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# Dietary Fiber Modulates Colon Cell Proliferation by Altering Luminal Concentrations of Short-Chain Fatty Acids*

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**To compare the effects of various types of dietary fiber on microbial production of short-chain fatty acids (SCFA) and on colon cell proliferation which is used as an intermediate biomarker for colon carcinogenesis, groups of 10 male Sprague-Dawley rats were fed one of four fiber-supplemented diets (6% cellulose, 6% pectin, 6% polydextrose, and a mixture of 3% cellulose and 3% polydextrose) for three weeks. As a control, a fiber-free diet was fed to a separate group of 10 rats. Cell proliferation was measured by in vivo incorporation of bromodeoxyuridine into DNA in the proximal and distal colon, respectively. Luminal concentrations of SCFA were measured by gas chromatography. Dietary fiber significantly influenced microbial production of SCFA in the colon; pectin supplementation produced the highest concentrations of luminal SCFA in both the proximal and distal colon ($p<0.05$). The degree of individual SCFA production was characterized by a relatively higher increase in butyrate production by the pectin-supplemented diet, and in propionate production by the polydextrose-supplemented diet, resulting in alterations of the molar ratios of acetate, propionate and butyrate. There were significant differences in colon cell proliferation among the diet groups; the pectin-supplemented diet produced a significantly higher effect on cell proliferation of distal colonic epithelial cells ($p<0.05$), and the polydextrose-supplemented diet produced an intermediate effect compared to the fiber-free or cellulose-supplemented diet. Increased cell proliferation was correlated to increased luminal concentrations of butyrate in the proximal colon and to increased luminal concentrations of propionate and butyrate in the distal colon ($p<0.05$). Therefore, these data suggest that dietary fiber may modulate colon cell proliferation by altering luminal SCFA concentrations, particularly butyrate and perhaps propionate. In addition, the present study is the first finding that has demonstrated a relative increase in colon cell proliferation due to supplementation with polydextrose, suggesting that the overuse of this artificially synthesized polysaccharide in food processing technology needs to be carefully evaluated from the public health point of view.**

**Key words :** colon, cell proliferation, dietary fiber, short-chain fatty acids

## INTRODUCTION

Although epidemiological studies generally support a protective role of dietary fiber in colon carcinogenesis,[16)40)] experiments in animal models using a variety of dietary fiber and chemical carcinogens have produced conflicting results.[19)32)] The controversy in the literature may be in part explained by the fact that dietary fiber is a heterogeneous group of compounds with different physicochemical properties. Poorly fermentable fiber such as cellulose and wheat bran generally protect against experimentally induced colon cancer,[5)17)42)43)] while the more fermentable fiber such as pectin, oat bran and guar do not protect and may actually enhance tumorigenesis.[6)19)42)]

One of the physicochemical properties of fermentable fiber is the microbial production of short-chain fatty acids (SCFA), such as acetate, propionate and butyrate in the colon. Of these, the role of butyrate in colon physiology and colonic disease has been a focus of intense investigation in recent years.[18)29)33)36)38)41)] Colonocytes are unique, in that, of all the cells in the non-ruminant body, butyrate is the preferred metabolic fuel source.[4)9)31)] In addition to its primary role as an energy source for colonocyte metabolism, butyrate has been found, *in vivo and in vitro*, to influence other crucial cellular events, including proliferation, differentiation, and apoptosis.[27)] However, the *in vivo* results are often opposite to those obtained from immortalized or transformed cell lines. For example, butyrate stimulates cell proliferation *in vivo*,[34)35)] while simultaneously depressing cell division and promoting differentiation and apoptosis *in vitro*.[11)13-15)]

Since health effects of dietary fiber have been widely recognized, there have been attempts to produce functional foods using a variety of dietary fibers; one of these

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is polydextrose, an artificially synthesized polysaccharide, which has been the focus of attention by many food technologists as a fat replacer because of its poor digestibility, similar to naturally-occurring dietary fiber.[1] Polydextrose is a randomly-bonded polymer of glucose with sorbitol end groups and citric acid, and is more resistant to acid or enzyme hydrolysis than a polymer such as starch.[45] Therefore, polydextrose is poorly digested in the small intestine; after oral administration, approximately 60% of the polydextrose is excreted in feces and 30% is fermented in the lower gut by intestinal microflora, producing volatile fatty acids and $CO_2$.[10]

We compared the microbial fermentation of an artificially-synthesized fiber, polydextrose, to that of naturally-occurring dietary fiber (cellulose and pectin), in the lumen of the rat colon. Also, we investigated whether these different types of dietary fiber exerted differential effects on colon cell proliferation (used as an intermediate biomarker for colon carcinogenesis), and whether those effects were related to the luminal SCFA concentrations. Because the fermentation of dietary fibers has been noted to vary in different sites of the colon, we determined the luminal SCFA concentrations and their correlations to cell proliferation in the proximal and distal colon, respectively.

## MATERIALS AND METHODS

### 1. Materials

The internal standard, 2-ethylbutyric acid, for quantification of short-chain fatty acids (SCFA), and standards for peak identification of individual SCFAs, were purchased from Sigma Chemical (Seoul, Korea). Normal goat serum, goat anti-mouse immunoglobulin serum, and peroxidase-labeled mouse immunoglobulins were purchased from Signet Laboratories (Dedham, MA). Mouse monoclonal antibody to bromodeoxyuridine (BrDU), normal mouse serum, BrDU, $H_2O_2$ (30%), albumin imidazole, $CoCl_2$, and 3,3'-diaminobenzidine tetrahydrochloride were purchased from Sigma Chemical (Seoul, Korea).

### 2. Animals and Diets

The male Sprague-Dawley rats were stratified by their body weights and assigned to one of five experimental diets (10 animals/diet), following a one-week acclimatization period during which they consumed standard rat chow. Consequently, mean initial body weights were not different among the diet groups. The composition of the basal fiber-free control diet, on a weight basis, is shown in Table 1. The fiber-supplemented diets were prepared by uniformly diluting each basal fiber-free diet by the addition of 6% cellulose, 6% pectin, 6% polydextrose, or a mixture of 3% cellulose and 3% polydextrose (Table 2). The diets were designed by this uniform dilution technique, so that if rats had equivalent energy intakes from carbohydrate, protein and fat, they would have equivalent amounts of all nutrients except fiber. Food and water were allowed ad libitum and renewed daily to provide fresh diets. Experimental diets were provided for three weeks. Twenty-four-hour food intakes for two consecutive days were measured twice during the study. Body weights were recorded weekly. Feces were collected for three consecutive days and pooled to measure 72-hour fecal output twice during the study. The experimental rats were individually housed in hanging wire cages to minimize coprophagy and consumption of bedding.

**Table 1.** Composition of the Basal Fiber-free Diet on a Weight Basis[1]

| Ingredients[2] | Basal Diet |
|---|---|
| Dextrose | 52.6 |
| Casein | 23.6 |
| DL-Methionine | 0.4 |
| Corn oil | 15.0 |
| Mineral mix[3] | 6.0 |
| Vitamin mix[4] | 2.0 |
| Choline bitartrate | 0.4 |

1 : Values are expressed as g per 100g diet.
2 : All ingredients (dry components) were purchased from ICN Biochemicals (Costa Mesa, CA).
3 :AIN-76 mineral mixture(29).
4 : AIN-76 vitamin mixture(30).

**Table 2.** Composition of the Fiber Supplemented Diets

| Components1 | Basal Diet(%) | Fiber Supplement(%) |
|---|---|---|
| Fiber-free | 100 | 0 |
| Cellulose | 94 | 6 |
| Pectin | 94 | 6 |
| Polydextrose | 94 | 6 |
| Polydextrose + Cellulose | 94 | 3 + 3 |

1 : Source of each fiber supplement was as follows: cellulose(no. 900453, ICN), pectin(no. 102587, ICN), polydextrose(Pulmowon Cooperation, Seoul, Korea)

### 3. Fecal Wet and Dry Weight

Feces from each of the 50 rats were collected twice a day on days 18 to 20 of the study. They were collected into pre-weighed vials, reweighed and stored at -20℃ until dry weights were taken by drying at 100℃ for 24 hours. Dried samples were placed in a desiccator until cooled (approximately 2 hours). The process was repeated until a constant weight was obtained for each sample.

### 4. Colon Length and Weight

Immediately after the animals were killed by $CO_2$ gas,

the colon was resected from the junction between the cecum and the colon just above the anus. The colon length was then immediately measured and divided into proximal and distal segments, which were equal in length. The two colon segments were flushed clean with phosphate-buffered saline solution (PBS), pH 7.4, and then weighed respectively.

### 5. Luminal Short-Chain Fatty Acid (SCFA) Analysis

After the resected colon was divided into proximal and distal segments, the luminal contents from each of the two colon segments were placed into separate cryotubes, which were immediately plunged into liquid nitrogen for 20 seconds and then stored at -70℃ until SCFA analysis. The procedure used for SCFA analysis has been described previously.[7] Briefly, the frozen luminal contents were ground to a fine powder. Approximately 0.3 g of the powdered sample were weighed accurately. Five hundred microliters of 3mM 2-ethylbutyric acid in 70% alcohol (as first internal standard) were added to the sample tube. The samples were then vigorously vortexed and stored at 4℃ for eight hours to extract SCFA. To prepare the extracted samples for gas-liquid chromatography, the samples were first shaken vigorously for 20 minutes and centrifuged at 11,500 *g* at 4℃ for 20 minutes. A 100-㎕ aliquot of the supernatant was removed and combined with 100 ㎕ of 3mM heptanoic acid (second internal standard), prepared in 70% ethanol. Immediately before injection into the gas chromatograph (GC), 20 ㎕ of 0.1mM phosphoric acid was added to the sample which was then vortexed. One microliter of the sample containing phosphoric acid was then injected into a Hewlett Packard 5890 Series II GC with a 30 m, 0.53 mm i.d. deactivated glass capillary precolumn. The data were integrated and plotted with a Hewlett Packard Series II Integrator. The conditions used for gas chromatography were as follows: injector temperature, 165℃; detector temperature, 220℃; column flow, 2.21 ㎖/min of helium; make-up flow, 28 ㎖/min of nitrogen; and oven temperature, 185℃(gradient). Standards and a blank were run before and after the daily sample runs.

### 6. In Vivo Measurements of Cell Proliferation

Exactly one hour before sacrifice, each animal was given an intraperitoneal injection of BrDU (5mg/kg body weight) in phosphate-buffered saline (PBS), pH 7.4, to measure *in vivo* cell proliferation.[23] All animals in this experiment were killed at the same time of day (8:00-12:00) to avoid any major effects arising from diurnal variations.[39] After the colon length and weight were measured, a 1-㎝ length of colon was taken from each of the two colon segments. The colon tissue was then placed in 70% ethanol overnight, a graded series of ethanol from 80% to 100%, and finally xylene before it was embedded in paraffin wax. Paraffin sections (4㎛ thick) were cut perpendicular to the mucosal surface and affixed to albumin-coated slides.

The incorporation of BrDU into DNA was localized using the monoclonal anti-BrDU antibody, and bound antibody was detected using the peroxidase-conjugated antibody to mouse immunoglobulin. The whole procedure of immunoassay is described in detail elsewhere.[23]

### 7. Analysis of Crypt Size and Proliferative Activity

All slides were coded so that observers were unaware of their identity. Only well-oriented crypts in which the base, lumen, and the top of the crypt could be seen were selected. Twenty-five crypt columns were read per animal. Crypt height measured in terms of the number of cells, and number and position of labeled cells, were recorded, counting upward from Position 1 at the base to the mouth of the crypt. Ten crypt circumferences were measured by counting the number of cells appearing in transverse sections of crypts.[44] The mean of 25 crypt heights and the mean of 10 crypt circumferences were calculated from each of the animals at each site. The mean crypt height was multiplied by the mean of crypt circumference to estimate the total number of cells per crypt.[44] The labeling index (%) was determined by dividing the number of labeled cells by the total cells in the crypt column and multiplying by 100. The proliferative zone (%) was calculated by dividing the position of the highest labeled cell by the total cells in the crypt column and multiplying by 100. The means of 20 labeling indexes and proliferative zones were calculated from each of the animals. Individual animal means were then used to determine means ± SE for each dietary group.

### 8. Statistical Analyses

All data were analyzed using one-way analysis of variance (ANOVA). When the $p$ values were less than 0.05, means were separated using Duncan's new multiple range test. Correlations of short-chain fatty acids with cell proliferation were determined using simple regression analysis.

## RESULTS

### 1. Food Intake, Energy Intake and Weight Gain

The final body weight tended to be lower in the groups fed pectin- and polydextrose-supplemented diets, resulting in lower body weight gain compared to the other groups. However, these differences were not statistically significant (Table 3). No significant differences were

**Table 3.** Effect of Dietary Fibers on Body Weight, Food Intake, and Energy Intake[1,2]

| | Diets | | | | | *p* value |
|---|---|---|---|---|---|---|
| | FF | CE | PE | PO | PC | |
| Body wt, g | | | | | | |
| Initial | 330± 6 | 325± 8 | 325± 8 | 328±6 | 331± 9 | 0.9665 |
| Final | 389±13 | 386±12 | 377±15 | 375±7 | 385±10 | 0.8958 |
| Wt gain, g/3 wk | 66± 8 | 61± 8 | 51± 9 | 47± 6 | 54± 4 | 0.3590 |
| Food intake, g/day | 22.9±0.9 | 19.7±2.3 | 22.6± 3.2 | 19.7±1.4 | 19.7±2.4 | 0.6786 |
| Energy intake,[3] kcal/day | 100.9±4.2 | 81.5±9.6 | 93.6±13.2 | 81.6±6.0 | 81.5±9.9 | 0.4333 |

1 : Values are means±SE of 10 rats/group.
2 : Abbreviations are as follows: FF, fiber-free; CE, cellulose; PE, pectin; PO, polydextrose; PC, polydextrose + cellulose.
3 : Energy intake(kcal) = (g of carbohydrate intake × 4kcal/g) + (g of protein intake × 4kcal/g) + (g of fat intake × 9kcal/g).

**Table 4.** Effect of Dietary Fibers on Fecal Output[1,2]

| | Diets | | | | | *p* value |
|---|---|---|---|---|---|---|
| | FF | CE | PE | PO | PC | |
| Fecal output, g/72h | | | | | | |
| Wet wt | $4.5\pm0.1^{c}$ | $8.9\pm0.4^{a}$ | $5.9\pm0.5^{b}$ | $8.2\pm0.5^{a}$ | $8.9\pm0.3^{a}$ | 0.0000 |
| Dry wt | $3.2\pm0.1^{d}$ | $6.8\pm0.3^{a}$ | $4.1\pm0.2^{c}$ | $4.8\pm0.3^{c}$ | $5.9\pm0.1^{b}$ | 0.0000 |
| Moisture content,[3] % | $26.7\pm1.3^{b}$ | $24.2\pm1.5^{c}$ | $28.0\pm3.0^{b,c}$ | $40.2\pm1.0^{c}$ | 32.0±1.7b | 0.0000 |

1 : Vllues lre melns±SE of 10 rats/group. Abbreviations as in Table 3.
2 : Means in the same row with different symbols(a, b, c, d) are significantly different($p<0.05$).
3 : Moisture content(%)={(g of wet feces - g of dry feces)/g of wet feces} × 100.

**Table 5.** Effect of Dietary Fibers on Colon Length and Weight[1,2]

| | Diets | | | | | *p* value |
|---|---|---|---|---|---|---|
| | FF | CE | PE | PO | PC | |
| Colon length, cm | $20.0\pm0.2^{b}$ | $21.3\pm0.4^{a}$ | $21.5\pm0.3^{a}$ | $22.2\pm0.4^{a}$ | $21.8\pm0.5^{a}$ | 0.0020 |
| Colon weight, g | | | | | | |
| Proximal | $0.66\pm0.01^{b}$ | $0.82\pm0.03$ | $0.81\pm0.04^{a}$ | $0.80\pm0.03^{a}$ | $0.78\pm0.04^{a}$ | 0.0057 |
| Distal | $0.64\pm0.02^{b}$ | $0.76\pm0.02^{b}$ | $0.79\pm0.02^{a,b}$ | $0.86\pm0.03^{a}$ | $0.78\pm0.05^{a,b}$ | 0.0002 |

1 : Values are means±SE of 10 rats/group. Abbreivations as in Table 3.
2 : Means in the same row with different symbols (a,b) are significantly different($p<0.05$).

found in daily food intake and daily energy intakes among the diet groups (Table 3).

## 2. Fecal Output and Fecal Moisture Content

Seventy-two-hour fecal output was significantly different among the diet groups ($p<0.05$), as shown in Table 4. Both wet and dry weights of feces were significantly greater in all the fiber-supplemented diet groups compared to the fiber-free diet group. Among the types of dietary fiber supplemented to the diets, cellulose supplementation resulted in the greatest fecal output, whereas pectin supplementation resulted in the least fecal output. In the groups fed polydextrose-supplemented diets, wet weight of feces was similar to that in the group fed the cellulose-supplemented diet. However, when feces were dried, because of a high fecal moisture content in the group fed polydextrose-supplemented diets, fecal weight was reduced by 40% in the group fed 6% polydextrose-supplemented diet and by 32% in the group fed the mixture of 3% polydextrose and 3% cellulose-supplemented diet.

## 3. Colon Length and Weight

As shown in Table 5, colons were significantly longer in the groups fed fiber-supplemented diets than in the group fed the fiber-free diet ($p<0.05$). However, there were no significant differences among the groups fed fiber-supplemented diets. The effects of feeding rats with the fiber-supplemented diets on colon weight were dependent on the site of the colon. That is, the proximal colon was significantly heavier in all the groups fed fiber-supplemented diets compared to the group fed

**Table 6.** Effect of Dietary Fibers on short-chain fatty acids(SCFA) in Proximal Colon[1-3]

| | Diets | | | | | *p* value |
|---|---|---|---|---|---|---|
| | FF | CE | PE | PO | PC | |
| Acetate | $12.08\pm1.63^{b}$ | $0.06\pm0.01$ | $19.04\pm1.55^{a}$ | $11.58\pm1.06^{b}$ | $11.14\pm1.04^{b}$ | 0.0000 |
| Propionate | $5.82\pm0.62^{b}$ | $0.02\pm0.01$ | $8.73\pm1.14^{a}$ | $7.65\pm0.97^{a}$ | $7.87\pm1.10^{a}$ | 0.0000 |
| Isobutyrate | $1.43\pm0.09^{a}$ | *N.S* | $1.10\pm0.12^{a,b}$ | $0.86\pm0.04^{b}$ | $1.00\pm0.08^{a,b}$ | 0.0059 |
| Butyrate | $2.24\pm0.29^{b}$ | $0.01\pm0.00$ | $5.28\pm0.77^{a}$ | $2.88\pm0.46^{b}$ | $2.73\pm0.34^{b}$ | 0.0000 |
| Isovalerate | $1.28\pm0.07^{a}$ | *N.S* | $0.73\pm0.08^{b}$ | $0.78\pm0.19^{b}$ | $1.58\pm0.55^{a}$ | 0.0016 |
| Valerate | $2.36\pm0.10^{a}$ | *N.S* | $2.25\pm0.21^{a}$ | $1.67\pm0.09^{b}$ | $1.73\pm0.10^{b}$ | 0.0038 |
| Total SCFA | $25.21\pm2.64^{b}$ | $0.09\pm0.02$ | $37.13\pm3.45^{a}$ | $25.42\pm2.39^{b}$ | $26.05\pm2.61^{b}$ | 0.0000 |

1 : Values are means±SE of 10 rats/group and expressed as umol/g of feces. Abbreviations as in Table 3.
2 : Values less than 0.01 are considered as non-significant(N.S).
3 : Means in the same row with different symbols(a, b, c) are significantly different($p<0.05$).

**Table 7.** Effect of Dietary Fibers on SCFA in Distal Colon[1-3]

| | Diets | | | | | *p* value |
|---|---|---|---|---|---|---|
| | FF | CE | PE | PO | PC | |
| Acetate | $8.74\pm1.16^{b}$ | $0.04\pm0.01^{c}$ | $14.04\pm1.14^{c}$ | $10.37\pm1.07^{b}$ | $11.40\pm1.67^{a,b}$ | 0.0000 |
| Propionate | $2.79\pm0.51^{b}$ | $0.01\pm0.00^{c}$ | $8.18\pm1.43^{a}$ | $6.00\pm0.92^{a}$ | $5.65\pm0.94^{a}$ | 0.0000 |
| Isobutyrate | $1.04\pm0.08^{a,b}$ | *N.S* | $1.33\pm0.18^{a}$ | $0.82\pm0.03^{b}$ | $0.91\pm0.07^{b}$ | 0.0020 |
| Butyrate | $1.36\pm0.20^{b}$ | $0.01\pm0.00^{c}$ | $5.16\pm0.83^{a}$ | $1.61\pm0.23^{b}$ | $1.98\pm0.41^{b}$ | 0.0000 |
| Isovalerate | $1.10\pm0.14^{a,b}$ | *N.S* | $1.47\pm0.29^{a}$ | $0.51\pm0.04^{b}$ | $0.91\pm0.15^{b}$ | 0.0071 |
| Valerate | $1.89\pm0.09^{a,b}$ | *N.S* | $2.22\pm0.30^{a}$ | $1.59\pm0.04^{b}$ | $1.73\pm0.11^{b}$ | 0.0030 |
| Total SCFA | $16.81\pm2.01^{b}$ | $0.08\pm0.01^{c}$ | $32.10\pm4.32^{c}$ | $19.66\pm1.66^{b}$ | $21.25\pm2.27^{b}$ | 0.0000 |

1: Values are means±SE of 10 rats/group and expressed as umol/g of feces. Abbreviations as in Table 3.
2: Values less than 0.01 are considered as non-significant(N.S).
3: Means in the same row with different symbols(a, b, c) are significantly different($p<0.05$).

fiber-free diet($p<0.05$), whereas the distal colon was significantly heavier only in the group fed polydextrose-supplemented diets ($p<0.05$).

## 4. Luminal Short-Chain Fatty Acid (SCFA) Concentrations

In the proximal colon, as shown in Table 6, cellulose was almost not fermented, resulting in the lowest luminal concentrations of total SCFA (sum of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate) in the group fed cellulose-supplemented diet ($p<0.05$). In contrast, the pectin-supplemented diet produced significantly higher concentrations of total SCFA, compared to the other fiber-supplemented diets and fiber-free diet ($p<0.05$). The pectin-supplemented diet produced significantly higher concentrations of acetate, propionate, and butyrate compared to the other fiber-supplemented diets and fiber-free diet, whereas the supplementation of diets with polydextrose by 6% or 3% produced significantly higher concentrations of propionate only compared to the fiber-free diet ($p<0.05$). Although luminal concentrations of SCFA were significantly different among the diet groups, the degree of individual SCFA production was very similar; that is, concentrations of acetate were the highest in all diet groups, followed by propionate and butyrate, respectively ($p<0.05$).

Although luminal SCFA concentrations were lower in the distal colon than in the proximal colon, the effects of different diets on SCFA production were similar to those in the proximal colon; that is, compared to SCFA concentrations in the fiber-free diet group, significantly increased concentrations of acetate, propionate and butyrate through pectin supplementation, and significantly increased concentrations of propionate through the polydextrose supplementation, were found ($p<0.05$)(Table 7).

## 5. Colon Cell Proliferation

As shown in Table 8, there were no significant differences in crypt size and proliferative activity of the proximal colon. Although the pectin-supplemented diet seemed to produce the highest labeling index (LI) followed by the polydextrose-supplemented diet, the differences were not statistically significant.

Unlike in the proximal colon, dietary fiber significantly affected proliferative activity of the distal colon ($p<0.05$), but no differences were found in crypt size among the

**Table 8.** Effect of Dietary Fibers on Proximal Colon Crypt Size and Proliferative Activity[1,2]

| | Diets | | | | | *p* value |
|---|---|---|---|---|---|---|
| | FF | CE | PE | PO | PC | |
| Crypt height, no. of cells | 32.2±1.8 | 32.5±1.6 | 34.3±3.6 | 31.9±2.3 | 34.6±2.1 | 0.8894 |
| Crypt circumference, no. of cells | 18.2±1.0 | 18.1±0.9 | 16.4±0.8 | 18.3±1.6 | 19.3±0.6 | 0.4073 |
| Total no. of cells in crypt | 590±59 | 594±55 | 582±64 | 603±97 | 673±61 | 0.8829 |
| LI, % | 10.2±0.7 | 10.8±0.5 | 13.8±2.9 | 13.2±1.5 | 11.8±1.1 | 0.4545 |
| PZ, % | 49.6±1.2 | 48.8±1.7 | 53.1±3.1 | 46.7±1.8 | 50.1±1.5 | 0.2560 |

1 : Values are means±SE of 10 rats/group.
2 : Abbreviations are as follows: LI, labeling index; PZ, proliferative zone; other abbreviations as in Table 3.

**Table 9.** Effect of Dietary Fibers on Distal Colon Crypt Size and Proliferative Activity[1-3]

| | Diets | | | | | *p* value |
|---|---|---|---|---|---|---|
| | FF | CE | PE | PO | PC | |
| Crypt height, no. of cells | 37.7±1.9 | 38.1±2.5 | 39.0±3.3 | 41.3±2.0 | 41.8±1.8 | 0.6506 |
| Crypt circumference, no. of cells | 20.2±1.4 | 19.5±0.8 | 15.9±1.3 | 21.2±1.0 | 20.5±1.3 | 0.1045 |
| Total no. of cells in crypt | 774±83 | 752±70 | 781±56 | 879±73 | 868±91 | 0.6646 |
| LI, % | $10.9\pm0.7^{b}$ | $11.8\pm0.8^{b}$ | $17.9\pm2.6^{a}$ | $16.2\pm1.4^{a,b}$ | $11.9\pm1.0^{b}$ | 0.0041 |
| PZ, % | $28.2\pm3.6^{b}$ | $31.4\pm1.7^{a,b}$ | $41.0\pm3.1^{a}$ | $37.5\pm2.8^{a,b}$ | $31.5\pm1.5^{a,b}$ | 0.0097 |

1 : Values are means±SE of 10 rats/group. 17
2 : Abbreviations are as follows: LI, labeling index; PZ, proliferative zone; other abbreviations as in Table 3 and 8.
3 : Means with different symbols (a, b) are significantly different ($p < 0.05$).

diet groups. As shown in Table 9, the pectin-supplemented diet produced significantly higher LI than any of the fiber-free diet, the cellulose-supplemented diet, or the mixture of cellulose and polydextrose-supplemented diet. The pectin-supplemented diet also resulted in a larger proliferative zone (PZ) in the distal colon compared to the fiber-free diet ($p<0.05$). The polydextrose-supplemented diet produced intermediate effects on LI and PZ.

## 6. Correlations between cell proliferation and short-chain fatty acids (SCFAs)

As shown in Table 10, concentrations of luminal SCFAs were significantly correlated with proliferative activity in both the proximal and distal colon ($p<0.05$). Among the SCFAs, butyrate was significantly correlated with the labeling index (LI) in the proximal colon ($p$= 0.0503), and both propionate and butyrate were significantly correlated with the LI ($p$=0.0498, 0.0409 respectively) and with the proliferative zone (PZ) ($p$=0.0517, 0.0538 respectively) in the distal colon, indicating that colon cell proliferation was increased as these SCFAs were produced in greater amounts. Although acetate was produced in the greatest amount in both the proximal and distal colon, its correlations to cell proliferation were not as strong as those of propionate and butyrate.

**Table 10.** Correlations between SCFA and colon cell proliferation[1,2]

| Cell Proliferation | Proximal colon | | Distal colon | |
|---|---|---|---|---|
| | LI | PZ | LI | PZ |
| Acetate | 0.63 (0.1478) | 0.56 (0.3899) | 0.66 (0.1932) | 0.63 (0.2111) |
| Propionate | 0.65 (0.1522) | 0.32 (0.5806) | 0.77 (0.0498) | 0.75 (0.0517) |
| Butyrate | 0.77 (0.0503) | 0.61 (0.2926) | 0.76 (0.0409) | 0.75 (0.0538) |
| Total SCFA | 0.61 (0.1980) | 0.49 (0.4969) | 0.69 (0.1298) | 0.66 (0.2036) |

1 : Values are correlation coefficients (r). Abbreviations as in Table 8.
2 : Values in the parenthesis are p values

## DISCUSSION

Dietary fiber is a heterogeneous group of compounds with different physicochemical properties, contributing in part to a diversity in colonic microbial fermentation. Generally, soluble fiber is found to be more fermentable in the colon compared to insoluble fiber, so they exert less of a bulking effect.[46)47)] In addition, the degree of microbial fermentation in the colon appears to be depen-

dent on the specific fiber and on the specific region of the colon being considered. For example, even if most soluble fibers are fermentable in the colon, the degree of fermentation varies significantly among those. With regard to differences of fermentation at different sites of the colon, dietary fiber like pectin and oat bran is fermented to a greater degree in the proximal colon than in the distal colon,[46-48] whereas wheat bran appears to be more fermentable in the distal colon.[28)29)] The results of the present study support the general findings from similar studies: that is, 1) soluble fiber, pectin and polydextrose were more fermentable in the colon than the insoluble fiber, cellulose, which was almost not fermented; 2) pectin was more fermentable compared to polydextrose in both the proximal and distal colon; and 3) both pectin and polydextrose were fermented to a greater degree in the proximal colon than in the distal colon.

Major short-chain fatty acids (SCFAs) produced as a result of the microbial fermentation of dietary fiber in the colon are acetate, propionate and butyrate. However, the concentrations of these individual SCFAs are significantly influenced by the types of dietary fiber. With regard to the molar ratios of acetate, propionate and butyrate, we found that rats consuming the fiber-free diet produced ratios of 60 : 29 : 11 in the proximal colon and ratios of 68 : 22 : 10 in the distal colon. Because pectin supplementation produced relatively higher concentrations of butyrate in the proximal colon compared to the other SCFAs, and of both propionate and butyrate in the distal colon compared to acetate, the ratios of the three SCFA were altered to 58 : 26 : 16 in the proximal colon and to 51 : 30 : 19 in the distal colon. The alterations in the ratios of these SCFAs through polydextrose supplementation were quite different from those alterations resulting from pectin supplementation; polydextrose supplementation produced relatively higher concentrations of propionate compared to the other SCFAs in both the proximal and distal colon, resulting in ratios of 52 : 35 : 13 in the proximal colon and of 58 : 33 : 9 in the distal colon. Breves and Stuck[8)] have reported that acetate, propionate and butyrate are typically produced within the colonic lumen in the molar ratios of 60 : 25 : 15, respectively. However, these ratios were altered to 45 : 15 : 35 in the rats consuming the oat bran diets and to 65 : 10 : 20 in the rats consuming the wheat bran diets, suggesting that different dietary fiber sources affect both the absolute amounts of each SCFA produced in the colon as well as the relative ratios of the SCFA concentrations.[48)]

In this study, we noted that the diet supplemented with 6% pectin produced a significantly increased effect on cell proliferation of the distal colon, and the diet supplemented with 6% polydextrose produced an intermediate effect. When the supplemented level of polydextrose was reduced to 3%, and the other 3% of dietary fiber was supplemented with cellulose, however, the relatively increased effect of polydextrose disappeared. The hyperproliferative effects of pectin on rat colon have been found in several other studies,[20)21)23)24)46)] but the effects of polydextrose on colon cell proliferation have not been investigated. This is the first study demonstrating what the effects of polydextrose on colon cell proliferation are. Because colonic epithelial cell proliferation has been used as a biomarker of colon carcinogenesis, the hyperproliferative effects of pectin and polydextrose can be interpreted in such a way that, unlike cellulose, these highly fermentable dietary fibers may be promotive rather than protective against colon cancer. Although an increased consumption of dietary fiber has generally been recognized as protective against colon cancer,[16)40)] not all dietary fiber exerts such effects. Some soluble fiber such as pectin, oatbran and guar have been reported to enhance experimentally induced colon carcinogenesis.[6)19)21)42)]

There have been several suggested mechanisms by which specific types of dietary fiber exert differential effects on colon carcinogenesis; that is, differential effects on dilution of carcinogens and co-carcinogens,[12)] gastrointestinal transit time,[26)] microbial production of SCFAs resulting from microbial fermentation of dietary fiber,[28)46-48)] and alteration of luminal pH.[25)] Among those, the theory which is the most recent focus of attention is that butyrate, one of the major SCFAs produced by microbial fermentation of dietary fibers, protects against colon cancer by inhibiting colonic cell proliferation and promoting differentiation and perhaps apoptosis.[27)] After a very thorough review of the chain of scientific evidence, however, Lupton[27)] suggested that this theory is not warranted by the current data because *in vitro* data with human biopsies or *in vivo* data in animals do not support this theory. Although butyrate clearly inhibits cell growth and promotes differentiation in a variety of cell lines,[22)30)] including those derived from colon carcinomas,[11)14)13)15)] it appears to have the opposite effect *in vivo*.[35)34)] Sheppach et al.[37)] found a hyperproliferative effect of butyrate on nomal human colonic biopsies, suggesting that the disparity between *in vitro* and *in vivo* data is more likely related to the difference between transformed cells and normal colonocytes. In the present study, we found that an increased production of butyrate was significantly correlated with an increased cell proliferation in both the proximal and distal colon, supporting a hyperproliferative effect of butyrate found in most *in vivo* studies using experimental animals. In the distal colon, the luminal concentrations of propionate, another type of major SCFA, were also positively correlated to cell proliferation. However, its effect on colon cell proliferation is not well understood, and may need to be further ex-

plored.

In summary, the results of the present study showed that 1) microbial fermentation of dietary fiber varied according to the type of dietary fiber and to the sites of the colon: the highest SCFA production was found in the pectin-supplemented diet group, followed by the polydextrose-supplemented diet group, and a greater degree of fermentation was found to be in the proximal colon compared to the distal colon; 2) the concentrations of individual SCFAs were significantly influenced by the types of dietary fiber: relatively higher levels of butyrate were produced by the pectin-supplemented diet and higher levels of propionate were produced by the polydextrose-supplemented diet; 3) the highly fermentable dietary fiber, pectin, significantly increased cell proliferation in the distal colon; 4) the artificially synthetic polysaccharide, polydextrose, produced a relatively increased effect on cell proliferation compared to cellulose; and 5) increased cell proliferation was correlated with increased luminal concentrations of butyrate in the proximal colon and with increased luminal concentrations of propionate and butyrate in the distal colon. In conclusion, these results suggest that dietary fibers may modulate colon cell proliferation by altering luminal SCFA concentrations, particularly for butyrate and perhaps propionate. Additionally, the present study is the first finding which demonstrated a relatively increased effect of polydextrose on colon cell proliferation, suggesting that the overuse of this artificially synthesized polysaccharide in food processing technology needs to be carefully evaluated regarding potentially undesirable health consequences.

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