Speed of onset of adaptive mucosal hypoplasia and hypofunction in the intestine of parenterally fed rats

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Summary

1. To establish the speed of onset of jejunal and ileal mucosal hypoplasia and hypofunction in parenterally fed rats, we measured three indices of mucosal mass, three mucosal enzymes and quantitative histology after 3, 6, 10 and 15 days of total parenteral nutrition and compared the results with those in two orally fed control groups, one with and one without intravenous catheters and metabolic cage restraint. The kinetics of galactose absorption in vivo were also measured after 10 days of total parenteral nutrition and in both control groups.

2. The most striking decrease in both jejunal and ileal mucosal wet weight and protein and DNA content per 10 cm length of intestine, occurred after only 3 days of total parenteral nutrition; thereafter the mean values showed only a slight further decrease.

3. The results of the morphometric studies showed that the hypoplasia affected the villi slightly more than the crypts. Within 3 days of starting total parenteral nutrition, mean jejunal mucosal thickness decreased by 16% and after 15 days it had fallen by 28%. The ileum showed similar, although less marked, changes. In the jejunum (not the ileum) modest cellular hypotrophy accompanied the mucosal hypoplasia; there were more epithelial cells/unit length of mid-villus and there was more DNA per g of mucosa in the total parenteral nutrition group than in the control group of rats.

4. Jejunal galactose absorption from the 16, 32 and 64 mmol/l solutions was significantly less in the 10-day total parenteral nutrition rats than in the controls, the apparent $V_{\text{max}}$ being five times greater in the orally fed animals. The apparent Michaelis constant ($K_{m}$) was also significantly less than normal in the jejunum of the parenterally fed rats, suggesting increased affinity of the hypothetical carrier for galactose, perhaps as a result of functionally hypermature cells.

5. Mucosal alkaline phosphatase and catalase activities per unit length of intestine decreased and $a$-D-glucosidase activity increased in the jejunum and ileum of the total parenteral nutrition rats.

6. These results show that during total parenteral nutrition, the ileum and particularly the jejunum show marked reductions in mucosal mass and function after only 3 days of total parenteral nutrition and that there is a more gradual and progressive loss of mucosal mass thereafter up to 15 days.

Key words: galactose absorption, hypofunction, hypoplasia, intestinal adaptation, mucosa, total parenteral nutrition.

Introduction

There is now considerable evidence that luminal nutrition influences normal small bowel structure and function (Dowling, 1976). It is known, for example, that when exogenous luminal nutrition is completely excluded from the intestine of animals maintained with exclusive parenteral nutrition, mucosal hypoplasia develops in both jejunum and ileum (Levine, Deren, Steiger & Zinno, 1974;...
gut structure and function changed in animals. Hormones acted indirectly by stimulating aerobic and anaerobic bacteriology were carried out. The results of these studies will be reported.

We wished to extend these studies in the rat but, since the speed of onset of the intestinal hypoplasia during total parenteral nutrition is unknown, we first had to establish how rapidly gut structure and function changed in animals nourished exclusively by the intravenous route. This paper reports our findings of jejunal and ileal structure, intestinal mucosal immunocytochemistry (gut-hormone profile) and quantitative aerobic and anaerobic bacteriology were carried out. The results of these studies will be reported separately.

This paper was presented in part at the European Society for Clinical Investigation, Rotterdam, 1977 (European Journal of Clinical Investigation, 7 (Abstract), 230–231).

Materials and methods

Study design

'Charles River' male albino Cobb–Wistar rats were used throughout. There were eleven controls, five of which were housed individually in conventional rat cages and were fed orally with a commercial rat-diet (Thompson diet 1/2 inch). The remaining six were also fed orally but in addition were subjected to the same procedure as that used for the rats nourished exclusively by the parenteral route (see below) in that they were housed in metabolic cages and had both harnesses and intravenous catheters which were kept patent for 15 days with 2 ml of sodium chloride solution (0.15 mol/l, saline)/day (sham intravenously fed controls).

Thirty rats received their total nutrition parenterally. Of these, seven were killed after 3 days, six after 6 days, two groups of six after 10 days (one group for studies of gut structure and mucosal enzymes and one for galactose absorption studies in vitro) and five rats after 15 days of total parenteral nutrition when quantitative intestinal histology, indices of mucosal mass, kinetics of galactose absorption and mucosal enzyme activities were studied.

Nutrition

Total parenteral nutrition. The technique used was modified from that described by Steiger, Vars & Dudrick (1972). Briefly, an indwelling silastic catheter was passed through the jugular vein into the right atrium. Its other end was then guided through a subcutaneous tunnel to exit at the interscapular area. The intravenous feeding-line was protected by a flexible spiral wire hose which was attached at its lower end to the rat by a pectoral harness, and at its upper end through a water-tight swivel to the nutrient perfusion pump.

The intravenous nutrients were infused throughout the 24-h period with a Scientific Research Instruments (C. E. Payne and Sons Ltd, Clapham, London) 'syringe-pusher' infusion pump, at rates ranging from 1.3 to 2.6 ml/h. Over the first 1–2 days, 24 ml of a 5% amino acid mixture in 32% glucose [10% glucose/Vamin (Vitrum, Stockholm, Sweden) to which additional glucose, potassium and vitamins were added] was infused over 18–20 h and 1 g of a stabilized triglyceride/phospholipid emulsion (10–20% Intralipid, Vitrum) was given over the remaining 4–6 h. The metabolic tolerance to this regimen was monitored by the general appearance of the animal and by urine testing to exclude glycosuria, proteinuria and aciduria. When tolerance was acquired the volume of the Vamin/glucose infusate was increased to 50 ml/day and the Intralipid load remained constant. This provided a total of 45 kcal (0.188 MJ) per rat/day over the first 24–48 h, which ultimately increased to 82 kcal (0.343 MJ) per rat/day (16 g of carbohydrate, 1 g of fat and 2.5 g of protein). The parenterally fed rats were allowed free access to water by mouth.

As a crude index of the nutrional state of the animals and to enable us to compare the effects of total parenteral nutrition on the gut and on other organs the fresh weights of kidneys, adrenals and testes were noted in all groups of rats.
Oral nutrition. The orally fed rats were fed ad libitum, the mean food intake in both the conventionally fed and the sham intravenously fed groups being the same at $23.3 \pm 2.9$ g of the commercial rat diet/day. This provided roughly comparable total metabolizable energy to that of the intravenously fed group (71 kcal, 0.297 MJ) but the contributions from carbohydrate (10.6 g), fat (0.5 g) and protein (3.85 g) in the oral diet were somewhat different from those in the total parenteral nutrition group.

Body weights

The initial body weights were $181 \pm 1.0$ g in the orally fed rats, $188 \pm 3.0$ g in the sham intravenously fed controls and $194 \pm 9.7$ g in the total parenteral nutrition group. These differences were not statistically significant.

After 3 and 6 days of total parenteral nutrition there was no increase in body weight from the initial control values but, thereafter, the mean weight gain of $2.5 \pm 0.9$ g/day compared with that of $3.2 \pm 0.9$ g/day in the sham intravenously fed rats and $5.7 \pm 0.4$ g/day in the orally fed controls. As a result, the mean final weight in the orally fed group of $267 \pm 5.9$ g was significantly greater than that in both the sham intravenously fed control ($236 \pm 8.8$ g; $P < 0.02$) and in the 15-day total parenteral nutrition group of rats ($214 \pm 4.3$ g; $P < 0.001$). However, even after 15 days of exclusive parenteral feeding, all the rats appeared healthy and active.

Studies of intestinal structure and function

The sampling site for quantitative histology (and bacteriology), indices of mucosal mass, mucosal enzymes and the cannulation sites for the perfusion/absorption studies in vivo are shown schematically in Fig. 1.

At the end of the experiment, the entire small bowel was removed, stripped of its mesentery and 10 cm lengths of jejunum and ileum (Fig. 1) were measured against a vertical scale with a 5 g stretch. These intestinal segments were placed on a glass plate over ice, split longitudinally, the mucosa was scraped into tared vials, and the weight of the mucosal scrapings was recorded. The mucosa was then frozen at $-20^\circ$C until analysed for protein, DNA and the activities of α-D-glucosidase, alkaline phosphatase and catalase.

For these analyses, the mucosa was thawed and immediately homogenized on 10 ml of ice-cold sodium chloride solution (0.075 mol/l) with both an Ultra-Turrax homogenizer and an MSE P1600 sonicator. Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) and DNA by the technique of Prasad, Mouchell, Koniuch & Oberleas (1972).

Intestinal structure

Mucosal mass. This was estimated from the measurements of mucosal wet weight and protein and DNA content expressed/unit length of intestine.

Quantitative histology. Lengths of jejunum (2 cm) taken from 10 cm below the ligament of Treitz and of ileum taken from 15 cm proximal to the ileocaecal valve (Fig. 1) were processed for measurements of villus height, crypt depth and epithelial cell density as previously described (Feldman, Dowling, MacNaughton & Peters, 1976). Results in μm were the mean readings from the 10 tallest, well-orientated villi in each section.
Intestinal function

Galactose absorption in vivo. Galactose absorption was measured by a modified recirculation–perfusion technique in vivo as previously described by Batt & Peters (1976). Galactose was chosen as the test monosaccharide since it is actively transported but is not metabolized by rat intestine.

Approximately 20–25 cm lengths of mid-jejunum and mid-ileum were perfused simultaneously (Fig. 1). The initial galactose concentrations were 8, 16, 32 and 64 mmol/l in the jejunum and 4, 8, 16 and 32 mmol/l in the ileum. The osmolality of all solutions, measured with an EEL advanced osmometer, was adjusted to 350 mmol/kg by varying the concentration of sodium chloride.

A fixed, ascending sequence of galactose concentration was used, each sugar being perfused for 30 min, with 1 ml samples being drawn at 5, 10, 20 and 30 min. At the end of each perfusion period the intestinal segment and its attendant cannulae were drained by gravity and the circuit was flushed with air before the next solution was introduced.

Absorption, as defined by luminal disappearance of substrate from the perfusion medium corrected for fluid transfer on the basis of change in \(^{14}\)C-polyethylene glycol (mol. wt. 4000) concentration, was expressed as \(\mu\) mol of galactose absorbed h\(^{-1}\) cm\(^{-1}\) of intestine. As well as calculation of absorption for the different galactose concentrations in individual animals the mean values were also plotted by using the Eisenthal & Cornish-Bowden (1974) direct linear-plot to obtain the apparent \(V_{\text{max}}\) and \(K_{\text{m}}\) values.

Mucosal enzymes. The activities of two brush border enzymes, \(\alpha\)-d-glucosidase (EC 3.2.1.20) and alkaline phosphatase (EC 3.1.3.1) and of the peroxisomal marker enzyme, catalase (EC 1.11.1.6), were calculated in units and expressed both per cm length of intestine and as enzyme specific activity (units/mg of DNA).

\(\alpha\)-d-Glucosidase and catalase were estimated by Peters, Muller & de Duve’s modification (1972) of Baudhin, Beaufay, Rahman-Li, Selliger, Wattiaux, Jaques & de Duve’s method (1964) and alkaline phosphatase was measured by the technique of Bowers & McComb (1966).

Statistical methods

Results are expressed as mean values ± SEM. The significance of differences between mean values was tested by Wilcoxon’s rank-sum test and Student’s non-paired \(t\)-test as appropriate.

Results

Intestinal structure

Mucosal mass. The results for the three indices of mucosal mass for both jejunum and ileum are shown in Fig. 2. Despite the fact that the oral food intake was comparable in both control groups for all three indices jejunal and ileal values were slightly less in the animals with the intravenous cannulae and harnesses than in the group fed orally alone. However, with the exception of amount of DNA/unit length of ileum (\(P < 0.01\)), none of these differences was statistically significant.

After only 3 days of total parenteral nutrition, there was a marked reduction in mean jejunal mucosal protein, which fell from 28.6 ± 1.5 mg/10 cm of intestine in the sham intravenously fed controls to 22.7 ± 2.2 in the total parenteral nutrition group and this 21% reduction was statistically significant (\(t = 2.248; P < 0.05\)). Thereafter, there was little further change in the mean values from the rats studied after 6, 10 and 15 days of total parenteral nutrition, all these values being significantly less than those in the sham intravenously fed controls (\(P < 0.005–0.001\)), but none of the results in the four subgroups of total parenteral nutrition rats was significantly different from each other. A similar pattern of results was seen in the ileum where the mean values fell by 53, 49, 62 and 59% after 3, 6, 10 and 15 days of exclusive intravenous feeding respectively.

The results for mucosal wet weight and amount of DNA/unit length of intestine showed a similar pattern to that described for protein. After only 3 days of total parenteral nutrition there was already a significant fall in the mean values of both these variables in the jejunum and ileum. Thereafter, the mean values, although fluctuating slightly, again tended to fall more gradually.

When the results for protein and DNA content were expressed per unit weight of mucosa (instead of per unit length) there was a different pattern of results.

The mean protein content/g wet weight of mucosa actually increased from 84.9 ± 4.5 to 108.4 ± 14.0 in the hypoplastic jejunum of the 3-day total parenteral nutrition rats although this difference was not statistically significant. Thereafter, the mean levels fell slightly and again these were not significantly different from those of the control. Similarly with DNA, despite the fact that mucosal wet weight decreased per unit length of intestine, when the DNA content was expressed per g wet weight of jejunal mucosa the mean level increased significantly (\(P < 0.02–0.001\)) above that in the sham intravenously fed controls.
Intestinal changes during parenteral nutrition

Fig. 2. Effect of 3–15 days of total parenteral nutrition on mucosal wet weight, protein and DNA/unit length of jejunum (a) and ileum (b) compared with those in conventionally housed, orally fed control rats and in a second group of orally fed controls which had intravenous catheters and harnesses and were housed in metabolic cages (sham intravenously fed controls). Results are means ± SEM for five to seven rats in each group. *P* values refer to the significance of differences between total parenteral nutrition groups and the sham intravenously fed control group of rats. N.S., Not significant.

The results for ileal protein and DNA/g mucosal wet weight showed a less consistent pattern than was seen for the jejunum. In fact, although most of these mean values were less than those of the controls, the differences were not statistically significant except for the 3-day total parenteral nutrition rats (49.2 ± 3.2 mg/g wet wt. for protein, *P* < 0.05; 2.83 ± 0.27 for DNA, *P* < 0.01), which were significantly less than those in the sham intravenously fed controls (71.6 ± 9.9 and 4.7 ± 0.49 respectively).

**Histological measurements of villus height and crypt depth.** The quantitative histological measurements of total mucosal thickness are given in Fig. 3. The ratio of villus height/crypt depth and the number of epithelial cells/200 μm length of mid-villus are given in Table 1. Once again the measurements of villus height and crypt depth were essentially the same in the two control groups and there was no significant difference between the orally fed alone and the sham intravenously fed subgroups.

In keeping with the indices of mucosal mass after only 3 days of total parenteral nutrition there was a significant fall in jejunal mucosal thickness when compared with both the orally fed and the sham intravenously fed control groups (*P* < 0.01).

With more prolonged intravenous feeding the mean jejunal values fell more gradually, these differences all being significantly less than the values for both the orally fed and sham intravenously fed control groups (*P* < 0.001). This hypoplasia affected villi more than crypts so that
Fig. 3. Effect of 3–15 days of total parenteral nutrition on histological measurements of total mucosal thickness for jejunum (a) and ileum (b) showing mean values ± SEM for both villus height (□) and crypt depth (□) (see legend to Fig. 2). P values refer to the significance of differences between the total parenteral nutrition group and the sham intravenously fed control group of rats. N.S., Not significant.

Table 1. Effect of 3–15 days of total parenteral nutrition on the number of epithelial cells/200 μm length of mid-villus and on the ratio of villus height/crypt depth in the jejunum and ileum

Results are mean values ± SEM for five to seven rats in each group. The P values refer to the significance of difference between mean values for the total parenteral nutrition groups and the sham intravenously fed control groups (oral + intravenous). N.S., Not significant.

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<th>Controls</th>
<th>Total parenteral nutrition</th>
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<td></td>
<td>Orally fed</td>
<td>Sham intravenously fed</td>
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<td>Jejunum</td>
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<td>No. of epithelial cells/200 μm length of mid-villus</td>
<td>45.8 ± 1.1</td>
<td>47.3 ± 1.4</td>
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<td>Ratio of mean villus height/crypt depth</td>
<td>2.4:1</td>
<td>2.6:1</td>
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<td>Ileum</td>
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<tr>
<td>No. of epithelial cells/200 μm length of mid-villus</td>
<td>52.8 ± 1.0</td>
<td>51.2 ± 1.5</td>
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<tr>
<td>Ratio of mean villus height/crypt depth</td>
<td>1.7:1</td>
<td>1.8:1</td>
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the ratio of mean villus height/crypt depth fell slightly during total parenteral nutrition (Table 1). As suggested by the DNA content, there were slightly more cells/unit length of mid-villus in the hypoplastic jejunal mucosa of the total parenteral nutrition group than in the control group of rats. However, the major change was in villus height and crypt depth with a corresponding reduction in the total number of epithelial cells even though each individual cell was slightly smaller so that more were required to fill a 200 μm space on the villus side (cellular hypotrophy combined with mucosal hypoplasia).

In the ileum the pattern of results was similar to that seen in the jejunum except that the magnitude of the changes was very much less. For example, the reduction in mean total mucosal thickness was only 6–15% after 3–15 days of total parenteral nutrition: these differences were not statistically significant. However, after 15 days of total parenteral nutrition there was a 17% decrease in mean villus height and this did reach statistical significance when compared with the sham intravenously fed control group (P < 0.05). The ratio of villus height/crypt depth remained relatively constant and there was no appreciable change in cell size, the cell number/200 μm length of mid-villus being comparable in the controls and in the total parenteral nutrition groups.

**Intestinal function**

**Galactose absorption in vitro.** The results of jejunal galactose absorption are shown in Fig. 4. In the jejenum mean galactose absorption by the 10-day total parenteral nutrition rats was less than that of the sham intravenously fed controls at every concentration studied, the difference between the two groups being significant at the 16 (P < 0.05), 32 (P < 0.01) and 64 mmol/l (P < 0.001) galactose concentrations. As a result, there was an 81% reduction in the apparent Vmax for jejunal galactose absorption, which fell from 65.0 ± 3.1 μmol h⁻¹ cm⁻¹ in the control to 12.3 ± 2.1 in the total parenteral nutrition groups of rats (P < 0.001). The apparent Km was also significantly reduced from 50.0 ± 8.9 in the controls to 15.3 ± 3.2 mmol/l in the 10-day total parenteral nutrition group (P > 0.01).

In the ileum the differences between the control and total parenteral nutrition groups of rats were much less marked, the apparent Vmax value of 12.8 ± 5.5 μmol h⁻¹ cm⁻¹ being only slightly less than that in the controls (14.4 ± 2.2) and this difference was not statistically significant. Similarly, there was no significant difference in the apparent Km values for the controls (11.6 ± 2.9 mmol/l) and for the 10-day total parenteral nutrition rats (19.9 ± 6.9 mmol/l).

**Mucosal enzymes.** These results are given in Fig. 5 (activity/unit length of intestine) and in Table 2 (specific activities). With the exception of α-D-glucosidase specific activity in the ileum (Table 2), there were no significant differences between the two control subgroups for all three enzymes whether expressed per unit length or per mg of DNA in jejenum and ileum.

In the jejunum, when the enzyme activities were related to intestinal length, there were significant reductions in the mean values for all three enzymes after 3, 6, 10 and 15 days of total parenteral nutrition when compared with the sham intravenously fed control group except for alkaline phosphatase in the 3- and 6-day total parenteral nutrition rats. In contrast, there were no changes in the specific activities of the two brush border enzymes for any of the total parenteral nutrition groups although the mean catalase activities (units/mg of DNA) were less in the parenterally fed rats.

In the ileum the pattern of results was, with few exceptions, similar to that seen in the jejunum. For example, the alkaline phosphatase and catalase activities/10 cm of intestine in the 3–15-day total parenteral nutrition groups were all significantly less than the results in the controls, but the α-D-glucosidase activity was not significantly different. Similarly, there was no significant difference in alkaline phosphatase and catalase specific activities in the total parenteral nutrition groups (with the exception of alkaline phosphatase in the 6-day total parenteral nutrition
Fig. 5. Effect of 3–15 days of total parenteral nutrition on the activities of α-D-glucosidase, alkaline phosphatase and catalase expressed per unit length of intestine for jejunum (a) and ileum (b) compared with that in conventionally housed, orally fed control rats and in a second group of orally fed controls which had intravenous catheters and harnesses and were housed in metabolic cages (sham intravenously fed controls). N.S., Not significant.

Table 2. Effect of 3–15 days of total parenteral nutrition on the amounts of mucosal α-D-glucosidase, catalase and alkaline phosphatase expressed as enzyme specific activity (units/mg of DNA) for both jejunum and ileum.

Results are mean values ± sem for five to seven rats in each group. *P* values refer to the significance of differences between mean values when compared with the sham intravenously fed control group. N.S., Not significant.

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<td>α-D-Glucosidase</td>
<td>71.8 ± 8.5</td>
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<td>Alkaline phosphatase</td>
<td>20.0 ± 3.2</td>
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<td>Catalase</td>
<td>76.8 ± 2.3</td>
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<td>α-D-Glucosidase</td>
<td>13.9 ± 1.3</td>
<td>26.7 ± 4.0</td>
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<td><em>P</em> &lt; 0.01</td>
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<td>Alkaline phosphatase</td>
<td>7.0 ± 0.8</td>
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<tr>
<td>Catalase</td>
<td>59.7 ± 3.9</td>
<td>74.1 ± 10.0</td>
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Intestinal changes during parenteral nutrition

The results of this study have defined, for the first time, the speed of change in jejunal and ileal structure and function when exogenous luminal nutrition is completely excluded from the intestine of rats maintained with total parenteral nutrition. Even though exogenous nutrients are excluded, the intestine continues to receive saliva, mucus, bile and protein-rich gastric and pancreatic secretions (albeit probably in reduced amounts), which, theoretically at least, could provide a source of endogenous luminal nutrition. The results have shown that marked mucosal hypoplasia and hypofunction develop rapidly during exclusive parenteral feeding and that by 3 days there are already significant reductions in the indices of mucosal mass and in the histological measurements of mucosal thickness. Thereafter, there is a more gradual further decrease in the indices of mucosal mass. These findings provide essential background information for future studies on the mechanisms of the intestinal adaptive changes which are seen after parenteral feeding. Furthermore if these results can be extrapolated to man they stress the importance of luminal stimuli in the maintenance of normal gut structure and function in patients undergoing short- and long-term parenteral nutrition.

Discussion

The results of this study have defined, for the first time, the speed of change in jejunal and ileal structure and function when exogenous luminal nutrition is completely excluded from the intestine of rats maintained with total parenteral nutrition. Even though exogenous nutrients are excluded, the intestine continues to receive saliva, mucus, bile and protein-rich gastric and pancreatic secretions (albeit probably in reduced amounts), which, theoretically at least, could provide a source of endogenous luminal nutrition. The results have shown that marked mucosal hypoplasia and hypofunction develop rapidly during exclusive parenteral feeding and that by 3 days there are already significant reductions in the indices of mucosal mass and in the histological measurements of mucosal thickness. Thereafter, there is a more gradual further decrease in the indices of mucosal mass. These findings provide essential background information for future studies on the mechanisms of the intestinal adaptive changes which are seen after parenteral feeding. Furthermore if these results can be extrapolated to man they stress the importance of luminal stimuli in the maintenance of normal gut structure and function in patients undergoing short- and long-term parenteral nutrition.

Previous studies of gut structure and function during total parenteral nutrition

The results of many previous studies have shown that when the intestinal mucosa is deprived of exogenous luminal nutrition it generally becomes hypoplastic with diminished function. This happens, for example, in self-emptying and in Thiry–Vella by-pass loops. However, in these experimental models, as well as excluding luminal nutrients, one is also examining the effects of starvation and nitrogen depletion on the gut. The advent of long-term total parenteral nutrition provides a means of completely excluding exogenous luminal nutrients from the intestine whilst maintaining the nutritional state of the animal at virtually normal levels. Indeed we (Dowling, Feldman, MacNaughton & Peters, 1973; Feldman et al., 1976; Hughes et al., 1978) and others (Levine et al., 1974; Bury et al., 1975; Johnson et al., 1975; Koga et al., 1975; Eastwood, 1977) have previously studied the effects of total parenteral nutrition on the small bowel but until now most investigators have examined only gut structure and there has been no information about the speed of onset of the mucosal hypoplasia and hypofunction. Our previous studies in the dog, for example, were carried out after 6 weeks of intravenous feeding (Hughes et al., 1978). Levine et al. (1974) studied the rat after only 7 days of total parenteral nutrition. Bury et al. (1975) chose 3 weeks, Johnson et al. (1975) 8–19 days, Koga et al. (1975) 4–8 weeks and Eastwood (1977) 10 days of intravenous feeding to examine the effect on intestinal mucosal structure.

Experimental design

Since there were already marked changes in mucosal structure and function after only 3 days of total parenteral nutrition, in retrospect one can say that it would also have been of interest to examine the intestinal changes 24 and 48 h after starting parenteral feeding. However, such studies are open to the objection that at these times one may also be examining the effects of anaesthesia and the minor trauma of inserting an indwelling caval catheter through a skin tunnel. Indeed, two lines of indirect evidence suggest that these
procedures result in stress to the animals. First, despite the fact that they received identical total calorie intake to the control rats, in the absence of obvious complications such as sepsis the parenterally fed rats did not grow as rapidly as the orally fed controls. Secondly, if adrenal wet weight can be taken as a crude index of stress, the mere insertion of the intravenous feeding-line itself and the housing of the animal in a metabolic cage seems to be enough to increase adrenal weight in the sham intravenously fed controls and to produce significant further increases in adrenal weight in the 3- and 6-day total parenteral nutrition groups. Fortunately, however, these changes in adrenal weight/100 g body weight were no longer significantly different from the sham intravenously fed control group after 10 days of total parenteral nutrition (the time chosen for the detailed studies of galactose absorption in vivo).

In the present studies we chose two control groups, both of which received solid food orally. It might be argued that one group of controls should have been given identical nutrient fluids orally to those used for intravenous feeding but this would have necessitated gavage, which in itself probably causes stress. Besides, the aim of the study was to look at the effects of excluding normal luminal stimuli provided by food and not to study the effect of a no-residue diet. In the event, there were only minor differences for the various indices of intestinal structure and function between the orally fed alone and the orally plus sham intravenously fed control groups and of the variables studied; only ileal α-D-glucosidase specific activity and the amount of mucosal DNA/10 cm of ileum were significantly different between the two control subgroups.

Present results

Differences between jejunum and ileum. If normal structure and function of the duodenal and jejunal mucosa are constantly stimulated by the presence of luminal nutrition (or other luminal stimuli) and the ileum, which receives much less nutrition in its chyme because of proximal absorption, is less stimulated, then one might expect the jejunum to show more marked effects of deprivation of exogenous luminal stimuli than the ileum (Gleeson, Cullen & Dowling, 1972a; Gleeson, Dowling & Peters, 1972b). The present results of quantitative histology and of galactose absorption support this hypothesis since the jejunal changes were more marked after 3–15 days of total parenteral nutrition than those seen in the distal small bowel.

Enterocyte size and mucosal enzymes. In the present study the major changes in mucosal thickness occurred as a result of fewer crypt and villus epithelial cells, in other words hypoplasia. There were, however, additional changes in cell size with somewhat smaller enterocytes.

In general, when mucosal hypoplasia occurs there is a slower epithelial cell migration with the result that there is more time for enteroblast maturation in the crypts and the villus becomes populated with a greater proportion of more mature enterocytes than normal. Indeed, this hypothesis has been advanced to explain the apparent paradox of increased mucosal enzyme specific activities in hypoplastic mucosa, such as that seen here in three of the four total parenteral nutrition groups for ileal α-D-glucosidase activity. Usually when intestinal adaptation develops in the rat, however, the most marked changes in mucosal enzymes occur as a result of the hyper- or hypo-plasia with more or less enzyme activity/unit length of intestine respectively despite the subtle changes in enzyme specific activity. Therefore with the mucosal hypoplasia of total parenteral nutrition one would have expected less enzyme activity/unit length of intestine, as happened with all three enzymes in the jejunum and with alkaline phosphatase and catalase in the ileum of the total parenteral nutrition group of rats. But why this did not happen for ileal α-D-glucosidase, and why jejunal catalase and ileal α-D-glucosidase specific activities did not show the same pattern as that seen for the other enzymes, is unknown.

Galactose absorption in vivo. As already mentioned, the most marked changes in absorption during total parenteral nutrition were seen in the jejunum where there were obvious differences in segmental galactose absorption (as defined by luminal disappearance of substrate from the perfusion fluid) at every concentration studied.

The object of studying different galactose concentrations was to obtain apparent $V_{\text{max}}$ and $K_m$ values for control and experimental animals. The markedly reduced apparent $V_{\text{max}}$ and unit length of jejunum was comparable with the values seen for normal ileum. This finding is compatible with the observed reduction in villus height and mucosal mass with a reduced number of epithelial absorptive cells. The reduction in the apparent $K_m$ for jejunal galactose absorption suggests that the cells required less galactose to satisfy the hypothetical carrier for galactose than normal. Again such a phenomenon could be explained by the villus being populated with relatively hypermature cells as was postulated to explain the increased α-D-glucosidase specific
activity in the ileum of the 3-, 10- and 15-day total parenteral nutrition rats. In the ileum, in keeping with the very much less marked reductions in villus height and crypt depth during total parenteral nutrition, there were only minor and non-significant changes in both the apparent \( V_{\text{max}} \) and \( K_m \) values for galactose absorption.

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References


