

Systemic factors are trophic in bypassed rat small intestine in the absence of luminal contents

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Abstract

Mucosal histology, crypt cell proliferation and brush border enzymes were measured in rats with varying degrees of jejunioleal bypass, in order to compare the effect of systemic and luminal factors on adaptive growth and differentiation (brush border enzymes) in small intestinal epithelium. Eighty five percent jejunioleal bypass caused a functional short gut; in intestine remaining in continuity there were significant increases in segmental weight, villus area and crypt depth, compared with sham operated controls and 25% jejunioleal bypass rats. Despite villus cell hyperplasia in 85% bypass rats, mucosal sucrase and alkaline phosphatase fell in jejunum and remained low in ileum, while leucine amino peptidase rose in ileum. There was a significant fall in villus area ($p < 0.01$) and crypt cell production ($p < 0.001$) in self emptying loops of 25% bypass rats not exposed to luminal contents compared with control segments of sham operated rats. In contrast, self emptying loops of 85% bypass rats were not atrophied despite the much greater distance from luminal nutrients; the villus area ($p < 0.01$) and crypt cell production ($p < 0.005$) were higher than in 25% bypass rats, and at least as great as in sham operated rats. These results indicate that adaptive hyperplasia has a variable effect on expression of brush border enzymes which might reflect villus cell immaturity. The atrophic effect of diversion of luminal contents can be counteracted by systemic growth factors released as part of the adaptive response; thus systemic growth factors are not dependent on a permissive effect of luminal contents.

Compensatory structural and functional changes occur in the small intestine after bypass or resectional surgery. In segments left exposed to luminal contents after extensive resection there is an increase in both epithelial cell proliferation rate in the crypts and migration rate of these cells onto the villi, resulting in enlarged villi.¹⁻³ Functionally, these segments exhibit increased absorptive capacity per unit length.¹

The mechanisms by which these adaptive changes take place are complex and only understood in broad physiological terms. Luminal factors such as nutrients and pancreaticobiliary secretions are important in maintenance of cell proliferation in the small intestine in mammals.⁴⁻⁸ Systemic, circulating factors may also influence mucosal morphology and cell proliferation.⁹⁻¹² As yet, no specific factor(s) has been conclusively identified although enteroglucagon has been implicated.¹³ Epidermal growth factor has a potential role,¹⁴ but may act through the

lumen rather than systemically.¹⁵ While it has been concluded that both systemic and luminal factors are involved in adaptation of the shortened gut, the relative importance of each is unknown.¹⁶ It has been concluded that systemic factors play only a permissive role,¹¹ but it remains uncertain whether systemic factors are trophic in the absence of any luminal contents.

Jejunioleal bypass in which bypassed intestine is retained as a self-emptying loop, and similar models of intestinal adaptation, offer the opportunity to test the influence of systemic factors on intestinal epithelial proliferation relative to luminal factors, as all intestine remains exposed to the same systemic factors. Jejunioleal bypass produces hypertrophy of the gut left in continuity and exposed to luminal contents, the extent being proportional to the degree of bypass.¹⁷ The histology of self emptying loops created by the bypass has been reported to show a variety of changes: either atrophy^{2,17-20} or hypertrophy.^{3,21-22} Whether these changes result from an alteration in cell proliferation or in life span of villus cells is not clear. The relationship between the degree of atrophy or hypertrophy of the self emptying loop and the extent of the jejunioleal bypass - that is, the degree of shortening of discontinuity gut, has not been studied. If systemic growth factors were present in jejunioleal bypass, then these might stimulate cell proliferation in self emptying loops and so counteract the atrophic effect of diversion of luminal contents on mucosa of self emptying loops, depending on the relative importance of each influence.

The aim of this study was to determine the importance of systemic factors versus luminal factors and their influence on epithelial morphology and cell proliferation of rat small intestine. Villus area and crypt cell production were measured and compared in intestinal segments in rats subjected to 85% and 25% jejunioleal bypass. The maturity or extent of differentiation of epithelial cells was also determined by measuring the brush border enzymes, alkaline phosphatase, sucrase, and leucine aminopeptidase.

Methods

RATS

Male Sprague-Dawley rats of approximately 200 g were divided into three groups of seven which underwent the following operations (Fig 1):

SHAM

The jejunum was transected 6 cm distal to the

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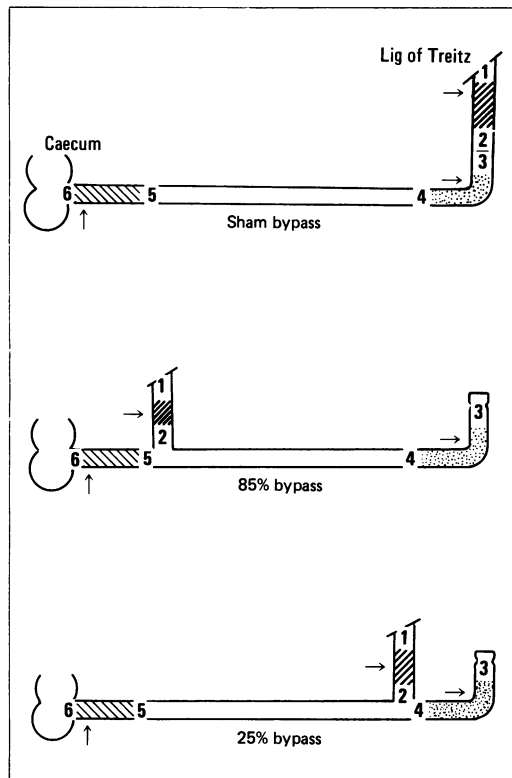


Figure 1: Diagrammatic representation of gut segments studied in the sham operated, 85% and 25% jejunoileal bypass rats. The 85% bypass has an extensive bypass creating a long self emptying loop, relative to the shorter 25% bypass loop. Segment 1-2 represents the segments designated jejunum, 3-4 the self emptying loop (SEL) and 5-6 the ileum. Arrows in each region indicate the 1 cm segments taken for histology with the adjacent 3 cm segment being used for enzyme assay. In the SEL the segments for histology and measurement of crypt cell production rate were taken 1 cm away from the blind end and then a further 15 cm closer to the anastomosis.

duodenojejunal flexure and simply reanastomosed.

EIGHTY FIVE PERCENT JEJUNOILEAL BYPASS

The jejunum was transected 6 cm distal to the duodenojejunal flexure and the proximal end of the transected jejunum anastomosed, end-to-side, to the ileum 10 cm proximal to the caecum. The distal cut end was oversewn.²³

TWENTY FIVE PERCENT BYPASS

As for 85% bypass, but the anastomosis was established approximately 25 cm distal to the site of transection.

Rats drank only 5% glucose and water for 24h before surgery. Surgery was performed under barbiturate anaesthesia (Nembutal, 60 mg/kg, Ceval Chemicals Australia Pty Ltd, Hornsby, NSW). Postoperatively, rats fed freely on rat chow (Clarke King and Co, North Melbourne, Australia). The rate of postoperative weight gain in 85% bypass rats was never less than 90% that of sham operated rats; 25% bypass rats gained weight in parallel with sham operated rats. The 85% bypass rats were weight and age matched to control rats and weighed every two days for 80 days postoperatively. Rats were killed 12 weeks after surgery by carbon dioxide asphyxiation and the entire length of small intestine removed and

irrigated with ice cold saline. The weight per unit length of standardised segments of jejunum, ileum and the end of self emptying loops was estimated by measuring ice cooled segments allowed to hang with a 10 g weight attached to the end. After opening the intestine longitudinally, standardised 1 cm segments were taken for histologic examination (Fig 1). This study was approved by the animal ethics committee of The Royal Melbourne Hospital.

HISTOLOGY

Tissue was fixed and processed for paraffin sectioning. Serial sections (3 μ m) were cut both longitudinal and transverse to the long axis of the gut, and were stained with H & E.

VILLUS AREA INDEX

Villus height (VH), crypt depth (CD) and villus base width, measured in planes both longitudinal (BWL) and transverse (BWT) to the direction of flow down the gut, were determined using a micrometer eyepiece. An index of the villus area (VAI) as a measure of villus cell mass²⁴ was calculated according to:

$$VAI = (BWL + BWT) \times VH$$

Measurements were made on 20 complete villi and 20 entire crypts randomly selected from histological sections from each gut segment. Only optimally sectioned crypts were counted that is, those with crypt lumen in full view from the base to the mouth of the crypt. The crypt mouth was defined as the position at which cell nuclei changed from a midcellular to basal position.

CRYPT CELL PRODUCTION RATE INDEX

Vincristine sulphate (David Bull, Laboratory, Melbourne, Australia) was given to rats by ip injection (1 mg/kg of body weight) to arrest cells in metaphase. The rats were killed at 1, 1.5, 2, 3 and 3.5 or 4 hours after injection. The injection times were staggered to enable the animals to be killed over a period of two hours and so minimise circadian variations in both enzyme activity and crypt cell production rate. Counts of arrested metaphases per crypt were obtained from longitudinally orientated histological sections; 50 crypts were counted per segment per rat. The number of arrested metaphases per crypt was plotted against time and the crypt cell production rate index calculated from the slope of this line. The crypt cell production rate index was determined close to the blind end of the self emptying loops and in the mid-self emptying loops region (Fig 1).

ASSAYS

Brush border enzyme activities were determined in standardised segments adjacent to those taken for histology from proximal jejunum, distal ileum and blind end of the self emptying loops in bypass rats and corresponding segments in sham operated rats (Fig 1). Intestinal segments were laid on an ice-cold glass plate and then the external surface scraped with a glass slide to

TABLE I Weight per unit length in intestinal segments from bypass and sham operated rats

Site	Weight				
	Sham (mg/cm)	85%-bypass (mg/cm)	p	25%-bypass (mg/cm)	p
N=	7	7		6	
Jejunum	81.23* (4.00)	125.60 (13.80)	<0.01	83.82 (3.22)	NS
Ileum	72.01 (5.53)	195.00 (18.90)	<0.001	84.40† (4.52)	NS
SEL‡	84.88 (4.90)	81.11 (9.62)	NS	53.22§ (4.12)	<0.01

*Mean (SEM); p refers to comparison with sham group, NS – not significant (>0.05).

†25% v 85% p<0.001, ‡SEL – self emptying loop, §25% v 85% p<0.01.

extrude the mucosa. A 5% homogenate was then prepared in 50 mmol/l mannitol, 10 mmol/l Tris. HCl, pH 7.4, using a Waring blender.²³ Alkaline phosphatase and protein were assayed as described.²⁵ Sucrase was assayed by the method described by Dahlqvist²⁶ and leucine aminopeptidase according to Muira *et al.*²⁷

STATISTICAL ANALYSIS

Small intestinal segment weights, villus height, crypt depth and villus area results were analysed by analysis of variance, and intergroup comparisons were tested with the Studentised Newman-Keuls test. The CCPR was determined by regression against time of number of arrested metaphases per sectioned crypt, using the Minitab computer program (Minitab Inc, University Park, PA). Crypt cell production rate slopes were compared using an analysis of covariance.²⁸

Results

SEGMENTAL WEIGHT

Intestinal segments in 85% jejunoileal bypass rats remaining exposed to luminal contents, showed a 55% increase in the weight/unit length

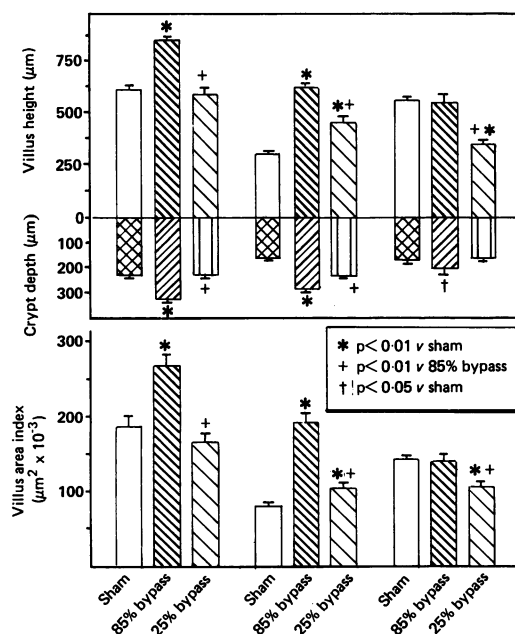


Figure 2: Morphometric measurements in comparable gut segments of 85% bypass, 25% bypass and sham operated (Sham) rats. Vertical bars show the SEM.

of the jejunum ($p < 0.01$) and a 170% increase in the ileum ($p < 0.001$) when compared with sham operated rats (Table I). No significant increases were observed in comparable segments remaining in continuity of 25% bypass rats. Unlike the 25% bypass self emptying loops which atrophied, as shown by 37% decrease in weight per unit length ($p < 0.01$, Table I), the 85% bypass self emptying loops retained a similar weight to the sham operated rats. Macroscopic examination indicated that the self emptying loops were empty and the anastomoses clear from obstruction.

MORPHOLOGY

In the 85% bypass rats, histological examination confirmed adaptive hypertrophy in both jejunum and ileum (Fig 2); this was proportionally more marked in the ileum. The villus area index increased by 42% in the jejunum ($p < 0.001$) and 133% in the ileum ($p < 0.001$) compared to the sham operated rats. The villus area index in the self emptying loops of 85% bypass rats was similar to control segments in sham operated rats, however note that the crypt depth increased by 22% ($p < 0.05$).

In rats subjected to 25% bypass there was a mild, but significant, adaptive hypertrophy in ileum, with a 28% increase in villus area index ($p < 0.01$). The jejunum remained similar to the sham operated rats (Fig 2). In the self emptying loops of 25% bypass rats there was 25% decrease ($p < 0.01$) in the villus area index and no significant change in crypt depth in comparison to sham operated rats.

CRYPT CELL PROLIFERATION

Metaphase accumulation was linear with time over the experimental period at both the end of the self emptying loops, which originally was proximal jejunum, (Fig 3a) and the mid-self emptying loops region (Fig 3b). There was no apparent lag in the onset of vincristine metaphase arrest. The rate of metaphase accumulation, or crypt cell production rate, was determined from the gradient of the regression line. The rates at the end of the self emptying loops were: 85% bypass – 3.58 (1.57) metaphase arrests/crypt/hour/(±95% confidence interval), sham operated – 3.01 (0.50), 25% bypass – 1.88 (0.27). The rates in the mid-self emptying loops were: 85% bypass – 3.39 (1.08), sham operated – 3.20 (0.37), 25% bypass – 2.25 (1.57). In each experimental situation, the crypt cell production rates at the end of the self emptying loops were similar to those at the mid-self emptying loops region. The crypt cell production rate in 85% self emptying loops was increased compared to 25% bypass rats at both the end of the self emptying loops ($p < 0.005$) and the mid-self emptying loops region ($p < 0.05$). In both sites the crypt cell production rate index in 85% bypass rats was equal to or higher than that of sham operated rats.

ENZYME ASSAYS

The relationship between brush border enzyme

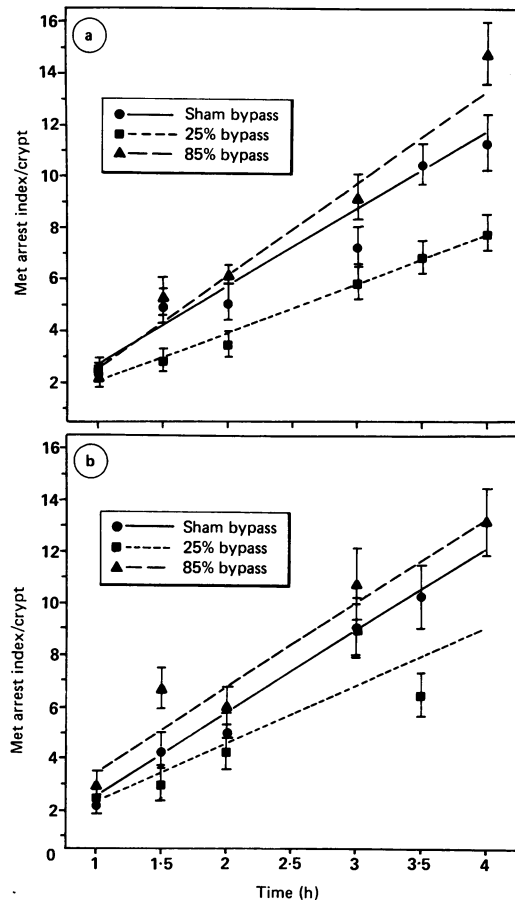


Figure 3: Metaphase accumulation with time at the blind end of the self emptying loop (A) and the mid-loop region (B). The slopes of the regression lines represent the rate of accumulated metaphases or crypt cell production rate, in each experimental group. Vertical bars represent 95% confidence intervals.

activity in mucosal homogenates and villus area index of 85% bypass and sham operated rats is shown in Figure 4. In jejunum of 85% bypassed rats, activities of alkaline phosphatase ($p < 0.05$), and sucrase ($p < 0.01$) fell (Table II). In ileum, despite the doubling of villus area index to levels normally encountered in the jejunum, there was no significant increase in alkaline phosphatase or sucrase activity from the normally low ileal activities. Alkaline phosphatase and

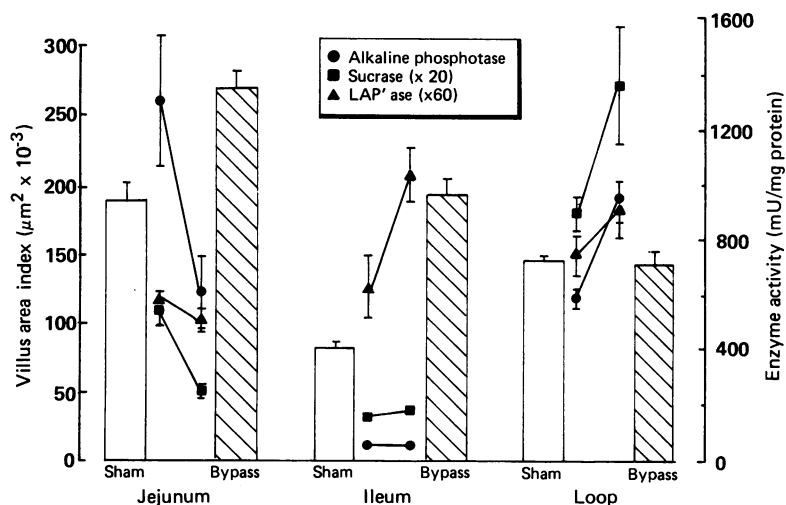


Figure 4: Relationship between villus area index and brush border enzyme activities in 85% bypass and sham operated rat intestinal segments. Vertical bars represent SEM.

sucrase activities increased slightly, but significantly, in the 85% self emptying loops, even though the villus area index was similar to the sham operated rats. Leucine aminopeptidase activity was affected differently in that it increased in hypertrophied ileal mucosa, but remained essentially unchanged in other segments. Rats with a 25% bypass had increased activity of alkaline phosphatase in the jejunum ($p < 0.05$ relative to sham operated rats, with all other enzyme activities remaining similar to controls (Table II).

Discussion

The findings show that diversion of luminal contents during the creation of self emptying bypassed loops does not necessarily cause atrophy. Significant atrophy did occur in self emptying loops of animals subjected to a modest 25% bypass, but not in the longer self emptying loops of rats subjected to extensive 85% bypass. Low crypt cell production rates and relatively shallow crypts were seen in self emptying loops of 25% bypass rats, while crypt cell production rate and crypt depth were maintained at normal and above normal respectively in self emptying loops of the 85% bypass rats. Thus, atrophy in these self emptying loops is prevented by maintenance of cell proliferation.

Rats subjected to 85% bypass showed the typical changes created by surgical shortening of the gut without bypass^{1,7,29,30} of marked ileal and modest jejunal hypertrophy. Shortening of the gut in 25% bypass rats caused mild hypertrophy only in ileum. It seems likely, therefore, that the growth stimulus responsible for adaptive hypertrophy in 85% bypass rats is also the factor which prevents atrophy by maintaining cell proliferation in self emptying loops. Thus, variation in extent of bypass could explain varying reports of histology in self emptying loops in previous studies.^{2,3,17-22}

The mechanism responsible for increased crypt cell proliferation in short gut syndrome is uncertain. One hypothesis is that malabsorbed nutrients release growth factors from distal intestine, these enter the systemic circulation and so are delivered to the entire gut.^{11,31,32} Enteroglucagon has been implicated as the growth factor by a body of indirect evidence,^{13,31} but a recent study in which antibodies were used to block its action have not supported this.³³

An alternative mechanism involves the local release of high concentrations of paracrine growth factors by nutrient rich luminal contents presented to mucosa not normally exposed to such substances – namely distal ileum in short gut syndrome. It is known that ingestion of food maintains normal morphology and prevents atrophy³⁴⁻³⁵ and that epithelial cells directly obtain nutrients from the lumen.³⁶ The proliferative effect of nutrients, however, need not simply be a matter of ready availability of metabolic fuels as indicated by the work of Weser *et al.*⁸ They showed that segmental intraluminal administration of nutrients to fasted rats caused an increase in mucosal cell mass in perfused segments, but growth effects also occurred in more proximal segments not being

TABLE II Brush border enzyme activities in mucosal homogenates* obtained from intestinal segments of bypass operated and sham operated rats

	Alk phosphatase (mU/mg)	p	Sucrase (mU/mg)	p	Amino- peptidase (mU/mg)	p
Jejunum						
85% bypass	615 (130)	0.005	13 (1)	0.009	9 (1)	0.15
25% bypass	1565 (68)	0.03	28 (2)	0.59	8 (1)	0.14
sham	1301 (234)	—	28 (2)	—	10 (1)	—
Ileum						
85% bypass	66 (11)	0.41	9 (1)	0.17	17 (2)	0.08
25% bypass	66 (16)	0.28	6 (2)	0.16	9 (1)	0.29
sham	61 (13)	—	8 (1)	—	10 (2)	—
SEL						
85% bypass	964 (92)	0.02	68 (11)	0.035	15 (2)	0.16
25% bypass	868 (170)	0.06	71 (15)	0.05	15 (2)	0.18
sham	593 (33)	—	45 (3)	—	13 (1)	—

*Expressed as mU/mg mucosal protein (mean (SEM)). p value refers to comparison with sham group.

perfused.⁸ While luminal nutrients had a local growth effect, be it direct or indirect, they also led to growth factor entering the systemic circulation. Thus systemic growth factor might simply be an epiphenomenon of the adaptive response, and not the main mechanism.

If the nutrient value of luminal contents were to be the principal mechanism for stimulation of crypt cell production rate in short gut syndrome, then we would expect atrophy and decreased crypt cell production rate in diverted self emptying loops no matter how short the functional gut. In this study crypt cell production rate and villus morphology were maintained in the long self emptying loops of rats with an 85% anatomically shortened gut. Thus, systemic, non-luminal growth factors are able to overcome the lack of luminal nutrients and other stimuli. This has a number of important implications. Systemic administration of a growth factor for therapeutic reasons in short gut syndrome even before commencing enteral nutrition, may prove of use. More importantly, it is feasible to test putative systemic growth factors by infusion into parenterally nourished animals, since the presence of luminal nutrients is not critical for a proliferative response.

Adaptive enlargement of villi, creating more enterocytes, need not necessarily imply optimisation of functional capacity of these cells. While absorption of glucose is increased per unit length,⁷ this may simply result from increased villus cell mass as reflected in the increased villus area seen in this study. One indicator of villus cell functional capacity is the expression of brush border enzymes. Alkaline phosphatase is a good indicator of differentiation as cells towards the tip of the villus show highest levels of enzyme.³⁷⁻

³⁹ Sucrase-isomaltase is well expressed by villus cells of all ages, but not crypt cells. In this study, expression of each of these differentiation markers altered with changes in morphology, especially after 85% bypass. Alkaline phosphatase and sucrase activity fell in hypertrophied jejunum when expressed relative to mucosal protein. Similar effects were observed by McCarthy *et al.*⁴⁰ for sucrase and by Wilson *et al.*⁴¹ and Menge *et al.*³⁰ for disaccharidases and/or alkaline phosphatase. As previously observed,³⁰⁻⁴¹ neither sucrase or alkaline phosphatase fell in ileum despite the marked increase in villus height; these enzymes are normally very low in activity in ileum. These changes suggest that villus cells are relatively immature in this model

of adaptive hypertrophy and that growth factor-induced cell proliferation does not commit cells to improved functional differentiation.

In contrast with sucrase and alkaline phosphatase, leucine aminopeptidase activity increased in hypertrophied ileum, although not in jejunum. This would suggest a selective effect on that enzyme, possibly because of increased luminal protein in the ileum. It has been shown that brush border peptide hydrolase activity is induced by protein rich diets.⁴²

Pancreatic enzymes are known to hasten turnover of brush border enzymes and reduce their activity.⁴³ This might explain the modest increase in activities in the self emptying loops, but is unlikely to explain the falls in jejunal alkaline phosphatase and sucrase, as this region is normally exposed to pancreatic secretions anyway, and there was no apparent adverse effect on leucine aminopeptidase in the ileum.

While multiple studies³⁰⁻⁴¹ have supported our findings of reduced expression of sucrase and alkaline phosphatase in hypertrophied jejunum, Chaves *et al.*,⁴⁴ using quantitative histochemical measures, have shown apparent increases in enzyme activities in hyperplastic jejunum in the short gut syndrome. While such a technique provides relative measures within a single tissue section, there is uncertainty about the validity of comparison between sections obtained from different animals under different experimental conditions. Keelan *et al.*⁴⁵ found alkaline phosphatase activity to be increased in jejunal brush border preparations isolated from hypertrophied rabbit gut, although sucrase was unchanged. They concluded that cells of hypertrophied villi were not immature, even though their data gave no indication concerning the amount of enzyme per cell. Until cellular transport capacities or content of brush border enzymes are measured in cells isolated from along the length of the villus in each experimental setting, it will not be possible to determine the functional differentiation of villus cells.

Both cell proliferation and differentiation might be affected in bypassed loops by bacterial activity.^{7,46,47} In a study of luminal bacteria in rats subjected to jejunoileal bypass, Viddal *et al.*⁴⁸ found the proximal part of the self emptying loops (near the blind end) to contain very low numbers of bacteria, while the distal part close to the anastomosis was populated by bacteria similar to those in the gut remaining in continuity. In that study, enzyme activities in the self emptying loops were slightly raised compared with control segments, confirming our findings and those of Menge *et al.*^{2,49} Thus the changes in proliferation observed in the self emptying loops were unlikely to have been caused by luminal flora.

In conclusion, atrophy does not occur in jejunal self emptying loops after extensive (85%) jejunoileal bypass because crypt cell proliferation is maintained in the self emptying loops. Thus, the atrophic effect of diversion of luminal nutrients can be counteracted by growth factors which must be systemic. Consequently, luminal factors are not essential for maintenance of normal cellular turnover when there is a powerful stimulus to cell proliferation. Adaptive

crypt cell proliferation does not appear to commit cells to full functional differentiation on the villi, although additional studies are needed to establish this with certainty.

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