenol red was obtained by comparison with standard solutions prepared from mixtures of the dye in urine from rats fed a stock diet.

All data were analyzed for statistical significance by using analysis of variance (BMDP Program for analysis of variance with repeated measures; Health Science Computing Facility, University of California, Los Angeles, CA). Homogeneity of variance of untransformed and log-transformed data was evaluated by the Cochran-C and Bartlett-Box methods. No significant heterogeneity of variance was found in urinary or fecal phenol red data, and therefore analysis of variance was performed without data transformation. However, variances of the ratios of urinary to fecal phenol red recoveries showed significant ($P < 0.05$) heterogeneity. Logarithms of these ratios had homogeneous variances and were therefore used in analysis of variance. Multiple comparisons among means were made with the Tukey B method (40). Statistical significance was determined by setting the aggregate type I error rate at 5% ($P = 0.05$) for each set of comparisons.

## RESULTS

Food intake and weight gain are summarized in table 2. Total food intake was highest for rats fed type I carrageenan (carrageenan I), type II carrageenan (carrageenan II), and cellulose diets; intermediate for those fed pectin, fiber-free and 5% guar gum diets; and lowest for those fed 15% guar gum diet. Food intakes in the highest groups (carrageenan I, carrageenan II and cellulose were significantly higher than that of the lowest group (15% guar

TABLE 2

*Food intake, weight gain, and ratio of urinary to fecal dye recovery from rats fed various diets*[1,2]

| | Food intake | | | Urinary/fecal dye recovery ratio | |
|---|---|---|---|---|---|
| | Total intake | Fiber-free portion | Weight gain | At wk 2 | At wk 4 |
| | g | g | g | | |
| Fiber-free | 520$^{ab}$ | 520$^{abc}$ | 95$^{ab}$ | 0.113 ± 0.066$^{c}$ | 0.067 ± 0.022$^{b}$ |
| | (501) | (501) | (96) | | |
| 5% carrageenan II | 679$^{b}$ | 645$^{a}$ | 133$^{b}$ | 0.054 ± 0.019$^{abc}$ | 0.016 ± 0.004$^{a}$ |
| 15% carrageenan II | 691$^{b}$ | 587$^{ab}$ | 111$^{b}$ | 0.092 ± 0.034$^{bc}$ | 0.025 ± 0.001$^{a}$ |
| | (603) | (512) | (90) | | |
| 5% cellulose | 619$^{b}$ | 588$^{ab}$ | 130$^{b}$ | 0.033 ± 0.007$^{a}$ | 0.015 ± 0.006$^{a}$ |
| 15% cellulose | 692$^{b}$ | 588$^{ab}$ | 123$^{b}$ | 0.040 ± 0.005$^{abc}$ | 0.020 ± 0.001$^{a}$ |
| | (687) | (584) | (132) | | |
| 5% carrageenan I | 639$^{b}$ | 607$^{a}$ | 110$^{b}$ | 0.031 ± 0.013$^{a}$ | 0.015 ± 0.004$^{a}$ |
| 15% carrageenan I | 607$^{b}$ | 516$^{abc}$ | 95$^{ab}$ | 0.043 ± 0.009$^{abc}$ | 0.023 ± 0.002$^{a}$ |
| 5% pectin | 546$^{ab}$ | 519$^{abc}$ | 115$^{b}$ | 0.036 ± 0.011$^{ab}$ | 0.024 ± 0.012$^{a}$ |
| 15% pectin | 509$^{ab}$ | 433$^{bc}$ | 83$^{ab}$ | 0.045 ± 0.007$^{abc}$ | 0.025 ± 0.008$^{ab}$ |
| 5% guar gum | 537$^{ab}$ | 510$^{abc}$ | 85$^{ab}$ | 0.043 ± 0.012$^{abc}$ | 0.030 ± 0.016$^{ab}$ |
| 15% guar gum | 420$^{a}$ | 357$^{c}$ | 24$^{a}$ | 0.065 ± 0.046$^{abc}$ | 0.035 ± 0.015$^{ab}$ |
| Pooled SD | 63 | 57 | 25 | | |

[1]Data are means or mean ± SD ($n$ = 3). Data in parentheses are from a partial replication of the experiment several months later; they were not included in the statistical analysis indicated in this table. [2]Means in a given column differ significantly ($P < 0.05$) if they do not share a common superscript letter.

gum). When food intakes were calculated on the basis of the fiber-free portion, those of the 5% carrageenan diet groups were significantly higher than those of the 15% pectin and 15% guar groups. However, there were no significant differences between the fiber-free diet group and any of the groups fed fiber-containing diets.

Weight gain followed the same general pattern that food intake did: gain was high on cellulose and most carrageenan diets, intermediate on fiber-free, and lowest on 15% guar gum. The weight gain of rats fed the 15% guar gum diet was significantly lower than those of rats fed cellulose, 5% carrageenan I, carrageenan II or 5% pectin. When the experiment was partially replicated several months later, similar food intake and weight gain data were obtained (table 2).

Amounts of phenol red recovered from urine and feces are given in figure 1. As expected, dye recoveries in feces were much higher than those in urine. In many diet groups, most of the 60-mg dose was found in feces. When rats were fed their respective diets for 2 wk, urinary phenol red recovery was highest for the fiber-free and carrageenan II diet groups, intermediate for the cellulose and carrageenan I groups, and lowest for the guar gum and pectin groups. Differences between the groups giving the highest (fiber-free and 15% carrageenan II) and lowest (guar gum and pectin) urinary recoveries were statistically significant. By 4 wk, a somewhat different picture emerged. Urinary recovery of phenol red had dropped in every diet group, and dye recovery in the fiber-free group was significantly higher than that in any other group.

The possible role of phenol red binding directly to dietary fiber was investigated by mixing the dye at a final concentration of 0.140 mM with 2.5% cellulose, 0.5% guar gum, and 1% carrageenan I or II suspensions at pH 7.0. Then the mixtures were centrifuged to remove the polysaccharide, and the phenol red in the supernatant was measured by its 560 nm absorbance. In a similar experiment, 2.5% pectin and dye

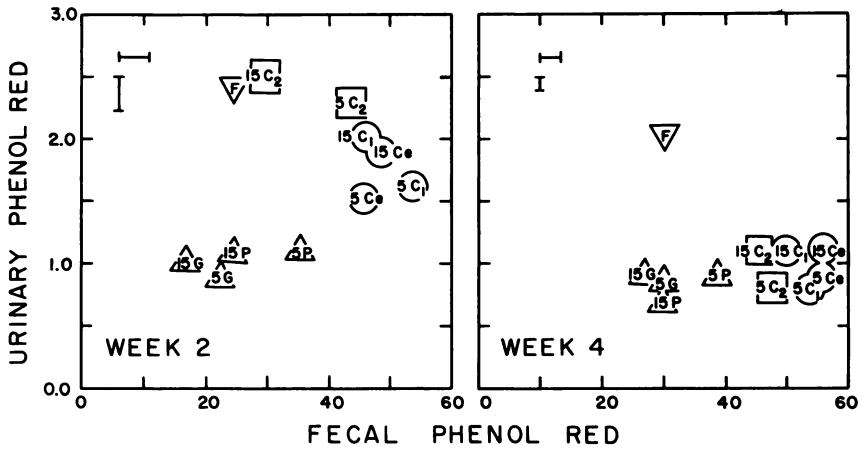


Fig. 1 Amount of phenol red recovered in urine and feces after administration of 60-mg doses after 2 and 4 wk of feeding. Symbols represent the means for each diet group, expressed as milligram per rat. Diet abbreviations are: F, fiber-free; 5 $C_1$ and 15 $C_1$, 5 and 15% carrageenan I; 5 $C_2$ and 15 $C_2$, 5 and 15% carrageenan II; 5 Ce and 15 Ce, 5 and 15% cellulose; 5 G and 15G, 5 and 15% guar gum; 5 P and 15 P, 5 and 15% pectin, respectively. Error flags indicate pooled standard errors of the mean.

were mixed and then two volumes of ethanol were added to precipitate the pectin before measuring the 560 nm absorbance of the supernatant. The dye was recovered quantitatively in the supernatant in each case (average recovery of 100.2 ± 0.5%).

Fecal recoveries of phenol red after 2 wk of feeding were high in the cellulose, carrageenan I and 5% carrageenan II groups, intermediate in the 5% pectin and 15% carrageenan II groups, and low in the fiber-free, guar gum and 15% pectin groups (fig. 1). Significant differences were found between the lowest group (15% guar gum) and most of the poorly digestible fiber diet groups (cellulose, carrageenan I and 5% carrageenan II) and also between the highest group (5% carrageenan I) and most diet groups fed either no fiber or easily digestible fiber (fiber-free, guar gum and 15% pectin). Similar results were obtained at 4 wk. The consistent differences in fecal recovery of phenol red among diet groups suggested that phenol red metabolism in the gut or body may have been altered by dietary fiber. To test the latter hypothesis, 2 mg of phenol red in 1 ml of normal saline were injected i.p. into a separate group of 16 rats fed various fiber-free and carrageenan-containing diets. Urinary recoveries averaged 0.94 ± 0.14 (mean ± pooled standard deviation) with no significant differences among diet groups.

The data in figure 1 fell into distinct clusters. Rats fed fiber-free diet for 2 wk had relatively high urinary and low fecal recoveries of phenol red; animals fed either of the readily fermentable dietary fibers (pectin or guar gum) had low urinary recoveries and low fecal recoveries; and animals fed with less readily fermentable cellulose or carrageenan I had intermediate urinary and high fecal recoveries of phenol red. Carrageenan II seemed to be more like the other poorly fermentable fiber at the 5% level, but at the 10% level its effects were similar to those of the fiber-free diet.

The ratio of phenol red recovery from urine to that from feces gives another index of apparent intestinal permeability. This ratio was consistently higher in the fiber-free diet group than in any other group (table 2). At 2 wk the ratio in the fiber-free group was significantly higher than those in the 5% pectin, 5% carrageenan I and 5% cellulose groups. Furthermore, the ratios tended to be higher with each of the 15% fiber-containing diets than with the corresponding 5% fiber-containing diets. This observation was substantiated by a significant $F$-value ($F_{1,24} = 8.47$, $P < 0.01$) for fiber level in a two-way analysis of variance among fiber-containing diets. At 4 wk the ratios of urinary to fecal phenol red recovery were lower than they were at 2 wk. The ratio in the fiber-free group was higher than

that in any other group, and significantly higher than those of all but the guar gum groups. The effect of the level of dietary fiber was similar to that seen at 2 wk: rats fed 15% dietary fiber had higher average ratios than those fed the same fiber at 5% ($F_{1,24}$ = 6.41, $P < 0.02$).

To confirm the decrease in urinary phenol red excretion between wk 2 and 4, the experiment was partially replicated several months later with fiber-free, 15% carrageenan II and 15% cellulose diets. The results shown in table 3 are generally similar to those shown in figure 1 and table 2. Urinary excretion of phenol red was highest in the fiber-free, intermediate in the 15% carrageenan II, and lowest in the 15% cellulose diet groups, with the only significant difference being that between the fiber-free and 15% cellulose groups after wk 2 of feeding. Urinary recovery of phenol red decreased between wk 2 and 4, with the largest decrease in the fiber-free diet group. When the data for the three diets from both this and the earlier experiment were analyzed together, a highly significant effect of weeks of feeding was obtained (table 4).

Fecal recoveries of phenol red in the original and replicated experiment were also similar, with the cellulose diet giving consistently higher recoveries than the fiber-free diets, and the carrageenan II diet group behaving more like the fiber-free group at 2 wk and more like the cellulose group at 4 wk. Fecal dye recovery increased between wk 2 and 4 in all diet groups; with the largest increase in the carrageenan II group. This resulted in a highly significant effect of weeks of diet and no significant interactions (table 4). Fecal dye recoveries were consistently lower in the later experiment, giving rise to a significant effect of replication.

The ratio of urinary to fecal dye recovery in the replicated experiment also showed consistent responses to diet and the number of weeks the animals were fed, with the fiber-free diet giving the highest, the carrageenan II diet giving intermediate, and the cellulose diet giving the lowest ratios. Differences between the fiber-free and cellulose diets were statistically significant. The ratios decreased consistently from wk 2 to 4, and there was a highly significant effect of weeks of feeding in analysis of variance of the replicated portions of the experiment (table 4). A slight decrease in fecal dye recoveries in the later replication of the experiment gave rise to a significant effect of replication of the ratio of urinary to fecal dye recoveries.

## DISCUSSION

Weight gain and food consumption by the rats in this study were similar to those found earlier (38): rats fed 15% guar gum or 15% pectin diets gained less weight than did rats fed other diets. These findings are similar to those of Graham et al. (41) who reported

TABLE 3

*Recovery of phenol red after intragastric administration in a partial replication of the first experiment*[1,2]

| Diet | Urinary recovery | Fecal recovery | Urinary/fecal dye recovery ratio |
|---|---|---|---|
| | | *mg* | |
| | | *After 2 wk of feeding* | |
| Fiber-free | 3.2 ± 0.8[a] | 18.6 ± 4.6[a] | 0.177 ± 0.090[a] |
| 15% carrageenan II | 1.9 ± 0.03[ab] | 27.7 ± 4.7[a] | 0.069 ± 0.011[ab] |
| 15% cellulose | 1.7 ± 0.4[b] | 42.4 ± 0.8[b] | 0.041 ± 0.009[b] |
| | | *After 4 wk of feeding* | |
| Fiber-free | 2.0 ± 0.2[a] | 20.3 ± 4.6[a] | 0.103 ± 0.018[a] |
| 15% carrageenan II | 1.7 ± 0.9[a] | 37.0 ± 5.6[b] | 0.047 ± 0.027[ab] |
| 15% cellulose | 1.3 ± 0.3[a] | 44.6 ± 0.8[b] | 0.030 ± 0.007[b] |

[1]Data are means ± SD. [2]Means in a given column and feeding period differ significantly ($P < 0.05$) if they do not share a common superscript letter.

TABLE 4

*Summary of analysis of variance of the replicated portions of the experiment*

| Dependent variable | Source of variation[1] | df | *F* | Statistical significance[2] |
|---|---|---|---|---|
| Urinary dye recovery | Diet | 2 | 12.71 | 0.001 |
| | Replication | 1 | 0.83 | NS |
| | Weeks of feeding | 1 | 27.65 | <0.001 |
| Fecal dye recovery | Diet | 2 | 56.15 | <0.001 |
| | Replication | 1 | 15.44 | 0.002 |
| | Weeks of feeding | 1 | 9.56 | 0.009 |
| Urinary/fecal dye recovery ratio[3] | Diet | 2 | 49.75 | <0.001 |
| | Replication | 1 | 7.71 | 0.017 |
| | Weeks of feeding | 1 | 28.55 | <0.001 |

[1]None of the interaction terms was statistically significant ($P > 0.05$). There were 12 degrees of freedom in each error mean square. [2]NS means not statistically significant ($P > 0.05$). [3]Analysis of variance was actually performed on the logarithms of the ratios.

that rats fed 15% guar gum diets actually lost weight and Cullen et al. (42) who found that pectin at 15% depressed rat growth.

Although it would have been preferable to study the permeability of the actual carcinogens or promoters, their structures are unknown. Therefore we chose to use phenol red, which is not only easy to measure in extracts of urine, feces, and tissue, but also very poorly absorbed by the gut (43). This meant that the background level of absorption would be low, and that increased intestinal absorption could be easily recognized by increased dye recovery in urine.

Use of gastric administration of phenol red as a permeant has the disadvantages that the site(s) of absorption are unknown, as are the extent of metabolism and storage within tissues. Nevertheless we felt that even the kind of simple experiment described in this report would yield valuable insight into the possible effects of diet on intestinal permeability. The question of sites of absorption and regions of changed intestinal permeability are the subject of subsequent investigation.

Interpretation of these experiments could be complicated by differences in the viscosity of intestinal contents, the binding of phenol red to dietary fiber residues, or host tissues, or by metabolism in the gut or the body. For instance glucuronidation of phenol red has been observed in cultured hepatocytes and may be important in the intact rat (44). Nevertheless, control experiments in which the dye was administered i.p. suggest that neither metabolism nor storage of the dye were changed by dietary carrageenan. In vitro control experiments showed that phenol red was not bound to any of the dietary fiber materials used.

The choice of "dietary fiber" for this experiment was a compromise between the complexity of natural foods and the simplicity of homogeneous polysaccharides. The materials we used are either food additives or natural constituents of foods. Of the materials used, carrageenan deserves special mention. The carrageenans belong to a heterogeneous group of sulfated polygalactans extractable from various red seaweeds. Carrageenan type I (carrageenan I) consists mainly of $\kappa$ and $\lambda$ polymers, and type II (carrageenan II) of $\iota$-polymer. The $\iota$-polymer is readily hydrolyzed in acid to produce degraded carrageenan, which is reported to cause ulceration of the rat cecum following oral administration (45). Therefore we included both types of carrageenan in this study.

The clustering of phenol red recovery data (fig. 1) and the ratios of urinary to fecal dye recovery (table 2) after 2 wk of feeding can be explained by a model in which *1*) a large portion of the phenol red absorption occurs in the cecum or colon, 2) the amount of phenol red available for absorption in the

cecum and colon is diminished by bacterial metabolism of the dye, and 3) phenol red absorption is increased by destruction of gut mucins, erosion of the mucosa, or other damage.

Rats fed the fiber-free diet, for example, would be expected to have slow intestinal transit times (46–48) and ample opportunity for bacterial metabolism of phenol red, resulting in the low fecal dye recoveries found in these experiments (fig. 1). However, the fiber-free diet also supports high levels of intestinal mucinase and may enhance intestinal permeability in a variety of ways, resulting in the large urinary recoveries of phenol red and the large ratio of urinary to fecal dye recovery.

Poorly digested dietary fiber such as cellulose and carrageenan would be expected to support rapid intestinal transit and short transit times, resulting in only limited dye destruction and the high fecal dye recoveries observed (46–48). The low urinary recoveries of dye might be attributable to the more rapid transit reducing the time available for dye absorption, the larger fecal bulk providing physical limitations on absorption rates, or a reduction in physical damage to the mucosa (49, 50). The combination of low urinary recovery and large fecal recoveries of phenol red could give rise to the low ratios in table 2.

Readily fermented dietary fiber such as guar gum or pectin presents a more complicated picture. These kinds of fiber would be expected to support the same slow intestinal transit rates and extensive dye destruction that fiber-free diets do (46–48), giving rise to similarly low fecal dye recoveries as observed. However, urinary dye recoveries were much lower in the guar gum and pectin diet groups than they were in the fiber-free group, suggesting that the lower intestines of these fiber-fed animals were less permeable than those of the fiber-free group. Ratios of urinary to fecal dye recovery were comparable to those of rats fed less readily fermentable dietary fiber, suggesting that the unmetabolized dye was absorbed at least as readily as it was in the cellulose and carrageenan diet groups.

When type II (*i*) carrageenan was fed at 15% of the diet, it supported a large urinary recovery of phenol red and a high ratio of urinary to fecal recovery, suggesting that it rendered the intestine much more permeable than did any of the other dietary fiber supplements. Carrageenan II may have done this by physically damaging the intestine. Even though we did not see any signs of gross intestinal or colonic ulceration on autopsy, there may have been the same sort of subtle damage that has been reported in rats fed pectin or agar (40, 50). Such damage would be consistent with the effects of degraded carrageenan mentioned earlier and the report that native carrageenans induce chronic ulcerative disease of the colon in rabbits (51).

Further support for the hypothesis that bacterial mucinases may influence intestinal permeability by degrading the mucin permeability barrier can be obtained by comparing data from this experiment with that from an earlier report (38). When urinary dye recoveries (fig. 1) were compared with fecal mucinase activities measured in rats fed comparable diets for a similar period, Pearson correlation coefficients of 0.70 ($P = 0.03$) and 0.75 ($P = 0.02$) were obtained, depending on whether specific activity or total daily enzyme output was used. This implies that about half the variance in urinary phenol red recovery is associated with variance in mucinase. However, it must be noted that these calculations involve data from two separate experiments, and that statistical association does not prove that there is a causal relationship.

Despite the correlation between fecal mucinase and one index of permeability, urinary dye recovery, there was no clear association between mucinase and the other index, the ratio of urinary to fecal dye recovery. The correlation between mucinase specific activity and dye recovery ratio was either 0.50 or −0.79, depending on whether the fiber-free diet group was included. The corresponding correlation coefficients between total daily output of mucinase and dye recovery ratio were 0.099 or −0.58, respectively, for data including or excluding the fiber-free group. These results emphasize the provisional nature of both the mucinase hypothesis and the use of the dye recovery ratio as an index of apparent intestinal permeability.

That an adaptation has taken place between wk 2 and 4 of feeding is clear from changes in the clustering of data in figure 1,

the urinary recovery of phenol red, and the ratios of urinary to fecal recovery (table 2). The same general changes were seen in two separate experiments performed several months apart, and they may indicate important adaptive changes in intestinal permeability. The observation that chronic ingestion of guar gum leads to an increased cecal and colonic weight is consistent with this hypothesis (52, 53).

Despite the unanswered question of whether the actual carcinogens or promoters of the gut share permeability properties with phenol red, the results of this study are consistent with the hypothesis that certain dietary fibers may alter the permeability of the intestine to foreign substances.

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