Small intestinal response to 'elemental' and 'complete' liquid feeds in the rat: effect of dietary bulk

D G MAXTON, E U CYNK, AND R P H THOMPSON

From the Gastrointestinal Laboratory, The Rayne Institute, St Thomas' Hospital, London

SUMMARY   The effect of oral isocaloric feeding on small intestinal structure and function was studied in the rat. The liquid 'elemental' enteral feed Vivonex HN, the liquid 'complete' feed Ensure and the same liquid complete feed with 9% bulk Enrich were compared with solid chow containing 21% bulk (normal rat chow), all given for four weeks. Weight gain was significantly less in the group fed Vivonex HN than that of any other groups. The bulkless Vivonex HN and Ensure increased proximal jejunal mass compared to Enrich with 9% bulk or to normal rat chow. Jejunal mucosal DNA and protein levels also tended to be higher in Ensure and Vivonex HN fed animals, as was jejunal sugar absorption. In the terminal ileum, however, total weight was decreased by both elemental and complete feeds with and without bulk, but particularly by the elemental diet. Bulkless feeds therefore increase jejunal and reduce terminal ileal mass. The striking atrophy of the terminal ileum produced by the elemental diet may be important for its efficacy in treating inflammatory bowel disease.

Elemental diets may be as effective as steroids in the treatment of Crohn's disease, but how they influence the disease is unknown.[1-3] Improved nutritional status, exclusion of a toxic dietary factor and 'bowel rest' have all been suggested.[4] 'Bowel rest' is used to mean a reduced mucosal energy expenditure for absorption, and does not imply lack of gut motor activity. It is thought to occur because elemental feeds contain protein as individual amino acids, carbohydrate as glucose or oligosaccharides and either a low lipid content or medium chain triglycerides, and so they require little enzymatic hydrolysis in the lumen or mucosa. Elemental diets, however, are unpalatable and expensive.[5] 'Complete' enteral feeds are cheaper and more palatable, but contain 'whole' undigested casein or soya proteins rather than amino acids, long chain rather than medium chain triglycerides, and hydro- lysates of starch with larger oligosaccharides rather than small glucose polymers. Neither contain fibre or bulk. Greater intestinal 'work' is therefore required

for the digestion of complete compared with elemental feeds.

We therefore investigated the effects of an ele- mental diet, a 'complete' enteral feed, the same 'complete' enteral feed with additional bulk, and finally normal chow upon intestinal structure and function, all given in isocaloric amounts over four weeks, in groups of rats to determine whether 'complete' enteral feeds produce similar intestinal changes to 'elemental' diets and also to study the particular role of dietary bulk.

Methods

ANIMALS AND FEEDS
Thirty young female Wistar rats weighing 240–250 g were divided into four groups and fed simultaneous- ly. Group 1 (n=8) received normal solid rat chow (Modified Maintenance Diet No 1, Scientific Diet Supplies), containing 20.7% unabsorbable bulk by weight; group 2 (n=7) Enrich (Abbott Labora- tories), a liquid complete enteral feed with 9% added bulk; group 3 (n=8) Ensure (Abbott) a liquid com- plete feed similar to Enrich, but containing no bulk; group 4 (n=7) Vivonex HN (Norwich-Eaton Ltd), a

liquid elemental diet. Their main ingredients are given in Table 1.

Animals were housed individually in wire bottom cages to prevent coprophagia, and all groups received 54 calories/day for four weeks, excluding the unavailable calories in the dietary bulk. Animals on liquid feeds (Enrich, Ensure, Vivonex HN) fed from non-spill valved containers, while those on solid chow from special non-spill solid dispensers designed in this laboratory.[6] Water was available *ad libitum*. This regime ensured minimal spillage and coprophagia, isocaloric diets between the groups, and weight gain, but was sufficiently limited for all feeds to be completely eaten daily. Body weights were measured weekly before the daily feed.

EXPERIMENTAL PROTOCOL
Each animal was anaesthetised with intramuscular Thalaminol (fentanyl and droperidol; Janssen Pharmaceuticals Ltd) 0·4 ml/100 g bw, and the small intestine exposed through a ventral midline incision.

PERFUSION TECHNIQUE
Sugar absorption was measured by the method of Batt and Peters.[7] A 30 cm segment of jejunum was cannulated with the proximal cannula 3 cm distal to the ligament of Treitz, and the isolated segment gently flushed with warmed isotonic saline, followed by air. A peristaltic pump proximal to the cannulated loop maintained a flow of 2·4 ml/min and circulated the perfusion fluid through the intestine to a reservoir, which was constantly stirred. This flow rate was used by Batt and Peters[7] in similar experimental conditions. It produces measurable differences in sugar concentration without macroscopic damage to the perfused segment, and allows early recognition of blockage, which is less apparent with slower flow rates.

The perfusion fluid contained D-galactose (BDH Chemicals Ltd) 4·5 mmol/l, 3-0-methyl glucose (Koch-Light Laboratories) 4·1 mmol/l, and D-glucose (BDH Chemicals Ltd) 1·1 mmol/l in water made isotonic with 128·3 mmol/l sodium chloride(BDH). Ten microcuries (0·37 mBq) ^{51}Cr-ethylenediamine tetra-acetate (EDTA) (Radio-chemical Centre, Amersham) was added as a non-absorbable marker to correct for water flux. D-galactose and 3-0-methyl glucose were used to include actively absorbed sugars that respectively can and cannot be metabolised by the intestinal mucosa. Thus while disappearance of galactose from the lumen may represent mucosal utilisation, disappearance of 3-0-methyl glucose reflects absorption alone.

Twenty millilitres of perfusion fluid was placed in the reservoir and pumped through the proximal cannula and small intestine until it flowed from the

Table 1 *Composition of diets*

	Chow	Enrich	Ensure	Vivonex HN
Carbohydrate				
g/1000 cal	183·9	137·2	137·2	211·0
% by weight	61·5	58·9	66·1	81·5
Type	corn starch and oligo-saccharides	hydrolysed corn starch	hydrolysed corn starch	glucose oligo-saccharides
Fat				
g/1000 cal	10·0	33·8	35·2	0·87
% by weight	2·7	15·6	17·8	0·70
Type	oleic and linoleic	oleic and linoleic	oleic and linoleic	linoleic
Essential fatty acids				
g/1000 cal	2·0	17·9	18·7	0·7
% of total fat	20·0	53	53	80
Amino acids or protein				
g/1000 cal	55·9	36·2	35·2	44·4
% by weight	15·1	16·7	17·0	17·7
Type	soya	casein	casein	amino acids
Unabsorbable fibre				
g/1000 cal	76·6	19·2	nil	nil
% by weight	20·7	8·8		
Type	hemi-cellulose and cellulose	soya poly-saccharide		

distal cannula, when the perfusion time was started and maintained for 40 min. During the experiment the isolated loop was maintained at 37°C with overhead lamps, and a thermometer placed in the abdominal cavity on the perfused loop. 0·5 ml samples were taken from the reservoir at 0, 25, and 40 minutes for sugar estimations and 0·1 ml samples at corresponding times for ^{51}Cr-EDTA levels. At the end of the perfusion the loop was gently flushed with air, and the animal killed by intracardiac Thalaminol injection without regaining consciousness.

LABORATORY METHODS
The perfused jejunal segment was carefully dissected free from mesentery, laid flat and its length recorded without tension, and weighed. The mucosa was scraped off with glass slides, weighed, and stored at −20°C.

The terminal ileum was also isolated, cannulated and flushed with cold isotonic saline, and a 10 cm segment adjoining the caecum also measured laid flat without tension and weighed.

Mucosal DNA and protein were measured by standard methods.[8,9] Sugar concentrations were estimated by quantitative thin layer chromatography.[10] Sugar absorption was measured from the total sugar content in the perfusion fluid at the start and during

the experiment after correction for water fluxes and for the reducing perfusion volume after removal of samples.

Results are expressed as means ±SEM. Statistical comparison was made by unpaired Student's t test.

Results

WEIGHT GAIN

Initial body weights in all four groups were similar. Fifty four calories/day increased weight in most animals, but the gain varied on the different isocaloric diets (Fig. 1). Animals fed Vivonex HN gained significantly less weight than those fed Enrich, Ensure or normal rat chow whose weight gains were similar. Consequently all results are expressed per 100 g body weight as smaller animals would necessarily be expected to have lower gut weights, although only Vivonex HN animals are affected. The correction does not alter the significant findings of the study.

JEJUNAL SEGMENT AND MUCOSAL WEIGHTS

The total weight of the perfused jejunal segment for each dietary group is shown in Table 2. Animals fed Ensure had significantly greater jejunal total weights than those fed normal rat chow ($p<0·01$) or Enrich ($p<0·02$). Rats receiving Vivonex HN had jejunal weights similar to those on Ensure but significantly higher than normal rat chow fed animals ($p<0·05$). Gut segment weights of Enrich animals were comparable with normal rat chow.

Similar to total weight, jejunal mucosal weight, corrected for total body weight, was higher in Ensure and Vivonex HN fed rats (Table 2) compared with normal rat chow and Enrich although this only
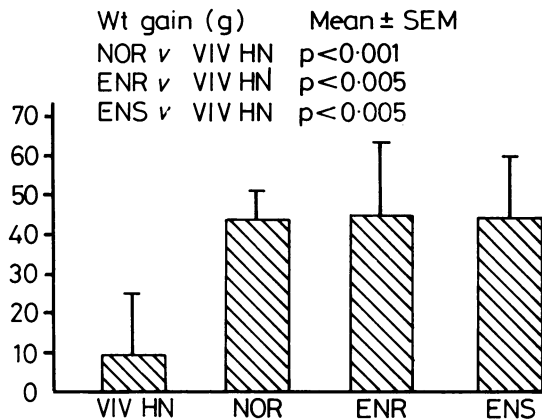
Table 2   *Jejunal segment and mucosal weights: mean±SEM*

	VIV HN	NOR	ENR	ENS
Jejunal segment weight mg/cm/100 g bw	29·5±1·9	23·3±1·1	23·7±1·2	28·5±1·0
Significance NOR v VIV HN p<0·05, NOR v ENS p<0·01, ENR v ENS p<0·02				
Jejunal mucosal weight mg/cm/100 g bw	11·8±0·7	10·3±0·2	10·0±0·6	12·4±0·7
Significance NOR v ENS p<0·02, ENR v ENS p<0·02				
Jejunal mucosal DNA ug/cm/100 g bw	36·9±2·4	35·3±3·0	39·8±3·6	55·4±7·9
Significance All NS				
Jejunal mucosal protein ug/cm/100 g	1295±73	1176±34	1076±84	1283±90
Significance All NS				
Mucosal protein/ mucosal DNA ratio ug/cm/100 g	35·8±2·0	34·4±3·1	28·6±2·7	28·0±4·2
Significance VIV HN v ENR p<0·05				

VIV HN=Vivonex HN; NOR=normal rat chow; ENR=Enrich; ENS=Ensure.

reached significance for Ensure (Ensure v normal rat chow: $p<0·02$. Ensure v Enrich: $p<0·02$).

JEJUNAL MUCOSAL DNA AND PROTEIN

Jejunal mucosal DNA µg/cm/100 g bw for Ensure was greater than normal rat chow and Enrich, although the differences were not significant (Table 2). Vivonex HN levels, however, were comparable with normal rat chow and Enrich.

Mucosal protein content was not significantly different between groups (Table 2), although mean Ensure values were again higher than normal rat chow and Enrich. Vivonex HN protein levels were comparable with Ensure and greater than normal rat chow. Jejunal protein/DNA ratios were higher in Vivonex HN and normal rat chow groups compared with Ensure and Enrich, although the only significant increase was between Vivonex HN and Enrich (Table 2).

TERMINAL ILEUM WEIGHT

Changes of weight of the 10 cm terminal ileal segment were in contrast with those of the jejunal segment (Fig. 2). Thus rats fed normal rat chow had significantly greater terminal ileal weights than the three groups fed liquid complete or elemental diets (normal rat chow v Vivonex HN $p<0·001$, normal rat chow v Enrich $p<0·005$, normal rat chow v Ensure $p<0·05$). The addition of 9% bulk to a complete feed made no difference (Enrich v Ensure, NS). Animals

Wt gain (g)   Mean ± SEM
NOR v VIV HN   p<0·001
ENR v VIV HN   p<0·005
ENS v VIV HN   p<0·005

Fig. 1   *Animal weight gain after four weeks isocaloric feeding with elemental Vivonex HN (VIV HN), normal chow (NOR), Enrich (ENR) and Ensure (ENS) with 54 cals/day.*

TI Wt (mg/cm/100g bw)   Mean ± SEM

VIV HN v NOR   p<0·001   NOR v ENR   p<0·005
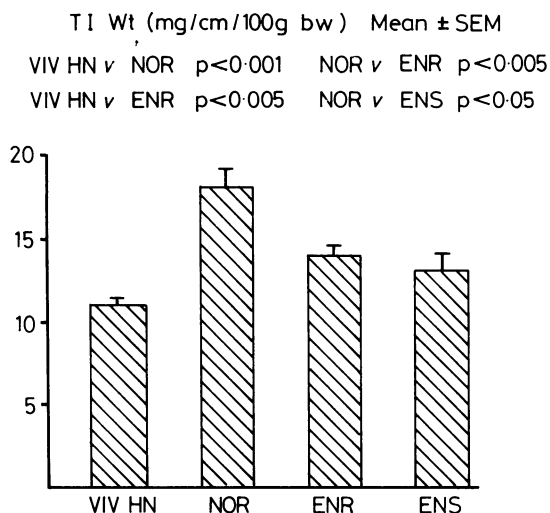VIV HN v ENR   p<0·005   NOR v ENS   p<0·05

Fig. 2   *Comparison of total weight of the terminal ileal segment in animals fed Vivonex HN (VIV HN), normal chow (NOR), Enrich (ENR) and Ensure (ENS).*

receiving Vivonex HN, however, had significantly lower terminal ileal weights than those on Enrich (p<0·001) and particularly than normal rat chow (p<0·001). Elemental Vivonex HN feeding also reduced terminal ileal weight even compared with Ensure, but this did not reach significance.

JEJUNAL ABSORPTION OF SUGARS (Table 3)
Galactose absorption, expressed as μmol/h/cm/100 g bw was highest in Vivonex HN animals. The only significant difference between groups, however, was that Ensure-fed animals absorbed more than Enrich (p<0·02).

3-0-methyl glucose absorption showed similar trends to galactose. Thus absorption was greatest in Vivonex HN fed animals, followed by Ensure. Vivonex HN animals absorbed significantly more 3-0-methyl glucose than Enrich (p<0·05), but there were no other significant differences between groups (Table 3).

Table 3   *Sugar absorption: mean ± SEM*

	VIV HN	NOR	ENR	ENS
Galactose μmol/ h/cm/100 g bw	0·69±0·10	0·51±0·05	0·43±0·03	0·59±0·04
Significance ENR v ENS p<0·02				
3-0-methyl glucose μmol/h/cm/ 100 g bw	0·55±0·08	0·34±0·05	0·34±0·03	0·45±0·04
Significance VIV HN v ENR p<0·05				

VIV HN=Vivonex HN; NOR=Normal chow; ENR=Enrich; ENS=Ensure.

Discussion

'Luminal nutrition' is an important trophic factor to the small intestine, maintaining its normal structure and function and allowing it to adapt to changing circumstances.[11 12] Luminal nutrition, however, includes many dietary constituents and the relative importance of lipid, protein, carbohydrate and vitamin content, or of total calorific value of the diet, is unclear. The effect of bulk on small intestinal mucosal structure and function is especially controversial.

Isocaloric feeding is therefore particularly important because hyperphagia alone induces mucosal hyperplasia,[13] presumably by increasing luminal nutrition or trophic hormone levels, while starvation induces hypoplasia.[14 15] All animals in this study received isocaloric diets orally for four weeks, although no allowance was made for the conversion of oral dietary fibre into nutrients such as short chain fatty acids in the colon. All diet provided was eaten each day, thus avoiding pair-feeding experiments and weight gain was similar in three of the four experimental groups. Precautions against coprophagia were taken, although some faecal ingestion may still have occurred unobserved despite wire bottom cages. All groups tolerated the diets well and body weights increased, but this was significantly less in animals fed Vivonex HN than in those receiving normal rat chow, Enrich or Ensure. Similar observations have been recorded in other studies with animals fed Vivonex HN and other elemental diets,[16 17] and may represent the low biological value of these feeds or a growth stimulating effect of bulk. The complete but bulkless Ensure, however, produced similar weight gain to both normal rat chow and Enrich, the same liquid feed with additional bulk. Further, a study in man[18] showed reduced nitrogen retention in patients receiving Vivonex HN compared with those on normal food. It is therefore probable that Vivonex HN provides nitrogen of low biological value.

The total and mucosal weights, corrected for body weight, of the 30 cm perfused segment of proximal jejunum were increased in animals fed Ensure and Vivonex HN compared with normal rat chow and Enrich. Jejunal protein levels were also higher in both Ensure and Vivonex HN than normal rat chow and Enrich, but mucosal protein/DNA ratios tended to be higher in Vivonex HN and normal rat chow compared with Ensure and Enrich fed animals. The increased protein in Vivonex HN fed animals may thus represent mucosal cell hypertrophy while in the Ensure group the low protein/DNA ratio suggests an hyperplastic response. Conversely, in the terminal ileum Vivonex HN produced a substantial reduction in total weight below normal rat chow levels. Both

Ensure and Enrich also reduced terminal ileal weight compared with normal rat chow. These results suggest that, compared with normal chow, elemental diets and liquid complete feeds without bulk increase small intestinal mucosal mass proximally and reduce it distally. This may be because of an increase in available 'luminal nutrition' proximally from such diets, especially elemental feeds, with consequently less nutrients available in the ileum. Complete liquid feeds without bulk, however, do not produce such striking distal atrophy as elemental diets. A possible explanation for this discrepancy is that delayed gastric emptying of the hypertonic elemental diet may reduce the rate of flow of nutrients into the jejunum, increase proximal absorption, and therefore again reduce ileal luminal nutrients. Alternatively liquid diets may fail to stimulate trophic gastrointestinal hormones or pancreatiobiliary secretions. The composition of the diets may also be critical for stimulating or maintaining mucosal mass. Thus chow differs from the other diets in lipid, protein and carbohydrate content in addition to bulk.

Supplementation of the complete feed with 9% bulk returned jejunal total intestinal and mucosal weights to normal chow level and below the levels with the complete feed without bulk. This may be because of the unabsorbed fibre in normal rat chow and Enrich physically paring off the villus tips, as described in ultrastructural studies.[19]

These results contrast with those of some other studies. Thus Young *et al*[16] fed rats four different liquid bulkless diets including complete and elemental feeds for two weeks and found reduced mucosal weight, DNA and protein throughout the intestine compared with normal chow. Liquid diets were given intragastrically, however, while chow was eaten orally. Intragastric feeding bypasses the cephalic phase of intestinal motility and secretion and so comparison between those diets is difficult. Ecknauer *et al*[17] fed rats orally isocaloric amounts of either chow, Vivonex HN or Vivonex HN with 24% bulk as cellulose, but for only nine days. Total small intestinal weight was reduced in animals receiving Vivonex HN compared with chow, although this was partly reversed by the additional bulk. No distinction was made, however, between a jejunal or ileal effect. Morin *et al*[20] compared an intragastric elemental diet (Vivonex) with both isocaloric intravenous feeding and oral chow. The elemental diet reduced proximal mucosal weight, but the hypoplasia was maximal in the distal ileum and similar to that of animals fed intravenously with complete exclusion of luminal nutrients. This suggests that few nutrients reached the terminal ileum from such feeds, closely agreeing with our own conclusions.

Although the distal ileal changes are therefore consistent, the proximal increase demonstrated is not. As with our study, however, Nelson *et al*[21] did find that, compared with solid food, jejunal villus height was increased rather than reduced in animals fed on elemental diet (Vivonex) for three months. Both solid and liquid diets were given *ad libitum*, however, without controlling calorie intake.

Intestinal function was investigated by absorption of D-galactose and 3-0-methyl glucose. For Ensure, Enrich, and normal rat chow fed animals absorption simply reflected the mucosal weight pattern, but absorption of both sugars per centimetre was greatest in Vivonex HN fed animals, when corrected for body weight. The 80% carbohydrate content of Vivonex HN is high, however, compared with 59–66% hydrolysed starch in the other dietary groups. Feeding animals high carbohydrate diets induces an increase in the transport capacity for glucose.[22] Thus Young *et al*[16] showed increased maltase activity in animals fed high carbohydrate Vivonex HN compared with normal chow. It is likely therefore that dietary constituents influence transport of their specific hydrolysis products and the higher carbohydrate absorption with Vivonex HN reflects a higher dietary load. Bulk in the 9% and 21% dietary weight amounts given here did not increase absorptive capacity.

In conclusion, an elemental diet and a 'complete' enteral feed given orally in isocaloric amounts altered small intestinal structure and function. Weight gain was significantly less in the elementally fed group than in other groups. When corrected for body weight, both an 'elemental' diet and a 'complete' enteral feed increased proximal jejunal mass compared with animals fed either the complete feed with additional bulk or normal chow. Terminal ileal weight, however, was decreased by both elemental and complete feeds with and without bulk, but most strikingly by the elemental diet. Adding 9% bulk to a complete enteral feed decreased rather than increased proximal small intestinal mass, but did not affect the terminal ileal atrophy. The effects of elemental diets on the ileum may be important for their efficacy in treating inflammatory bowel disease affecting this site.

References

1 O'Morain C, Segal AW, Levi AJ. Elemental diets in treatment of acute Crohns' disease. *Br Med J* 1980; **281:** 1173–5.

2 O'Morain C, Segal AW, Levi AJ. Elemental diet as primary treatment of acute Crohns' disease: a controlled trial. *Br Med J* 1984; **288:** 1859–62.

3 Sanderson IR, Boulton P, Menzies I, Walker-Smith JA. Improvement of abnormal lactulose/rhamnose permeability in active Crohn's disease of the small bowel by an elemental diet. [Abstract]. *Gut* 1985; **26:** A1114.

4 Saverymuttu S, Hodgson HJF, Chadwick VS. Controlled trial comparing prednisolone with an elemental diet plus non-absorbable antibiotics in active Crohn's disease. *Gut* 1985; **26:** 994–9.

5 Anonymous. Enteral feeding. *Drug Ther Bull* 1980; **18:** 20.

6 Senapati A, Johnson C, Brown IMH, Thompson RPH. A method to reduce spillage of food in pair-fed rats. *Animal Technol* 1984; **35:** 123–4.

7 Batt RM, Peters TJ. Absorption of galactose by the rat small intestine in vivo: proximal-distal kinetic gradients and a new method to express absorption per enterocyte. *Clin Sci* 1976; **50:** 499–509.

8 Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956; **62:** 315–23.

9 Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagent. *J Biol Chem* 1951; **193:** 265–75.

10 Menzies IS, Mount JN, Wheeler MJ. Quantitative estimation of clinically important monosaccharides in plasma by rapid thin layer chromatography. *Ann Clin Biochem* 1978; **15:** 65–76.

11 Williamson RCN. Intestinal Adaptation. *N Engl J Med* 1978; **298:** 1444–50.

12 Dowling RH. Small bowel adaptation and its regulation. *Scand J Gastroenterol* 1982; **17:** suppl 74: 53–74.

13 Jacobs LR, Bloom SR, Harsoulis P, Dowling RH. Intestinal adaptation in hypothermic hyperphagia. *Clin Sci* 1975; **48:** 13P.

14 Steiner M, Bourges HR, Freedman LS, Gray SJ. Effect of starvation on the tissue composition of the small intestine in the rat. *Am J Physiol* 1968; **215:** 75–7.

15 Aldiwachi HS, Wright NA, Appleton DR, Watson AJ. The effect of starvation and refeeding on cell population kinetics in the rat small bowel mucosa *J Anat* 1975; **119:** 105–21.

16 Young EA, Cioletti LA, Winborn WB, Traylor JB, Weser E. Comparative study of nutritional adaptation to defined formula diets in rats. *Am J Clin Nutr* 1980; **33:** 2106–18.

17 Ecknauer R, Sircar B, Johnson LR. Effect of dietary bulk on small intestinal morphology and cell renewal in the rat. *Gastroenterology* 1981; **81:** 781–6.

18 Smith JL, Artega C, Heymsfield SB. Increased ureagenesis and impaired nitrogen use during infusion of a synthetic amino acid formula. *N Engl J Med* 1982; **306:** 1013–8.

19 Cassidy MM, Lightfoot FG, Like LE, Story JA, Krichevsky D, Vahouny GV. Effect of chronic intake of dietary fibre on the ultrastructural topography of rat jejunum and colon: a scanning electron microscopy study. *Am J Clin Nutr* 1981; **34:** 218–28.

20 Morin CL, Ling V, Bourassa D. Small intestinal and colonic changes induced by a chemically defined diet. *Dig Dis Sci* 1980; **25:** 123–8.

21 Nelson LM, Carmichael HA, Russell RI, Lee FD. Small intestinal changes induced by an elemental diet (Vivonex) in normal rats. *Clin Sci* 1978; **55:** 509–11.

22 Scharrer E, Wolfram S, Raab W, Amann B, Agne N. Adaptive changes of amino acid and sugar transport across the brush border of rat jejunum. In: Robinson JWL, Dowling RH, Riecken E-O, eds. *Mechanisms of intestinal adaptation.* Lancaster: MTP Press, 1982: 123–37.