Dietary fibre on cell proliferation in large bowel mucosal crypts near or away from lymphoid nodules and on mineral bioavailability

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Abstract. The effect of consumption for 24 weeks of different amounts (0%, 5% or 10% w/w) of fermentable (pectin and guar gum) or nonfermentable (cellulose and lignin) dietary fibres on cell proliferation and other parameters in large bowel mucosal crypts was studied in rats. In all 12 dietary groups, the crypts located over the distal aggregate of lymphoid nodules (ALN) had more colchicine arrested metaphase figures per midaxial crypt section (MC) and a longer crypt column height than crypts located three to four cm away from this ALN. These differences are attributed to the tropic influence of nodular cells in the ALN. Consumption of fermentable fibre decreased pH in the lumen of the caecum, and glucose, Zn and Cu in serum but increased Ca and Mg in serum. The decrease in caecal pH and serum glucose was significantly correlated with a decrease in MC. Increased intake of the nonfermentable fibre types increased faecal bulk but had no significant correlation with the other measured crypt parameters. Multiple regression analyses was used to model the relationships between the mucosal crypt criterion variables and the two measured predictor variables, caecal pH and serum glucose. Relationships between dietary fibre, ALN, MC, bioavailability of dietary minerals and risk of colorectal cancer are discussed.

INTRODUCTION

Dietary modification can cause adaptive changes in the structure and function of the mucosa of the large bowel. For example, the mitotic index in the distal large intestine is responsive to: parenteral *vs.* enteral feeding, caloric intake, and the level of dietary cellulose (Cameron *et al.* 1990), the ratio of dietary sucrose to fermentable starch (Bianchini *et al.* 1992), and the ratio of dietary fat to protein (Lin & Visek 1990).

The study reported here deals with the long-term (24 weeks) consumption of different types and amounts of two fermentable dietary fibres, pectin (P) and guar gum (GG), and of two nonfermentable dietary fibres, cellulose (C) and lignin (L), on the mitotic index in the distal large bowel crypts and on the bioavailability of dietary minerals. Fermentable dietary fibre

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types are decomposed into short chain fatty acids by microflora, especially in the proximal segment of the large bowel, resulting in acidification of the luminal contents. The lowering of luminal pH may directly or indirectly influence cell proliferation of intestinal crypt epithelial cells and the bioavailability of dietary minerals. Nonfermentable dietary fibres are not digested by gut microflora but can increase the volume of faeces, speed gut transit time and dilute the faecal contents.

To help determine how dietary fibre works to modify bowel mucosa structure and function, measures were made on body weight, faecal dry mass, caecal luminal pH, levels of serum glucose, calcium (Ca), magnesium (Mg), copper (Cu) and zinc (Zn) and on: mitotic index, crypt cell column height and mean proliferative zone height in midaxial histological sections of large bowel mucosal crypts taken over the distal aggregate of lymphoid nodules (ALN) or 3–4 cm proximal to this ALN. All of the data collected were subjected to regression analyses and the results used to account for the observed changes.

MATERIALS AND METHODS

Animals

One hundred and twenty, male Sprague–Dawley rats were obtained from Harlan (Houston, TX, USA). Rats were housed two per cage in solid plastic cages with high wire mesh false bottoms to minimize access to the bedding or faeces. The animal housing room was well ventilated (20 air changes per hour) with a temperature of 25°C, and automatically controlled light/dark cycle of 14/10 h. All animals had free access to food and deionized water during the entire experiment. Approval of the Institutional Animal Care and Use Committee at the UTHSCSA was received prior to commencing any animal experimentation.

Diets

Compositions of the experimental diets are listed in Table 1. The diets were formulated so that each kilocalorie (kcal) of food consumed would provide an equal quantity of protein, complex carbohydrates, vitamins and minerals. This balance was accomplished by decreasing the sucrose content of the food as the fermentable fibre or corn oil content was increased.

Diet components

Purified high nitrogen casein, pure corn starch, AIN-76 vitamin mixture, AIN-76 mineral mixture, choline bitartrate (99% pure) and Alphacel (non-nutritive bulk cellulose) were obtained from ICN Nutritional Biochemicals, Cleveland, OH, USA. Wesson brand corn oil (100% pure, no additives) and Imperial brand (Sugarland, TX, USA) extra fine pure cane sugar were purchased locally. D.L. methionine (cell culture quality, M.W. 149.2) and citrus pectin (without sucrose, acid content 86%, methoxy content 9.6%) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Autohydrolysed lignin was obtained from Iogen Corp. (Ottawa, Canada).

Experimental design

Ten- to 12-week old rats were randomly paired, assigned cages and ears were marked for identification. Ten days were allowed for adjustment to their new environment.

Pairs of rats were then randomly divided into 12 groups, 10 rats per group. During the adjustment period all rats were fed standard AIN-76 formula food (with 5% cellulose and 5% corn oil) and deionized water. Food consumption for each cage of two rats and individual rat body weights were recorded weekly.

Table 1. Composition of the diet (g/100 g)

	0% fibre	5% pectin or guar gum	10% pectin or guar gum or 5% P/5% GG	5% cellulose or lignin	10% cellulose or lignin	10% corn oil, 10% pectin	20% corn oil, 10% pectin
Ingredient							
Corn oil	5.0	5.0	5.0	5.0	4.7	10.0	20.0
Sugar	53.0	48.6	43.8	50.0	47.4	36.4	21.5
Casein	21.0	20.7	20.6	20.0	18.9	21.8	24.3
Cornstarch	15.7	15.6	15.4	15.0	14.2	16.4	18.2
Vitamin and minerals	5.2	5.1	5.0	5.0	4.7	5.5	6.1
Fibre	0.0	5.0	10.0	5.0	10.0	10.0	10.0
Energy content of diet (kcal/g)*	4.05			3.90	3.66	4.21	4.69
Pectin diet (kcal/g)*	4.01	3.97					
Guar gum diet (kcal/g)*		4.00	3.94				
5% P, 5% GG (kcal/g)*			3.96				

*Caloric content is calculated at 4 kcal/g for protein and carbohydrate, 9 kcal/g for fat, 3.2 kcal/g for pectin and 2.9 cal/g for guar gum. For each kcal ingested the rats received equal vitamins, minerals, protein and complex carbohydrates (starch), thus equal calorie intake yields equal nutrient intake.

Diet components and chemicals: purified high nitrogen casein, pure corn starch, Alphacel (non-nutritive bulk cellulose) AIN-76 vitamin mixture, AIN-76 mineral mixture and choline bitartrate (99% pure) was obtained from ICN Nutritional Biochemicals, Cleveland, OH, USA. Imperial brand (Sugarland, TX, USA) extra fine pure cane sugar and 100% pure corn oil (Wesson) was purchased locally. D.L. methionine (cell culture, M. W. 149.2) was purchased from Sigma, St. Louis, MO, USA.

At 22 weeks after the start of the experiment, six randomly selected rats from each experimental group were assigned individually to a metabolic cage for careful monitoring of faecal output. Readings did not begin until the third day in the metabolic cage so that the rats would have time for adaptation to the new environment. Faecal samples were collected daily for the next 3 days, pooled for each individual rat and were used to determine mean faecal dry weight per day per rat.

Faecal dry weight

Faeces were dried in a vacuum oven (100 °C) until constant weight was obtained for 3 consecutive days.

Tissue preparation

The rats were sacrificed after consuming the experimental diets for 26 weeks. Sacrifice was between 0900 and 1200 h each day to minimize the effect of diurnal variation on the proliferative parameters. Exactly 3 h before scheduled sacrifice, each rat was weighed and given an intraperitoneal injection of colchicine (1 mg/kg body weight dissolved in normal saline). This drug arrests all cells entering the metaphase stage of mitosis during the 3 h following the colchicine injection.

The rats were ether anaesthetized, then killed by decapitation. Blood for serum glucose and mineral analyses was collected from the carotid artery. The entire large bowel of each rat was immediately removed.

Luminal pH in the caecum

The caecum was split open to expose the contents for pH measurements. A calibrated Beckman pH meter (model $\Phi 40$) with a Fisher combination surface electrode was quickly immersed into the intestinal contents until pH equilibration (< 60 s). The electrode was rinsed with Scope mouthwash (Proctor and Gamble, Cincinnati, OH, USA) to remove mucous and distilled water between the pH readings. The pH measurement was repeatedly validated using a pH standard.

Analysis of cell proliferation in the large bowel crypts

Descending colon and rectum were gently stretched to remove longitudinal folds, pinned flat serosal side down onto corkboard, then fixed in 10% buffered formalin. Distal segments of large bowel from five rats were randomly selected from each diet group for analyses of cell proliferation in the mucosal crypts. Tannin, leached from the corkboard onto which the bowel was pinned, differentially stained the rectal aggregate of lymphoid nodules (ALN); ALN were confirmed by histological sections. Histological sections of segments that were excised from either over the rectal ALN or from 5.5 to 6.5 cm proximal to the anus (where no ALN occur) were used to score the cell proliferation parameters of colonic crypts located over or away from the rectal ALN, respectively. The bowel segments were embedded on edge in paraffin blocks. Sections of paraffin blocked tissue were cut 4 μ m thick, placed on microscope slides then stained with haematoxylin and eosin.

Complete mid-axially sectioned crypts were defined as those having: (1) the base adjacent to the muscularis mucosa (2) the lumen visible from the base to the mouth of the crypt and (3) a single column of epithelial cells from the base to the mouth. Complete, midaxial crypts in each region of the colon were scored as they were encountered on scanning the histological slides without any other selection.

To score the cell proliferation parameters, cells were counted from the base up each side of the complete mid-axially sectioned crypts to the mouth of the crypt. The recorded parameters were: the total height of the crypt in number of cells from the base to the mouth (CH), the total number of colchicine arrested, metaphase figures per mid-axial crypt section (MC) and the position of each metaphase figure along the cell column. The proliferative zone height (PZH) was calculated as the mean of the heights of the metaphase figures. At least three crypts over and five crypts away from the rectal ALN were scored from each of the rats.

Serum mineral analyses

Blood serum concentrations of Ca, Cu, Mg and Zn were determined with an atomic absorption spectrophotometer equipped with a doublebeam background corrector and a microprocessor. The flow rates of acetylene and air were controlled at 2.1 and 3.2 L/min, respectively. The solutions of metal standards were prepared from reference standards (Fisher Scientific, Dallas, TX, USA). Serum samples were diluted with deionized-distilled water.

Serum glucose

Rat serum specimens were sent to University Hospital Clinical Pathology Laboratory (San Antonio, TX, USA) for routine serum glucose analyses. Glucose standards were run for quality assurance of the assay. The laboratory is certified by the College of American Pathologists.

Statistical analyses

Linear regression analyses (SAS Statistical Package, SAS Institute, Cary, NC, USA) were used to determine the correlations between the mucosal crypt parameters: PZH, CH, or MC and other measured variables: pH, glucose, Ca, Cu, Mg or Zn. The correlation matrix between all variables was used to deduce biologically relevant correlations for entry into a model. Graphs and linear regression analyses were generated using Prism® (GraphPad Software, Inc., San Diego, CA, USA). Analyses of variance (ANOVA) followed by a Student–Newman–Keuls (SNK) multiple range test (SAS software) was used to determine significant differences in mean caecal pH of groups of rats which consumed 0%, 5% or 10% fermentable fibre. A student's *t*-test was used to test for significant differences in serum glucose or caecal pH between groups which consumed or did not consume fermentable dietary fibre and was also used to test for significant differences in the proliferative parameters of mucosal crypts located over or away from the ALN. A probability value < 0.05 was used to indicate significant differences between groups. A MannWhitney (nonparametric *t*-test) test was used to determine significant differences in the median levels of serum Ca, Mg, Cu or Zn in groups which consumed or did not consume fermentable fibre.

RESULTS

The mean $\pm$ SE of caecal pH, body weight, and faecal dry weight for each group of rats is presented in Table 2.

ANOVA followed by SNK showed that the mean body weight of the three heaviest groups (Table 2) was significantly more than the mean body weight of the two lightest groups of rats. However the weight difference did not seem to be related to type or amount of dietary fibre.

Rats were grouped by fermentability of the dietary fibre for analyses of faecal dry weight. ANOVA followed by SNK revealed that the mean faecal dry weight of the rats which

Table 2. Effects of dietary fibre type and quantity, and sucrose content (complete diet composition in Table 1) on means of body weight, caecal pH and faecal dry weight*

Dietary type and percentage of fibre [†]	Percentage sucrose in diet	Caecal pH (means $\pm$ SE)	Body weight (mean g $\pm$ SE)	Faecal dry weight (mean g/day $\pm$ SE)
5% corn oil diets				
0% fibre	53	7.08 $\pm$ 0.06	498 $\pm$ 11	0.50 $\pm$ 0.05
5% C	50	7.06 $\pm$ 0.03	479 $\pm$ 15	1.37 $\pm$ 0.04
10% C	47	6.90 $\pm$ 0.06	474 $\pm$ 15	2.35 $\pm$ 0.08
5% L	50	7.21 $\pm$ 0.06	473 $\pm$ 5	1.36 $\pm$ 0.03
10% L	47.4	7.11 $\pm$ 0.07	489 $\pm$ 7	2.62 $\pm$ 0.12
5% P	48.6	6.82 $\pm$ 0.08	449 $\pm$ 9	0.44 $\pm$ 0.04
10% P	43.8	6.69 $\pm$ 0.09	458 $\pm$ 11	0.71 $\pm$ 0.02
5% GG	48.6	6.94 $\pm$ 0.07	500 $\pm$ 8	0.61 $\pm$ 0.08
10% GG	43.8	6.27 $\pm$ 0.16	467 $\pm$ 12	0.86 $\pm$ 0.01
5% P/5% GG	43.8	6.70 $\pm$ 0.11	435 $\pm$ 12	0.53 $\pm$ 0.10
> 5% corn oil diets				
10% P/10% CO	36.4	6.58 $\pm$ 0.10	475 $\pm$ 15	0.78 $\pm$ 0.05
10% P/20% CO	21.5	6.41 $\pm$ 0.07	504 $\pm$ 8	0.78 $\pm$ 0.01

*The faecal dry mass data of the 0% fibre and lignin groups are from (Cameron *et al.* 1997) whereas the other faecal dry mass data are reported here for the first time. The body weight and pH data are from (Carter *et al.* 1998), except the 5% P/5% GG data. The 5% corn oil data are from (Hardman & Cameron 1995). $n = 8$ –12 per group.

[†]C, cellulose; P, pectin; GG, Guar gum; L, lignin; CO, corn oil.

consumed the no fermentable fibre diets (cellulose and lignin) was significantly ($P < 0.05$) greater than the mean faecal dry weight of rats which consumed the 0% fibre and the fermentable fibre (pectin and guar gum) diets.

ANOVA followed by SNK of the caecal pH of rats grouped by amount (0%, 5% or 10% w/w) of fermentable dietary fibre, presented in Fig. 1, reveals that the mean caecal pH of the groups of rats which consumed 10% fermentable dietary fibre (pectin or guar gum) was significantly lower than the mean caecal pH of rats which consumed 0% or 5% fermentable dietary fibre. The group which consumed 5% fermentable fibre had a mean caecal pH that was intermediate between the pH values of the 0% and 10% fermentable fibre groups

Figure 1. Amount of fermentable fibre in the rat food with pH in the lumen of the caecum (mean $\pm$ SE). ANOVA followed by SNK revealed that the means of caecal pH of groups of rats fed either 0 or 5% fermentable fibre were significantly greater than the mean caecal pH of the group of rats fed 10% w/w fermentable fibre.

Figure 2. Correlation between caecal pH and serum glucose concentration (mean $\pm$ SE). $\square$ indicate the means $\pm$ SE from groups of rats fed fermentable fibre; $\blacksquare$ indicate the means $\pm$ SE from groups of rats fed no fermentable fibre. The results of the statistical analysis of the data in this figure are reported in the text.

indicating that pH decreased in response to an increased dosage of fermentable dietary fibre.

When rats were grouped by whether or not the diet contained fermentable fibre, results of a student's *t*-test revealed that the mean caecal pH of rats fed no fermented fibre (open squares in Fig. 2) was significantly ($P < 0.05$) higher than the mean caecal pH of the rats fed fermentable fibre, solid squares in Fig. 2.

Further analysis revealed the relationships of caecal pH to the serum levels of glucose, Ca, Mg, Cu and Zn. Linear regression analysis of data for all groups (Fig. 2), indicated a significant positive relationship between caecal pH and serum glucose ($r = 0.72$, $P < 0.01$). Figure 3 illustrates the relationship between caecal pH and the serum concentration of: Ca, Mg, Cu and Zn. Linear regression analyses indicated that the slopes of the linear regression between pH and serum Ca, Mg or Cu were not significantly different from a slope of zero (for Ca: correlation coefficient (r) = 0.48, $P < 0.11$ and for Mg, $r = 0.47$, $P = 0.11$; for Cu, $r = -0.55$, $P = 0.09$). The slope of the linear regression between pH and zinc was negative and significantly different from zero ($r = -0.70$, $P = 0.01$). However, when the median serum mineral levels of groups of rats fed fermentable fibre were compared by Mann-Whitney test to the medians of groups of rats fed no fermentable fibre, serum Zn and Cu were found to be significantly lower ($P \leq 0.05$) in groups which did not consume fermentable fibre; Ca was significantly ($P \leq 0.05$) higher and Mg was marginally significantly higher ($P = 0.07$) in groups which consumed no fermentable fibre. Thus, serum glucose was found to be linearly related to pH but serum minerals were related in a stepwise manner with an apparent transition point about pH 6.9 (see Fig. 3).

A statistical analyses of the data in Fig. 4 reveals that the mucosal crypts located over the rectal ALN had more cells in the crypt column (CH), a higher number of metaphase figures per midaxial crypt section (MC) and a higher mean proliferative zone height (PZH) than the crypts located 3–4 cm away from the ALN in all 12 dietary groups. Student's *t*-tests of the pooled means by location of the crypt revealed significant ($P < 0.001$) differences in each parameter due to the location of the crypt.

Figure 3. Relationship between caecal pH and serum concentration of four divalent cations: calcium, magnesium, copper and zinc. The mean caecal pH ($\pm$ SE) and the corresponding mean serum cation concentration ($\pm$ SE, $n = 10$) of rats from each dietary fibre group is presented. $\square$ indicate the means $\pm$ SE from groups of rats fed fermentable fibre; $\blacksquare$ indicate the means $\pm$ SE from groups of rats fed no fermentable fibre. The results of the statistical analysis of the data in this figure are reported in the text.

Linear regression analyses showed that the caecal pH was significantly and positively correlated with both MC and the PZH in crypts located either over or away from the ALN (Fig. 4). However, the mean height of crypts located away from the ALN was not significantly correlated with pH while the mean height of crypts located over the ALN was significantly and negatively correlated with pH.

DISCUSSION

Relationships between: type and amount of dietary fibre, pH in the lumen of the proximal large bowel and cell proliferation index in the distal large bowel mucosal crypts

Addition of increasing amounts of highly fermentable fibre to the diet has previously been demonstrated to lower the pH in the lumen of the caecum and proximal colon (Jacobs & Lupton 1986; Lupton, Coder & Jacobs 1988; Carter *et al.* 1998) and this is confirmed by the data in this report. There are, however, conflicting reports in the literature concerning the relationships between luminal pH as measured in the proximal large bowel and epithelial cell proliferation in the distal large bowel mucosal crypts. Results from two studies reveal a significant positive relationship between pH in the proximal large bowel and the epithelial cell proliferation index in the crypts of the distal large bowel (Bianchini *et al.* 1992; Lin & Visek 1990). Likewise, data from the present study demonstrates a highly significant ($P < 0.001$) positive linear relationship between pH in the proximal portion of the large bowel and colchicine arrested metaphase figures per midaxial section

Figure 4. The mean pH of each dietary group, as listed in Table 2 and the mean SE of rectal crypt cell proliferation index, i.e. number of metaphase figures per midaxial crypt section (1 mg colchicine/kg body weight was injected into the rat 3 h prior to killing to arrest and therefore to collect the metaphase figures). Also included in Figure 4 are data on the mean proliferative zone height and the crypt column height of each dietary group. See the text for results of statistical analyses of the data in this figure.

in the distal large bowel mucosal crypts of rats fed increasing levels of fermentable fibre (Fig. 4).

However, Lupton and colleagues reported that a decreased luminal pH in the proximal large bowel, due to feeding of dietary fermentable fibres, was accompanied by an increased epithelial cell proliferation index in the distal large bowel mucosal crypts (Jacobs & Lupton

1984; Jacobs & Lupton 1986; Newmark & Lupton 1990). Linear regression analyses of the crypt cell tritiated thymidine labelling indices in crypts located in the distal colon [listed in Table 6 of Jacobs & Lupton (1986)] and the data on luminal pH of the proximal colon of rats fed the same dietary fibre types and not treated with a carcinogen [using data taken from Fig. 1 of Lupton *et al.* (1988)], showed that there was not a significant linear relationship between luminal pH and the crypt cell labelling index, i.e. the slope was not significantly different from zero (slope = 0.0097, $r = 0.09$, P -value = 0.89, degrees of freedom = 3). Thus, in contrast to the claim of Newmark & Lupton (1990) the finding from the present study strongly supports the claim that more fermentable fibre in the diet lowers the cell proliferation index in the crypts of the distal large bowel mucosa.

The relationships between pH in the lumen of the caecum of the 12 groups of rats fed the different diets and the mucosal crypt proliferation parameters in the distal large bowel, as assessed by multiple linear regression analyses, are summarized in Fig. 5. The correlation coefficient (r) and the positive or negative (minus sign in Fig. 5) correlations between measured parameters are indicated. A decrease in pH in the lumen of the caecum, attributable to increased amounts of fermentable fibre in the diet, is significantly correlated with MC in crypts located over and in crypts located away from the ALN. Caecal pH is significantly and inversely correlated with CH in crypts located over the ALN but not in the crypts located away from the ALN. This indicates that crypts over the ALN are responding differently to the dietary modification than crypts located away from the ALN. Also notice in Fig. 5 the significant inverse correlation between CH and MC in crypts over the ALN but not in crypts located away from the ALN, again indicating the site-specific differences in response to the dietary modifications. The basis of these differences may be due to the fact that the crypt epithelium over the ALN is in close proximity to and in paracrine communication with the tropic influence of lymphoid nodule cells.

The results of this study make clear that MC in the distal large bowel mucosal crypts was significantly and positively correlated with caecal luminal pH and with serum glucose in crypts located over and in crypts located away from the ALN (summarized in Fig. 5). Thus the diet with increased fermentable dietary fibre decreased both caecal pH and serum glucose. This finding makes it questionable which predictor variable, caecal pH or serum glucose, better explains MC in the colorectal crypts.

Relationships between intake of dietary fibre types, changes in luminal pH and the serum levels of Ca, Mg, Zn and Cu

Clearly the increase in fermentable dietary fibre resulted in a significant decrease in luminal pH and significant changes in the morphokinetic parameters in the colorectal crypts. To test whether these changes were also associated with changes in intestinal function, such as altered mineral absorption, we measured the serum levels of four minerals, i.e. Ca, Mg, Cu and Zn. Serum levels of Ca and Mg were positively related to caecal lumen pH but the serum levels of Cu and Zn were negatively related to caecal pH. As food was the sole source of the minerals, the dietary modifications must be responsible, perhaps by altering intestinal luminal pH, for the observed changes in mineral balance. Change in the luminal pH may influence: the activity of enzymes within the lumen or within the epithelial cells lining the luminal surface, the type and metabolic activity of microflora, the pH dependent solubility of minerals, the absorption of particular luminal constituents such as the dietary minerals and the level of these minerals in the serum (Newmark & Lupton 1990). However, the mechanisms involved remain unresolved. To our knowledge, a reciprocal relationship between the serum level of Ca and Mg verses the serum level of Cu and Zn brought about by modification of dietary

Figure 5. (Left panels) Relationships between pH in the lumen of the caecum of the 12 groups of rats fed the different diets and the mucosal crypt proliferation parameters measured in the distal large bowel. MC, mean number of metaphase figures per midaxial crypt section; PZ, mean proliferative zone height; CH, mean crypt column height. (Upper left panel) Data from mucosal crypts located 3–4 cm proximal to the rectal aggregate of lymphoid nodules (away from the ALN). (Lower left panel) Data collected from mucosal crypts located over the rectal ALN. The correlation coefficient (r) and the positive or negative relationship between measures are indicated. With 12 groups (thus, 10 degrees of freedom), a P -value for correlation coefficients ≥ 0.576 , is ≤ 0.05 . The consumption of increasing amounts of dietary fermentable fibre resulted in a decrease in caecal pH which is significantly correlated with a decrease in MC both away from and over the ALN. (Right panels) Relationships between serum glucose and the mucosal crypt proliferation parameters measured in the distal large bowel. A decrease in serum glucose level due to consumption of the fermentable fibre containing diet was significantly correlated with a decrease in MC both away from and over the distal most ALN.

fibre types and amounts and the resulting modification in luminal pH, has not previously been reported.

Importance of mitosis in the large bowel crypts to colorectal cancer

Colorectal cancer is generally agreed to arise through a multistage process of at least five or six mutations in tumour suppressor genes or oncogenes (Fearon & Vogelstein 1990). Mutations in cells that do not proliferate in the adult body, i.e. mature neurones, cannot give

rise to cancer whereas mutations in cells that do proliferate can give rise to cancer (Ames, Gold & Willett 1995). The key factor for carcinogenesis appears to be mutagenesis of cells capable of proliferation, such as the stem cell(s) located towards the bottom of the mucosal crypts in the large bowel, accompanied by chronic stimulation of mitosis of these cells. Evidence is mounting to indicate that each crypt is derived from a single stem cell (Wright 1997). Mutations that occur in nonstem cells in the crypts may display focal atypia and dysplasia but these nonstem cells can be discarded by cell turnover which would seem to make nonstem cells less likely than stem cells to express themselves as an adenocarcinoma.

It follows from this logic that, exposure to a mutagen/carcinogen followed by a sustained increase or promotion of cell division, would increase the risk of cancer. There is evidence to support this idea in the large bowel and that a sustained increase of cell division in the mucosal crypts increases the risk of cancer in the large bowel. For example, Hardman & Cameron (1994) have reported that large bowel mucosal crypts located over ALN in rats have an increased mitotic index compared to crypts located away from ALN, a finding confirmed in the present study, and that crypts over ALN are at higher than normal risk of forming adenocarcinomas following exposure to a colorectal carcinogen (i.e. dimethylhydrazine, DMH). Likewise, Barthold & Jonas (1977) induced an increase in cell proliferation in the colon crypt epithelial cells by injection of mice with *Citrobacter freundii* which inflamed the bowel and resulted in a decreased time for DMH-induced colorectal tumours to develop.

The results from the present study demonstrate that an increase in the level of fermentable dietary fibre decreases the metaphase index in the crypts of the large bowel which would in turn be expected to lower risk of colorectal cancer. Heitman, Hardman & Cameron (1992) reported that dietary supplementation with either pectin or guar gum significantly reduced the incidence of DMH-induced colorectal cancer in rats. In a separate study, Hardman & Cameron (1995) again found that supplementation of the diet with pectin following exposure to the DMH suppressed colorectal cancer in DMH-treated rats. It is therefore concluded that cell proliferation in the colorectal crypts is a factor that can influence carcinogenesis and that an increase in the amount of fermentable fibre in the diet results in a decrease in the mitotic index in the crypts and reduces risk of colorectal cancer when fed to rats following injection of a colorectal carcinogen (Heitman *et al.* 1992; Hardman & Cameron 1994).

It is also concluded from the results of the present study that supplementation of the diet with fermentable fibre results in systematic modifications in the bioavailability of dietary minerals, i.e. serum levels of Ca and Mg decrease while serum levels of Cu and Zn increase. Thus, consumption of different types and amounts of dietary fibre for 24 weeks resulted in different adaptive changes in the structure and in the function of the intestinal mucosa.

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