Wheat bran- but not oat bran-enriched diets increase the mucosal height of the cecum and colon of newly weaned and aged rats

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Abstract

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The effect of diets enriched with oat or wheat bran (prepared by the addition of 300 g of each fiber to 1000 g of the regular diet), given for 8 weeks, on the mucosal height of the colon and cecum was investigated. Newly weaned (21 days old) and aged (12 months old) male Wistar rats were used in this study. As compared to controls, diets enriched with wheat bran provoked a significant increase in the mucosal height, whereas oat bran did not cause any effect. In newly weaned rats (21 days old), wheat bran increased the mucosal height (µm) in the cecum by 20% (mean ± SEM for 8 rats; 169.1 ± 5.2 and 202.9 ± 8.0 for control and wheat bran, respectively) and in the colon (218.8 ± 7.2 and 264.5 ± 18.8 for control and wheat bran, respectively). A similar effect was observed in aged rats (12 months old), with an increase of 15% in the mucosal height (µm) of the cecum (mean ± SEM of 8 rats; 193.2 ± 8.6 and 223.7 ± 8.3 for control and wheat bran, respectively) and of 17% in the colon (300.4 ± 9.2 and 352.2 ± 15.9 for control and wheat bran, respectively).

Introduction

The greatest protection associated with high fiber intake is that against gastrointestinal disorders. In 1980, the Royal College of Physicians (1) reviewed the potential protective role of fibers against gallstones, functional bowel disorders, diverticular disease of the colon, colon cancer, appendicitis, hemorrhoids, and hiatal hernia. Although a full link between these conditions and fiber-depleted diets has not been established, populations with a high fiber intake have a lower incidence of these gastrointestinal disorders.

Total parenteral nutrition or the administration of a fiber-free liquid diet leads to colonic atrophy (2). The addition of fibers to a fiber-free defined diet increases colonic DNA synthesis and DNA content as well as mucosal weight (3). The response of the mucosa to fibers is assumed to occur due to the bulk effect (4) and to fermentation to short-chain fatty acids (SCFA; acetic, propionic, and butyric acids) (5). In fact, Pell et al.

(6) postulated that dietary fiber has a viscosity-related trophic effect in the small bowel and a fermentation-related trophic effect in the colon. SCFA are mainly produced by fermentation of water-soluble fibers and play a role as a metabolic fuel for the colon (5), stimulate mucosal proliferation (7), and increase sodium and water absorption (8). Because the generation of SCFA has been assumed to be the main mechanism for the intestinal function of fibers, a number of studies have been developed to examine the effect of these acids on cell proliferation and differentiation. Butyrate provokes a marked inhibition of the growth of several tumor cell lines in culture (9). A recent study from our laboratory has shown that propionate, at concentrations ranging from 1 to 5 mM, also inhibits the proliferation of tumor (HeLa-155 and KB-172) and normal (MA-104) cell lines *in vitro* (10). In addition, propionate

Table 1 - Basic composition of the regular diet.

Protein	18%
Fat	4.0%
Carbohydrates	45.5%
Minerals	8.0%
Insoluble fiber	18.0%
Soluble fiber	3.9%
Calcium	1.8%
Phosphorus	0.8%

Vitamin mixture (expressed by kg of diet)

Vit A	20,000 IU
Vit D3	6,600 IU
Vit E	30.0 mg
Vit K	6.0 mg
Vit B12	10.0 mg
Vit B2	8.0 mg
Niacin	95.0 mg
Pantothenic acid	24.0 mg
Thiamin	4.0 mg
Choline	2,000 mg
Pyridoxine	6.0 mg
Biotin	0.1 mg
Folic acid	0.5 mg
Manganese	50.0 mg
Iodine	2.0 mg
Iron	65.0 mg
Zinc	35.0 mg
Copper	26.0 mg
Antioxidant	100.0 mg

has been shown to stimulate lymphocyte proliferation at low concentrations (40 μM and 0.1 mM) but to inhibit it at concentrations above 1 mM (11). Intracolonic infusion of SCFA increases mucosal weight and the protein and DNA content of the proximal colon (12). Experiments carried out on parenterally nourished rats have shown that SCFA infusion into the colon promotes a modest increase in mucosal height and mucosal DNA content but does not influence absorption (13).

In spite of the reports mentioned above, it is not known whether the effect of a water-soluble fiber-enriched diet (the main source of SCFA) on the mucosa of the large bowel is more pronounced than that of an insoluble fiber-enriched diet. In the present study, we determined the effect of fiber-rich diets on the mucosal height of the colon and cecum. Rats received two types of fiber-rich diets presenting different soluble/insoluble ratios for 8 weeks, i.e., oat bran (0.3) and wheat bran (0.14). To better characterize the effect of the fiber-rich diets on the intestinal mucosa, the experiments were carried out on newly weaned (21 days old) and aged (12 months old) rats.

Material and Methods

Animals and diet preparation

Newly weaned (21 days old) male Wistar rats weighing 50-60 g and aged rats (12 months old) weighing 310-330 g were obtained from the Animal House of the Institute of Biomedical Sciences, USP, São Paulo. During the experiment, the rats were kept in groups of 5 animals at 23ºC on a 12-h light/ 12-h dark cycle (lights on at 7:00 a.m.). Newly weaned and aged rats were divided into three groups: 1) control rats were fed a regular diet whose composition is shown in Table 1, 2) the oat bran-enriched diet was prepared by the addition of 300 g of oat bran to 1000 g of the regular diet, and 3) the wheat

bran-enriched diet was prepared by the addition of 300 g of wheat bran to 1000 g of the regular diet. The soluble/insoluble fiber ratio was 0.22, 0.30 and 0.14 for control, oat bran and wheat bran-enriched diets, respectively. During the 8 weeks on the respective diets, body weight, daily food intake and feces elimination were evaluated weekly. After this period, now mature (previously called newly weaned) and aged rats were killed by decapitation without anesthesia and the colon and cecum were removed. The experiments were always carried out between 8:00 and 11:00 h.

Measurement of mucosal height of the colon and cecum

Segments of the cecum and colon were kept in 10% formalin solution and processed for paraffin embedding, and 7-μm thick slices were stained with hematoxylin-eosin. The height of the cecum and colon mucosa was determined in 8 rats, 3 sections per rat, and 10 different areas per section. The mucosal height was measured in areas formed by a single cell layer with the base of the crypt being in direct contact with the muscular mucosa. All measurements were carried using an ocular micrometer (Ernest Leitz, Wetzpar, Germany).

Statistical analysis

Data were analyzed statistically by ANOVA and by the Student t-test. The level of significance was set at $P<0.05$.

Results

The body weight gain of mature rats fed the oat bran-enriched diet did not differ from the control group. However, the body weight of mature rats fed the wheat bran-enriched diet was 18% lower than control at the end of the 8-week period (data not shown). The body weight gain of aged rats was not modi-

Table 2 - Effect of soluble (oat bran) and insoluble (wheat bran) fiber-rich diets on the mucosal height of colon and cecum of mature and aged rats.

For details of diet preparation and measurement of the mucosal height see Material and Methods. Data are reported as the mean ± SEM of 8 rats. *$P<0.05$ compared to the control group (Student t-test).

	Mucosal height (μm)		
	Control	Oat bran	Wheat bran
Mature rats			
Cecum	169.1 ± 5.2	165.5 ± 3.0	202.9 ± 8.0*
Colon	218.8 ± 7.2	233.2 ± 10.5	264.5 ± 18.8*
Aged rats			
Cecum	193.2 ± 8.6	188.2 ± 11.1	223.7 ± 8.3*
Colon	300.4 ± 9.2	267.8 ± 13.3	352.2 ± 15.9*

fied by either fiber-rich diet. Daily food intake and feces dry weight did not differ between groups ($P<0.05$).

The wheat bran-enriched diet provoked a significant increase in mucosal height, whereas oat bran did not cause any effect compared to control (Table 2). In mature rats, wheat bran increased the mucosal height in cecum and colon by 20%. A similar effect was observed in aged rats, with an increase of 15% in the cecum and of 17% in the colon. Photomicrographs of cecum and colon mucosa provide additional evidence of these findings (portions 3 and 6 of Figures 1 and 2).

Discussion

Volatile short-chain fatty acids are the major solute fraction of fecal water produced by fermentation of fibers by anaerobic bacteria normally residing in the large bowel (14). These fatty acids are known to have a trophic effect on the large bowel and the response to the different fibers suggests that the more readily fermentable fibers have the greatest effect (6). Several studies have presented evidence that acetate, propionate and mainly butyrate raise DNA synthesis in the
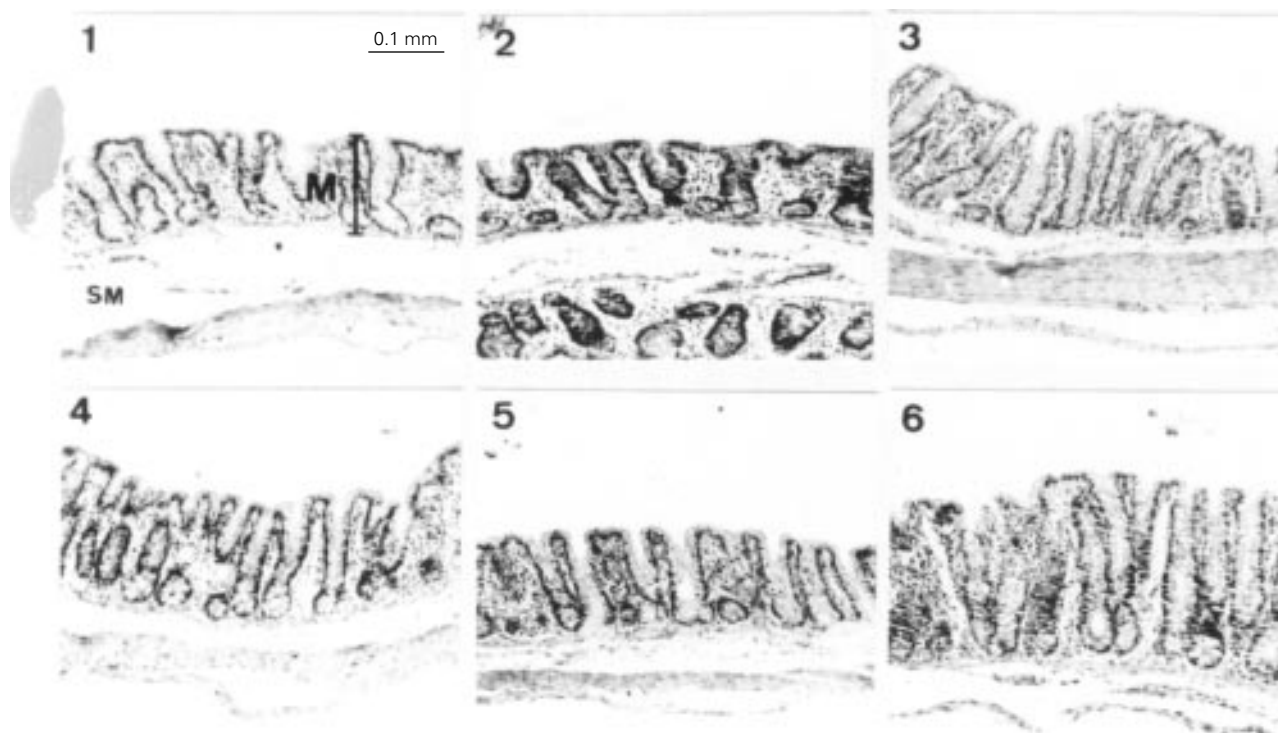
Figure 1 - Photomicrographs of the cecum mucosa from mature (1, 2 and 3) and aged (4, 5 and 6) rats fed a control diet (1 and 4) or diets enriched with oat bran (2 and 5) or wheat bran (3 and 6). The tissues were prepared as described in Material and Methods and stained with hematoxylin and eosin (X100). The mucosal height is indicated by the vertical bar. The horizontal magnification bar corresponds to 0.1 mm. M, Mucosa; SM, submucosa.

intestinal mucosa (15). In addition, these SCFA are also fuels for these cells (5) and this fact might be important in the control of epithelial cell proliferation. In spite of this, however, there are several trophic factors which can effectively control the maintenance of the intestinal epithelium such as 1) blood flow to the tissue (16), 2) motility of the intestine (17), 3) trophic hormones such as enteroglucagon (18), 4) conditions of the lumen such as the presence of biliary salts (19), and 5) amines produced by bacteria metabolism.

The stimulating effect of the SCFA on cell proliferation should be considered with concern. In fact, there is evidence that these fatty acids are potent inhibitors of cell proliferation at high concentrations. Butyrate has been shown to reduce tumor development (9) and to promote apoptosis of colonic carcinoma cells (20,21) and of the myeloid HL-

60 cell line (22). Also, the development of colorectal cancer has been inversely correlated with the amount of butyric acid in the feces (23). Propionate stimulates lymphocyte proliferation at concentrations up to 1 mM but provokes a marked inhibition at concentrations above 2 mM (11). This SCFA also inhibits the proliferation of several tumor cell lines (10). Several mechanisms have been postulated to be involved in the control of cell proliferation by SCFA such as inhibition of oncogene expression (24,25), stimulation of polyamine biosynthesis (26) and reduction of lipid synthesis (27).

The production of SCFA in the gastrointestinal tract results mainly from water-soluble dietary fibers. The soluble/insoluble fiber ratios of the fiber-rich diets used in this study were 0.30 for oat bran and 0.14 for wheat bran. Therefore, the oat bran-enriched diet presents a 2-fold higher capacity than
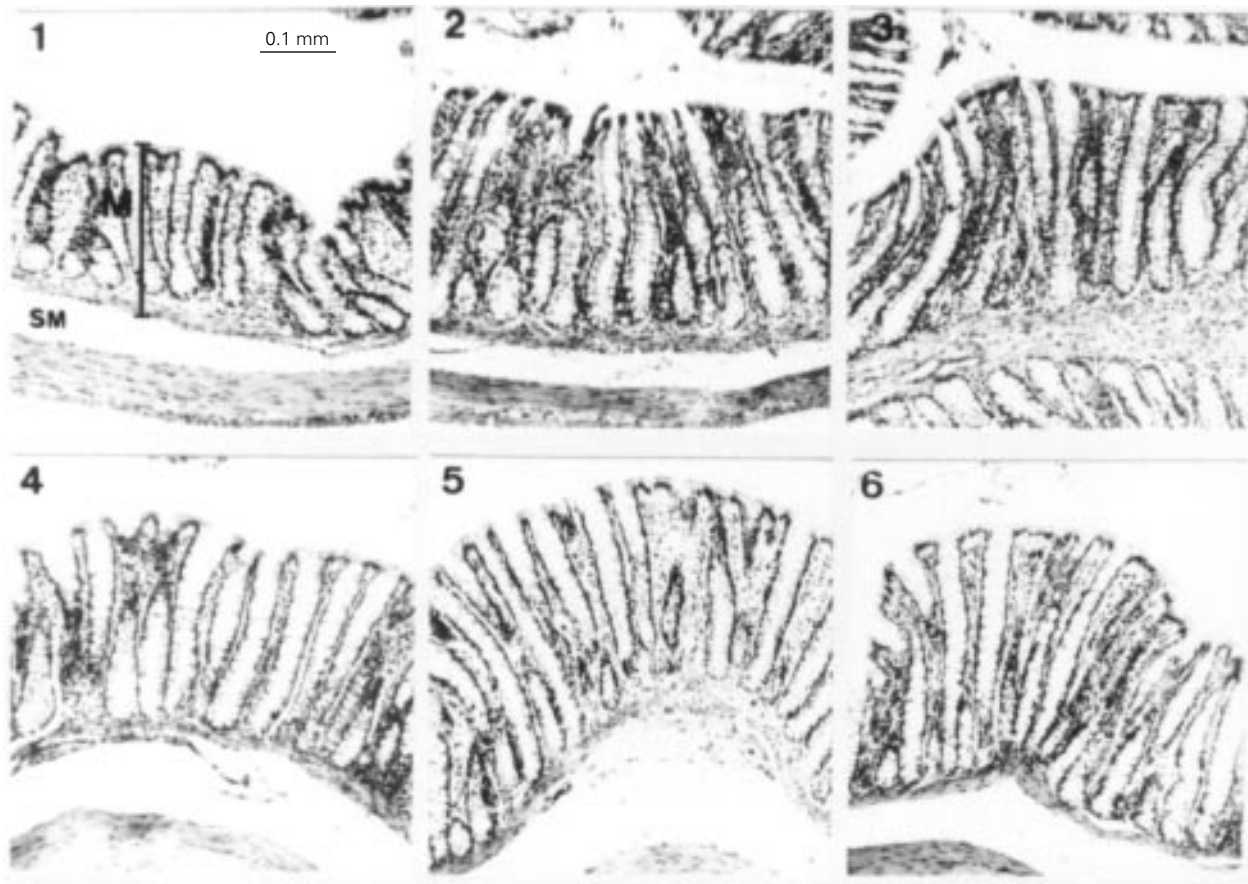
Figure 2 - Photomicrographs of the colon mucosa from mature (1, 2 and 3) and aged (4, 5 and 6) rats fed a control diet (1 and 4) or diets enriched with oat bran (2 and 5) or wheat bran (3 and 6). The tissues were prepared as described in Material and Methods and stained with hematoxylin and eosin (X100). The mucosal height is indicated by the vertical bar. The horizontal magnification bar corresponds to 0.1 mm. M, Mucosa; SM, submucosa.

the wheat bran-enriched diet for the production of SCFA.

Jacobs and White (28) showed that in rats fed wheat bran (20% dietary supplementation) for 9 weeks there is decreased exfoliation and cell migration in the cecum, whereas cell proliferation and cell migration increase in the colon. Indeed, wheat bran did present a significant effect on the large intestine mucosa which was clearly more pronounced than that of oat bran (Table 2 and Figures 1 and 2). Whether these changes are due to decreased exfoliation and cell migration or to increased cell proliferation and cell migration remains to be investigated. This effect of wheat bran was observed in both newly weaned and aged rats. Similarly,

Goodlad et al. (29) showed that refeeding of 3-day starved rats with wheat bran-enriched diet causes a large proliferative response in the proximal, mid-, and distal colon. These findings led us to postulate that the changes in the large intestine epithelium induced by fiber-rich diets do not depend exclusively on the amount of short-chain fatty acids produced. In fact, wheat bran (a poor SCFA producer) did increase the mucosal height of the colon and cecum, whereas oat bran (a high SCFA producer) did not significantly affect the mucosa. Other factors may play a role in the present observations such as an increase in the motility and blood flow of the intestine, and secretion of enterohormones.

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