Effects of an elemental diet, inert bulk and different types of dietary fibre on the response of the intestinal epithelium to refeeding in the rat and relationship to plasma gastrin, enteroglucagon, and PYY concentrations

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SUMMARY Refeeding starved rats with an elemental diet resulted in a marked increase in crypt cell production rate (CCPR) in the proximal small intestine but not in the distal regions of the gut. Little effect on CCPR was noted when inert bulk (kaolin) was added to the elemental diet. Addition of a poorly fermentable dietary fibre (purified wood cellulose) had little effect on intestinal epithelial cell proliferation except in the distal colon where it significantly increased CCPR. A more readily fermentable fibre (purified wheat bran) caused a large proliferative response in the proximal, mid, and distal colon and in the distal small intestine. A gel forming fibre only significantly stimulated proliferation in the distal colon; the rats in this group, however, did not eat all the food given. There was no significant correlation between CCPR and plasma gastrin concentrations, but plasma enteroglucagon concentrations were significantly correlated with CCPR in almost all the sites studied. Plasma PYY concentrations also showed some correlation with CCPR, especially in the colon. Thus while inert bulk cannot stimulate colonic epithelial cell proliferation fermentable fibre is capable of stimulating proliferation in the colon, and especially in the distal colon: it can also stimulate proliferation in the distal small intestine and it is likely that plasma enteroglucagon may have a role to play in this process.

The intestinal epithelium can respond to a wide variety of stimuli by altering its proliferative rates.^{1,2} Feeding animals with low bulk chemically defined semisynthetic (elemental) diets causes atrophy and reduced intestinal cell proliferation.³⁻⁹ This has been attributed to lack of non-absorbable bulk,¹⁰ but more recent work strongly suggests that it is because of lack of dietary fibre.¹¹⁻¹⁴ The term dietary fibre encompasses a wide range of diverse substances which

cannot be digested without the action of the gut microflora.¹⁵ The proliferative effects of the various types of fibre also appears to vary,¹⁴ this may be because of the differences in the ease with which they can be fermented in the hind gut, as there is evidence that the products of hind gut fermentation can have a trophic effect on the gastrointestinal epithelium.¹⁶ Such an effect could be direct or indirect, and if the latter, a hormonal mechanism may be implicated. Two of the hormones which have most often been implicated in the control of intestinal epithelial cell proliferation are enteroglucagon and gastrin.¹⁷

One way of studying the mechanisms involved in the regulation of intestinal epithelial cell proliferation without recourse to grossly unphysiological

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stimuli is to investigate the response of the gut to starvation and refeeding.^{18 19}

The hypothesis that hind gut fermentation stimulates colonic epithelial cell proliferation was tested in the present study by comparing the effects on the intestinal epithelium of refeeding starved rats with an elemental diet supplemented with inert bulk (kaolin), a poorly digestible fibre (wood cellulose), a digestible bran concentrate (Trifyba) and a soluble non-starch polysaccharide which forms a gel in water (prepared from the ispaghula husk). Refeeding with the elemental diet alone or with a standard rat chow was also studied.

The proliferative rates of the above treatments were also compared with the plasma hormone concentrations of gastrin, enteroglucagon, and peptide YY (PYY), a new candidate hormone²⁰ which appears to be colocalised with enteroglucagon.²¹⁻²³

Methods

ANIMALS

Male Wistar rats were used (Olac Ltd, Blackthorn, Oxon, UK). Their mean body weights are shown in Table 1. They were kept on wire bottomed cages and fed a powdered version of a standard laboratory diet (Labshure PRD, Christopher Hill, Poole, Dorset) for two weeks before the start of the experiment and were then fed or starved as described in the experimental design below. They had constant access to water.

EXPERIMENTAL DESIGN

Eight groups of 10 rats were used. Group A was fed the powdered laboratory rodent diet PRD throughout the investigation, group B was starved for three days and then killed. Groups C, D, E, F, G, and H

were starved for three days and then refed for two days. All refed groups were given 15 g/rat/day of their respective diets at 0900 hours. Group G was refed the PRD diet and group H was refed with the elemental diet Flexical (Mead Johnson, Slough). The diet for group C was a 1:1 mixture of Flexical and kaolin. Diet D was a 1:1 mixture of Flexical and cellulose (Solka Flocc grade BW-100, Johnsen Jorgensen and Wettre Ltd, London). Diet E was a 1:1 mixture of Flexical and a fibrous extract of wheat grain Trifyba (Labaz Sanofi UK Ltd, Wythenshawe, Manchester; hemicellulose 40%, cellulose 20%, lignin 15% and pectin 5%). Diet F was a 1:1 mixture of Flexical and the dietary mucilage prepared from the ispaghula husk²⁴ (Reckitt & Colman, Hull).

The rats were weighed at the beginning of the experiment, after starvation and after refeeding. They were injected with vincristine at the end of the experiment so that the crypt cell production rate (CCPR) could be determined.

PROXIMATE ANALYSES OF THE DIETS (g/kg)

Labshure PRD

Barley, oats, wheat, wheatfeed, maize meal, soya bean extract, dried skim milk, dried yeast, white fish meal minerals and vitamins (crude protein 201, crude oil 28, crude fibre 52 energy content 9341 kJ (2254 kcal)).

Flexical

Sucrose, maize syrup solids, enzymically hydrolysed casein, tapioca starch, soya bean oil, medium chain triglycerides (fractionated coconut oil) citric acid, mono- and diglycerides, lecithin, amino acids (L-methionine, L-tyrosine L-tryptophan) vitamins and minerals (protein equivalent 99, fat 150, carbohydrate 678, ash 27, moisture 15 energy content 17782 kJ (4250 kcal)).

Crypt cell production rate (CCPR)

The use of valid methods for the quantification of proliferation is vitally important and a rate measure such as the crypt cell production rate (CCPR) method is one of the few methods which is robust enough to withstand criticism.^{25 26} The rats were given an intraperitoneal injection of vincristine sulphate (Tillots laboratories, Henlow Bedfordshire UK) at 0900 hours and were killed at timed intervals 30 to 180 minutes later. Samples of the small intestine and colon (as defined by their position in the small intestine and colon relative to length) were removed and fixed in Carnoy's fluid. The tissue was later stained with the Feulgen reaction and the intestinal or colonic crypts were displayed by microdissection (Fig. 1); the number of arrested metaphases in 10 crypts was then counted and the mean values plotted

Table 1 Body weight changes and per cent weight changes of the various groups of rats during the course of the investigation.

Group	Start weight (g)	Weight after 3 days starvation	Weight decrease (g) (%)	Weight at end of experiment	Weight increase upon refeeding (%)
	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE
A	236.6 2.8			252.2 2.9	
B	237.8 3.0	192.0 3.1	18.7 0.5		
C	236.7 3.8	186.7 3.7	21.1 0.9	194.4 2.6	4.3 1.2
D	235.0 2.4	184.0 2.1	21.7 0.6	183.5 2.6	-0.3 0.5
E	238.0 2.3	189.5 1.6	20.4 0.6	198.5 2.1	4.8 1.0
F	227.0 2.8	181.5 2.1	20.0 0.8	187.0 3.2	3.1 1.5
G	242.0 4.5	194.1 4.0	19.6 2.1	198.0 3.6	2.1 0.5
H	239.5 2.9	193.5 2.4	19.2 0.5	213.0 2.4	10.1 0.6*

*Significantly greater than all the other refed groups p<0.001

Fig. 1 Microdissected small intestinal crypt squash preparation. The tissue was stained with the Feulgen reaction and then gently teased apart to display intact crypts. Note the dense hyperchromatic vincristine arrested metaphase nuclei.

against time after injection. The slope of the line, fitted by least squares linear regression, gave the CCPR.²⁶

HORMONE ASSAYS

Gut hormones were measured using previously described radioimmunoassays for gastrin²⁷ pancreatic glucagon²⁸ enteroglucagon²⁹ and PYY.³⁰ Enteroglucagon was calculated by subtracting specifically measured pancreatic glucagon (C-terminal immunoreactivity measured with antiserum RCS5 from total N-terminal glucagon immunoreactivity (measured with antiserum R59)). The assays were capable of detecting the following plasma changes with 95% confidence gastrin 2 pmol/l, pancreatic glucagon 2 pmol/l, enteroglucagon 5 pmol/l, and PYY 2.5 pmol/l.

STATISTICAL ANALYSIS

Crypt cell production rate and weights are shown $\pm$ standard errors. Differences between groups were tested by a two-sided Student's *t* test. Crypt cell production rates were compared by a computer based *t* test using a pooled estimate of standard error. Correlations between CCPR's and hormone levels were tested by the Pearson product moment correlation method and Spearman's rank correlation method.

Results

All groups, except those fed diet F (Flexical plus ispaghula) ate all their food (15 g/rat/day). The rats in group F consumed only 8 g/rat/day. The intestines of this group was found on necropsy to be distended with the gel like ispaghula, suggesting that the

physical distension of the gut had inhibited food intake.

After one days refeeding groups C (Flexical + kaolin) and D (Flexical + wood cellulose) produced a considerable amount of white faeces, group E (Flexical + Trifyba) produced large dark faeces, group F (Flexical + ispaghula) only produced a few pale gelatinous faeces. The faeces of the rats fed the standard diet (G) were normal in colour and quantity whilst those fed Flexical on its own (H) had very few faeces and some of the rats in this group showed signs of diarrhoea.

The body weights of the various groups at different phases of the investigation are shown in Table 1. Analysis of variance showed that there were no significant differences in the start weights, weights after starvation and the percent weight losses. The mean weight loss for all the groups after three days starvation was $20.1\% \pm 0.4\%$. All groups except group D (Flexical + wood cellulose) showed a modest increase in body weight upon refeeding and the weight increase of group H (Flexical) was significantly ($p < 0.001$) greater than that of the other groups. There was no significant interaction between groups for the other refeed groups.

CRYPT CELL PRODUCTION RATES

The crypt cell production rates (CCPR's) of the sites studied in the small intestine are shown in Figures 2–4 and the CCPR's of the colonic sites are in Figures 5–7. Starvation significantly decreased CCPR in all sites ($p < 0.001$) except for the mid colon where $p < 0.05$.

Refeeding the rats led to a general increase in CCPR but this was only significant for group H (Flexical diet) in the 25% SI and 75% SI sites, for group G (Standard diet) in the 75% SI site and for group E (Flexical + Trifyba) in all sites except 25% SI; thus diet E had by far the most pronounced proliferative effect.

The CCPR of the refeed groups were still significantly lower than that of the fed group for all groups in the small intestine except group E in the 75% SI site. The CCPR in the colon was still less than that of the fed group (A) except for those of groups E (refed Flexical + Trifyba) and G (refed standard diet) for the 10% colon site, most groups except E in the 50% colon site and all the groups except D, E, and F in the 90% colon site. See Table 2. The CCPR of group E (refed Flexical + Trifyba) in the distal colon was significantly greater than that of the fed group (A) ($p < 0.01$).

The CCPR's of the various groups expressed as a percentage of the values in group H (Flexical alone) are shown in Table 3. The CCPR of group H in the 25% SI site was greater than that of all the other refeed

Fig. 2 Crypt cell production rates in the proximal small intestine (25% of the distance from pylorus to ileocaecal valve). Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refed - Flexical + kaolin. Group D = Starved then refed - Flexical + wood cellulose. Group E = Starved then refed - Flexical + Trifyba. Group F = Starved then refed - Flexical + ispaghula mucilage. Group G = Starved then refed - Powdered standard diet. Group H = Starved then refed - Flexical. * = significantly different than group B $p < 0.05$. ** = significantly different than group B $p < 0.01$. *** = significantly different than group B $p < 0.001$.

Table 2 Crypt cell production rate of the various groups expressed as a percentage of the values obtained in group A (fed rats) and the p values for these when tested against group A. For errors see Figs. 1-6

	25% SI		75% SI		90% SI	
	CCPR	p <	CCPR	p <	CCPR	p <
Group B	34.8		18.7	0.001	19.1	0.001
C	27.1	0.001	32.9	0.001	24.9	0.001
D	38.2	0.001	17.9	0.001	17.8	0.001
E	49.6	0.001	83.7		73.3	0.05
F	41.2	0.001	42.9	0.001	31.0	0.001
G	46.2	0.01	57.3	0.01	37.0	0.001
H	69.9	0.01	52.3	0.01	33.8	0.001
	10% colon		50% colon		90% colon	
	CCPR	p <	CCPR	p <	CCPR	p <
B	0.6	0.001	21.5		2.9	0.001
C	13.2	0.001	79.7	0.01	36.5	
D	13.4	0.001	34.2		82.5	
E	64.2		96.2		189.4	0.01
F	25.8	0.01	11.4	0.01	96.1	
G	52.2		72.2		40.4	
H	7.1	0.001	35.4		38.5	

groups and this was significantly so for all groups other than group E. In the 75% SI site the CCPR of group G (standard diet) was the same as that of the elemental diet and that of group E was greater. The response to the elemental diet was less in the 90% SI site whilst that of group E (Flexical + Trifyba) was significantly greater ($p < 0.01$). The CCPR's of the proximal colon (10% of length) showed that all the refed groups had greater CCPRs and that of group E was significantly ($p < 0.05$) greater than that of Flexical alone (H). The pattern of response in site 50% colon was more confused but groups C, E, and G seemed to have more proliferative activity. The results for the distal (90%) colon indicated that groups refed with Flexical plus cellulose, Trifyba and ispaghula (D, E, and F) had greater CCPR's than those fed Flexical alone (H) and the CCPR of group E was significantly ($p < 0.001$) greater.

PLASMA HORMONE CONCENTRATIONS

The effect of the various treatments on plasma enteroglucagon, gastrin and PYY are shown in

Fig. 3 Crypt cell production rates in the mid ileum (75% of the distance from pylorus to ileocaecal valve). Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refed - Flexical + kaolin. Group D = Starved then refed - Flexical + wood cellulose. Group E = Starved then refed - Flexical + Trifyba. Group F = Starved then refed - Flexical + ispaghula mucilage. Group G = Starved then refed - Powdered standard diet. Group H = Starved then refed - Flexical. * = significantly different than group B $p < 0.05$. ** = significantly different than group B $p < 0.01$. *** = significantly different than group B $p < 0.001$.

Figures 8, 9, and 10. Starvation significantly lowered the plasma concentrations of all hormones ($p < 0.001$). Plasma enteroglucagon increased significantly after refeeding in all groups except group C (Flexical + kaolin) (where $p < 0.055$) see Figure 8. Analysis of variance showed that plasma gastrin concentrations were not significantly altered after refeeding. There were, however, significant differences (by analysis of variance) between the enteroglucagon concentra-

tions of the refeed groups ($p < 0.01$) the highest levels being in groups E (Flexical + Trifyba) and H (Flexical alone) respectively. Plasma concentrations of PYY also showed significant ($p < 0.001$ by analysis of variance) differences between the refeed groups, mainly due to there being a marked increase in PYY levels in groups refeed with Flexical + Trifyba or isphagula (E and F).

Pearsons product moment correlation showed

Fig. 4 Crypt cell production rates in the terminal ileum (90% of the distance from pylorus to ileocaecal valve). Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refeed - Flexical + kaolin. Group D = Starved then refeed - Flexical + wood cellulose. Group E = Starved then refeed - Flexical + Trifyba. Group F = Starved then refeed - Flexical + isphagula mucilage. Group G = Starved then refeed - Powdered standard diet. Group H = Starved then refeed - Flexical.

* = significantly different than group B $p < 0.05$ ** = significantly different than group B $p < 0.01$

*** = significantly different than group B $p < 0.001$

Fig. 5 Crypt cell production rates in the proximal colon (10% of the distance from caecum to rectum). Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refeed - Flexical + kaolin. Group D = Starved then refeed - Flexical + wood cellulose. Group E = Starved then refeed - Flexical + Trifyba. Group F = Starved then refeed - Flexical + isphagula mucilage. Group G = Starved then refeed - Powdered standard diet. Group H = Starved then refeed - Flexical.

* = significantly different than group B $p < 0.05$ ** = significantly different than group B $p < 0.01$

*** = significantly different than group B $p < 0.001$

Fig. 6 Crypt cell production rates in the mid colon (50% of the distance from caecum to rectum). Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refeed - Flexical + kaolin. Group D = Starved then refeed - Flexical + wood cellulose. Group E = Starved then refeed - Flexical + Trifyba. Group F = Starved then refeed - Flexical + isphagula mucilage. Group G = Starved then refeed - Powdered standard diet. Group H = Starved then refeed - Flexical.

* = significantly different than group B $p < 0.05$ ** = significantly different than group B $p < 0.01$

*** = significantly different than group B $p < 0.001$

Table 3 Crypt cell production rate of the various groups expressed as a percentage of the values obtained in group H (fed the elemental diet Flexical). For standard errors see Figs. 1-6.

	25% SI		75% SI		90% SI	
	CCPR	p<	CCPR	p<	CCPR	p<
Group A	143.0	0.01	177.8	0.01	296.2	0.001
B	49.8	0.01	33.3	0.05	56.7	
C	38.6	0.001	58.5		73.9	
D	54.6	0.01	31.9		52.9	
E	70.9		148.8		217.2	0.01
F	59.0	0.02	76.3		91.7	
G	66.1	0.05	101.9		109.5	
<hr/>						
	10% colon		50% colon		90% colon	
	CCPR	p<	CCPR	p<	CCPR	p<
A	1413.0	0.01	282.1		260.0	
B	8.7		60.7		7.5	
C	187.0		225.0		95.0	
D	189.1		96.4		215.0	
E	906.5	0.05	271.4		492.5	0.001
F	365.2		3.2		250.0	
G	737.0		203.6		105.0	

Table 4 Spearman's rank correlation coefficient for crypt cell production rates and plasma hormone levels

	Plasma enteroglucagon	Plasma gastrin	Plasma PYY
25% small intestine	0.881†	-0.095	0.667*
75% small intestine	0.738*	-0.024	0.571
90% small intestine	0.738*	-0.024	0.571
10% colon	0.690*	0.381	0.643*
50% colon	0.500	0.452	0.310
90% colon	0.833†	0.333	0.810*

*p<0.05; †p<0.01

significant correlations between CCPR and plasma enteroglucagon in four sites (p<0.001) to p<0.02) and between CCPR and plasma PYY in four sites (p<0.01) and between CCPR and gastrin in three sites (p<0.05); however, these correlations were unduly influenced by the high CCPRs and hormone concentrations in group A (fed) as Figures 11 and 12 show. Spearman's rank correlation was thus used to reduce this bias, see Figures 13 and 14. The results for the Spearman's correlation are shown in Table 4. Plasma enteroglucagon concentrations were significantly correlated with the CCPRs of all sites except the mid colon. Plasma PYY concentrations were significantly correlated with the CCPR in three sites, two of these being in the colon. There was no significant correlation between plasma gastrin concentrations and CCPR or between plasma gastrin level and enteroglucagon or PYY (rs=0.190, 0.286) Plasma PYY was, however, significantly correlated with enteroglucagon (rs=0.905, p<0.01).

Discussion

The results of the present study show that a low residue elemental diet is capable of stimulating an adaptive response after refeeding, as was observed after intestinal resection³¹ and that the effects of such diets are mainly confined to the proximal regions of the gastrointestinal tract.^{7,8} These effects may also vary with the type of elemental diet³² and Flexical can maintain small bowel mass more than some other elemental diets.³³ Elemental diets require minimal digestion and are mainly absorbed in the proximal regions of the small intestine, thus the trophic responses reported here are compatible with the effects of luminal nutrition or mucosal workload.¹⁷

It is worth noting that the CCPR's of the fed group (A) were considerably greater than the control values from other experiments done in this laboratory such as¹⁸ which suggests that giving a standard diet in powdered form also stimulates proliferation, perhaps because of it being readily digested or to changes in intestinal transit time.

The diminished proliferative response seen in the distal gut of group H (refed with Flexical), which occurred despite the considerably greater calorific value of this diet confirms that such diets deprive the distal gut of luminal nutrition and/or functional demand. The greater calorific value of this diet was reflected in the significantly greater weight gain of group H.

Reduced rates of hind gut epithelial proliferation have been linked with lack of bulk.^{10,34} An increase

Fig. 7 Crypt cell production rates in the distal colon (90% of the distance from caecum to rectum).

Fig. 8 Plasma enteroglucagon concentrations. Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refed - Flexical + kaolin. Group D = Starved then refed - Flexical + wood cellulose. Group E = Starved then refed - Flexical + Trifyba. Group F = Starved then refed - Flexical + ispaghula mucilage. Group G = Starved then refed - Powdered standard diet. Group H = Starved then refed - Flexical. * = significantly different than group B $p < 0.05$ ** = significantly different than group B $p < 0.01$ *** = significantly different than group B $p < 0.001$

Fig. 9 Plasma gastrin levels. Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refed - Flexical + kaolin. Group D = Starved then refed - Flexical + wood cellulose. Group E = Starved then refed - Flexical + Trifyba. Group F = Starved then refed - Flexical + ispaghula mucilage. Group G = Starved then refed - Powdered standard diet. Group H = Starved then refed - Flexical. * = significantly different than group B $p < 0.05$ ** = significantly different than group B $p < 0.01$ *** = significantly different than group B $p < 0.001$

in colonic weight has been reported after feeding inert bulk (kaolin),³⁵ however, colonic weight also includes the muscle layer, and we have previously shown⁷ that kaolin can increase colonic muscle weight without affecting the mucosa. Bulky wheat bran can increase the size of colonic muscle cells whilst oat bran and pectin supplemented diets decrease the size of muscle cells.³⁶ The CCPR's obtained for group C (Flexical + kaolin) confirm that inert bulk has little effect on mucosal proliferation.

The CCPR's obtained on refeeding diet C (Flexical + kaolin) confirm that bulk (kaolin) has little effect on mucosal proliferation.⁷ All the groups except group F (Flexical + ispaghula) ate all the food given. The intestine of group F were distended with the swollen gel from stomach to colon and it is probable that this inhibited food intake. The amounts of fibre given in this study were far greater than those expected in normal diets, but such levels have been tolerated by rats in other studies.³⁷ Despite the reduced food intake of group F a significant effect on CCPR was noted in the distal colon and there was some indication of a proliferative effect in the small intestine 75% site.

Diet D (Flexical + wood cellulose) also had a significant effect on the CCPR of the distal colon despite the poor digestibility of this fibre compared with the more natural bran like fibres.¹⁵ Addition of

Fig. 10 Plasma Peptide YY (PYY) levels. Group A = Fed group (powdered laboratory rat diet). Group B = Starved for 3 days. Group C = Starved then refed - Flexical + kaolin. Group D = Starved then refed - Flexical + wood cellulose. Group E = Starved then refed - Flexical + Trifyba. Group F = Starved then refed - Flexical + ispaghula mucilage. Group G = Starved then refed - Powdered standard diet. Group H = Starved then refed - Flexical. * = significantly different than group B $p < 0.05$ ** = significantly different than group B $p < 0.01$ *** = significantly different than group B $p < 0.001$

Fig. 11 Plot of plasma enteroglucagon concentrations versus crypt cell production rates for the proximal small intestine (25% of the distance from pylorus to ileocaecal valve).

Fig. 12 Plot of plasma gastrin levels versus crypt cell production rates for the proximal small intestine (25% of the distance from pylorus to ileo-caecal valve).

Fig. 13 Rank plot of plasma enteroglucagon concentrations versus crypt cell production rates for the proximal small intestine (25% of the distance from pylorus to ileocaecal valve).

Fig. 14 Rank plot of plasma gastrin concentrations versus crypt cell production rates for the proximal small intestine (25% of the distance from pylorus to ileocaecal valve).

the purified wheat bran fibre Trifyba (diet E) had a dramatic stimulatory effect on the CCPR of almost all sites. This was most pronounced in the distal colon. The response of the colon to fibre in other experiments¹² also suggests that the distal colon is more susceptible than the proximal colon to such stimuli. It is interesting to note that most experimentally induced colonic tumours occur in the mid to distal zones of the large bowel.^{38,39} Colonic epithelial hyperplasia has been associated with increases in carcinogenesis which is consistent with the two step model of carcinogenesis where proliferation can promote a previously initiated cell.³⁹ Enhancement of colon carcinogenesis in the rat has been reported after supplementation with wheat bran,⁴⁰ this was especially so when given at the same time as the carcinogen which was also the regime that caused the maximum increase in hyperplasia;⁴¹ however, when the bran diet was given after the carcinogen there were less tumours.⁴⁰ The maximum yield of tumours was, however, obtained when a fibre free diet was given after the carcinogen; fibre free diets have been associated with reduced levels of enzymes which can detoxify certain xenobiotic chemicals.⁴²

Dietary fibre has several effects on the bowel, all of which may affect carcinogenesis. Fibre can delay

gastric emptying and impede or delay absorption, alter transit times, dilute or bind carcinogens, alter bile acid levels and cholesterol levels and induce xenobiotic metabolising enzymes.^{42,43} Hind gut fermentation plays an important role in the energy balance of most single stomached mammals including man,⁴⁴ and the products of this fermentation are the short chain fatty acids (SCFA's) or volatile fatty acids (VFA's). The release of these SCFA's also has several effects on the colon such as lowering the luminal pH, increasing mucosal blood flow, altering ammonia concentrations and increasing water absorption.⁴⁵⁻⁴⁷

The main SCFA's are acetic acid, propionic acid and butyric acid. Butyric acid is preferentially metabolised by isolated colonocytes.⁴⁸ SCFA's have been reported to have a trophic effect in the large and small intestine^{16,49,50} and the response to the different fibres suggests that the more readily fermentable fibres had the greatest effect. It is thus likely that rodent hind gut mucosal cell proliferation can also be stimulated by SCFAs as is the forestomach of ruminants where inert sponges had no effect on epithelial development but SCFAs stimulated proliferation.^{11,51} A considerable proportion of SCFA's are metabolised by the epithelial cells of the colon or by the

liver.^{37,52,53} Trophic effects could be caused by either the SCFAs or by their metabolites or by some other systemic factor.

Plasma enteroglucagon concentrations have previously been linked with increased CCPR¹⁷ and the correlation between the plasma concentrations of this hormone and the CCPR in this investigation suggest that enteroglucagon may be involved in moderating this effect. Enteroglucagon cells are present throughout the gut but most are localised in the terminal ileum and colon⁵⁴ which is a strategic position for monitoring SCFA release (or the presence of undigested food). Peptide YY distribution in the gut is similar to that of enteroglucagon²¹⁻²³ and it is interesting that most of the correlation between PYY and CCPR was due to the high levels of PYY in groups E and F which had the most readily fermented diets and that two of three of the PYY correlations observed occurred in the colon. Peptide YY can inhibit gastric acid secretion and stomach emptying^{55,56} thus active fermentation in the colon may be a signal for delaying gastric digestion and emptying mediated *via* PYY. A role for PYY in the control of gastrointestinal epithelial cell proliferation is less certain as although PYY levels have been shown to correlate with CCPR in resected rats, direct infusion of PYY has little effect on CCPR.⁵⁷

The conclusions of the present study are thus; that while a fibre free diet can stimulate epithelial cell proliferation in the proximal small intestine of refed rats, cell proliferation in the distal gut and colon requires other factors. The hypothesis that inert bulk is required is not borne out. Readily fermentable fibre can be effective in stimulating proliferation, the release of SCFAs into the lumen could account for the proliferative effects noted. These proliferative effects were also noted in the small intestine and plasma enteroglucagon was implicated in this.

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