Nutrient Metabolism

Wheat Bran Diet Reduces Tumor Incidence in a Rat Model of Colon Cancer Independent of Effects on Distal Luminal Butyrate Concentrations1,2

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ABSTRACT To investigate the effects of dietary fibers in colonic luminal physiology and their role in the prevention of colon cancer, a study was conducted using two diet groups and two treatment groups in a $2 \times 2$ factorial design. The two diets differed only in the type of dietary fiber, wheat bran and oat bran, and the two treatments were injection with the colon-specific carcinogen azoxymethane, or saline, as a control. There were 34 rats in the carcinogen-injected and 11 saline-injected rats per diet group. The goal of the study was to determine if a moderate consumption (6 g/100 g diet) of wheat bran or oat bran would alter the development of colonic tumors in this rat model of colon cancer, and if the differences in tumor incidence were correlated to luminal butyrate concentrations, luminal pH or fecal bulk. Short-chain fatty acid concentrations (SCFA) were measured in feces during the first half of the study (the promotion phase of tumor development) and again at the end of the study. Rats consuming oat bran had greater body weights ($P < 0.002$), produced much larger concentrations of all SCFA, including butyrate, in both the proximal and distal colon ($P < 0.0001$), had more acidic luminal pH values ($P < 0.0001$), but also had significantly more development of colon tumors ($P < 0.03$). Alternatively, rats consuming wheat bran produced more typical molar ratios of the SCFA (65:10:20), had a relatively greater concentration of butyrate than propionate, and produced a larger volume ($P < 0.05$) and more bulky stool than the rats fed oat bran. The results of this study support other evidence that an acidic luminal pH is not protective in and of itself, and that diets containing wheat bran are protective against colon cancer development. In addition, these data show that large luminal butyrate concentrations in the distal colon alone, as were present in the rats consuming oat bran diets, are not protective of tumor development. J. Nutr. 127: 2217–2225, 1997.

KEY WORDS: • colon cancer • luminal pH • butyrate • dietary fiber • rats

The role of dietary fiber in the prevention of colon cancer is still not completely understood despite numerous investigations that stemmed from Burkitt’s (1971) first suggestions concerning the importance of dietary fibers in preventing colon cancer in humans. The differences observed in colon cancer incidence with ingestion of different dietary fibers are due, at least in part, to the fact that dietary fibers are not a single entity, but are a complex group of substances with distinct physicochemical properties and thus exert different effects within the colon. One of the major differences between dietary fibers is their fermentability, or susceptibility to degradation by the colonic microflora. The fermentation of dietary fiber, and other substrates present in the colonic lumen, results in the production of short-chain fatty acids (SCFA), $\text{H}_2\text{O}, \text{CO}_2$, methane and $\text{H}_2$ gases (Macfarlane and Gibson 1995). Of the SCFA produced, the four-carbon monocarboxylic acid butyrate is of particular importance for several reasons. First, butyrate is the primary fuel source of colonocytes; it is utilized preferentially over glucose, glutamine and the other SCFA (Roediger 1982). Second, butyrate has been shown to increase cellular proliferation in normal colonocytes (Lupton and Kurtz 1993, Sakata 1987), a property that has been suggested as a risk factor for colon tumor development (Preston-Martin et al. 1990). Finally, butyrate has been shown in cell culture systems to increase markers of differentiation and induce apoptosis (Hague et al. 1995). These effects suggest that butyrate may be protective of tumor development because it appears to have contrasting effects on proliferation, differentiation and apoptosis in normal vs. neoplastic tissues. Thus, the role of butyrate in colon carcinogenesis remains incompletely understood; however, because its concentrations can be manipulated by changing the amount or type of fiber in the diet, its effects within the colon can be readily evaluated.

The fibers chosen for this study, wheat bran and oat bran, are two important fiber sources that have different compositions and physicochemical properties. Oat bran is a fermentable fiber that is readily digested in the proximal colon to produce large quantities of SCFA (Zhang and Lupton 1994). However, the extensive fermentation of the fiber reduces its dilution potential and its fecal bulking capacity, a property that is believed to play an important role in the prevention of colon cancer independent of effects on SCFA concentrations.

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2 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.
3 To whom correspondence and reprint requests should be addressed.
4 Abbreviations used: AOM, azoxymethane; B/P, ratio of butyrate to propionate; ESEM, environmental scanning electron microscopy; SCFA, short-chain fatty acids.

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of colon cancer (Cummings 1993). In contrast, wheat bran is fermented more slowly, resulting in greater concentrations of butyrate in the distal colon (Folino et al. 1995, Lupton and Kurtz 1993). However, because of the decreased fermentability of wheat bran, it maintains a greater dilution potential and fecal bulking capacity. Dilution potential is believed to be important in preventing colon cancer by reducing the exposure of the colonic surface epithelium to carcinogens and promoters (e.g., bile acids and ammonia) (Newmark and Lupton 1990). The presence of butyrate in the distal colon is also believed to be important in the prevention of colon cancer, because the majority of tumors in both humans and experimentally induced rodent cancer models occur in the distal colon (Buffle 1990, Holt et al. 1996, Reddy et al. 1975).

This study was conducted to shed further light on the role of dietary fiber in colon cancer. In particular, the goal of this study was to determine if moderate consumption (6 g/100 g diet) of oat bran or wheat bran fiber would alter tumor incidence in a rodent model of colon cancer, and if the tumor incidence was correlated to concentrations of butyrate in the distal colon. In addition, the change in SCFA concentrations during the promotion phase of tumor development, the critical period during which carcinogen-exposed cells either control the mutagenic event or progress toward development of tumors, was also studied. Finally, tumor incidence and concentration of SCFA were also compared to alterations in the luminal microenvironment (e.g., luminal pH) and dilution potential (e.g., fecal weight).

**MATERIALS AND METHODS**

**Animals and diets.** The animal use protocol for these experiments was approved by the University Laboratory Animal Care Committee at Texas A&M University. All animals were treated in accordance with the NIH guidelines (NRC 1985). Ninety male Sprague-Dawley rats (Harlan Sprague Dawley, Houston, TX) weighing 40–60 g were included in the study. The rats were randomly divided into four groups in a 2 × 2 factorial design, i.e., two diets differing only in the type of dietary fiber (wheat bran or oat bran) and two treatment groups [injection with the colon specific carcinogen, azoxymethane (AOM) or saline]. Each carcinogen-injected group consisted of 34 rats; the saline-injected controls had 11 rats per group. The rats were individually housed in hanging wire cages to minimize coprophagy and consumption of bedding. They were maintained in temperature- and humidity-controlled animal facilities with a 12-h daily light-dark cycle for the duration of the study. Food consumption and fecal output data were obtained over a 48-h period at wk 4 and at the end of the study. Food and water were consumed ad libitum throughout the study. Body weights were recorded weekly for all animals.

Composition of the diets is shown in Table 1. The diets met all of the nutritional requirements of the laboratory rat and were well tolerated. The diets were specifically designed to exploit the basic differences of the two types of dietary fibers present; wheat bran is poorly fermented, but can be degraded to produce significant amounts of butyrate, especially in the distal colon, whereas oat bran is readily fermented by the colonic microflora to produce large quantities of SCFA. The amount of fiber was chosen to approximate a moderate fiber intake (6 g/100 g diet, which corresponds to 30 g fiber/d in humans), an amount within the currently recommended 20–30 g/d.

**Carcinogen administration.** The AOM (Sigma Chemical, St. Louis, MO) was diluted in 9 g/L sodium chloride, pH 7.2, and administered in two weekly subcutaneous injections at a dose of 15 mg/kg. The injections were given during the second and third weeks of the study. Each of the control rats also was injected with an equal volume of the saline vehicle. During the injection periods, rats were housed in isolation units with negative pressure ventilation, and a full face respirator was worn when the animals were fed or handled. The study was scheduled to extend 36 wk postinjection to allow tumor development. However, criteria for determining when rats were to be killed before the designated end of the study included, but was not limited to substantial weight loss, persistent diarrhea and/or abnormal feces, rectal bleeding or blood in the stool, or other clinical evidence of tumor development.

**Fecal dry weights and fecal infrastructure.** Fecal outputs were determined during wk 4 (~28–30) of the study and then again the week before each rat was killed. Feces from each of the rats were collected for a 48-h period into preweighed vials and then reweighed. The feces were dried at 60–80°C for 24–48 h and dried samples were placed in a dessicator until cooled (2–4 h). The process was repeated until a constant weight was obtained for each sample. Fecal pellets were examined by environmental scanning electron microscopy (ESEM) to determine the infrastructure of fecal material obtained from rats consuming diets containing wheat bran or oat bran. The samples were collected and stored as described for determination of SCFA concentrations. On the day of analysis, the pellets were fractured along the long axis to examine the distribution of fibers within the pellet using an Electroscan E-3 ESEM (Electroscan, Wilmington, MA) at an operating voltage of 20.0 kV. Preparation for ESEM does not require fixation, drying or coating (Turner et al. 1995). A total of five regions from each pellet were examined for comparison, and pellets from both diet and carcinogen groups were evaluated.

**Short-chain fatty acid analysis.** Short-chain fatty acids were quantified in feces collected during wk 4, 8, 12 and 16 from 10 rats per group and at wk 38 (the time of killing). Feces obtained from the rats during the first half of the study were collected within 20 min of being passed and were immediately placed into liquid nitrogen and stored at −80°C until analysis. At the end of the study, fecal contents from the proximal and distal colon were collected into cryovials immediately after the rat was killed and then placed in liquid nitrogen and stored at −80°C. All fecal collections occurred between 0900 and 1200 h to minimize any differences resulting from diurnal variation. The luminal contents and fecal pellets were prepared for analysis by grinding in a mortar and pestle chilled by immersion in liquid nitrogen. The powdered sample (~0.20 g) was weighed.

TABLE 1

<table>
<thead>
<tr>
<th>Item</th>
<th>Wheat bran</th>
<th>Oat bran</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet ingredients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>60.4</td>
<td>49.6</td>
</tr>
<tr>
<td>Casein</td>
<td>18.1</td>
<td>13.7</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>4.5</td>
<td>1.7</td>
</tr>
<tr>
<td>D-L-Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Mineral mix (AIN-93-M-MX)</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix (AIN-93-VX)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>TTHQ (antioxidant)</td>
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<td>0.01</td>
</tr>
<tr>
<td>Fiber supplement</td>
<td>12.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>g/100 g diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber</td>
<td>49.65</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>13.3</td>
</tr>
<tr>
<td>Protein</td>
<td>16.0</td>
</tr>
<tr>
<td>Lipid</td>
<td>4.34</td>
</tr>
</tbody>
</table>

1. Ingredients other than the fiber supplements were supplied and mixed by Harlan Teklad, Madison, WI. TTHQ, tertiary butyhydroxyquinone.

2. Fiber supplements were obtained from the American Association of Cereal Chemists (AACC); AACC Certified Hard Red Wheat Bran, Lot 195, and AACC Certified Oat Bran.
TABLE 2

Effect of fiber on food intake, body weight and fecal weight in rats fed diets containing either wheat bran or oat bran and injected with azoxymethane (AOM) or saline.\(^1\,2\)

<table>
<thead>
<tr>
<th>Category</th>
<th>Wheat bran</th>
<th>Oat bran</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOM</td>
<td>Saline</td>
<td>AOM</td>
</tr>
<tr>
<td>24-h food intake (wk 4), g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>23.5 ± 0.4a</td>
<td>22.9 ± 0.7a</td>
<td>21.2 ± 0.4b</td>
</tr>
<tr>
<td>S</td>
<td>20.4 ± 0.6</td>
<td>21.9 ± 0.6</td>
<td>20.6 ± 0.5</td>
</tr>
</tbody>
</table>

\(^1\) Abbreviations used: AOM, azoxymethane-injected rats; saline, saline-injected rats; Carc, treatment group of either carcinogen- or saline-injected rats; D × C, diet and carcinogen interaction.

\(^2\) Means ± SEM, n = 33 AOM rats and n = 11 saline rats. Within rows, values with different letters are different, P < 0.05.

Results

Food intake, weight gain, and physical data. Food and energy intakes were not different among the groups, except at wk 4 in rats consuming oat bran and injected with AOM (Table 2). Rats fed the oat bran diet weighed more (P < 0.0021) than rats consuming wheat bran at both wk 16 and 36, but were not different at wk 4. Rats consuming wheat bran had a larger (P < 0.05) fecal output than the rats consuming oat bran (Table 2). There were no differences between diet or treatment groups for colon length or width. Figure 1 illustrates the gross physical differences between the two diet groups; the fecal pellets from the wheat bran rats are substantially larger and have a mucus outer coat, whereas the fecal pellets from the rats consuming oat bran are smaller, denser and darker. These differences in fecal consistency are further illustrated by ESEM photomicrographs of the pellets (Fig. 2). The fecal pellets from the wheat bran–fed rats clearly showed the large amounts of remaining fiber and numerous open spaces within the structure of the pellet. Conversely, the fecal pellets obtained from oat bran–consuming rats were of a more amorphous nature, with little evidence of remaining fiber; they contained a large amount of cellular debris and digested luminal contents with few open spaces observed.

Short-chain fatty acid concentrations. The effect of diet and carcinogen treatment on the fecal SCFA concentration over time is illustrated in Table 3 and Figure 3. Fecal SCFA concentrations have been shown in previous studies to be closely related to the luminal SCFA concentration of the distal colon (McIntyre et al. 1991). In general, the fecal concentrations of SCFA in rats consuming wheat bran decreased steadily
all time points examined during the first 16 wk of the study. Further, rats consuming oat bran diets had SCFA concentrations, especially acetate, propionate and butyrate, that changed differently over time. Figure 3 illustrates the changes in the concentration of propionate and butyrate that occurred between wk 4 and 16 in rats from both diet and treatment groups. In rats injected with AOM and consuming oat bran, fecal concentrations of acetate and propionate declined from wk 4 to 8, leveled out at wk 12, and then decreased to the lowest concentration at wk 16. Butyrate concentrations over the 16-wk period in rats consuming oat bran did not differ over the first 3 wk but declined notably at wk 16. Rats injected with AOM and consuming wheat bran tended to have a rather steady decline in the concentration of each of these SCFA over the 16-wk time period. Rats injected with saline and consuming oat bran had substantial fluctuation in the concentration of each SCFA over the 16-wk period. Rats injected with saline and consuming oat bran had substantial fluctuation in the concentration of each SCFA over the 16-wk period. Rats injected with AOM and consuming wheat bran tended to have a rather steady decline in the concentration of each of these SCFA over the 16-wk time period. Rats injected with saline and consuming oat bran had substantial fluctuation in the concentration of each SCFA (especially propionate and butyrate), but by wk 16, the concentration was at its lowest value for both propionate and butyrate. Acetate concentrations in the oat bran control rats were at the same levels at wk 16 as they were at wk 4. Wheat bran control rats followed a similar pattern for all three SCFA as was observed in the AOM-injected rats. Despite the different patterns of fecal SCFA concentrations that were observed during the intermediate time points, significant decreases in the concentrations of acetate ($P < 0.04$), propionate ($P < 0.004$) and butyrate ($P < 0.019$) occurred from wk 4 until wk 16 for both diet and carcinogen groups (Table 3). No differences were found between the AOM- and saline-injected rats with respect to the total or individual SCFA concentrations at any time point. To examine the potential effects of differences in body weight on these values, body weight was included in the model as a covariate. Body weight was found to be a significant ($P < 0.05$) cause of variation primarily at wk 8. Acetate, propionate, butyrate and total SCFA concentrations were each negatively affected; therefore, as body weight increased, the concentration of those SCFA decreased. Body weight also had a significant negative effect on the concentrations of acetate ($P < 0.04$) and total SCFA ($P < 0.05$) at wk 4. Finally, body weight had a positive effect ($P < 0.04$) on the concentrations of butyrate in the rats at wk 16.

Luminal SCFA concentrations obtained from the proximal and distal colon of the rats are shown in Table 4. In all cases, SCFA concentrations from the distal colon are higher than the levels found in feces at the earlier time points. In addition,
several significant differences were identified among the groups. First, there were differences ($P < 0.0001$) in SCFA concentrations between the diet groups, with the exception of acetate; in each instance, the concentration of SCFA was higher in the rats consuming oat bran. Further, there were differences ($P < 0.007$) between the proximal and distal colon for total SCFA concentration. Consistent differences between the carcinogen-injected and saline-injected groups were not found. However, there were some notable exceptions to this in the rats consuming oat bran diets (Table 4). In those rats, the concentrations of propionate, isobutyrate, valerate and total SCFA in the proximal colon were significantly ($P < 0.05$) higher in the AOM-injected rats than in the saline controls. These same differences were not present in the distal colon, but a trend ($P = 0.101$) for greater concentrations of these SCFA in the AOM rats was still observed. In the rats consuming wheat bran, the pattern was opposite, with AOM-injected rats tending ($P = 0.08$) to have lower concentrations of SCFA than the saline-injected control rats. Data were analyzed with body weight as a covariate to determine if the differences in body weight among the groups would account for the differences observed between the diet groups, but no differences were found (data not shown). Finally, the relationship of the butyrate to propionate ratio (B/P ratio) between the different diet and carcinogen treatment groups was examined. In the rats consuming wheat bran, the mean $\pm$ SEM of the B/P ratio was 1.97 $\pm$ 0.11, but in the rats fed oat bran, the ratio was 2.50 $\pm$ 0.11 ($P < 0.0009$). No differences were found between the carcinogen groups or for location in the colon. However, a nearly significant interaction existed between the diet and carcinogen groups ($P = 0.06$), suggesting that diet and carcinogen together have an important effect on the ratio. In particular, the AOM-injected rats consuming wheat bran had a B/P ratio (2.03 $\pm$ 0.14) that was equivalent to that of the saline-injected rats (1.90 $\pm$ 0.07) consuming the same diet. However, the AOM-injected rats consuming oat bran had a lower B/P ratio (2.27 $\pm$ 0.12) than their saline counterparts (2.72 $\pm$ 0.17). Body weight had no effect on the B/P ratio when it was included as a covariate in the statistical analysis.

### Luminal pH measurements
For both the proximal and distal sections of the colon, the luminal pH in the rats consuming the wheat bran diet was higher ($P < 0.0001$) than the luminal pH in rats consuming the oat bran diet (Fig. 4). There were no differences in luminal pH between the carcinogen- and saline-injected groups or for location within the colon.

### Tumor incidence
There was a lower ($P < 0.021$) tumor incidence in rats consuming wheat bran diets compared with those consuming oat bran. Twenty-seven percent of rats consuming wheat bran developed tumors (9/33), compared with 52% of the rats consuming oat bran (17/33). One rat from each diet group was lost to the study for the following reasons: one rat in the wheat bran group was injured and had to be killed, and one of the rats from the oat bran group was found dead (postmortem examination revealed a large lung tumor). There were no significant differences in tumor location, with 9/17 tumors occurring in the distal colon of rats fed oat bran diets, whereas 5/9 tumors in the rats fed wheat bran diets were found in the distal colon. There were also no differences in the number of tumors that developed per rat (23% of the rats consuming oat bran developed multiple tumors, whereas 22% of the rats consuming wheat bran had multiple tumors present). However, there was a difference ($P < 0.041$) between the diets in the relative size of the tumors that developed, with rats consuming oat bran diets having visible (macroscopic) tumors, whereas the rats consuming wheat bran had a greater number of microscopic tumors (i.e., the tumors were visible only by microscopic examination of the tissue, usually within Peyer's patches).

### DISCUSSION
This study has addressed a key issue in the debate surrounding the role of dietary fiber in the prevention of colon cancer: is it the luminal butyrate concentration within different segments of the colon that most determines whether a fiber will be protective or not? Previous work has shown that poorly fermented fibers, such as wheat bran, cellulose and lignin, are protective against colon cancer (Heitman et al. 1989, McIntyre et al. 1993, Young et al. 1996), whereas the readily...
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Rats that consumed oat bran diets weighed more than rats consuming wheat bran at both wk 16 and 36, despite similar feed intakes. The reasons for the weight differences are not known. However, it is possible that the increased energy available to the rats consuming oat bran as a result of the larger quantities of SCFA produced could play an important role. Luminal acetate, and a large proportion of propionate, is not utilized by the colonic epithelial cells, but is instead transported to the liver for further metabolism or use in lipid synthesis (Remy et al. 1995). This may be an important issue because there may be a relationship between excess body weight and development of colon cancer (Albanes and Taylor 1990). In addition, many investigators have suggested that a relationship between energy intake and colon carcinogenesis exists as well (Lasko and Bird 1995, Reddy et al. 1987b). Thus, the potential to increase energy intake is an effect of soluble fibers that will require additional examination to completely appreciate. A further effect of large concentrations of SCFA being presented to the liver is the potential for these substances to influence the metabolic activation of AOM in the liver. Finally, neither wheat bran nor oat bran had a significant influence on colon length or width, which are two indices that tend to mirror cellular proliferation measurements (Sakata 1986). However, proliferation was not addressed in this particular study, and thus this connection cannot be confirmed.

Rats consuming wheat bran diets had larger fecal outputs (fecal dry weights) than their oat bran–consuming counterparts at both early (4 wk) and late (36 wk) time points. This illustrates an important difference in the two fiber types: dilution potential and fecal bulking capacity are greater with wheat bran (Gazzaniga and Lupton 1987). The ability of wheat bran to alter the luminal microenvironment by adsorbing substances such as secondary bile acids (particularly lithocholic acid), ionized long-chain fatty acids and ammonia may be one of the most important facets of its protective effects (Reddy et al. 1987a, Sakata 1987, Visek 1978). The presence of large amounts of undigested fiber in the lumen of the rats fed wheat bran diets is readily illustrated both grossly and via electron microscopy (Fig. 1, 2) and serves as physical evidence of the dilution potential of the wheat bran fiber.

Tumor development in the colon is proposed to be the consequence of a series of genetic insults that occur after a primary event that initiates the carcinogenic process (Staley et al. 1995). Colon tumorigenesis is further enhanced by tumor promoters present in the colonic lumen during the promotion phase of carcinogenesis. To further examine the influence of dietary fibers during the promotion phase of tumorigenesis, we chose to assess fecal SCFA production during the first 16 wk after carcinogen injection. As expected, rats consuming the oat bran diets had significantly higher concentrations of SCFA than their wheat bran counterparts. This point may be important in that previous studies have shown that differences in microbial populations within the colon can alter the effect

fermented fibers, such as oat bran, pectin, and guar gum, are not protective and may possibly be promotive of tumor development (Jacobs and Lupton 1986, McIntyre et al. 1993). Several issues have been raised as possible explanations for these differences, including the differences in fecal bulk or dilution potential (Cummings 1993), differences in production of SCFA, which relates to increases in cell proliferation (Folino et al. 1995, Lupton and Kurtz 1993, Zhang and Lupton 1994), differences in the location of greatest butyrate concentration (McIntyre et al. 1991 and 1993) and alterations in luminal pH (Newmark and Lupton 1990). Wheat bran and oat bran were chosen for this study for two major reasons: 1) they are common and readily available dietary fiber sources, and 2) they are very different fibers with respect to their fermentability and dilution potential. Finally, we chose to use an amount of dietary fiber that was conservative (6 g/100 g diet or ~ 30 g/d) but was within the range recommended for adult humans to consume in a healthy diet, rather than study an amount of fiber that might give distinct results, but might also be an unrealistic goal for human consumption.

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fermented fibers, such as oat bran, pectin, and guar gum, are not protective and may possibly be promotive of tumor development (Jacobs and Lupton 1986, McIntyre et al. 1993). Several issues have been raised as possible explanations for these differences, including the differences in fecal bulk or dilution potential (Cummings 1993), differences in production of SCFA, which relates to increases in cell proliferation (Folino et al. 1995, Lupton and Kurtz 1993, Zhang and Lupton 1994), differences in the location of greatest butyrate concentration (McIntyre et al. 1991 and 1993) and alterations in luminal pH (Newmark and Lupton 1990). Wheat bran and oat bran were chosen for this study for two major reasons: 1) they are common and readily available dietary fiber sources, and 2) they are very different fibers with respect to their fermentability and dilution potential. Finally, we chose to use an amount of dietary fiber that was conservative (6 g/100 g diet or ~ 30 g/d) but was within the range recommended for adult humans to consume in a healthy diet, rather than study an amount of fiber that might give distinct results, but might also be an unrealistic goal for human consumption.

Rats that consumed oat bran diets weighed more than rats consuming wheat bran at both wk 16 and 36, despite similar feed intakes. The reasons for the weight differences are not known. However, it is possible that the increased energy available to the rats consuming oat bran as a result of the larger quantities of SCFA produced could play an important role. Luminal acetate, and a large proportion of propionate, is not utilized by the colonic epithelial cells, but is instead transported to the liver for further metabolism or use in lipid synthesis (Remy et al. 1995). This may be an important issue because there may be a relationship between excess body weight and development of colon cancer (Albanes and Taylor 1990). In addition, many investigators have suggested that a relationship between energy intake and colon carcinogenesis exists as well (Lasko and Bird 1995, Reddy et al. 1987b). Thus, the potential to increase energy intake is an effect of soluble fibers that will require additional examination to completely appreciate. A further effect of large concentrations of SCFA being presented to the liver is the potential for these substances to influence the metabolic activation of AOM in the liver. Finally, neither wheat bran nor oat bran had a significant influence on colon length or width, which are two indices that tend to mirror cellular proliferation measurements (Sakata 1986). However, proliferation was not addressed in this particular study, and thus this connection cannot be confirmed.

Rats consuming wheat bran diets had larger fecal outputs (fecal dry weights) than their oat bran–consuming counterparts at both early (4 wk) and late (36 wk) time points. This illustrates an important difference in the two fiber types: dilution potential and fecal bulking capacity are greater with wheat bran (Gazzaniga and Lupton 1987). The ability of wheat bran to alter the luminal microenvironment by adsorbing substances such as secondary bile acids (particularly lithocholic acid), ionized long-chain fatty acids and ammonia may be one of the most important facets of its protective effects (Reddy et al. 1987a, Sakata 1987, Visek 1978). The presence of large amounts of undigested fiber in the lumen of the rats fed wheat bran diets is readily illustrated both grossly and via electron microscopy (Fig. 1, 2) and serves as physical evidence of the dilution potential of the wheat bran fiber.

Tumor development in the colon is proposed to be the consequence of a series of genetic insults that occur after a primary event that initiates the carcinogenic process (Staley et al. 1995). Colon tumorigenesis is further enhanced by tumor promoters present in the colonic lumen during the promotion phase of carcinogenesis. To further examine the influence of dietary fibers during the promotion phase of tumorigenesis, we chose to assess fecal SCFA production during the first 16 wk after carcinogen injection. As expected, rats consuming the oat bran diets had significantly higher concentrations of SCFA than their wheat bran counterparts. This point may be important in that previous studies have shown that differences in microbial populations within the colon can alter the effect
of carcinogens, including AOM, in the colon (Reddy et al. 1975). Because diet can alter fecal microbial populations (Maciorowski et al. 1997), the potential exists for a diet to influence the metabolism of AOM via this mechanism. However, the rats were started on the diets <1 wk before their first carcinogen injection, and because the development of a stable luminal microenvironment (luminal pH, microbacterial population and SCFA concentrations) following a diet change requires at least 2 wk (Zhang and Lupton 1996), it is unlikely that this significantly influenced the metabolism of AOM in the colon. Fecal SCFA concentrations were not affected by carcinogen treatment at any of the four time points examined. However, there was a significant decrease in the concentration of each SCFA over time (Fig. 3). This decrease in concentration was observed in both diet groups and in both carcinogen- and saline-injected rats. The decrease in individual and total SCFA concentrations over time is interesting, because it illustrates an apparently universal event in the development of these rats from weanlings to young adults. The reduction in fecal SCFA probably represents a combination of increased SCFA absorption as the colon matures and lengthens, and an increase in utilization of SCFA for energy as the animal is reaching its mature size and body weight. However, it could also be due to changes in the luminal microflora that occur as the animal matures. Of the three primary SCFA, there were no significant trends in either the wheat bran or oat bran rats that would suggest a possible link to increased or decreased tumor development other than the primary differences in overall concentrations of the individual SCFA. However, there was an inverse relationship between the concentration of the three major fecal SCFA and body weight at the earliest time points (4 and 8 wk). This early period is purportedly critical in the process of tumor development, and if a greater decrease in the fecal concentration of SCFA occurs as a result of excess body weight, this could potentially alter colonocyte health by reducing the availability of normal energy substrates and render the cells more susceptible to mutagens and other tumor-promoting substances.

The protective value of a fiber has often been linked to the production of butyrate and especially the concentration of butyrate in vivo (Folino et al. 1995, Sakata 1987). However, butyrate has also been shown to have different effects on cell proliferation in vitro and on tumor cells; these effects include increasing markers of differentiation and inducing apoptosis (Hague et al. 1995). Because the colonic epithelium has proven refractory to culture, the differences between the response to butyrate in vivo and effects in cell lines have been examined.

### TABLE 4

<table>
<thead>
<tr>
<th>Distal colon</th>
<th>Wheat bran</th>
<th>Oat bran</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOM</td>
<td>Saline</td>
<td>AOM</td>
</tr>
<tr>
<td>µmol/g wt feces</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>18.3 ± 2.2</td>
<td>24.2 ± 2.6</td>
<td>23.0 ± 1.9</td>
</tr>
<tr>
<td>Propionate</td>
<td>3.2 ± 0.5</td>
<td>4.6 ± 0.6</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>Butyrate</td>
<td>6.24 ± 1.5</td>
<td>8.2 ± 1.8</td>
<td>16.9 ± 1.3</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>0.43 ± 0.07</td>
<td>0.60 ± 0.09</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>0.53 ± 0.10</td>
<td>0.63 ± 0.12</td>
<td>1.63 ± 0.90</td>
</tr>
<tr>
<td>Valerate</td>
<td>0.42 ± 0.09</td>
<td>0.54 ± 0.11</td>
<td>1.21 ± 0.08</td>
</tr>
<tr>
<td>Total</td>
<td>29.1 ± 3.5</td>
<td>38.7 ± 4.1</td>
<td>51.5 ± 3.1</td>
</tr>
</tbody>
</table>

1 Abbreviations used: Carc, treatment groups consisting of either AOM- or saline-injected rats; Loc, location within the colon; D x C, diet/ carcinogen interaction; D x L, diet/location interaction. Values are means ± SEM, n = 33 rats/AOM group, 11 rats/saline group. Significance was set at P < 0.05. There were no significant carc x loc or three-way interactions found.
difficult to reconcile. However, the question remains: Does butyrate have antineoplastic effects in vivo, and if it does, is there an optimum concentration or location of butyrate production for butyrate to have a protective effect? In naturally occurring human colon cancer, and in the majority of rodent studies using carcinogens, the distal colon is the primary site of tumor occurrence (Buflill 1990, Holt et al. 1996). Typically, the distal colon has the lowest concentrations of SCFA, including butyrate compared with the cecum and proximal colon, which are the primary sites of fiber fermentation. Thus, if butyrate is protective, then it seems reasonable to suggest that the diet that results in the production of a greater concentration of butyrate in the distal colon would be more desirable. In previous studies in which wheat bran diets were fed, the concentrations of butyrate in the distal colon were very high (15–20 mmol/L) compared with that produced from fermentation of other fibers (Lupton and Kurtz 1993, McIntyre et al. 1993). However, in this study, the concentration of butyrate in the distal colon of the rats consuming wheat bran was lower than that in the proximal colon. Further, the concentrations of butyrate in the rats consuming oat bran was significantly higher in both proximal and distal colon than that in the rats consuming wheat bran. This finding is somewhat difficult to reconcile, but may be due to the modest concentration of fiber we chose to test in this study. Other studies examining the effects of wheat bran have frequently used 8–10% fiber (Folino et al. 1995, Lupton and Kurtz 1993, McIntyre et al. 1993), and it is possible that a large quantity of the fiber must be consumed to achieve an increased concentration of butyrate in the distal colon. Alternatively, there are many different types of wheat bran available, and the type and grind of the wheat bran does have a significant effect on the SCFA produced (Folino et al. 1995). For this study, the wheat bran was coarsely ground hard red winter wheat and thus would be slowly fermented and expected to produce large concentrations of butyrate in the distal colon. However, it appears that in rats fed diets containing 6 g of fiber/100 g diet, a significant increase in distal butyrate concentration was not achieved. Further, despite the major difference in distal butyrate concentration observed in this study, the rats consuming wheat bran still had the lowest incidence of colorectal tumors. This finding suggests that the concentration of butyrate in the distal colon alone does not explain the protection wheat bran offers against colon tumor development.

In addition to the differences in butyrate concentrations that are observed for rats fed the two different diets, there are major differences observed for the percentages of acetate, propionate and butyrate. Typically, acetate, propionate and butyrate are produced within the colonic lumen in the molar ratios of 60:25:15, respectively (Breves and Stuck 1995). However, in the rats consuming the oat bran diets, the ratios were 45:15:35 for acetate, propionate and butyrate, respectively. The rats consuming wheat bran had SCFA ratios that were very similar to that produced by the typical diet, i.e., 65:10:20. However, there was a greater concentration of butyrate and/or lower concentration of propionate present in the distal colonic lumen. Thus, the possibility exists that there are optimum ratios of SCFA for maintenance of normal colonic epithelium. Furthermore, butyrate or propionate concentrations that are too large may be detrimental to colonocyte health.

To examine the relationship of butyrate and propionate concentrations, butyrate to propionate (B/P) ratios were calculated. Although the difference in the B/P ratio between the two diets was significant, the difference was not unexpected because of the differences in the individual SCFA concentrations previously observed. However, there were no differences in the B/P ratios between treatment groups or for location within the colon.

The pH of the colonic lumen is influenced by a variety of factors including the microbial population and their metabolic activity, epithelial ion exchange processes, luminal bile acids, and undigested or unabsorbed carbohydrates, proteins and lipids (Feldman and Icks 1997, Newmark and Lupton 1990). Thus, diets that contain fibers that are fermented differently would be expected to have distinct effects on the luminal microenvironment, and ultimately, pH. In previous studies, a more alkaline luminal pH was associated with an increased incidence in colon cancer (Thornton 1981). Acidifying the colonic lumen is believed to be beneficial by reducing (protonating) bile acids, long-chain fatty acids and ammonia (Newmark and Lupton 1990), which are strong irritants and may act as tumor promoters. In addition, a more acidic colonic pH reduces the activity of the enzyme 7-α-dehydroxylase, an enzyme that catalyzes the conversion of primary bile acids to secondary bile acids in the colonic lumen (Reddy et al. 1992). Secondary bile acids are known tumor promoters (Reddy et al. 1987a); thus decreasing their formation is an important goal. However, Gallaher and Franz (1990) showed that although the overall activity of 7-α-dehydroxylase was reduced with a decrease in pH, the concentration of the enzyme was increased as a result of increased production by the luminal microflora. In addition, there have also been a number of studies that failed to show that merely acidifying the colon had a protective effect and suggested that it was the presence of the SCFA that was most important (Bartram et al. 1993, McIntyre et al. 1993). In this study, rats consuming the oat bran diets clearly had larger concentrations of SCFA (Table 4) and also more acidified colon contents, both in the proximal and distal colon (Fig. 4), yet they also had the greatest tumor incidence compared with the rats consuming wheat bran. Thus, these results support the conclusion that acidification of luminal contents is not protective against colon tumor development.

In summary, the group of rats consuming diets containing oat bran at a concentration of 6 g/100 g diet had greater body weights, produced larger concentrations of SCFA, including butyrate, in both the proximal and distal colon, had more acidic luminal pH values, but also had a significantly larger number of animals develop colon tumors than their wheat bran counterparts. Alternatively, rats consuming wheat bran produced concentrations of the three major SCFA in more typical molar ratios, had lower body weights throughout the study, and produced a larger quantity of feces (suggesting greater dilution potential and fecal bulking ability) than their oat bran counterparts. The results of this study support other evidence showing that an acidic luminal pH is not protective in and of itself. The data also show that distal colonic butyrate concentrations alone are not protective of colon cancer development, and although butyrate may still have antineoplastic properties, it is not the sole reason for a fiber’s protective effect. Thus in rats consuming wheat bran, a reduction in tumor development in this model of colon cancer may be associated with wheat bran’s not being a source of excess calories, producing a concentration of butyrate and propionate that are within “normal” limits, and retaining its dilution potential and thus reducing the exposure of the colonic epithelium to carcinogens and promoters that are presumed to be necessary for tumor development. Wheat bran is a complex fiber that contains many morphologic components, such as aleurone and the pericarp seed-coat (Cheng et al. 1987) and a number of other substances, such as phytates (Kirby and Nelson 1988) that may have antineoplastic properties of their own. Thus a clear
picture of the effects of wheat bran in prevention of colon cancer remains elusive, but the results of this study continue to support the suggestions that wheat bran is protective against colon cancer and should continue to be a focus of future research efforts.

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LITERATURE CITED