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Modulation of distal colonic epithelial barrier function by dietary fibre in normal rats

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Abstract

***Background*—Dietary fibre influences the turnover and differentiation of the colonic epithelium, but its effects on barrier function are unknown.**

***Aims*—To determine whether altering the type and amount of fibre in the diet affects paracellular permeability of intestinal epithelium, and to identify the mechanisms of action.**

***Methods*—Rats were fed isoenergetic low fibre diets with or without supplements of wheat bran (10%) or methylcellulose (10%), for four weeks. Paracellular permeability was determined by measurement of conductance and ^{51}Cr-EDTA flux across tissue mounted in Ussing chambers. Faecal short chain fatty acid (SCFA) concentrations were assessed by gas chromatography, epithelial kinetics stathmokinetically, and mucosal brush border hydrolase activities spectrophotometrically.**

***Results*—Body weight was similar across the dietary groups. Conductance and ^{51}Cr-EDTA flux were approximately 25% higher in animals fed no fibre, compared with those fed wheat bran or methylcellulose in the distal colon, but not in the caecum or jejunum. Histologically, there was no evidence of epithelial injury or erosion associated with any diet. The fibres exerted different spectra of effects on luminal SCFA concentrations and pH, and on mucosal indexes, but both bulked the faeces, were trophic to the epithelium, and stimulated expression of a marker of epithelial differentiation.**

***Conclusions*—Both a fermentable and a non-fermentable fibre reduce paracellular permeability specifically in the distal colon, possibly by promoting epithelial cell differentiation. The mechanisms by which the two fibres exert their effects are likely to be different.**

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Keywords: colon; differentiation; epithelium; fibre; paracellular permeability; proliferation

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The colonic epithelium performs an essential barrier function by acting to prevent proinflammatory macromolecules from gaining access to the internal milieu. Macromolecules that permeate the epithelium do so mainly via the paracellular pathway, for which the tight junction is the rate determining structure. Evidence from human disease, including ulcerative colitis[1] and Crohn's disease,[2] and experimental animals[3] suggests an association between the presence of intestinal mucosal inflammation and increased paracellular permeability. Whether impaired barrier function is the cause or effect (or both) of the mucosal inflammation is not known but impaired epithelial barrier function could lead to increased permeation of luminal proinflammatory macromolecules into the lamina propria, a process of potential pathogenic significance.

Despite the physiological importance of the maintenance of paracellular permeability in the colon, there are no published studies on whether variations in diet exert any influence on the permeability of the colonic epithelium. Some attention has been paid to the effect of dietary manipulation on small intestinal permeability. For example, removal of specific dietary macro- and micronutrients,[4 5] total parenteral nutrition,[6] and fasting[7] are reported to increase paracellular permeability in rat small intestine. The colon, however, has a different relation to diet than does the small intestine, in that the interaction of dietary factors with the luminal flora may exert more influence than the dietary components themselves. Fermentation of dietary undigestible carbohydrate (such as fibre) and unabsorbed protein yields products such as short chain fatty acids (SCFAs), phenols, and reducing sulphur compounds, all of which may have beneficial or detrimental effects on the epithelium (reviewed by Gibson[8]). Minimisation of luminal fermentation by the unphysiological surgical procedure of diverting the faecal stream from a segment of colon in rats causes notable atrophy of the diverted segment and is associated with increased paracellular permeability.[9] The mechanism for such an effect was not examined.

Of the fermentation products produced in the colonic lumen, only SCFAs have been investigated for their effects on paracellular permeability. In vitro, butyrate, acetate, and propionate all exert a concentration dependent reduction in paracellular permeability in the Caco-2 model of colonic epithelium, an effect that is maximal 48 hours after initial exposure.[10] In contrast, acute exposure to butyrate increases paracellular permeability in guinea pig or rat colonic mucosa mounted in Ussing chambers (Mariadason *et al*, manuscript submitted).[11] Luminal concentrations of SCFAs are readily amenable to modulation by diet. Altering the amount and type of dietary

Abbreviations used in this paper: CCH, crypt column height; DPPIV, dipeptidylpeptidase IV; MI, mitotic index; PD, potential difference; SCFA, short chain fatty acid.

carbohydrate alters colonic SCFA levels.[12] Furthermore, diets high in fermentable fibre or starch resistant to digestion not only increase colonic SCFA levels but also are able to alter physiological indexes such as epithelial turnover,[13 14] and to alter disease pathogenesis such as tumour formation following carcinogen administration.[14 15] Limited indirect[15] and direct data[16 17] suggest that SCFAs are causally related to some of these effects, particularly tumour formation.

The present study aimed to determine whether altering the amount and type of fibre in the diet of normal rats influenced paracellular permeability in the distal colon. Wheat bran was chosen because it has previously been shown to increase notably distal colonic concentrations of SCFAs.[12] As wheat bran is slowly fermented and considerable unfermented fibre remains in the faeces, the effect of a non-fermentable fibre, methylcellulose, which is associated with very low SCFA levels in the colonic lumen,[13] was also examined. Relations between changes in permeability with changes in morphology, epithelial turnover and differentiation, and luminal indexes were also sought.

Methods

ANIMAL HUSBANDRY

Male Sprague-Dawley rats weighing approximately 160 g were obtained from the Monash University Animal Services, Victoria, Australia. Animals were housed at the Royal Melbourne Hospital Animal facility, with diurnal lighting, and access to food and water provided ad libitum. Animals were housed in groups of four, in drop bottom wire cages to reduce coprophagy. Rat diets were based on the AIN-76 standard for purified diets for rats and mice,[18] and prepared as previously described.[17] For all dietary intervention studies, the feeding period was four weeks and each dietary group comprised 12 rats. Studies were approved by the Hospital Campus Animal Ethics Committee of the Royal Melbourne Hospital. The conduct was in accordance with the guidelines laid down in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes in a registered animal facility.

MEASUREMENT OF BODY WEIGHT, FOOD CONSUMPTION, AND LUMINAL INDEXES

The rats were monitored daily regarding their general health and signs of stress, and were weighed weekly. Twenty four hour food consumption, 24 hour faecal output (wet weight), and faecal and caecal pH were measured as previously described.[13] SCFA concentrations were determined in rat faecal and caecal contents by gas chromatography, as previously described.[10]

MEASUREMENT OF PARACELLULAR PERMEABILITY

For measurement of paracellular permeability, animals were anaesthetised with Nembutal (Boehringer Ingelheim, Artarmon, New South Wales, Australia); two segments of distal colon (approximately 1 and 4 cm from the rectum), and one segment from each of the caecum and jejunum (5 cm distal to the ligament of Trietz) were removed, and the animals were sacrificed under anaesthesia. The distal colon and jejunum were stripped of the underlying muscle layers, and mounted in 0.76 cm^2 Ussing chambers (CSIRO, Victoria, Australia). Due to difficulties in stripping, the caecum was mounted in Ussing chambers with the muscle layers intact. The apical and basolateral surfaces were bathed by separate reservoirs, each containing 15 ml Krebs buffer at 37°C and pH 7.4. The buffer contained Na 140 mM, K 10 mM, Mg 1.1 mM, Ca 1.25 mM, Cl 127.7 mM, H_2PO_4 2 mM, HCO_3^- 25 mM, and glucose 10 mM (BDH Chemicals, Poole, UK). Carbogen was bubbled through the chambers to oxygenate the tissue. A 30 minute equilibration period was allowed to elapse prior to commencement of measurement of paracellular permeability. Prior studies had shown this to be sufficient time for stabilisation of electrical indexes to occur. For measurement of transepithelial conductance, the spontaneous potential difference (PD) across the epithelium was determined, and the tissue clamped at zero voltage by introducing an appropriate short circuit current (*I*sc) with an automatic voltage clamp (DVC 1000; World Precision Instruments, New Haven, Connecticut, USA). The *I*sc was continuously monitored and PD measured by briefly removing the voltage clamp for 5–10 seconds every 15 minutes. Values at the end of the first, second, and third 15 minute periods were recorded. Transepithelial conductance (G) was calculated according to Ohm's law, and expressed as mS/cm^2. For measurement of the transepithelial flux of EDTA, 2 Mbq of ^{51}Cr-EDTA (Australian Radioisotopes, New South Wales, Australia) was added to the apical reservoir at the end of the 30 minute equilibration period, and serial aliquots of 0.5 ml and 2 ml, were taken at regular intervals from the apical and basolateral reservoirs respectively. Radioactivity was determined by gamma counting, and results expressed as rate coefficients in µl/min.[19] Occasionally, the agar bridges would become damaged due to wear but were readily repaired. However, minor spills of EDTA across the bridge invalidated the EDTA flux results for those experiments and these were subsequently omitted from the analyses. In preliminary experiments, administration of vincristine to a group of eight rats three hours prior to sacrifice had no effect on paracellular permeability compared with a group of eight rats receiving vehicle only (data not shown).

MEASUREMENT OF COLONIC EPITHELIAL CELL KINETIC INDEXES

Cell proliferation was assessed by the vincristine sulphate (David Bull Laboratory, Melbourne, Australia) method of counting metaphase arrests, as previously reported in detail.[13] Ten longitudinally sectioned crypts were evaluated by one investigator (JMM), blinded to the origin of the sections. Crypt column height (CCH) was calculated as the mean number of cells per crypt column, determined by counting under light microscopy.[13] The rate

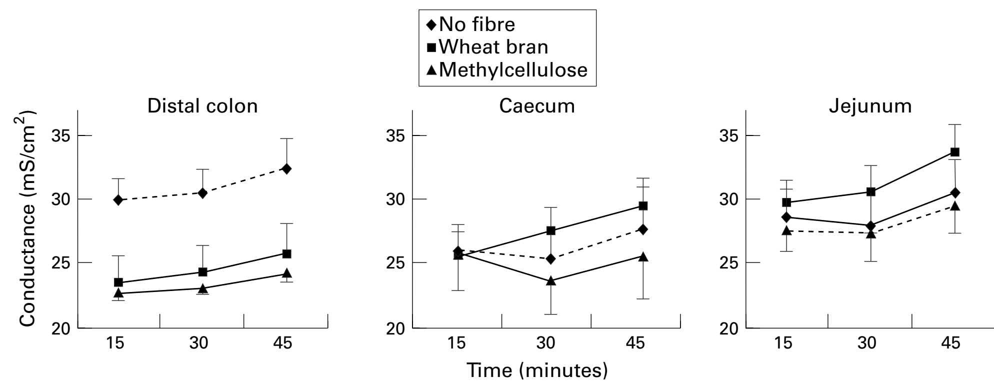

Figure 1 Effect of diet on transepithelial conductance in rat intestine. Data are expressed as mean (SEM) from 12 animals. ANOVA of area under the curve summary measures showed a significant difference across the dietary groups (p<0.001) for the distal colon only and, on multiple comparisons, wheat bran and methylcellulose groups were significantly different to the no fibre group (p<0.01).

of cell proliferation was determined by counting the cells arrested in metaphase and expressing it per crypt column. The mitotic index (MI) was calculated as a percentage by dividing the number of cells arrested in metaphase by the total number of cells and multiplying by 100.

MEASUREMENT OF ENZYME ACTIVITIES

Mucosal samples stripped of underlying muscularis were mechanically homogenised at 4°C in mannitol buffer (50 mM D-mannitol and 2 mM trizma base in dH_2O, pH 7.4) and Triton X-100 added to a final concentration of 0.1%. Alkaline phosphatase activity was measured according to the method of Young *et al*,[20] using *p*-nitrophenyl phosphate (Sigma-Aldrich, St Louis, Missouri, USA) as substrate. Dipeptidylpeptidase IV (DPPIV) activity was determined according to the method of Maroux *et al*,[21] using glycyl-L-proline-*p*-nitroanilide (Sigma-Aldrich) as substrate. Results were expressed relative to cellular protein content, measured using bovine gamma globulin as standard.[22] The coefficient of variation of all assays was less than 10%.

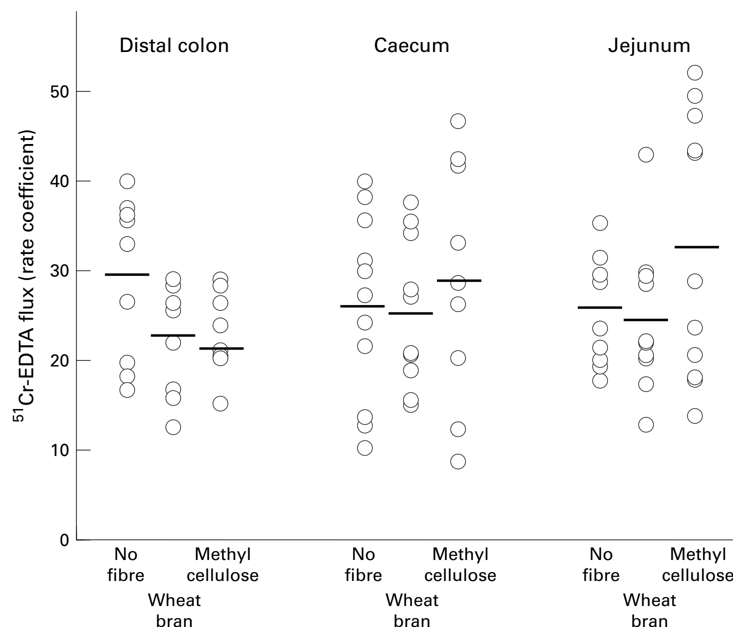

Figure 2 Effect of diet on the flux of ^{51}Cr-EDTA across distal colon, caecum, and jejunum mounted in Ussing chambers. The diets contained no fibre, wheat bran, or methylcellulose. For the distal colon only, differences were statistically significant across the dietary groups (p=0.004; ANOVA). EDTA flux in the wheat bran and methylcellulose groups was significantly different to that in the no fibre group on multiple comparisons (p<0.05).

STATISTICAL METHODS

Group data from all experiments are expressed as mean (SEM). The results across dietary groups were compared by analysis of variance (ANOVA). A p value of not more than 0.05 was considered statistically significant. All statistical analyses were performed using Minitab for Windows, version 9.2 (Minitab Inc., State College, Pennsylvania, USA).

Results

EFFECT OF DIET ON ANIMAL HEALTH

All rats remained healthy and showed no evidence of stress during the duration of the experimental period. Gain in body weight after four weeks was similar across all dietary groups. Those fed a no fibre diet gained 182 (9) g, those fed wheat bran 165 (7) g, and those fed methylcellulose 177 (6) g. Twenty four hour food consumption, measured at the end of the fourth week, was highest in the wheat bran (35 (4) g/rat) and methylcellulose fed groups (31 (1) g/rat), and least in the energy dense no fibre diet (26 (9) g/rat).

EFFECT OF DIET ON PARACELLULAR PERMEABILITY

As fig 1 shows, transepithelial conductance in the caecum or jejunum was similar across the three diets. However, significant differences were observed across the diets in the distal colon (p=0.004, ANOVA using area under the curve summary measure), due to the high conductance in rats fed a no fibre diet. Mean conductance was 24% less in both the wheat bran and methylcellulose groups.

The transepithelial flux of ^{51}Cr EDTA paralleled that of conductance. As fig 2 shows, the flux differed across the dietary groups in the distal colon (p=0.004) but not caecum or jejunum. The higher flux in animals fed the no fibre diet was responsible for this difference. Mean flux was 23% higher than in the wheat bran group and 28% higher than in the methylcellulose group.

Table 1 Effect of diet on faecal indexes

	No fibre	*Wheat bran*	*Methylcellulose*	*p Value**
24 hour faecal output (g/rat)	0.6 (0.1)†	6.0 (0.3)	4.5 (0.2)	<0.001
Faecal pH	7.1 (0.1)‡	5.7 (0.1)	6.9 (0.1)	<0.001
Caecal pH	6.9 (0.1)	6.5 (0.1)	7.2 (0.6)	<0.001
Faecal SCFA (μmol/g faecal water)				
Total	17.7 (1.9)	29.8 (4.1)	3.2 (0.7)	<0.001
Butyrate	2.1 (0.3)	3.8 (0.7)	0.9 (0.2)	<0.001
Caecal SCFA (μmol/g caecal water)				
Total	22.3 (3.9)	30.2 (2.1)	13.0 (1.6)	<0.001
Butyrate	2.1 (0.3)	6.0 (0.6)	1.4 (0.2)	<0.001

*ANOVA across the dietary groups.
†Animals housed four per cage and faecal output from each cage divided by 4; results are mean (SEM) of three cages.
‡Mean (SEM) of 12 rats.

Table 2 Effect of diet on epithelial kinetic indexes in distal colonic epithelium

	No fibre	*Wheat bran*	*Methylcellulose*	*p Value**
Crypt column height (cells)	30.5 (0.6)†	39.2 (0.8)	31.6 (0.8)	<0.001
No of metaphase arrests per crypt column	1.1 (0.2)	1.9 (0.3)	1.0 (0.3)	0.026
Mitotic index (%)	3.7 (0.5)	4.7 (0.8)	3.4 (0.7)	0.38

*ANOVA across dietary groups.
†Mean (SEM) of 12 rats.

EFFECT OF DIET ON FAECAL AND CAECAL INDEXES

The effects of diet on faecal and caecal indexes are shown in table 1. Twenty four hour faecal output, measured at the end of the fourth week, was considerably higher in the wheat bran and methylcellulose fed animals compared with those fed no fibre. Significant differences in faecal and caecal pH were observed across the dietary groups and these were due primarily to the lower pH in animals fed wheat bran. Total faecal and caecal SCFA concentrations were highest in animals fed wheat bran and lowest in those fed methylcellulose. Butyrate concentrations followed the same pattern (table 1) as did concentrations of acetate and propionate (data not shown).

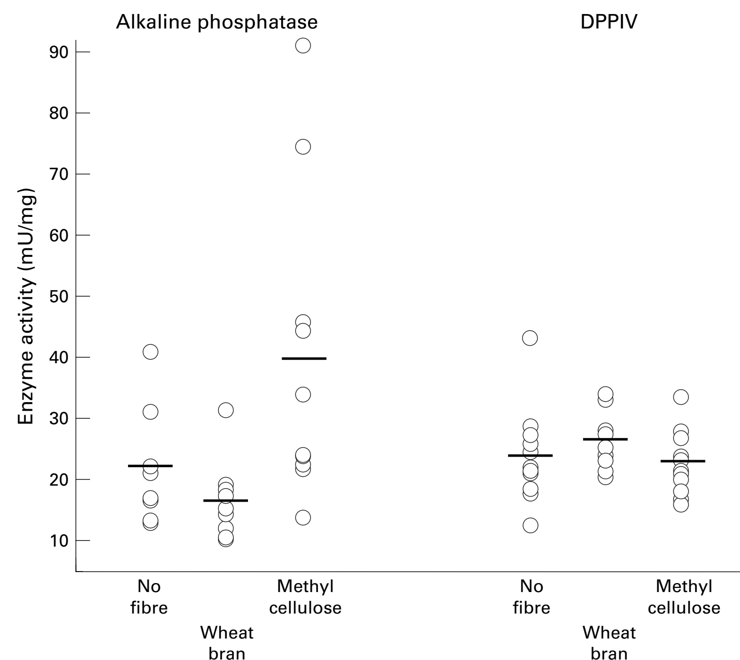

Figure 3 Effect of diet on brush border hydrolase activities of distal colonic mucosa. The diets contained no fibre, wheat bran, or methylcellulose. Alkaline phosphatase and dipeptidylpeptidase IV (DPPIV) activities differed significantly across the dietary groups (p<0.001 and p<0.05 respectively, ANOVA). Alkaline phosphatase activity in the methylcellulose group and DPPIV activity in the wheat bran group were significantly different to those in the other groups on multiple comparisons (p<0.05).

EFFECT OF DIET ON MUCOSAL CHARACTERISTICS IN THE DISTAL COLON

Morphology

There was no evidence of epithelial cell damage or erosions, or of abnormalities in crypt architecture in any dietary group. Likewise, no histological evidence of mucosal inflammation was seen.

Epithelial kinetics

Table 2 shows the effects of diets on epithelial kinetic indexes. Diet significantly altered crypt column height (CCH) in the distal colon (p<0.0001). Multiple comparisons showed that CCH was significantly increased in the wheat bran and methylcellulose fed animals compared with those fed no fibre (p<0.05). CCH was also significantly higher in the wheat bran fed animals compared with those fed methylcellulose (p<0.05). Statistically significant differences in the number of mitoses per colonic crypt column were also found across the dietary groups (p<0.05), due primarily to the increase induced by wheat bran. However, the mitotic index was similar across the groups.

Activities of brush border hydrolases

Significant differences were found across the dietary groups in mucosal alkaline phosphatase activities in the distal colon (p<0.001; fig 3). Mean alkaline phosphatase activities in the methylcellulose fed animals were 243% and 180% higher than in the no fibre and wheat bran groups, respectively, and were responsible for this difference. DPPIV activities also significantly differed across the dietary groups, but this was due to higher activities in the wheat bran group (fig 3). The activities of alkaline phosphatase and DPPIV were also measured in jejunal mucosa, but no differences across the dietary groups were observed (data not shown).

Discussion

This study shows, for the first time, changes in distal colonic epithelial permeability induced by alterations of dietary intake in normal rats. Thus, ingestion of dietary fibre reduced transepithelial conductance and the apical to basolateral flux of ^{51}Cr-EDTA by approximately 25% compared with ingestion of a no fibre diet for four weeks. EDTA, due to its hydrophilicity, permeates mainly via the paracellular route, indicating that the changes in permeability were due essentially to alterations in the paracellular pathway. A descending gradient of paracellular permeability from the jejunum to the colon has previously been reported using the techniques applied in the present study.[19] In the rats fed fibre, such a gradient was evident; in rats fed no fibre, permeability in the distal colon was comparable with that in the jejunum. Diet induced differences in paracellular permeability were not observed in either the caecum or jejunum. Such localised sensitivity of the distal colon to the intake of dietary fibre has also been

reported with regard to epithelial cell kinetics, as the effects of fibre on reversing epithelial atrophy are confined primarily to the distal colon.[13 23]

In an attempt to identify pathogenic mechanisms underlying diet induced changes in paracellular permeability, four aspects were considered. Firstly, the changes in permeability may have reflected epithelial erosion or ulceration associated with the lack of dietary fibre and not alterations in the function of tight junctions. Such a possibility was discounted, however, by the extensive morphological analysis of the distal colon, which showed no evidence of epithelial injury.

Secondly, the diets may have induced changes in permeability via mechanisms unrelated to colonic luminal and epithelial events. Small intestinal permeability may be influenced by deficiency of macronutrients[4] or by stress.[24] However, there was no evidence of such events occurring in the rats. The diets contained the recommended micronutrients and each diet was identical in that regard. Although 24 hour food intake was greater in fibre fed animals, this was expected as the fibre supplemented diets have a lower energy density. The rats gained weight to a similar degree across the three groups, and at no time showed evidence of stress.

Thirdly, associations between changes in luminal characteristics and paracellular permeability were investigated. Previous observations in Caco-2 monolayers suggested that SCFAs are key effectors in reducing paracellular permeability in vitro.[10] Consistent with that previously reported,[12] the wheat bran diet was associated with high levels of SCFAs in the faeces (an accurate indicator of the distal colonic environment[12]) and caecum. However, as also previously reported,[13] methylcellulose caused very low concentrations of SCFAs in the faecal and caecal contents, levels that were even lower than those observed in rats fed the no fibre diet. In parallel with SCFA concentrations, pH was lower in the faecal and caecal contents of wheat bran fed animals than in the other two groups. As no correlation was found between the indexes of fermentation measured and paracellular permeability in either the distal colon or caecum, the hypothesis that SCFAs were responsible for improved barrier function is not supported.

Both wheat bran and methylcellulose exert a notable bulking effect on luminal contents. As we have previously found,[12 13] faecal output in animals fed the fibre supplemented diets was approximately 10-fold that of the no fibre fed controls. The effects of wheat bran and methylcellulose on reducing paracellular permeability, therefore, paralleled their effects on faecal bulking. Fibre has previously been suggested to modulate colonic epithelial proliferation through its bulking effect, either by causing mechanical distension,[25] or by causing surface cell abrasion.[26] Other studies, however, have also shown that bulk alone is insufficient to modulate colonic epithelial cell proliferation,[27 28] suggesting that fibres may have other properties that are fundamental to their effect.

Finally, the possibility that alterations in permeability reflected alterations in epithelial turnover and/or differentiation was addressed. Epithelial atrophy itself has been suggested to impair barrier function,[9] and both wheat bran and methylcellulose have been shown to reverse such atrophy.[13] Even though methylcellulose exhibited only mild (but statistically significant) effects in the present study, it is possible that small changes in CCH may have large effects on paracellular permeability. Rather than the number of cells in the epithelium, the maturity of the tight junctions is more likely to be a central factor governing paracellular permeability. Morphological studies have shown well formed tight junctions between surface epithelial cells in comparison to poorly formed tight junctions in cells deep in the crypt,[11 29 30] and immunohistochemical studies have shown that the tight junction proteins, ZO-1 and occludin, are predominantly expressed in the surface epithelium (Mariadason *et al*, manuscript in preparation). These observations suggest that the differentiated surface epithelium is largely responsible for paracellular barrier function of the epithelium. Furthermore, the morphological observations are in keeping with studies of both spontaneous and butyrate induced differentiation in Caco-2 cells, which suggest that the maturation of tight junctions is part of the differentiation process.[10 31] In the present study, measurement of the activities of brush border hydrolases in the mucosa was used to assess the differentiation status of the epithelium.[32 33] This technique has been successful in, for example, identifying the loss of surface epithelial cells in colonic mucosa in Ussing chambers exposed to 10 mM butyrate (Mariadason *et al*, manuscript submitted). Both fibres significantly increased the activities of one hydrolase, DPPIV with wheat bran and alkaline phosphatase with methylcellulose. These different responses may each represent promotion of epithelial differentiation, although of differing phenotype. Studies in models of colonic epithelium in vitro, such as Caco-2 cell monolayers, have clearly shown that different stimuli can induce morphologically and functionally differentiated cells which exhibit different profiles of phenotypic markers of differentiation.[34 35]

The contrasting spectra of effects that the two fibres exert on epithelial kinetics and mucosal hydrolase activities raise concerns that the search for a single mechanism of action in altering paracellular permeability may be too simplified, and are more suggestive that separate mechanisms are at work. Wheat bran, for example, may be working predominantly via supply of SCFAs. It is known that butyrate or SCFAs, when infused into the colonic lumen, can totally mimic the trophic effect of wheat bran.[36 37] Butyrate and SCFAs also promote differentiation and improve barrier function in a colonic epithelial model system.[10] Methylcellulose may be acting via an unknown mechanism to correct, at least partly, the atrophy and to promote cell differentiation. One

possibility is that they represent the surface effect of the large amount of residual fibre in the lumen. Such hypotheses can only be addressed by further experimentation addressing the effect on barrier function of, for example, feeding inert bulking materials, or infusing SCFAs into the lumen of the colon.

Although a statistically significant difference in paracellular permeability was found across the groups, the biological significance of the observation is less easily ascertained. The rats in the present study did not suffer illness and there was no histological evidence of mucosal inflammation. However, rats are relatively resistant to the development of mucosal inflammation as exemplified by the lack of colitis in diverted colons, a feature that is invariable in the human equivalent.[38] Clinical studies in situations where compromised barrier function plays a pathogenic role (such as in the critically ill patient or in ulcerative colitis) may be the best way of determining whether the dietary changes to permeability are indeed clinically relevant.

In conclusion, this study shows that the amount of fibre in the diet significantly alters barrier function by reducing paracellular permeability in the distal colon, but not the caecum or jejunum of normal rats. Features shared by the fibres include faecal bulking, a trophic effect on the colonic epithelium, and promotion of at least one feature of colonic epithelial differentiation. Because of the differences in the spectra of effects the two fibres exert on luminal indexes, epithelial kinetics, and mucosal hydrolase activities, it is proposed that they are acting to reduce paracellular permeability by different mechanisms.

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