Modulation of Colonic Epithelial Cell Proliferation, Histone Acetylation, and Luminal Short Chain Fatty Acids by Variation of Dietary Fiber (Wheat Bran) in Rats

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ABSTRACT

The effect of increasing amounts of wheat bran (0, 5, 10, 20%) in AIN-76 semisynthetic diet on colonic luminal short chain fatty acids, epithelial cell histone acetylation, and cytokinetics, was studied for 2 weeks in groups of 10 male Sprague-Dawley rats. Luminal contents were removed from the colon at sacrifice, quick frozen, and analyzed for short chain fatty acids by gas-liquid chromatography. Histone acetylation was assessed in cells isolated from the same animals. Cell proliferation was measured after a short pulse in vivo with 3H-thymidine. Colonic luminal butyrate levels were lower in the 0 and 20% fiber groups, and higher in the 5 and 10% fiber groups. In contrast, cell proliferation, as determined by labeling index, was higher in the 0 and 20% fiber groups, and lower in the 5 and 10% fiber groups. This resulted in a significant inverse correlation between luminal butyrate levels and colonic cell proliferation. In addition, there was a positive linear correlation between luminal butyric acid levels and colonic epithelial cell histone acetylation. From these data it was concluded that colonic butyrate levels can be modulated by the addition of wheat bran to the diet and that butyrate can modulate DNA synthesis (as measured by labeling index) in the proliferative compartments of colonic crypts. The localization of dividing cells was unchanged and no induction of terminal differentiation was detectable (contrary to what has been observed for transformed cells in culture).

INTRODUCTION

Colonic epithelial cell proliferation and differentiation have been well characterized both in normal (1–3) and in pathological conditions (4, 5). Recently it was suggested that the measurements of these parameters in gastrointestinal cells might assist studies of nutritional intervention attempting to inhibit neoplasia (6).

Many recent epidemiological (7–9) and experimental (10–14) studies have focused on the role of dietary fibers and their gastrointestinal metabolites on colonic epithelial cell physiology and carcinogenesis. The widely divergent results of these studies could be explained, in part, by taking into account differences in fiber composition, particularly the fermentable components (15). Among the main fecal byproducts of fermentable fibers are SCFAs3 (16, 17). One of them, butyric acid, has been described to produce quite striking effects on all the tested cell lines of neoplastic or transformed origin (18, 19). These effects are seen even at concentrations obtainable in vivo, with fecal and cellular concentrations being correlated (20, 21). In fact, in several instances the expression of constitutive genes was increased (22, 23) together with a few usually silent genes (23–25). The net result was a marked inhibition of both DNA and protein synthesis (18, 19, 26), resulting in a preferential block of the cell cycle in early G1 (26), eventually leading to terminal cellular differentiation (18, 19). Butyric acid also has an effect on several enzymatic activities (18, 19, 27–29), including histone deacetylases (30). This class of enzymes is inhibited even by trace amounts of butyric acid with resultant histone hyperacetylation (30). Acetylation of the lysine residues in the basic unit of the histones neutralizes their positive charges, weakening the interaction with the phosphate group of the DNA strand, and therefore opening the structure of the basic unit of the chromatin, the nucleosome (31, 32). Association with hyperacetylated histones has been shown to be a necessary but insufficient condition for the expression of a gene sequence (33, 34). It also makes the DNA more prone to carcinogen damage and damaging repair (35, 36).

In this paper studies are presented involving the modulation of colonic epithelial cell proliferation by variable contents of fiber in the diet. We thought it would be helpful in understanding molecular mechanisms involved, to use a very well-defined type of semisynthetic diet (AIN-76) (37) with the addition of 0, 5, 10, and 20% of a very well-characterized fiber: AACC hard red wheat bran. The amount and composition of fecal SCFAs were carefully monitored and the level of histone acetylation was analyzed for each individual rat. Furthermore, the proliferative index was determined for 50 crypt columns/rat.

MATERIALS AND METHODS

Diet. The AIN-76 semisynthetic standard diet (59.6% glucose; 23.6% casein vitamin-free; 0.4% d,l-methionine; 8% corn oil USP; 0.4% choline bitartrate; 2% AIN-76 vitamin mix; 6% AIN-76 mineral mix; Dyets, Inc., Bethlehem, PA) was the basal diet for all studies (37). Note that glucose is substituting for starch, which may escape digestion in the small intestine and act like dietary fiber, and had to be eliminated as a variable. The diet was used in its standard formula or was uniformly diluted by the addition of 0, 5, 10, or 20% wheat bran (AACC certified hard red wheat bran, St. Paul, MN). The wheat bran supplement was analyzed by the AACC and contained 42.7% total dietary fiber (Association of Official Analytical Chemists Method 43.A14). Diets were freshly prepared every other day and stored at 4°C.

Animals. In each diet study a group of twenty 100-g male rats (Sprague Dawley; Charles River, Italy) was singly housed. The distance from the wire grid floor to the cage base was 10 cm to prevent rats from reaching their feces. They received the diet for 2 weeks with food and water ad libitum. During the study rats were weighed daily and monitored for food intake. Weight gain was calculated as the difference between initial and final body weight. Energy intake was evaluated from the food intake by using physiological fuel values of 4.0 Kcal/g for

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3 The abbreviations used are: AIN-76, American Institute of Nutrition semisynthetic diet; SCFA, short chain fatty acids; AACC, American Association of Cereal Chemists; 3Tmd, thymidine.
protein and carbohydrate and 9.0 Kcal/g for lipids, while dietary fiber was considered to provide 0 calories. Daily energy intake was calculated by multiplying the energy density of each diet by the respective daily food intake. Food intake, energy intake, and body weight were determined on 20 rats/diet group. Data were analyzed by one way analysis of variance. Where variance was significant (P < 0.05), means were separated by using Duncan’s test (38). At the end of the experiments, rats were sacrificed by cervical dislocation between 11 a.m. and 12 noon to minimize the effects of diurnal variation.

At the time of sacrifice the colon was quickly resected, opened lengthwise, and, after removal of fecal contents, was rinsed, frozen, and stored at −20°C until analysis for short chain fatty acids. The colon was placed immediately into ice-cold isotonic saline. Histones from 12 rats/diet group were analyzed and cell proliferation indexes were assessed in the remaining 8 animals.

Cell Proliferation Indexes. One h prior to sacrifice 8 animals/group were given injections of [3H]thymidine (Amersham International, United Kingdom; 2 Ci/mmol) 1 μCi/g of body weight. Sigmoid colon were fixed in 10% buffered formalin acetate, pH 6.9–7.1, and stabilized with 1.5% methanol (39). Paraffin sections were processed in a blinded fashion for autoradiography and were developed and stained with hematoxylin and eosin (39). In glands longitudinally sectioned from base to lumen, the total number of cells per “crypt column” (each side of the length of the crypt) and the number and position of labeled cells were scored (40). For each individual rat the labeling index is defined as the total number of [3H]TdT-labeled epithelial cells (in all the 50 crypts assayed) divided by the total number of cells counted in the same crypt column. This index was calculated not only for the total crypt but also for each of its five proliferative compartments of equal size (compartment 1 at the base of the crypt and compartment 5 at the crypt surface) (40). The significance of any apparent change in cell number, labeled cells, and labeling index of a group of rats, with the variation of the percentage of wheat bran supplement to the diet, was estimated by conventional analysis (using all the individual parameters in each group) with an updated version of a program based on the Mann-Whitney test (41).

Analysis of Level of Histone Acetylation. Histones were isolated from colon epithelial cells of 12 rats of each group. Colons were rinsed in KCl (25 mM), MgCl2 (5 mM), phenylmethylsulfonyl fluoride/1,2-epoxy-3-(p-nitrophenoxy)propane (0.1 mM), sodium butyrate (5 mM), Tris-HCl buffer (50 mM), pH 7.2. Epithelial cells were scraped from the mucosal layer and placed in chilled buffer-sucrose (0.32 M). Nuclei were prepared as previously described (42). Histones were extracted from washed nuclei with 0.25 M HCl, precipitated with 9 volumes of acetone at 4°C, and freed from nonhistone proteins and nucleic acid fragments by ion-exchange chromatography on Bio-Rex 70 (Bio-Rad) as already described (43). Electrophoretic separation of histone acetylated forms, mainly H4 and H3, was obtained on gels containing 15% polyacrylamide, 5.5% acetic acid, 8 M urea, and 0.2% Triton X-100 (43). Gels were stained with 0.25% Coomassie Brilliant Blue-R and analyzed directly with a scanning laser spectrophotometer (LKB 2202 Ultrascan) connected to a recorder-integrator (LKB). The statistical significance of the difference between the amount of acetylated forms of histone H4 and its total level of acetylation in the different dietary groups was evaluated in Table 5, using Duncan’s means separation (38).

Analysis of Short Chain Fatty Acids. Rat fecal samples were stored at −20°C until use. They were then placed in liquid nitrogen and ground to a fine powder in a chilled mortar and pestle. To approximately 0.3 g of powdered sample weighed accurately in a 1.5-ml microcentrifuge tube, 500 μl of 3 molar sodium butyrate acid (internal standard) in 70% ethanol were added. Each tube was then vortexed, stored briefly at 4°C, and subsequently shaken for 20 min (44). Samples were then centrifuged for 20 min at 11,500 x g at 4°C and 100 μl of the resulting supernatant were combined with 100 μl of 3 molar heptanoic acid in 70% ethanol (45). Immediately before injection in the gas chromatograph, 20 μl of 10% H3PO4 were added to the mixture and 1 μl was analyzed (46, 47). All solutions and samples were kept refrigerated during use. Chromatographic determinations were performed in a gas chromatograph Hewlett-Packard 5890 Series II, with flame ionization detector equipped with a 30-m, 0.53-mm inside diameter, HP-FFA-PTA-TPA capillary column (Hewlett-Packard) and a 1-m, 0.53-mm inside diameter, deactivated glass capillary precolumn (Supelco) (48). A Hewlett-Packard Series II Integrator was used to plot and integrate data. The chromatographic conditions used were: injector at 165°C, detector at 220°C, column flow, 2.21 ml/min helium, make-up flow, 28 ml/min N2, flame, 430 ml/min air, 30 ml/min H2. Samples were concentrated on the column head at 80°C for 30 s. The temperature was increased at 70°C/min to a temperature of 145°C for 5 min. A temperature gradient of 5°C/min was continued until the final temperature of 185°C was reached. After the analysis the column was allowed to regenerate at 200°C for 5 min and the temperature was reduced for the next run. Short chain fatty acid standards containing 2-ethylbutyric acid, heptanoic acid, and H3PO4 were made fresh, stored at −70°C, and chromatographed at the beginning and end of daily sample runs. Blanks containing only the internal standard solutions in 70% ethanol were also chromatographed daily. Response factors and retention times for the individual fatty acids were determined from the standard runs. Fatty acid composition of samples was calculated from the appropriate standard response factor. Data were obtained from a sample of 10 rats/dietary group. Statistical evaluation of the difference in amount of SCFAs for each group was performed by using Duncan’s mean separation.

Mathematical and Statistical Analysis of Data. Cell labeling indexes were fitted in a logistic regression model according to the equation (49):

\[ I_i = \exp(\beta, \chi_i) / 1 + \exp(\beta, \chi_i) \]

where \( I_i \) is the labeling index, \( \chi_i \) are the covariates (specific group of each individual; the fecal butyric acid concentration) and \( \beta \) the parameters, derived from the data, that indicate how \( I_i \) is modified with different values of \( \chi_i \). Data analysis was performed with GLIM package (50). This model is the most suitable for the statistical analysis of labeling index (as percentage) since it is based on a binomial distribution. It has been used in order to evaluate the diet, butyric acid, and their concurrent effect on \( I_i \). The importance of each variable is evaluated according to its deviance, defined as the difference between the variability not accounted for by the model in the presence and absence of the variable studied (Table 4). The deviance has approximately the trend of a \( x^2 \) with degrees of freedom equal to the number of independent parameter taken into consideration during model fitting. The percentage of variability accounted for by the model and covariates considered was estimated with the pseudo \( R^2 \) test (51).

In the study of a possible correlation between level of histone acetylation and fecal butyric acid concentration, the mean value of each group was fitted in a linear regression model by using the acetylation level as the dependent variable. The significance (P) of the model was also evaluated (52).

RESULTS

Effect of Diet on Food and Energy Intake and Weight Gain. Table 1 summarizes results of food and energy intake and weight gain in groups of rats ingesting 0, 5, 10, and 20% dietary wheat bran. At all fiber supplementation levels, rats consumed a greater weight of food than when wheat bran was not added to the diet. This was presumably due to the diet energy dilution by fiber supplementation. Energy densities of the diet were: 4.05 kcal/g, 0%; 3.93 kcal/g, 5%; 3.80 Kcal/g, 10%; 3.57 Kcal/g, 20%. When food energy values are calculated, all groups of rats ingesting 0, 5, 10, and 20% dietary wheat bran after 2 weeks there was no difference between the 0 and 20% groups. However, a greater weight of food than when wheat bran was not added to the diet. This was presumably due to the diet energy dilution by fiber supplementation.
10% wheat bran groups. Instead, rats fed 5% fiber supplemented diet gained approximately 5 g more and those fed 20% fiber gained 7 g less than the 0 and 10% groups (Table 1).

Effect of Diet on Colon Cytokinetics. Table 2 summarizes results of $[^3]$HIdThd labeling of colonic epithelial cells in the groups of rats ingesting 0, 5, 10, and 20% dietary wheat bran.

The principal findings observed with 0% fiber compared to 5, 10, and 20% fiber supplement were a lengthening of the colonic crypts which represents hyperplasia and an increased amount of epithelial cells in the crypt columns compared to 5% (P < 0.05), 10% (P < 0.03), and 20% (P = 0.005) fiber supplement intake. These findings indicate that the occurrence of hyperplasia and hyperproliferation were significant and somewhat less pronounced when the group receiving fiber-free diet was compared to the group receiving 20% wheat bran dietary supplement.

Effect of Diet on Luminal Short Chain Fatty Acids. As the amount of wheat bran in the diet increased from 0 to 5%, total SCFA concentration increased by a factor of 1.8 (P < 0.01) (Table 3). However, there was no difference in total SCFA concentrations between the 5 and 10% levels of supplementation, while with 20% wheat bran, SCFAs increased 3.4 times over the fiber-free control values (P < 0.001). Levels of both labeled and unlabeled for the group, tallied separately for each of five colonic crypt compartments; (c) the fraction of epithelial cells labeled or labeling index in each compartment of the colonic crypt columns and in the crypt columns as a whole, statistically averaged over all crypt columns measured for the group.

The results in Table 2B1 indicated: (a) The average number of labeled cells per crypt column increased by a factor, respectively, of 2.4, 2.5, and 1.7 when 0% was compared to 5, 10, and 20% dietary fiber supplement intake (P < 0.001 in each group). (b) In Table 2B2 the number of labeled epithelial cells per total cells by crypt compartment, increased in crypt compartments 1, 2, and 3 and for all crypt compartments when 0% was compared to 5, 10, and 20% fiber supplement and increased in compartment 4 when 0% was compared to 10 and 20% fiber supplement. (c) The labeling index increased in the crypt as a whole when 0% intake was compared to 5 and 10% (P < 0.001) and 20% (P = 0.005) fiber supplement intake.

<table>
<thead>
<tr>
<th>Table 1 Effect of diet on food and energy intake and rat body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values are expressed as mean ± SEM on a sample size of 20 rats/group. Means within a row not sharing the same superscript are significantly different at P &lt; 0.05.</td>
</tr>
<tr>
<td>Wheat bran</td>
</tr>
<tr>
<td>Food intake (g)</td>
</tr>
<tr>
<td>1st week</td>
</tr>
<tr>
<td>2nd week</td>
</tr>
<tr>
<td>Energy intake (Kcal)</td>
</tr>
<tr>
<td>1st week</td>
</tr>
<tr>
<td>2nd week</td>
</tr>
<tr>
<td>Rat weight (g)</td>
</tr>
<tr>
<td>Initial</td>
</tr>
<tr>
<td>After 1 week</td>
</tr>
<tr>
<td>After 2 weeks</td>
</tr>
<tr>
<td>Total gain</td>
</tr>
</tbody>
</table>

In Table 2B1 the number of labeled epithelial cells per total cells by crypt compartment, increased in crypt compartments 1, 2, and 3 and for all crypt compartments when 0% was compared to 5, 10, and 20% fiber supplement and increased in compartment 4 when 0% was compared to 10 and 20% fiber supplement. (c) The labeling index increased in the crypt as a whole when 0% intake was compared to 5 and 10% (P < 0.001) and 20% (P = 0.005) fiber supplement intake.

The labeling index increased in compartment 1 when 0% was compared to 5 and 10% fiber supplement (P < 0.001), in compartment 2 when 0% was compared to 5, 10, and 20% fiber supplement (P < 0.001), and in compartment 3 when 0% was compared to 5% (P ≤ 0.05), 10% (P ≤ 0.03), and 20% fiber supplement (P ≤ 0.02). These findings indicate that the occurrence of hyperplasia and hyperproliferation were significant and somewhat less pronounced when the group receiving fiber-free diet was compared to the group receiving 20% wheat bran dietary supplement.

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MODULATION OF COLONIC EPITHELIAL CELLS BY DIETARY FIBER

Table 3 Mean amount, in μm/g wet weight, of luminal SCFA in groups of 10 rats maintained with AIN-76 diet supplemented with different amounts of wheat bran

<table>
<thead>
<tr>
<th>Wheat bran</th>
<th>0%</th>
<th>5%</th>
<th>10%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>22.20 ± 2.6a</td>
<td>30.30 ± 2.9b</td>
<td>28.90 ± 1.4a</td>
<td>82.70 ± 7.6a</td>
</tr>
<tr>
<td>Propionic</td>
<td>5.30 ± 2.6a</td>
<td>10.20 ± 0.8b</td>
<td>9.60 ± 1.1a</td>
<td>7.70 ± 1.0ab</td>
</tr>
<tr>
<td>Isobutyric</td>
<td>0.07 ± 0.5a</td>
<td>0.99 ± 0.09a</td>
<td>0.67 ± 0.08b</td>
<td>0.26 ± 0.09a</td>
</tr>
<tr>
<td>Butyric</td>
<td>1.15 ± 0.15a</td>
<td>7.28 ± 0.86ab</td>
<td>9.53 ± 1.1a</td>
<td>6.23 ± 0.81a</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0.22 ± 0.10a</td>
<td>1.34 ± 0.11a</td>
<td>0.86 ± 0.12b</td>
<td>1.89 ± 1.56a</td>
</tr>
<tr>
<td>Valeric</td>
<td>0.13 ± 0.07a</td>
<td>1.17 ± 0.12a</td>
<td>0.76 ± 0.09a</td>
<td>0.16 ± 0.06a</td>
</tr>
<tr>
<td>Total</td>
<td>29.10 ± 3.2a</td>
<td>51.31 ± 3.9a</td>
<td>50.36 ± 2.5a</td>
<td>98.93 ± 7.1a</td>
</tr>
</tbody>
</table>

acetate followed the same pattern as total SCFA concentrations; however, the 5 and 10% levels of supplementation did not produce acetate values that were significantly higher than controls. In contrast, levels of propionate, isobutyrate, and valerate increased over control values with 5 and 10% but returned to control level with 20% wheat bran supplementation in the diet. There was no significant diet effect on levels of isovalerate.

Butyrate was the SCFA of interest because of its known effect on histone deacetylase (30). Its concentration increased in the colonic lumen with increasing amounts of wheat bran up to the 10% supplementation level, but the butyrate concentration of the 20% fiber dietary group was back to the 5% level (Table 3).

Mathematical Analysis of Data. On the basis of the logistic regression model we have established that all the covariates available have a significant effect on (/ labeling index), although the percentage of variability they account for when evaluated with the pseudo $R^2$ test, was found to be only 47%.

Table 4 Evaluation of effects of each covariate on labeling indexes as calculated by the model that takes into account the 2 covariates and their interaction

<table>
<thead>
<tr>
<th>Covariate</th>
<th>$b_i$</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (0%)</td>
<td>2.04700</td>
<td>0.06033</td>
<td>0.001</td>
</tr>
<tr>
<td>Group 2 (5%)</td>
<td>2.90700</td>
<td>0.09144</td>
<td>0.001</td>
</tr>
<tr>
<td>Group 3 (10%)</td>
<td>2.72200</td>
<td>0.08951</td>
<td>0.001</td>
</tr>
<tr>
<td>Group 4 (20%)</td>
<td>2.58800</td>
<td>0.07133</td>
<td>0.001</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>-0.05882</td>
<td>0.05008</td>
<td>0.8</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.10060</td>
<td>0.05142</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.05959</td>
<td>0.05088</td>
<td>0.8</td>
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<tr>
<td>Group 4</td>
<td>0.09574</td>
<td>0.05113</td>
<td>&lt;0.10</td>
</tr>
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</table>

DISCUSSION

In the literature there are several experimental (10–17) and epidemiological (7–9) reports on the influence of fiber (amount and composition) in the diet on colon epithelial cell metabolism and carcinogenesis. In many cases the results are conflicting or contradictory.

In this paper a clarification of some of the issues was attempted by using a well described model system: inbred rats, standardized housing conditions, well defined semisynthetic diets supplemented with different percentages of thoroughly characterized fiber, and the experiments were ended at the same time of the day.

In previous experiments we have seen that when diets are uniformly diluted by the addition of the fiber supplement the rats merely consumed a greater weight of diet in order to compensate. Since diets are designed on an energy basis, if rats have equivalent energy intakes they will have equivalent intakes of all nutrients except fiber.

In this study the highest fiber group (20%) did not eat a sufficiently greater weight of food to entirely compensate for the energy dilution (Table 1). Thus this group of rats had a lower energy intake than the other three groups; this was reflected also in a lower weight gain (Table 1). Although this is a complicating issue in our study, we do not feel that it should affect the interpretation of our results. In fact, in almost every diet-large bowel cancer study (53, 54), the group of animals that gains the least weight would develop the lowest number of tumors. In our case, however, the “marker” for cancer risk, an increase in cell proliferation, was highest not lowest in this group. It should also be noted that a 20% wheat bran-supplemented diet is not really a 20% fiber-supplemented diet but rather an 8.5% fiber diet (since wheat bran is only 42.7% dietary

quantitated since they have the highest electrophoretic mobility and no overlap with any other histone component. Scanning densitometry of every electrophoretic separation was carried out and was followed by computer-assisted analysis of the tracings. The percentage of each acetylated form (over total H4) for each preparation is reported in Table 5, together with the global level of acetylation.

The highest level of H4 acetylation was reached in the 10% fiber-supplemented group, where the most prominent form was the monoacetylated one (39.5%) >non- >di- >tetra- >triacetylated. The lowest level of acetylation was detected in the fiber-free diet group that had a similar amount of non- and monoacetylated form (44.2/46.4%), no tetraacetylated, 8.6% of tri- and traces of diacytated form (0.8%). The level of acetylation was slightly but significantly higher in the 5% than in the 20% bran-supplemented group, mainly because of a substantially higher amount of di- (17.7/11.4) and tri- (10.0/6.5) acetylated forms.

If compared to the level of acetylation of a colon-derived cell line (HT29), the fiber-free diet group was hypoacetylated while the other dietary groups (10 > 5 > 20%) were hyperacetylated.

In the study of a possible correlation between level of histone acetylation in colon epithelial cells and fecal butyric acid concentration, it was found that the mean value of each group (Table 5) can be fitted in a regression model where acetylation level is the dependent variable. The slope of the linear relation was 10.04 with SE of 4.52 and $P < 0.05$; the correlation therefore is significant and positive.
fiber). The 20% wheat bran-supplemented diet would correspond to a human diet of approximately 43 g/day. This is definitely a high fiber diet but not "nonphysiologically" nor "ordinarily high."

Before describing an overall evaluation of the data we would like to point out two peculiar effects of the fiber-free diet. The absence of fiber seems to cause a marked hyperplastic effect on colonic crypt cells of different groups of rats (Table 6). The correlative linearly with the percentage of wheat bran in the diet, leaving its origin so far undetermined.

When we analyzed the cellular parameters of the groups of rats in our studies, we noted that variation in results did not correlate linearly with the percentage of wheat bran in the diet. In particular, an increase in the percentage of wheat bran from 0 to 10% led to a progressive decrease of [3H]dThd-labeled crypt column cells and labeling index, but a 20% supplement causes an inversion of the trend (Table 2).

Butyric acid is the only one of the SCFAs produced by fiber fermentation, whose concentration has an effect that could account, at least in part, for the above noted labeling indexes (Tables 3 and 4). Since a mathematical evaluation of an appropriately chosen logistic regression model showed that the interpolation of the covariate analyzed (Table 4) can justify only 47% of the experimental distribution of labeling indexes, this means that other covariates are involved in the definition of the event. Although these covariates are necessary for the accurate definition of the model, it is unlikely that a single one of them has a determinant role as a modulator of cellular proliferation.

Butyric acid is known as an inducer of terminal differentiation of cultured cells of neoplastic origin (18, 19). Among the effects produced it causes a partial block of cells in early G1, with consequent inhibition of DNA synthesis. An inhibition of this type could be involved in modulating the labeling index of colonic crypt cells of different groups of rats (Table 6).

Table 5 Histone H4 acetylation in rat colon epithelial cells: correlation with percentage of wheat bran in the diet

<table>
<thead>
<tr>
<th>% of wheat bran</th>
<th>Acetyl groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.2 ± 0.9b</td>
<td>46.4 ± 0.9b</td>
<td>0.8 ± 0.7c</td>
<td>8.6 ± 0.8a</td>
<td>ND/ 73.8 ± 0.8a</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>23.9 ± 0.8a</td>
<td>40.4 ± 1.0a</td>
<td>17.7 ± 0.8a</td>
<td>10.0 ± 0.7c</td>
<td>8.0 ± 0.7b</td>
<td>137.4 ± 0.8a</td>
</tr>
<tr>
<td>10</td>
<td>24.0 ± 0.8a</td>
<td>39.5 ± 0.9a</td>
<td>16.2 ± 0.8a</td>
<td>8.5 ± 0.7a</td>
<td>11.8 ± 0.7a</td>
<td>144.5 ± 0.9a</td>
</tr>
<tr>
<td>20</td>
<td>28.2 ± 0.9a</td>
<td>41.8 ± 0.9a</td>
<td>11.4 ± 0.9a</td>
<td>6.5 ± 0.7a</td>
<td>12.1 ± 0.8a</td>
<td>132.5 ± 0.9a</td>
</tr>
</tbody>
</table>

a Amount of non-, mono-, di-, tri-, tetraacetylated forms as percentage of total histone H4 (average of 4 determinations).

b Global acetylation level of histone H4 = 1 x % mono- + 2 x % di- + 3 x % tri- + 4 x % tetraacetylated form ± SE.

c Not detectable or within SE.

d Luminal BA (butyric acid) concentration, in μM/g wet weight, in group of 10 rats maintained on diet supplemented with different amount of fiber (see footnote a).

e) Modulation of colonic epithelial cells by dietary fiber

The correlation coefficient between labeling index (J) and luminal butyrate concentration is strongly negative (—0.98; P < 0.01). It should be noted though that an opposite effect has been previously observed at very low butyric acid concentrations (in the range of 0.1 mM) in some normal primary cells in culture (56).

Cell accumulation (hyperplasia) in the colon of the fiber-free diet group of rats could not be accounted for simply by the absence of the brush-off effect of undigested fibrous material. In fact, hyperplasia appears, even if to a lesser extent, also in the group with the highest wheat bran supplementation (20%). This effect could be accounted for to some extent by the difference of crypt cell proliferation in the different groups (Table 2).

The state of morphological differentiation of colonic epithelial cells was not studied extensively, but no change was observed: this type of evaluation might show variations in differentiated cell distribution throughout the 5 crypt compartments with different proliferative capacity (Table 2).

Butyric acid is also a modulator of chromatin primary (nucleosome) structure because of its ability to inhibit histone deacetylation, therefore loosening the interaction between the DNA and these structural proteins. In colonic epithelial cells of different groups of rats the level of histone acetylation appeared to be modulated by the cellular concentration of butyric acid that is in equilibrium with its fecal concentration (Table 6) (44).

The correlation coefficient between the level of histone acetylation and butyric acid concentration in each group is positive (0.96; P < 0.05), while the labeling index (I) is negative (—0.97; P < 0.05) (Table 6). This means that butyric acid concentration controls to some extent not only colonic epithelial cell primary structure (and therefore its accessibility to carcinogen damage and damage repair), but also cell proliferation.

In the study of a possible correlation between luminal butyric acid concentration and histone acetylation, the mean value of this last parameter was conventionally evaluated for each group. In fact a fairly large number of rats is needed to obtain and analyze an electrophoretic separation of the different acetylated forms. Consequently there is an averaging effect of the data and the level of acetylation cannot be utilized as a covariate in Table 4.

A complicating factor in the interpretation of the results are the data for the 20% wheat bran diet group. There is no satisfactory explanation for the increase in cell number per crypt, labeled cells, and labeling index (corresponding to a lower concentration of luminal butyric acid and consequent lower degree of histone acetylation), with an increase of the wheat bran supplement from 10 to 20%. However, several parameters should be taken into account: the variation in concentration of luminal butyric acid itself and the decreased transit time through the intestine, with consequent shorter exposure of fecal material to intestinal flora and epithelial cells to the fermentation products.
MODULATION OF COLONIC EPITHELIAL CELLS BY DIETARY FIBER

These findings have further implications for both rodent and human studies. They can also explain some data in the literature, correlating amount of sodium butyrate to 1,2-dimethylhydrazine-induced rat intestinal neoplasia (57).

Thus the 5% wheat bran diet decreased both colonic epithelial cell hyperproliferation and hyperplasia, compared with the fiber-free diet. However, when wheat bran content was further enriched to 10 and 20%, increased hyperplasia and hyperproliferation gradually recurred. These findings may help to explain previous similar data in rodent models (10, 58–60). They also substantiate the hypothesis that complexities may arise in clinical human fiber trials (61) in which subjects are expected to begin clinical trials consuming different amounts of fiber, thereby contributing to lower, higher, or unchanged proliferative indices which are expected to be more uniform. Unfortunately, differences of this type have sometimes tended to be lumped together in the past.

From the results obtained in this study we postulate that, while diets containing moderate amounts of fiber may have protective effects on cell proliferation, differentiation, and carcinogenesis, fiber-free diets and diets supplemented with too much fiber would have the potential to promote colon carcinogenesis.

REFERENCES


Modulation of Colonic Epithelial Cell Proliferation, Histone Acetylation, and Luminal Short Chain Fatty Acids by Variation of Dietary Fiber (Wheat Bran) in Rats

Lidia C. Boffa, Joanne R. Lupton, Maria R. Mariani, et al.