Tissue hypertrophy and epithelial proliferation rate in the gut of rats fed on bread and haricot beans (*Phaseolus vulgaris*)

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The present study was designed to test the hypothesis that increasing short-chain fatty acid (SCFA) production in the large bowel increases gut epithelial proliferation rate (EPR). Two experiments were carried out in which rats were fed on bread (wholemeal or white)-based diets containing graded amounts of cooked haricot (*Phaseolus vulgaris*) beans; the latter are a rich source of fermentable carbohydrates. Consumption of beans was associated with several-fold increases in SCFA production with the greatest relative increase being for butyrate. Despite the very large increase in SCFA production, there was no evidence that this had any effect on EPR in the duodenum. Where the basal diet contained wholemeal bread (Expt 1) there was no effect of enhanced SCFA supply on EPR in either the caecum or colon, but with the white bread-based diet (Expt 2) adding beans produced increments in both SCFA supply and EPR in the caecum. Evidence that SCFA are responsible for enhanced EPR above normal levels is not convincing. In those instances where enhanced SCFA supply is associated with increased EPR, the increase may be (1) from a hypoproliferative state towards normal, (2) a transient phenomenon accompanying tissue hypertrophy or (3) a homeostatic response to increased cell loss by cell sloughing or apoptosis. It is not likely that there is any direct link with risk of colon cancer.

Epithelial proliferation rate: Short-chain fatty acids: Haricot beans: Tissue hypertrophy

Consumption of carbohydrates not digested in the small intestine (SI) is frequently associated with trophic effects in the large bowel (LB) of rats (Wyatt *et al.* 1988; Rémésy & Demigné, 1989; Seal & Mathers, 1989). The mechanism of this trophic effect has not been established. Since energy-yielding nutrients normally digested and absorbed from the SI are trophic, at least for the mucosa of that organ, it might be assumed that the major energy-containing products of LB fermentation, the short-chain fatty acids (SCFA), would stimulate growth of the LB tissues. Indeed, it has been established that SCFA are trophic for the rumen mucosa (Sakata & Tamate, 1978) and contribute to the development of the rumen during the weaning process in ruminant animals (Tamate *et al.* 1962). In rats direct infusion of SCFA into the distal intestine stimulates LB epithelial proliferation rate (EPR; Sakata, 1987) whilst isolation of the LB from its normal ileal supply of substrate, and therefore prevention of fermentation and SCFA production, suppresses EPR (Sakata, 1988). This led to the suggestion that the SCFA are lumen trophic factors (Sakata, 1987). A satisfactory explanation for the mode of action of SCFA putative trophic effect has not yet emerged (Rombeau *et al.* 1995; Sakata, 1995). A direct effect on the mucosal cell seems unlikely. Sakata (1987) reported that jejunal mucosal EPR was increased when SCFA were infused into the terminal ileum and suggested that an endogenous mediator may be involved. Candidates for the latter include the gastrointestinal peptides which have a trophic function (Goodlad & Wright, 1987) and especially enteroglucagon which is secreted

* For reprints.

mainly from the distal intestine and is present in higher concentrations in blood in some (Goodlad *et al.* 1987) but not all (Johnson *et al.* 1988; Goodlad *et al.* 1989; Malville-Shipen & Fleming, 1992) complex-carbohydrate feeding studies. An alternative mode of action was suggested by Mortensen *et al.* (1990) who found that SCFA have a dilating effect *in vitro* on the resistance arteries of the human colonic wall and speculated that improved microcirculation *in vivo* would have a trophic effect on the LB mucosa. Such an action might also explain the trophic effect on the intestinal mucosa of intravenously administered SCFA in the rat (Koruda *et al.* 1988). However, SCFA may not be essential for LB hypertrophy since caecal hypertrophy occurs when polysaccharides which are not (or are only poorly) fermented are fed to rats (Wyatt *et al.* 1988) and in the germ-free animal (Goodlad *et al.* 1989) in the absence of fermentation.

The objective of the present study was to test the hypothesis that production of additional SCFA would have a trophic effect on the gut and would be associated with increased EPR. To avoid problems of interpretation which occur when animals are fed on purified or elemental diets, the rats in these studies were fed on bread-based diets in which cooked haricot beans (*Phaseolus vulgaris*) were included to increase the intake of carbohydrates not digested in the SI and therefore stimulate LB fermentation and the production of SCFA. The choice of foods was to ensure greater relevance to human nutrition.

Brief accounts of parts of this study have already been published (Key & Mathers, 1989; Mathers *et al.* 1990).

MATERIALS AND METHODS

Two separate experiments were carried out. In the first (Expt 1), rats were fed on wholemeal-bread-based diets containing 0–450 g cooked, freeze-dried haricot beans (*Phaseolus vulgaris*)/kg diet as described by Key & Mathers (1993). Diets in the second experiment were based on white bread to reduce the intake of NSP with similar beans inclusion rates. The latter experiment consisted of two parts, an adaptation study (Expt 2a) and a balance study (Expt 2b) (Key & Mathers, 1995).

Diets and feeding

Bread for both experiments was purchased as standard 800 g sliced loaves from Robertsons Bakers, Carlisle, Cumbria, cut into approximately 20 mm squares, frozen at −20° within 4 h of delivery and then freeze-dried. The haricot beans (purchased from Health Fayre, Newcastle upon Tyne) were autoclaved to destroy heat-labile antinutritional factors, frozen at −20° and freeze-dried. Both breads and beans were milled to pass a 1 mm screen and stored at −20° until incorporated into the diets.

Expt 1. Four diets were formulated, each containing 500 g freeze-dried wholemeal bread/kg diet plus 0, 150, 300 or 450 g cooked and freeze-dried haricot beans/kg respectively (see Key & Mathers, 1993). The proportions of sucrose and casein + methionine were adjusted to maintain similar dietary protein concentrations and all diets contained the same proportion of vitamin and mineral mix (26 g/kg) with maize oil to provide essential fatty acids.

Expt 2. Again four diets were prepared but this time each contained 500 g white bread/kg diet and 0, 150, 300 or 450 g cooked and freeze-dried haricot beans/kg diet as described by Key & Mathers (1995). Adjustments to the proportions of sucrose and casein + methionine were made to keep the diets isonitrogenous and adequate supplies of all vitamins, mineral and essential fatty acids were included.

For all diets, the breads and beans provided all the polysaccharides whilst sucrose was the only other carbohydrate present. Cr_2O_3 (2 g/kg diet) was included in the diets as an

indigestible marker. Rats were offered 20 g (Expt 1) or 15 g (Expt 2) air-dry diet at 10.00 hours daily with uneaten feed removed at the same time the next morning. Water was available *ad libitum*.

Experimental protocols

Expt 1. Twenty-four Wistar rats were purchased (A. Tuck & Sons, Battlesbridge, Essex) and housed in individual perspex and stainless-steel metabolism cages (Thompson, 1970) with six rats allocated at random to each diet. After 7 d adaptation to the diets there followed two consecutive 7 d balance periods with total collection of faeces and urine and measurement of intake. Subsequently each animal was injected intraperitoneally with the metaphase arrest agent vincristine sulphate (1 mg/kg body weight provided in sterile saline (9 g NaCl/l)). After 2 h, anaesthesia was induced by inhalation of diethyl ether and blood, digesta and tissue samples were collected (Goodlad & Mathers, 1990). The portion of intestine 100 mm distal to the pyloric sphincter (duodenum) was removed, opened lengthways by cutting along the side opposite to the mesenteric vessels and a 10 mm length taken from the midpoint of the section. After the caecum was emptied and rinsed in saline, a 10 mm square was cut from the main body of the organ. The colon was opened and a 10 mm long portion of tissue was taken approximately halfway along its length. Tissue samples were immediately placed in fixative (40 g glutaraldehyde/l in phosphate buffer at pH 7·4) for 2 h before being transferred to phosphate buffer.

Expt 2. Fifty male Wistar rats were obtained from the Comparative Biology Centre, University of Newcastle upon Tyne, and housed in individual plastic metabolism cages. For 10 d, each rat was fed on the basal white-bread-based diet with no haricot beans (WH1) after which they were split at random into groups of thirty (adaptation study, Expt 2a) and twenty (balance study, Expt 2b) respectively. In Expt 2a, fifteen rats remained on diet WH1 whilst the other fifteen were changed abruptly to the diet containing 450 g haricot beans/kg (WH4). Five rats from each diet group were sampled on days 1, 2 and 3 thereafter. In Expt 2b the twenty rats were divided into four equal groups, five rats remained on diet WH1 whilst the other three groups were offered the diets containing 150 (WH2), 300 (WH3) or 450 (WH4) g cooked haricot beans/kg diet. A 7 d period was allowed for adaptation followed by a 7 d balance period. Vincristine sulphate was administered as described above and anaesthesia induced 2 h later by intraperitoneal injection of Hypnorm Midazolam cocktail (1·0 ml/300 g body mass; prepared by the Comparative Biology Centre). A 10 mm length of intestine from 100 mm distal to the pyloric sphincter and the entire caecal tissue (clipped to a glass microscope slide) were placed in formal saline fixative (100 ml formaldehyde/l saline).

Histological measurements

Expt 1. Samples were post-fixed in osmium tetroxide (10 g/l phosphate buffer, pH 7·4), rinsed in phosphate buffer and then dehydrated through a series of aqueous ethanol solutions (500, 700 and 900 ml/l respectively) for 15 min each followed by three 15 min washes in absolute ethanol and three 15 min rinses in propylene oxide. The samples were then immersed in epoxy resin–propylene oxide (1:1, v/v) for 6 h before embedding in pure resin at 60° for 16 h. Transverse sections (1 μm thick) were cut, stained with toluidine blue and mounted on microscope slides.

Expt 2. Samples were dehydrated in aqueous ethanol (700 ml/l) overnight, aqueous ethanol (900 ml/l) for 2 h and given two 1 h washes in absolute ethanol before being cleared by immersing in chloroform for 3 h and then 16 h. The fixed tissue was wax-embedded under vacuum at 58° and transverse sections (2–4 μm thick) cut, stained and mounted on microscope slides.

Intestinal sucrase (EC *3.2.1.48*) *assay* (*Expt 2*). Mucosal scrapes were suspended in 10 ml ice-cold mannitol (50 mM), Tris (hydroxymethyl) methylamine (2 mM, pH 7·4), homogenized with a Potter-Elvehjem homogenizer, diluted 1:10 or 1:20 with the same buffer and stored at −80°. Sucrase activity in these preparations was determined by a modification of the method of Dahlqvist (1968). Protein in the homogenates was assayed by a modified Folin–Lowry method (Lowry *et al.* 1951).

Calculation of organic matter flows and short-chain fatty acid production rates

Flow of organic matter (OM) from the terminal ileum to the large bowel was estimated by reference to the indigestible marker, Cr_2O_3, included in the diet and calculated as:

$$\frac{\text{daily } Cr_2O_3 \text{ intake}}{Cr_2O_3\text{:OM ratio in ileal digesta}}.$$

Faecal OM flow was estimated from quantitative collection of faeces. OM contents of digesta and faeces were determined by heating at 500° for 16 h and SCFA in caecal digesta by GLC as described by Mathers *et al.* (1990). SCFA production rates (or, more strictly, rates of apparent SCFA absorption from the LB) were determined from knowledge of OM disappearance within the LB (ileal OM flow − faecal OM output) and caecal SCFA molar proportions and using conventional assumptions about anaerobic stoichiometry (Demeyer & Van Nevel, 1975).

Statistical methods

Expt 1. The experiment was designed as a single-factor study with four treatments (diets) and six replicates (rats) per treatment. Values were examined by one-way ANOVA and orthogonal polynomials used to test for linear, quadratic and deviations from linear and quadratic responses to the inclusion of beans in the diet. Results are presented as means for each diet with a pooled standard error of the mean based on between-animals within-diets variation with 20 df.

Expt 2a, adaptation study. Two factor ANOVA was used to examine the effects of diet (WH1 *v.* WH4), of time of exposure to the diets (d) and of the interactions between diet and time. In each case the effects of time and the corresponding interactions term were split into (1) linear and (2) deviations from linear effects of time and each tested against the between-animals within-treatments variation with 24 df.

Expt 2b, balance study. Data from this study were analysed as for Expt 1 with the exception that the error term (between-animals within-diets) had 16 df.

RESULTS

Short-chain fatty acid absorption from the large bowel

Adding beans to the diet in place of sucrose and casein increased the flow of OM from the terminal ileum. Although OM output in faeces also increased, this was to a much smaller extent so that OM apparently fermented in the LB increased linearly (see Fig. 1 for Expt 1 and Key & Mathers (1995) for Expt 2). From knowledge of the proportions of SCFA in caecal digesta (Key & Mathers 1993, 1995), estimates of SCFA absorption from the LB were made using conventional assumptions about anaerobic stoichiometry (Demeyer & Van Nevel, 1975). For both wholemeal- and white-bread-based diets there were marked linear increases in estimated SCFA absorption with increases in beans inclusion, so that with the highest bean diets total SCFA absorption was five (Expt 1, Key & Mathers, 1993) or four

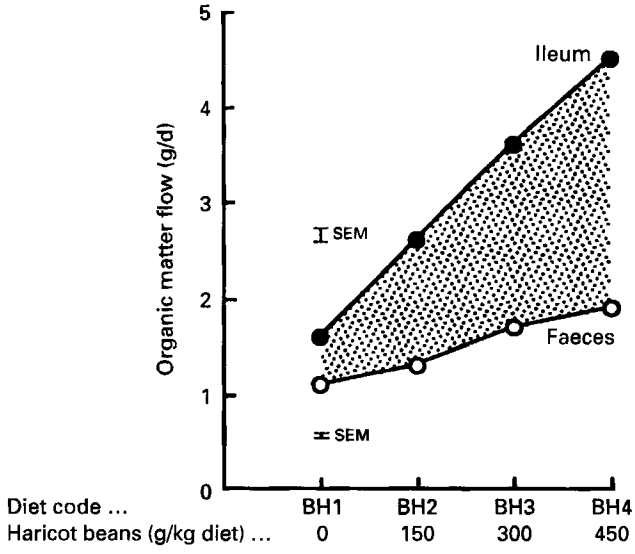

Fig. 1. Flow of organic matter (OM) from the terminal ileum (●—●) and output in faeces (○—○) of rats given wholemeal bread and cooked haricot beans (*Phaseolus vulgaris*) fed in mixed diets. Each value is a mean for six animals. The shaded area represents OM apparently fermented in the large bowel.

Table 1. *Expt 2b. Estimated absorption† of short-chain fatty acids (SCFA; mmol/d) from the large bowel of rats given white-bread-based diets containing 0–450 g cooked haricot beans* (Phaseolus vulgaris)*/kg diet*‡

(Mean values for six rats per diet with the pooled standard error of the mean)

Diet code...	WH1	WH2	WH3	WH4	Pooled	Statistical significance of dietary effects		
Beans in diet (g/kg)...	0	150	300	450	SEM	Lin	Quad	Dev
Acetate	3·8	7·5	7·0	14·6	1·14	***	NS	*
Propionate	0·7	1·7	1·6	2·1	0·34	*	NS	NS
Butyrate	0·2	1·0	1·1	2·3	0·33	***	NS	NS
Total SCFA	4·7	10·2	9·7	19·0	1·38	***	NS	*

Lin, linear; Quad, quadratic; Dev, deviations from linear and quadratic effects of dietary bean concentration.
* $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.
† Calculated from organic matter disappearance in the large bowel and caecal SCFA molar proportions (Key & Mathers, 1995) assuming conventional anaerobic stoichiometry (Demeyer & Van Nevel, 1975).
‡ For details of diets see pp. 274–275.

(Expt 2b, Table 1) times greater than with the corresponding basal (no beans) diet. All three major SCFA showed increases but this was most marked for butyrate (11-fold) in Expt 2b (Table 1).

Gastrointestinal compartment masses and large bowel transit time

Results for Expt 1 were reported by Key & Mathers (1993). In summary, adding beans to the diet resulted in linear increases in caecal mass due largely to an almost doubling of digesta mass but tissue mass also increased significantly. There was no effect on SI length.

Table 2. *Expt 2a. Adaptation study. Gastrointestinal tissue and contents masses and large bowel transit time (TT) in rats previously adapted to a white-bread-based diet (WH1; no beans) for 10 d and either continuing on this diet or changing to diet WH4 (450 g haricot beans/kg) and sampled on the following 3 d*†

(Mean values for five rats per treatment with the pooled standard error of the mean)

Diet code...	WH1			WH4				Statistical significance of treatments‡				
Beans in diet (g/kg)...	0			450								
Sampling day...	1	2	3	1	2	3	Pooled SEM	Diet	Time (Lin)	Time (Dev)	Diet × time (Lin)	Diet × time (Dev)
Stomach:												
Tissue (g)	1·48	1·46	1·61	1·41	1·37	1·34	0·061	**	NS	NS	NS	NS
Wet contents (g)	9·66	9·11	15·38	8·22	7·18	7·06	1·860	*	NS	NS	NS	NS
DM contents (g)	3·75	4·23	6·64	1·88	2·42	2·29	0·860	***	*	NS	NS	NS
SI length (m)	0·110	0·117	0·120	0·115	0·116	0·117	0·0018	NS	NS	NS	NS	NS
Caecum:												
Tissue (g)	0·81	0·87	0·81	0·98	1·22	1·24	0·047	***	*	*	*	NS
Wet contents (g)	1·71	1·40	1·64	2·89	3·27	3·40	0·168	***	NS	NS	NS	NS
DM contents (g)	0·31	0·26	0·34	0·45	0·52	0·59	0·035	***	*	NS	NS	NS
TT (d)	0·59	0·43	0·66	0·25	0·24	0·31	0·049	***	NS	*	NS	NS
Colon:												
Tissue (g)	0·92	0·91	0·89	1·29	1·08	1·11	0·057	***	NS	NS	NS	NS
Wet contents (g)	0·57	0·78	0·86	1·74	1·54	1·56	0·147	***	NS	NS	NS	NS
DM contents (g)	0·15	0·26	0·29	0·41	0·44	0·46	0·040	***	*	NS	NS	NS
TT (d)	0·34	0·65	0·78	0·26	0·31	0·32	0·086	***	**	NS	*	NS

SI, small intestine; Lin, linear; Quad, quadratic; Dev, deviations from Lin and Quad effects of dietary bean concentration.

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† For details of diets and procedures, see pp. 274–276.

‡ For details of statistical procedures, see p. 276.

Expt 2a. Rats fed on diet WH1 had on average more digesta (both wet and dry matter) in the stomach when killed than those given diet WH4 (Table 2). Stomach tissue mass was consistently lower for the animals adapting to the high-beans diet (WH4) but note the unusually large value for day 3 of the adaptation study for the WH1 rats. There were no significant treatment effects on SI length but masses of caecal tissue and contents both increased within 1 d when rats were changed to diet WH4 and continued to increase over the three sampling days (interaction of diet × time linear was significant, $P < 0·05$). A similar diet effect was observed for the colon but there was no statistically significant ($P > 0·05$) evidence of further change beyond the first day of adaptation. For both LB compartments, including beans in the diet reduced significantly ($P < 0·001$) the transit time (TT) of Cr_2O_3 through the organ. The apparent increase in colonic TT with diet WH1 during the 3 d study was unexpected.

Expt 2b. Whilst caecal and colonic tissue masses and SI length all increased linearly ($P < 0·01$) in rats given graded levels of dietary haricot beans, stomach tissue mass decreased linearly ($P < 0·05$) (Table 3). For the stomach there was a curvilinear response in digesta mass at the time of killing with the greatest amounts present for diet WH3. In contrast, caecal and colonic digesta masses (both wet and dry matter) increased linearly with increasing beans consumption. Large bowel TT declined as beans intake increased but the effect was not statistically significant for the colon because of relatively large inter-rat variation.

Table 3. *Expt 2b. Balance study. Gastrointestinal tissue and contents masses and large bowel transit time (TT) in rats given white-bread-based diets containing 0–450 g cooked haricot beans* (Phaseolus vulgaris)*/kg diet*†

(Mean values for five rats per diet with the pooled standard error of the mean)

Diet code...	WH1	WH2	WH3	WH4	Pooled	Statistical significance of dietary effects		
Beans in diet (g/kg)...	0	150	300	450	SEM	Lin	Quad	Dev
Stomach:								
Tissue (g)	1·59	1·54	1·51	1·34	0·075	*	NS	NS
Wet contents (g)	9·0	10·3	13·7	6·6	1·60	NS	*	NS
DM content (g)	3·2	4·0	4·8	2·2	0·57	NS	**	NS
SI length (m)	0·118	0·115	0·122	0·125	0·002	**	NS	NS
Caecum:								
Tissue (g)	0·82	0·93	1·17	1·29	0·062	***	NS	NS
Wet contents (g)	2·0	2·5	3·6	4·8	0·21	***	NS	NS
DM content (g)	0·41	0·52	0·62	0·83	0·045	***	NS	NS
TT (d)	0·77	0·54	0·49	0·41	0·050	***	NS	NS
Colon:								
Tissue (g)	0·92	0·97	1·06	1·21	0·059	**	NS	NS
Wet contents (g)	0·72	0·83	1·50	1·51	0·189	**	NS	NS
DM content (g)	0·24	0·27	0·44	0·46	0·078	*	NS	NS
TT (d)	0·55	0·40	0·42	0·37	0·101	NS	NS	NS

SI, small intestine; Lin, linear; Quad, quadratic; Dev, deviations from linear and quadratic effects of dietary bean concentration.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of diets and procedures, see pp. 274–276.

Mucosal morphology and duodenal sucrase activity

Expt 2a. Approximately 250 (duodenum) and 180 (ileum) mg of wet mucosa were collected by gentle scraping with the edge of a microscope slide (Table 4). The apparent increase in ileal mucosa wet weight over the 3 d of the study was unexpected and we have no explanation for this observation. Mucosal protein concentrations were similar at both intestinal sites (means 110 and 103 (SE 3·7) mg/g wet mucosa for duodenum and ileum respectively) but higher on day 2 than on days 1 or 3 of sampling for both diets and both sites. Sucrase activity was at least twice as great in duodenal than in ileal mucosa but there was little evidence that diet or time had any effect on this variable. When averaged across both diets, duodenal crypt depth decreased linearly ($P < 0·05$) over the three sampling days.

Expt 2b. The amounts of mucosa collected were similar to those in Expt 2a (Table 5). There was a marked linear ($P < 0·001$) decline in duodenal mucosal mass and a corresponding linear ($P < 0·05$) increase in sucrase activity per unit wet mucosa as the proportion of beans in the diet increased. The specific activity of ileal sucrase (IU/mg protein) showed a quadratic response to diet with a maximum on diet WH3 (300 g beans/kg diet). No effects of diet on duodenal mucosal morphometry were detected.

Gastrointestinal epithelial proliferation rate

Expt 1. EPR was approximately four times greater in the duodenum than in the caecum which, in turn, was slightly greater than for the colonic mucosa (Table 6). Values were similar for all four diets at all three intestinal sites.

Table 4. *Expt 2a. Adaptation study. Sucrase* (EC *3.2.1.48*) *activities in duodenal and ileal mucosa and duodenal villus heights and crypt depths in rats previously adapted to a white-bread-based diet (WH1, no beans) for 10 d and either continuing on this diet or changing to diet WH4 (450 g haricot beans/kg) and sampled on the following 3 d*†

(Mean values for five rats per treatment with the pooled standard error of the mean)

Diet code...	WH1			WH4				Statistical significance of treatments				
Beans in diet (g/kg)...	0			450			Pooled		Time	Time	Diet × time	Diet × time
Sampling day...	1	2	3	1	2	3	SEM	Diet	(Lin)	(Dev)	(Lin)	(Dev)
Mucosal mass (mg/100 mm intestine)												
Duodenum	234	266	288	270	239	231	21·8	NS	NS	NS	*	NS
Ileum	142	180	218	176	169	189	14·9	NS	**	NS	NS	*
Mucosal protein concentration (mg/g mucosa)												
Duodenum	99	114	93	98	141	112	10·2	NS	NS	**	NS	NS
Ileum	75	134	101	89	131	90	10·0	NS	NS	***	NS	NS
Sucrase activity (IU/g mucosa)												
Duodenum	8·4	6·0	7·6	8·8	9·4	6·7	1·03	NS	NS	NS	NS	NS
Ileum§	3·2	3·2	2·5	2·1	2·9	2·5	0·44	NS	NS	NS	NS	NS
Sucrase activity (IU/g protein)												
Duodenum	87	51	84	90	67	67	11·8	NS	NS	*	NS	NS
Ileum§	53	24	26	23	23	25	7·4	NS	NS	NS	NS	NS
Sucrase activity (IU/100 mm intestine)												
Duodenum	2·0	1·6	2·1	2·4	2·3	1·6	0·29	NS	NS	NS	NS	NS
Ileum§	0·44	0·57	0·53	0·37	0·46	0·39	0·079	NS	NS	NS	NS	NS
Duodenum												
Villus height (μm)	505‡	534	539	544	525	498	21·0	NS	NS	NS	NS	NS
Crypt depth (μm)	151‡	147	131	146	117	127	7·3	NS	*	NS	NS	NS
Villus:crypt ratio	3·36‡	3·67	4·19	3·87	4·52	3·96	0·328	NS	NS	NS	NS	NS

Lin, linear; Quad, quadratic; Dev, deviations from Lin and Quad effects of dietary bean concentration.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see pp. 274–276.
‡ *n* 3.
§ Ileal sucrase activities were significantly lower than for the duodenum.

Table 5. *Expt 2b. Balance study. Mucosal mass and protein concentrations and sucrase (EC 3.2.1.48) activity in duodenal and ileal muscosa and duodenal villus height and crypt depth in rats given a white-bread-based diet containing 0–450 g cooked haricot beans* (Phaseolus vulgaris)*/kg diet*†

(Mean values for five rats per diet with the pooled standard error of the mean)

Diet code...	WH1	WH2	WH3	WH4	Pooled	Statistical significance of dietary effects		
Beans in diet (g/kg)...	0	150	300	450	SEM	Lin	Quad	Dev
Mucosal mass (mg/100 mm intestine)								
Duodenum	278	277	244	178	17·5	***	NS	NS
Ileum	163	178	145	186	13·8	NS	NS	NS
Mucosal protein concentration (mg/g mucosa)								
Duodenum	113	107	104	126	13·1	NS	NS	NS
Ileum	136	94	89	104	17·2	NS	NS	NS
Sucrase activity (IU/g mucosa)								
Duodenum	7·9	9·6	9·7	12·4	1·21	*	NS	NS
Ileum	2·5	2·6	3·4	2·6	0·38	NS	NS	NS
Sucrase activity (IU/g protein)								
Duodenum	74	96	95	114	14·1	NS	NS	NS
Ileum	19	28	41	27	3·7	*	**	NS
Sucrase activity (IU/100 mm intestine)								
Duodenum	2·24	2·77	2·34	2·21	0·027	NS	NS	NS
Ileum	0·39	0·47	0·51	0·45	0·063	NS	NS	NS
Duodenum								
Villus height (μm)	509	483	496	511	21·3	NS	NS	NS
Crypt depth (μm)	128	132	122	126	9·8	NS	NS	NS
Villus: crypt ratio	4·0	3·8	4·1	4·1	0·24	NS	NS	NS

Lin, linear; Quad, quadratic; Dev, deviations from linear and quadratic effects of dietary bean concentration.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see pp. 274–276.

Table 6. *Expt 1. Epithelial proliferation rate (arrested cells/1000 crypt cells per 2 h) at various sites in the gastrointestinal tract of rats given wholemeal-bread-based diets containing 0–450 g cooked haricot beans* (Phaseolus vulgaris)*/kg diet**

(Mean values for six rats per diet except where indicated)

Diet code...	BH1	BH2	BH3	BH4	Pooled
Beans content of diet (g/kg)...	0	150	300	450	SEM‖
Duodenum	87	83	99†	85†	5·2
Caecum	19	19†	23‡	18§	3·0
Colon	16‡	13‡	13	17‡	3·4

* For details of diets and procedures, see pp. 274–275.
† n 5.
‡ n 4.
§ n 3.
‖ SEM assumes six rats per diet.

Expt 2a. Abrupt change from the white-bread-based diet without beans (WH1) to the highest bean diet (WH4) had no significant effect on duodenal EPR (means 116 and 121 arrested cells/1000 crypt cells per 2 h respectively) over the 3 d study but was associated with an immediate and significant ($P < 0·01$) increase in caecal EPR (Table 7). As in Expt

Table 7. *Expt 2a. Adaptation study. Epithelial proliferation rate (arrested cells/1000 crypt cells per 2 h) in the duodenum and caecum of rats previously adapted to a white-bread-based diet (WH1; no beans) for 10 d and either continuing on this diet or changing to diet WH4 (450 g haricot beans* (Phaseolus vulgaris)*/kg) and sampled on the following 3 d*†

(Mean values for five rats per treatment)

Diet code...	WH1			WH4				
Beans in diet (g/kg)...	0			450				
Sampling day...	1	2	3	1	2	3	Pooled SEM	Statistical significance of diet effect‡
Duodenum	119	117	112	124	114	124	8·8	NS
Caecum	38	39	30	58	64	43	6·4	**

** $P < 0.01$.
† For details of diets and procedures, see pp. 274–276.
‡ There were no statistically significant effects of sampling day.

Table 8. *Expt 2b. Balance study. Epithelial proliferation rates (arrested cells/1000 crypt cells per 2 h) in rats given white-bread-based diets containing 0–450 g cooked haricot beans* (Phaseolus vulgaris)*/kg diet*†

(Mean values for five rats per diet)

Diet code...	WH1	WH2	WH3	WH4	Pooled	Statistical significance of dietary effects		
Beans in diet (g/kg)...	0	150	300	450	SEM	Lin	Quad	Dev
Duodenum	91	103	123	107	12·0	NS	NS	NS
Caecum	26	25	39	53	6·0	**	NS	NS

Lin, linear; Quad, quadratic; Dev, deviations from linear and quadratic effects of dietary bean concentration.
** $P < 0.01$.
† For details of diets and procedures, see pp. 274–276.

2, duodenal EPR was greater than that for the caecum although the relative difference was less marked.

Expt 2b. Consumption of beans-containing diets for 2 weeks was without detectable effect on duodenal EPR (Table 8). In contrast, caecal EPR increased linearly ($P < 0.01$) as beans consumption increased so that with the highest bean diet (WH4) it was approximately twice the rate observed with the basal (no beans) diet (WH1).

DISCUSSION

Large-bowel substrate supply and short-chain fatty acid production

Adding cooked haricot beans to bread-based diets promoted substantial increases in OM flow to the LB, enhanced fermentation in that organ (Key & Mathers, 1993; Fig. 1), and resulted in greater SCFA production and absorption (Table 1). With the white-bread-based diets the greatest relative increase was in butyrate (11-fold) whilst acetate and propionate absorption increased by four and three times respectively with the highest bean diet. If SCFA and especially butyrate are important trophic factors for the gut as is suggested by the studies of Sakata (1987) and Koruda *et al.* (1988), then this experimental model should have provided suitable conditions to test the hypothesis.

Tissue hypertrophy

Hypertrophy of the gut following consumption of carbohydrates not digested in the SI is often reported as increases in the mass of gut organs or specifically of the tissue (Wyatt *et al.* 1988; Ikegami *et al.* 1990; Mathers & Dawson, 1991; Mathers & Fotso Tagny, 1994) and SI length is also sometimes increased (Ikegami *et al.* 1990). In the present study, animals fed on beans had longer SI and greater tissue mass for the caecum and colon (Tables 2 and 3) but not for the stomach tissue which was reduced in weight. Wyatt *et al.* (1988) argued that caecal enlargement was a response to the tendency for material to accumulate within it and this association between tissue mass and digesta contents was apparent for both the caecum and colon in the present study (Tables 2 and 3; Key & Mathers, 1993). Although with beans in the diet there was more wet (and dry) digesta in the large bowel, the digesta stayed in the organ for significantly shorter times (as assessed by Cr_2O_3 TT). Based on the hypothesis that the primary purpose of LB fermentation is energy salvage (Nordgaard *et al.* 1994), a possible explanation for these phenomena is that the LB (and especially the caecum) increased in size to permit proliferation of the bacteria required to ferment the extra OM supplied to the organ by the beans. This OM appears to have been extensively fermented (Key & Mathers, 1993, 1995) in a relatively short period of time. In contrast, when raw potato starch was the test carbohydrate, caecal enlargement was considerably greater and caecal TT increased significantly (Calvert *et al.* 1989; Mathers & Smith, 1993). Since non-fermented, non-viscous polysaccharides such as cellulose do not accumulate in the caecum and, therefore, have little effect on caecal mass (Wyatt *et al.* 1988), it may be supposed that the bacterial response to the supplied substrate provides a signal(s) for both organ growth and rate of movement of digesta through the organ. SCFA would be an obvious candidate signal since they are the major end-products of bacterial fermentation, are readily absorbed, and have been shown to stimulate ileal motility (Kamath *et al.* 1988). The caecal enlargement which occurs in germ-free animals (Goodlad *et al.* 1989) suggests that other factors must also be involved in hypertrophic responses.

Gut tissue hypertrophy could result from increased mass of the mucosa, the main metabolic tissue, or of the underlying muscle or both. The slightly but significantly ($P < 0{\cdot}01$) increased SI length with increasing consumption (Table 3) was associated with a reduced mucosal mass per unit length for the duodenum but not the ileum (Table 5) which might suggest the stretching and thinning of the organ (similar to the effect of stretching a rubber band). The lack of dietary effect on villus height, crypt depth or sucrase activity per unit length of duodenum whilst sucrase activity per g mucosa increased is in accord with this suggestion.

Does increased short-chain fatty acid production cause increased epithelial proliferation?

Whilst morphological measurements such as villus height and crypt depth and especially villus:crypt ratio provide some indication of epithelial proliferation (a fall in villus:crypt ratio may result from crypt elongation and increased cell production), more reliable measures are obtained by the use of stathmokinetic drugs, e.g. vincristine sulphate, which arrest cell division at metaphase and so provide a direct measure of the number of cells undergoing division between the time of drug administration and tissue collection. In the present study gut tissue from vincristine-treated animals was fixed and longitudinal sections through the crypts used to count the number of arrested cells per 1000 crypt cells. This procedure provides a measure of the proportion of crypt cells undergoing division only and is not a true estimate of crypt-cell production rate (Goodlad & Wright, 1982). Cells undergoing mitosis are found closer to the longitudinal axis of the crypt than are non-dividing cells (Wright & Alison, 1984) so that estimates of cell proliferation rate obtained

from axial sections of crypts, as in the present study, will overestimate the true proliferation rate by the factor b/a where b and a are the radial distances from the crypt axis to non-dividing and dividing cells respectively (Tannock, 1967). Tannock's factor is approximately 0·6 for the small bowel but over 0·7 for the colon (Wright & Alison, 1984). Changes in crypt dimensions in response to diet could, in theory, introduce bias into the estimate of cell proliferation but the failure to detect any between-treatments difference in crypt depth (Table 5) or in the total number of cells per crypt section (Key, 1990) suggests that the EPR estimates reported here (Tables 6–8) are unlikely to mislead.

In all three experiments (Tables 6–8) we found no evidence that diet had any effect on duodenal EPR despite the large increases in SCFA production resulting from addition of beans to the diet (Table 1). Sakata's (1987) hypothesis that SCFA are lumen trophic factors which can act at sites remote from that of SCFA production is not supported by these observations. This apparent conflict of observations could be reconciled if SCFA stimulates crypt-cell proliferation in conditions of hypoproliferation (as is found with elemental diets such as that used by Sakata (1987) or with total parenteral nutrition (Koruda *et al.* 1990)) but is not stimulatory in normo-proliferative states, e.g. when healthy animals are consuming normal feeds.

Results for LB EPR differed between experiments. In Expt 1 where wholemeal bread was the major component of the basal diet, adding beans did not change caecal or colonic EPR (Table 6). One interpretation of these results is that because wholemeal bread is rich in NSP, caecal SCFA production would be relatively high on the basal diet 'normalizing' EPR and so no effect of adding beans would be expected. In consequence, white bread (with a much lower NSP content) was the base for the diets in Expts 2a and 2b. Within 1 d of changing from diet WH1 (no beans) to WH4 (450 g beans/kg diet) there was a significant increase in caecal EPR which appeared to be maintained (Table 7). After 14 d of dietary exposure there was a significant ($P < 0·01$) linear increase in caecal EPR with increasing dietary bean content and, therefore, SCFA production (Table 8).

Bianchini *et al.* (1992) reported that starch-fed rats had higher caecal total SCFA and butyrate concentrations but reduced rectal crypt-cell proliferation than those fed with sucrose and there were strong negative correlations between caecal butyrate concentration (and molar proportion) and rectal proliferative activity. When oats were added to a semi-purified basal diet for rats, caecal SCFA concentrations (including that of butyrate) and crypt-cell proliferation throughout the SI and caecum were increased significantly but proliferation in the colon was reduced (Mathers *et al.* 1993). With pigs fed on several differentially fermentable carbohydrates to produce a range of caecal SCFA concentrations, Fleming *et al.* (1992) found no significant relationships between cell proliferation in the caecum or distal colon and any index of fermentation including butyrate concentration.

CONCLUSIONS

Increased cell division may be an early event in the development of many human cancers because it increases the risk of accumulation of molecular genetic errors (Preston-Martin *et al.* 1990). For example, inherited mutations in the *apc* gene which cause familial adenomatous polyposis leading to a very high risk of colorectal cancer are associated with altered crypt-cell proliferation (Mills *et al.* 1995). If this hypothesis is tenable, dietary components or strategies which increase gastrointestinal mucosal proliferation may be contraindicated. Current dietary advice to reduce the risk of cardiovascular diseases and cancer includes a recommendation to increase fermentable carbohydrates which will increase LB SCFA production. The present study provides some reassurance that with normal human foods, increased SCFA production does not inevitably result in increased

epithelial proliferation. The studies which have shown a stimulation of crypt-cell proliferation in response to direct administration of SCFA often appear to have used a hypoproliferative mucosa as the starting point (Sakata, 1995) so that an increase in proliferation may be viewed as a return to normal. Increased cell proliferation may be a transient phenomenon accompanying tissue hypertrophy and would be expected to return to normal when the animal adapted to the new situation. Finally, increases in cell proliferation following a dietary intervention may be a homeostatic response to increased cell loss by cell sloughing or apoptosis. If the latter is a central feature of the regulation of cell numbers in the gut mucosa (Hall *et al.* 1994), it will be important to obtain quantitative information on apoptosis *in vivo* in response to SCFA.

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