Fermentable carbohydrate modulates postprandial enteroglucagon and gastrin release in rats

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We studied the effects of a fermentable sugar-alcohol (lactitol) on the concentrations of enteroglucagon and gastrin in the blood of rats for 7·5 h after feeding. The control and treatment groups were fed on semi-purified diets containing either non-fermentable cellulose or lactitol respectively, at 100 g/kg. Compared with the cellulose-fed group, the animals fed with lactitol had higher levels of enteroglucagon (5–10 times higher than control; $P < 0·05$) and lower serum gastrin (70–80% of control; $P < 0·05$) for several hours after the withdrawal of feed. In contrast, varying the level of dietary lipid (maize oil) over a range of 8–120 g/kg had no effect on the release of either peptide. These results suggest that poorly absorbed fermentable dietary carbohydrate stimulates postprandial plasma enteroglucagon and inhibits serum gastrin release in the rat. The mechanism is uncertain but an endocrine response by the colon to fermentation products seems probable.

Enteroglucagon: Gastrin: Colonic fermentation

A number of gastrointestinal regulatory peptides are released from mucosal endocrine cells in response to the ingestion of food. Two such peptides are oxyntomodulin and glicentin, collectively termed 'enteroglucagon', both of which are derived by post-translational processing of proglucagon, and secreted into the circulation by the intestinal L cells of the ileal and colonic mucosa (Holst, 1982; Baldissera & Holst, 1984). There is increasing evidence that enteroglucagon is involved in the regulation of gastric motility and secretion. Oxyntomodulin interacts directly with the gastric oxyntic glands (Carles-Bonnet *et al.* 1992), and high levels can inhibit acid secretion (Holst *et al.* 1976), gastric emptying, and duodenal motility in man (Schjoldager *et al.* 1989). Glicentin has also been shown to inhibit gastric acid secretion in the rat (Kirkegaard *et al.* 1982). Abnormally high plasma enteroglucagon levels develop soon after proximal resection of the small bowel in the rat, and one or more of the peptides may be trophic to the intestinal mucosa (Jacobs *et al.* 1981).

In humans the diurnal pattern of serum enteroglucagon activity displays peaks and troughs determined by meal pattern (Le Quellec *et al.* 1992). Perfusion of the small bowel with emulsified fat selectively stimulates the release of enteroglucagon in the rat (Roberge & Brubaker, 1991), and it is often assumed that the small intestine is the main source of circulating enteroglucagon. However, as we have previously reported, replacing non-fermentable cellulose with readily fermentable polysaccharides in the diet of rats provokes a rise in plasma enteroglucagon to levels comparable with those caused by massive small-bowel resection (Johnson *et al.* 1988). This suggests that fermentation products derived

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from carbohydrate may stimulate the release of enteroglucagon from the colon. To test this hypothesis we have studied the effect of a fermentable sugar alcohol, lactitol, on serum enteroglucagon in the rat. In view of the putative role of enteroglucagon in the control of gastric secretion and motility, we also felt it was of interest to investigate the effect of lactitol on serum gastrin levels under the same experimental conditions. Lactitol is a palatable food additive (van Velthuisen, 1979) which is poorly absorbed (Metzger *et al.* 1988), but highly fermentable (Grimble *et al.* 1988).

MATERIALS AND METHODS

Animals and diets

Male Wistar rats (approximately 150 g) were obtained from a commercial supplier (R. Tuck & Sons, Huntingdon, Cambs.) and housed singly in polypropylene cages with wire bottoms and tops, in an air-conditioned small-animal room. The housing had an ambient temperature of 21°, and a 12 h–12 h light–dark cycle. All animals received a commercial pellet feed during an initial acclimation period and were then randomly assigned to the control or experimental groups for a feeding period of 10 d. All diets contained sucrose (300 g/kg), casein (200 g/kg), maize oil at 8, 80 or 120 g/kg, and starch at either 332, 260 or 220 g/kg. The control diet contained an insoluble form of cellulose at a concentration of 100 g/kg (Solkafloc; Johnson, Jorgensen Wettre, London). The treatment groups received an identical diet except that the cellulose was replaced with the non-digestible disaccharide, lactitol (4-β-D galactopyranosyl-D-sorbitol; Purac Biochem, Gorinchen, The Netherlands). All diets contained minerals and vitamins at levels appropriate for growing rats.

Experimental design

In all experiments sixty animals were randomly allocated to two groups of thirty and fed on either the control diet or a treatment diet. In Expts 1 and 2 the diets contained maize oil at 80 g/kg diet, whereas in Expts 3 and 4 the diets contained 8 g/kg or 120 g/kg of maize oil respectively. In Expts 1, 3 and 4 the animals were given free access to the control and treatment diets, but in Expt 2 the animals were trained to accept the diet as a single meal which was available for a period of 1 h in each 24 h.

After 10 d the feed was withdrawn at 09.00 hours, immediately after the dark phase of the cycle, or after the feeding period in the case of meal-fed rats. Five animals from the control group and five from the treatment group were then immediately anaesthetized with an intra-peritoneal injection of sodium pentobarbital. The abdomen was opened and a sample of blood (about 3 ml) was taken from the posterior vena cava for assay of intestinal peptides. The animal was then killed by cervical dislocation and the entire intestinal tract was removed and transected at the ileo-caecal and caeco-colonic junctions. The small intestine was extended on the bench with minimal stretching and measured. This procedure was repeated at 1·5, 3, 4·5, 6 and 7·5 h after feed withdrawal. For those animals killed at 4·5 h and 6·0 h the caecal sac was weighed full, the pH of the caecal contents was determined *in situ*, using a small pH electrode inserted through an incision, and then the sac was opened and the contents were removed by gentle scraping. The caecal tissue was then weighed again.

Analysis of enteroglucagon-like immunoreactivity

In the present study enteroglucagon was defined as 'enteroglucagon-like immunoreactivity' (ELI) and determined by radioimmunoassay using commercially available antibodies reacting to total glucagon-like immunoreactivity (GLI) and pancreatic glucagon

respectively. Total GLI was assayed using antiserum K 4023 (Novo Laboratories, Bagsvaerd, Denmark) which recognizes any peptide containing the glucagon mid-sequence and hence measures pancreatic glucagon, oxyntomodulin and glicentin. The glucagon-like peptides GLP-1 and GLP-2 were not detectable with our assay. Pancreatic glucagon was assayed using antiserum K 5563 which is specific for pancreatic GLI and is reported to show less than 1% cross-reactivity with intestinal GLI (Heding, 1971).

Blood samples ($2 \times 1{\cdot}0$ ml) were placed in tubes containing sodium citrate (100 g/l; 0·07 ml) and aprotinin (168 μg; 1–2 trypsin inhibitor units per 100 μg) and centrifuged at 12000 ***g*** for 4 min. Plasma samples were then separated and stored in liquid N_2 after the addition of aprotinin as above. Glucagon-like peptides were obtained by alcohol extraction (Andrews & Ronner, 1985). Plasma samples were precipitated with ethanol (Analar grade, 950 ml/l, 1·8 ml per 1 ml plasma) and centrifuged at 2000 ***g*** for 15 min). The supernatant fractions were transferred into fresh glass tubes and the solvent was removed on a vortex evaporator at 30°. The residues were redissolved in phosphate buffer (40 mmol/l; pH 7·3–7·5) containing NaCl (6 g/l), sodium merthiolate (0·24 g/l), human albumin (1·0 g/l) and aprotinin (40 mg/l). Pancreatic GLI and total GLI were measured by radioimmunoassay and ELI was then determined by difference and expressed in ng/l plasma.

Gastrin analysis

Each blood sample (1·0 ml) was placed in a tube without anticoagulant and centrifuged at 12000 ***g*** for 4 min, yielding approximately 0·5 ml serum, which was then stored at −20°. Gastrin levels were determined in 100 μl samples using a commercial radioimmunoassay kit. The assay is reported to give between 91% and 101% recovery within the concentration range relevant to the present study, to have a sensitivity of 3·3 pg/ml, and to show a cross-reactivity of 10·9% with CCK-8 (Beckton Dickinson Diagnostic Systems, Oxford, Oxon.).

Statistics

Means were compared using Student's *t* test for unpaired data, or by one-way ANOVA coupled with Tukey's test for the comparison of means. Means were considered significantly different at a probability level of 0·05. Analysis was carried out using Minitab (Minitab Corporation, State College, PA, USA). Data are presented as the mean and standard error of the mean.

RESULTS

Control and treatment diets were readily accepted, and animals fed with lactitol grew at a rate similar to those fed with cellulose. Considerable enlargement of the caecum was noted in the lactitol-fed rats in all the experiments. In Expts 3 and 4 the weights of both the caecal contents and the caecal tissue were measured in the animals killed at 4·5 h and 6·0 h. There were no significant differences within the groups at these time-points. For the pooled data, the wet weight of the caecal contents was 2–3-fold greater in the lactitol-fed rats compared with the controls, and the wet tissue weight of the caecal sac was approximately 2·5-fold greater (Table 1). These differences were statistically significant in both experiments. The pH of the caecal contents was significantly lower in lactitol-fed rats (Table 1).

The effect of prolonged consumption of lactitol on plasma ELI levels in the rat is summarized in Fig. 1. In Expt 1 the animals had free access to feed (upper panel). At the time of feed withdrawal the mean plasma ELI level in the group fed with lactitol was approximately five times higher than that of the cellulose-fed controls. The level remained

Table 1. *Caecal pH, weight of caecal tissue and contents, and small-intestinal length of rats fed with cellulose or lactitol in diets containing 8 or 120 g maize oil/kg*†

(Mean values with their standard errors for ten or thirty animals)

	pH‡		Caecal tissue (g)§		Caecal contents (g)§		Small-intestinal length (m)‡	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
8 g maize oil/kg								
Cellulose	6·91	0·06	0·714	0·038	2·982	0·135	1.217	0·014
Lactitol	6·34**	0·13	1·956***	0·087	5·156*	0·636	1·238	0·023
120 g maize oil/kg								
Cellulose	6·85	0·10	0·724	0·031	2·541	0·211	1·196	0·010
Lactitol	5·94***	0·14	1·746***	0·078	6·764***	0·712	1·242**	0·011

Mean values for lactitol-fed animals differed significantly from those for corresponding cellulose-fed animals: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† For details of diets and procedures, see p. 758.

‡ n 30.

§ n 10.

higher in the treatment group at every time point although the values for the groups tended to converge over the course of the day. The effect of meal-feeding on this pattern is illustrated in the lower panel, showing the results of Expt 2. Under these conditions the treatment and control groups both had similarly low plasma ELI levels immediately after consumption of the meal. The plasma ELI rose in both groups initially, but then declined to basal levels in the controls. In the lactitol group, however, the levels continued to rise after the 3 h time point, and remained approximately five times higher than those of the control group throughout the day. Both patterns seem consistent with release of ELI by fermentable material reaching the colon.

In both the previously described experiments all the diets contained maize oil at 80 g/kg diet. In Expts 3 and 4 the lactitol and cellulose feeds contained 8 g/kg and 120 g/kg maize oil respectively, so that the effects of dietary lipid and lactitol could be compared. To illustrate the effects of these diets on the ELI response, four sets of five curves of the type shown in Fig. 1(*a*) were generated using the individual values for the five rats at each time point. The areas under these curves were then calculated for the treatment and control groups, and the means of these integrated responses are given in Fig. 2. The integrated plasma ELI response was much higher in the lactitol-fed groups than in their respective controls at both levels of lipid intake. However, the magnitude of this response was significantly less ($P < 0·05$) in animals fed on the high-maize-oil diet. There was no significant difference in the ELI responses of the animals fed with cellulose at high or low levels of maize oil. Thus fermentable carbohydrate provides a strong stimulus for the release of intestinal glucagon-like peptides but an increase in the dietary lipid level does not.

Gastrin

Serum gastrin levels were determined in Expts 3 and 4. The time course for serum gastrin in animals fed with maize oil at 120 g/kg (Expt 3) is shown in Fig. 3. The areas under the curves were calculated as for ELI, and the integrated values obtained for diets containing maize oil at 120 g/kg and 8 g/kg are shown in Fig. 4. The gastrin levels were significantly lower in the lactitol-fed groups compared with the controls at both levels of lipid intake

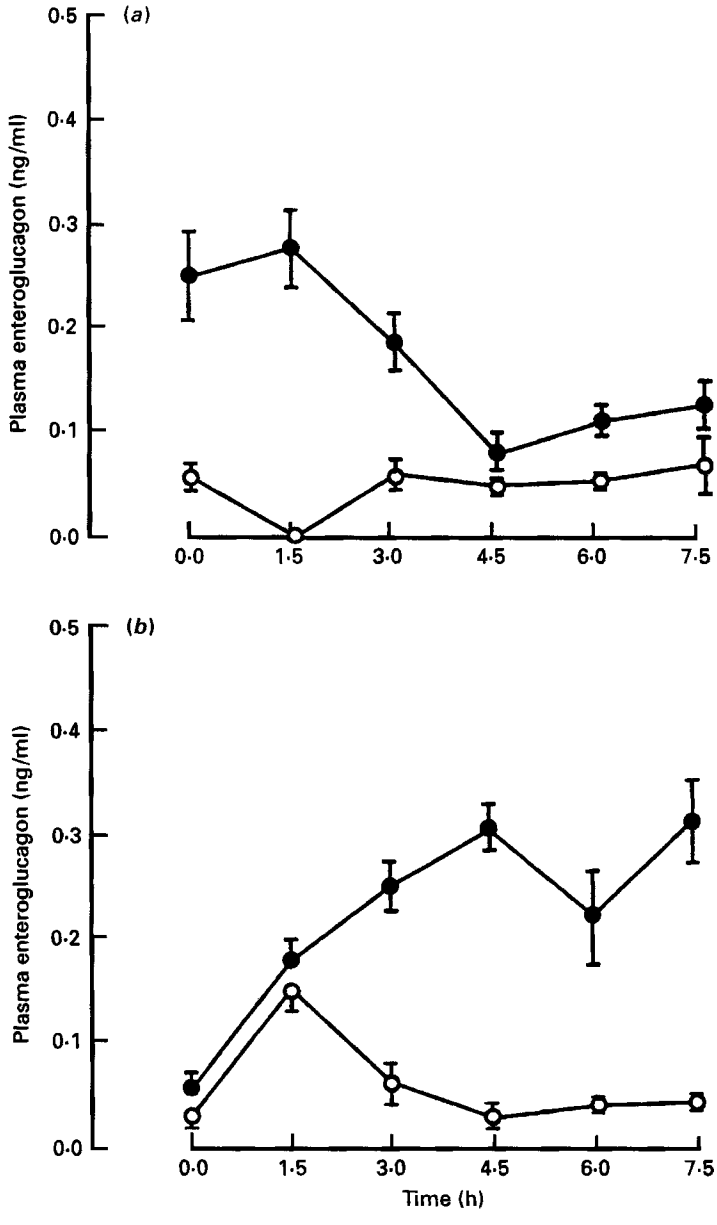

Fig. 1. Plasma enteroglucagon levels in rats fed with cellulose (○) or lactitol (●) at 100 g/kg diet. Animals received the feed either *ad lib.* (Expt 1; (*a*)) or as a single meal eaten over a period of 1 h (Expt 2; (*b*)) for 10 d before the experiment. In both cases the feed was withdrawn at time 0 on the day of the experiment. Each point represents the mean with its standard error for five animals killed at the times shown. Test and control means differed significantly ($P < 0{\cdot}05$) at all times, apart from 4·5 h and 7·5 h after removal of *ad lib.* feed (*a*), and 0 and 1·5 h after removal of the meal feed (*b*).

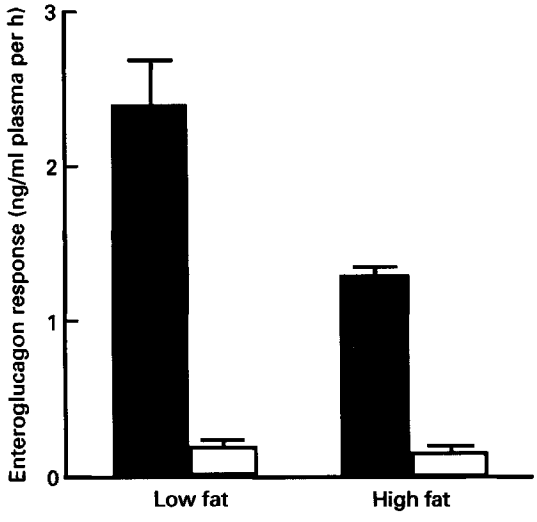

Fig. 2. Plasma enteroglucagon response to lactitol (■) or cellulose (□) fed *ad lib.* in conjunction with a low-maize-oil (Expt 3; 8 g/kg) or high-maize-oil (Expt 4; 120 g/kg) diet. For each column the plasma enteroglucagon concentration was integrated over 7·5 h following removal of feed. Values are means with their standard errors for the areas under five curves. Means for lactitol-fed rats differed significantly ($P < 0.05$) from cellulose-fed controls in each case. Lactitol-fed rats given a low-lipid diet had significantly higher plasma enteroglucagon levels than those given high lipid ($P < 0.05$).

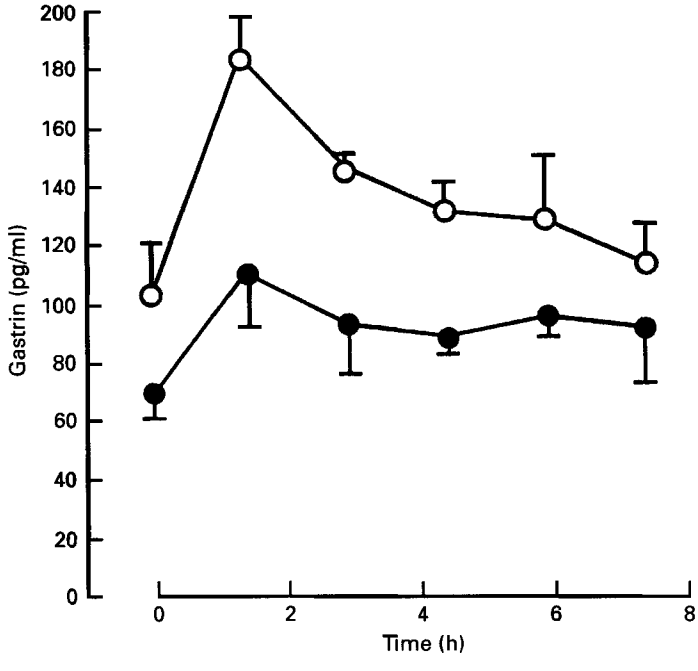

Fig. 3. Postprandial serum gastrin levels in rats from Expt 4 fed *ad lib.* on diets containing cellulose (○) or lactitol (●) together with maize oil at 120 g/kg. Serum concentrations differed significantly ($P < 0.05$) at all times other than 6 h and 7·5 h after removal of feed. Values are means for five animals, with their standard errors indicated by vertical bars.

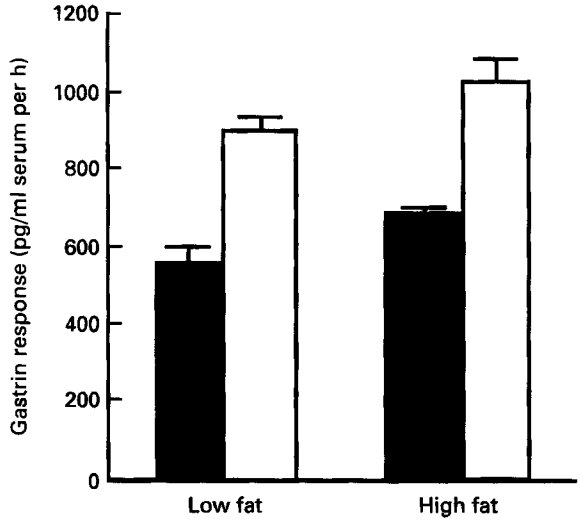

Fig. 4. Integrated (7·5 h) serum gastrin concentrations for rats in Expts 3 and 4 in response to lactitol (■) or cellulose (□). For both levels of dietary lipid, the postprandial serum gastrin response was significantly lower in the groups fed with lactitol ($P < 0·05$). Values are means for the areas under five curves, with their standard errors indicated by vertical bars.

($P < 0·05$), but there was no significant effect of maize oil on the gastrin response of the lactitol- or the cellulose-fed groups.

DISCUSSION

Most studies on the enteroglucagon response to food have focused on the small bowel. Perfusion studies in humans show that both glucose and lipid play some role in the release of enteroglucagon from the distal small intestine (Unger *et al.* 1968; Read *et al.* 1984; Spiller *et al.* 1988). Roberge & Brubaker (1991) studied the effect of perfusing the small-intestinal lumen of the rat with glucose or emulsified fat, and showed that lipid caused an increase in plasma enteroglucagon when introduced into the duodenum or ileum, but glucose was ineffective at both sites. The present study suggests that release of enteroglucagon from the distal alimentary tract by the products of carbohydrate fermentation makes a major contribution to the total plasma enteroglucagon titre.

In the present study animals given diets containing the same quantities of cellulose, but with greatly differing levels of fat, had similar plasma ELI levels in the fed state. In contrast, replacement of the cellulose with lactitol caused a substantial and prolonged rise in plasma ELI. Lactitol is virtually unabsorbed from the small intestine (Metzger *et al.* 1988) but is substantially fermented by the colonic microflora (Grimble *et al.* 1988). The fact that the high levels of ELI in the plasma persisted for several hours after the removal of food supports the hypothesis that colonic fermentation of lactitol was the main stimulus for the release of the peptides.

L-cells are distributed throughout the small intestine and colon but they occur at their highest density at distal sites. It has been shown that enteroglucagon and another gastrointestinal peptide, PYY, occupy two separate populations of secretory granules within the cytoplasm of L-cells from rabbit colon (Nilsson *et al.* 1991). The large bowel does not normally receive large quantities of unabsorbed nutrients from the small intestine, but carbohydrates and other residues reaching the caecum provide fermentable substrates

for the microflora. The present results suggest that the colon is adapted to provide an endocrine response to fermentable carbohydrate, and it seems probable that short-chain fatty acids (SCFA) provide the stimulus for this effect. This hypothesis is consistent with perfusion studies showing that the release of PYY from L-cells in the isolated rabbit colon is provoked by physiological concentrations of *n*-butyrate and acetate (Longo *et al.* 1991).

Our results are also consistent with the findings of Goodlad *et al.* (1989), who observed elevated levels of enteroglucagon and PYY in rats re-fed on a complex mixture of polysaccharides after starvation. Goodlad *et al.* (1989) also observed a very high level of enteroglucagon in germ-free rats which they attributed to the caecal enlargement associated with the germ-free state. In the present study lactitol consumption was associated with acidification of the caecal contents and enlargement of both the caecal contents and the caecal tissue. The caecum is likely to be the main site of lactitol fermentation and may therefore be the principal source of ELI in our animals. In previous work with viscous polysaccharides we have observed that high levels of plasma ELI are associated with an increase in small-intestinal length of about 15% (Johnson *et al.* 1988), but there was only a slight tendency for small-intestinal enlargement in the lactitol-fed rats in the present study (Table 1).

Gastrin-secreting cells are localized principally in the gastric antrum and the duodenum. Release of gastrin into the circulation is stimulated by endogenous and exogenous factors, including dietary proteins, peptides and amino acids. In the present study we observed that rats given free access to diets containing lactitol had serum gastrin levels 30–40% lower than the cellulose-fed controls throughout the postprandial period. To our knowledge, no similar effect of lactitol or other fementable carbohydrate on gastrin production has previously been reported. Indeed there are relatively few studies on the chronic effects of any food constituent on serum gastrin. In a recent study Reilly *et al.* (1995) investigated some effects of a mixture of SCFA (125 mM) infused into the colon of rats, and reported no effect on plasma gastrin, nor on plasma enteroglucagon. However, the rats had undergone caecectomy to reduce endogenous production of SCFA. This procedure may have eliminated most of the endocrine tissue responsible for the effects observed in the present study.

Sircar *et al.* (1980) have described a prolonged reduction in the serum gastrin response to food in the rat, commencing shortly after the introduction of a chemically defined diet, but the mechanism is unknown. Of more relevance perhaps is the study of Inoue *et al.* (1982), who observed a rise in the gastrin response to meals after resection of the colon in dogs and concluded that the intact colon may produce an endocrine factor that normally inhibits release of gastrin. Sasaki *et al.* (1987) studied the effects of ileo-jejunal transposition in totally colectomized dogs and observed a reduction in gastrin levels coupled with a prolonged increase in plasma enteroglucagon. They concluded from this that enteroglucagon may have an inhibitory effect on postprandial gastrin release. The present results are consistent with that proposal.

There is some evidence that the colonic mucosa plays an indirect role in the control of gastrin secretion in man. Patients with ulcerative colitis have elevated basal and postprandial gastrin levels (Besterman *et al.* 1983). If the colon does exert an inhibitory effect on gastrin secretion, and if the effect is susceptible to dietary manipulation in humans, there may be important implications. Apart from its function in the control of gastric acid secretion, gastrin has a trophic effect on the gastric mucosa (Ryberg *et al.* 1990) and perhaps on colonic tumours that possess gastrin receptors (Singh *et al.* 1985). Some patients with colorectal cancer have high postprandial gastrin levels and these may play an aetiological role in the disease (Wong *et al.* 1991).

The present study suggests that, like other regions of the gastrointestinal tract, the colon

is an important endocrine organ. There are several examples of effects on food intake and gastrointestinal motility in humans that may be mediated by fermentation of carbohydrate, and further studies are required to explore the possible involvement of gastrointestinal hormones. For example, an increase in satiety and a reduction in energy intake has been reported in human subjects, 5–6 h after consumption of a high-fibre meal (Burley & Blundell, 1990), and a sustained reduction in the rate of gastric emptying has been observed after prolonged consumption of pectin (Schwartz *et al.* 1982). Fermentable food ingredients such as lactitol may provide a novel method for the manipulation of proximal gastrointestinal motility and secretion. Finally it should be noted that we have not measured glucagon-like peptide 1 (GLP-1) in the present study. This newly recognized peptide is another product of post-translational proglucagon processing in the L-cell, and it is thought to play an important role as a stimulant for insulin secretion (Wang *et al.* 1995). Release of GLP-1 has been shown to have much in common with glicentin and oxyntomodulin in humans (Holst, 1994). The possibility that fermentable carbohydrate might stimulate GLP-1 production and could thereby influence carbohydrate metabolism deserves further study.

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