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Changes in proliferation and surface morphology in the rat ileum in response to total parenteral nutrition

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INTRODUCTION

Normal structure and function of the mammalian intestine are dependent upon food intake, digestive secretions and possibly gastrointestinal hormones. It is difficult, however, experimentally to separate the various mechanisms involved in regulating mucosal morphology and intestinal cytokinetic activity. For example, villus size and mitotic activity in the small intestinal mucosal epithelium change in response to starvation in the rat, but appear to change independently from one another (Altmann, 1972). Villus size was shown to be controlled by pancreatic and biliary secretions (Altmann & Leblond, 1970; Altmann, 1971, 1972; Clarke, Ecknauer & Feyerabend, 1976); however, epithelial cell proliferation in the small intestinal crypt was postulated as regulated by a negative feedback loop involving a tissue specific chalone (Tutton, 1973). One approach to understanding the mechanisms active in maintaining intestinal morphology has been to compare intestines from starved versus re-fed animals (Altmann, 1972; Clarke, 1975). Marked differences in both intestinal structure and mitotic activity are seen using this method. There are, however, certain limitations to starvation studies. For instance, amino acid transport, in addition to several other parameters, is decreased significantly in as brief a starvation period as 24 hours (McManus & Isselbacher, 1970). In experiments involving prolonged starvation, changes in intestinal structure may not be due entirely to the absence of intraluminal food and/or gastrointestinal secretions but may partly reflect the waning nutritional status of the animal. Studies using parenteral feeding (I.V.) may circumvent this limitation. Such parenteral feeding causes increases in total body weight accompanied by positive nitrogen balance (Steiger *et al.* 1975; Cameron, Pavlat, Stevens & Rogers, 1979) which indicated that the rats used were in good nutritional status. Previous studies using rats fed parenterally showed that intestinal weight drops 25 % to 40 % as compared to animals fed orally (Cameron, Pavlat & Urban, 1974; Johnson *et al.* 1975) but the ilea from these rats transport a neutral amino acid (valine) as efficiently as do those from orally fed control animals (Cameron *et al.* 1974). Apparently the large decrease in intestinal mass is not accompanied by a broad decrease in transport ability.

We therefore feel it is worth while to examine the surface morphology and proliferative activity of the ilea of rats given total parenteral feeding in comparison to rats fed solid food. The findings should help us to understand the relationships between intestinal structure, function and nutrition.

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MATERIALS AND METHODS

The techniques, solutions and equipment for continuous parenteral feeding (I.V.) of unrestrained rats have been described in detail elsewhere (Steiger, Vars & Dudrick, 1972). In brief, a 27 % solution of glucose and a 3·9 % solution of Fre-amine (McGaw Laboratories) with electrolytes and vitamins was continuously infused with a model 903 Holter pump. In the first experiment eight male Buffalo strain rats, eight weeks of age, were randomly divided into two equal groups. Both groups were housed individually in metabolic cages in a 25 °C room with 14 hours of light and 10 hours of darkness. The control group was fed Lab Blox (Wayne) *ad libitum*. Ten days after the start of parenteral feeding both groups of rats received an intraperitoneal injection of tritiated thymidine (^{3}HT) at 1 μCi/g body weight and were killed by an overdose of ether one hour later.

For light microscopy, segments of the lower ileum 1 cm in length, taken 1 cm above the ileocaecal junction, were removed from the rats, cut open and laid out serosal side down on paper cards. Mucus was washed from the luminal surface with a dilute solution of cetylpyridinium chloride prior to fixation in phosphate-buffered formaldehyde (10 %). Tissues for scanning electron microscopy were excised and prepared in a similar manner, but were fixed in 3 % glutaraldehyde buffered with sodium cacodylate (0.1 M, pH 7·1). The samples were post-fixed in cold 1 % osmium tetroxide and were dehydrated in an ascending series of acetones. The tissues were dried by the critical point method (Anderson, 1951) in a Tousimis Samdri PVT3 apparatus (Rockville, Md.) using liquid CO_2 as the transitional fluid. The samples were mounted on aluminium stubs and lightly coated with gold–palladium in a d.c. sputtering system (Commonwealth Scientific, Alexandria, Va.). The tissues were viewed in a JEOL JSM-35 scanning electron microscope operated at an accelerating voltage of 25 kV.

Histological sections of the ileum were prepared for radioautography by deparaffinization and dipping in Kodak NTB2 radioautographic emulsion. The slides were dried and placed in light tight slide boxes for a period of four weeks. At the end of this time, the slides were removed from 4 °C storage, developed in Dektol and stained in haematoxylin and eosin. Analysis of the radioautographs was done by counting the number of labelled cells per crypt. At least ten crypts per animal were counted for both groups and only those crypts sectioned so that the lumen was exposed from the top to the base of the crypt were scored.

Segments of the lower ileum 2 cm in length were also taken for biochemical analysis. This tissue was extracted by a modification of the Schmidt–Thannhauser method. The tissues were homogenized with a Tri-R stirrer and then extracted with 0·5 N perchloric acid ($HClO_4$). The acid-insoluble precipitates were washed with 0·5 N-$HClO_4$ and then 95 % ethanol containing 0·1 M sodium acetate. Each precipitate was then re-dissolved in 0·3 N KOH followed by incubation at 37 °C for 1 hour. The nucleoprotein fraction was re-precipitated by acidification of the KOH hydrolysate (0 °C) with nine drops of 10 N-$HClO_4$. The resulting precipitate was washed twice with 0·5 N-$HClO_4$ and once with 100 % ethanol. After the ethanol wash, the precipitate was incubated with 2 ml of 0·5 N-$HClO_4$ at 45 °C for 24 hours to hydrolyze the DNA fraction. The acid hydrolysis of the precipitate was repeated with 1 ml of 0·5 N-$HClO_4$. The two hydrolysates from each sample were then combined. A 1 ml aliquot of each combined hydrolysate was pipetted into a 9 ml test tube and diluted to 2 ml with 0·5 N-$HClO_4$. Four ml of diphenylamine solution

Table 1. *Summary of responses to total parenteral feeding in the rat ileum (values* ± S.E.M.)

	Oral	Parenteral	% increase (↑) or decrease (↓) from oral	Significance
(1) mg DNA cm of intestine	182·6±26·7	51·6 ±12·2	↓ 71·7	0·001
(2) ^{3}HT incorporation (CPM/μg DNA)	5·66±0·66	22·0 ± 1·2	↑ 289	0·001
(3) No. labelled cells/crypt in autoradiographs	26·2 ± 1·75	15·62± 3·77	↓ 40·4	0·01
(4) Mitotic figures per crypt	14·0 ± 0·52	5·7 ± 0·21	↓ 59·3	0·001
(5) Villus height in mm	0·33± 0·02	0·26± 0·01	↓ 21·2	0·05
(6) Crypt height in mm	0·26± 0·01	0·22± 0·002	↓ 15·4	0·01
(7) No. of cells from base to mouth of crypt	38·43± 0·35	29·22± 0·27	↓ 24	0·001

(Burton, 1956) was added to each sample. The resulting solutions were incubated at 30 °C for 24 hours and the DNA content in each sample was then determined by measuring the absorbance with a Zeiss PMQII spectrophotometer. The remaining 2 ml of each acid hydrolysate was pipetted into a scintillation vial and 15 ml of scintillation fluid added. The scintillation fluid contained 1 part Triton X-100 to every 2 parts of toluene. Four grams of PPO and 0·1 g of POPOP were dissolved in each litre of toluene. The radioactivity of each sample was determined in a Nuclear-Chicago Mark I beta liquid scintillation counter. Quenching was monitored by the channels ratio method.

Mean values between groups were subjected to Student's *t* test for statistical comparisons.

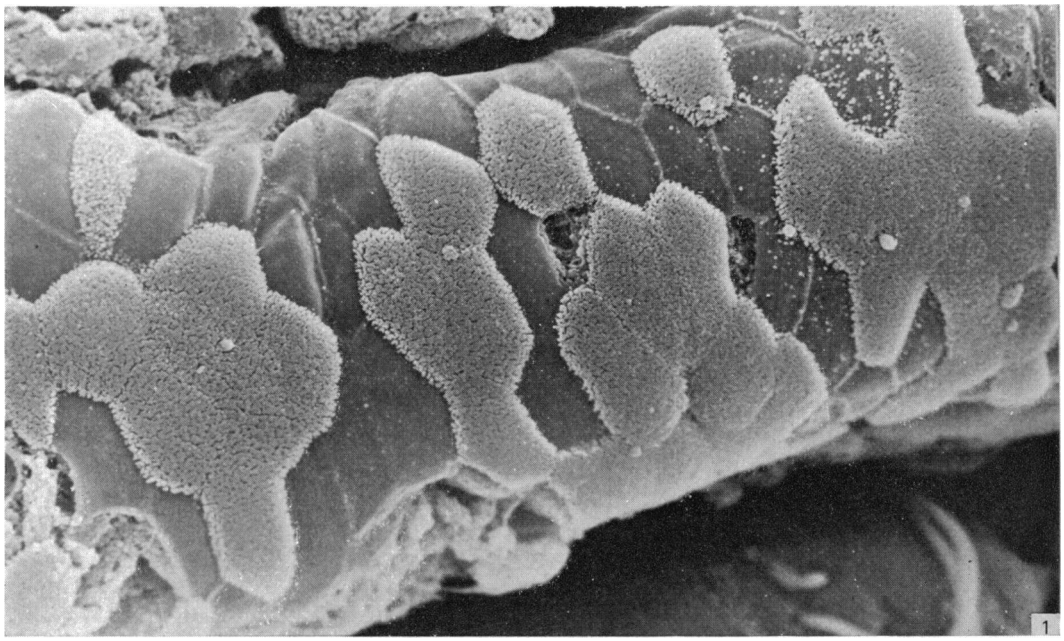
In the second experiment six male Sprague–Dawley rats, 12 weeks of age, were fed intravenously for ten days. The control group of four animals was fed Lab Blox (Wayne) *ad libitum*. Both groups were housed individually in metabolic cages at 25 °C room temperature, with 14 hours of light and 10 hours of darkness. Ten days after the start of parenteral feeding both groups of rats received an intraperitoneal injection of colchicine at 1 mg/kg of body weight and were killed by an overdose of ether three hours later.

For light microscopy, samples of the lower ileum were taken as in the first experiment and prepared for fixation as described above. These samples were fixed in 3 % paraformaldehyde in 0·1 m phosphate buffer (pH 7·3). Tissues were embedded in paraffin and sectioned at 4 μm. Sections were stained with haematoxylin and eosin. Analysis of the tissue was done by counting the number of metaphase figures per crypt. At least ten crypts per animal were counted for both groups and only those crypts sectioned so that the lumen was exposed from the top to the base were scored.

RESULTS

Experiment 1

At the beginning of the feeding treatment period, the mean weight of the group to receive parenteral feeding was 164·7 ± 6·8 g while that of the group to remain on solid food was 158.8 ± 5·7g. These values are not significantly different. At the termination of the experiment, the parenterally fed rats had gained an average of 13 g; this value is not statistically different from the solid fed rats as determined by Student's *t* test.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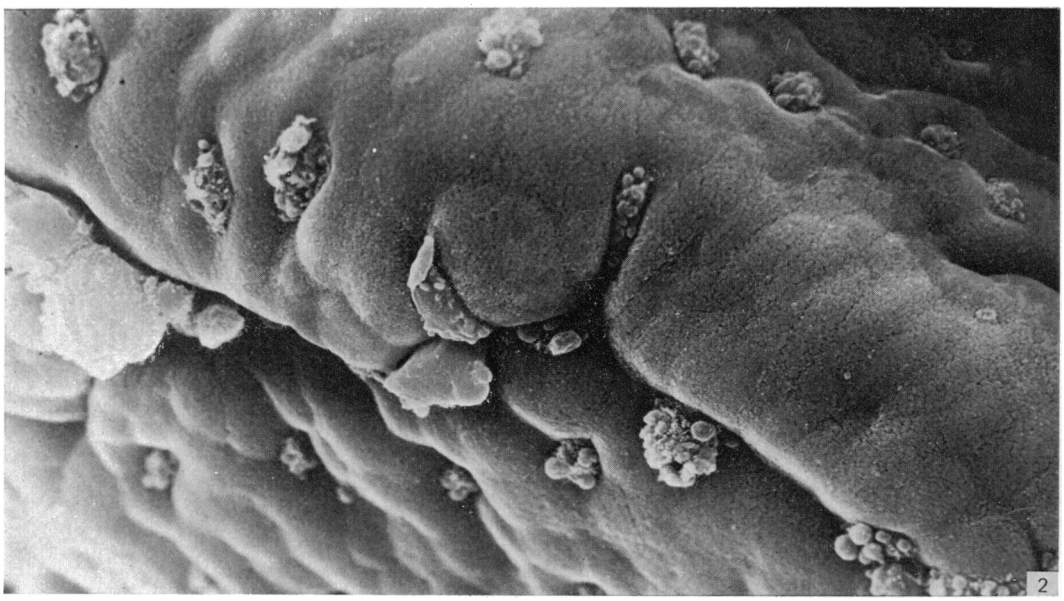
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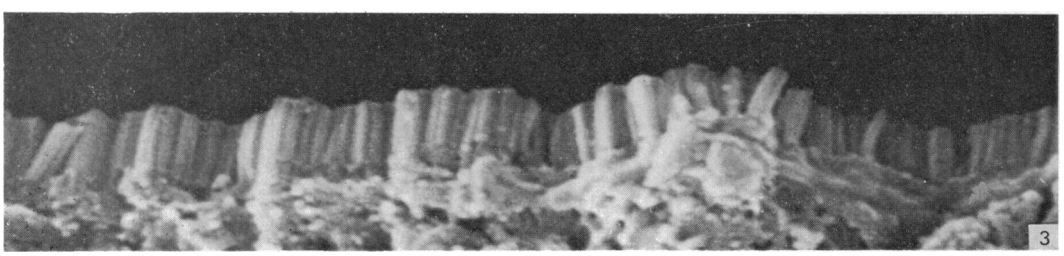
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Table 1 summarizes some of the responses of the rat ileum to total parenteral feeding observed during the present investigation. In a previous study (Cameron *et al.* 1974) parenteral feeding was observed to cause significant decreases in (1) total weight of the small intestine (% body weight), (2) ileal wet and dry weight, and (3) diameter of the small intestine. There was no significant difference in total length of the small intestine or total number of villi between orally fed and parenterally fed rats. The number of villi per unit area of ileum was greater in I.V. fed rats than in orally fed rats, indicating that ileal villi become more tightly packed in response to total parenteral feeding.

Cell proliferation

The DNA content per cm segment of ileum was markedly and significantly decreased in the parenterally fed rats; the amount of DNA per cm of ileum was reduced 72 % in the I.V. fed rats as compared to the orally fed animals. The incorporation of tritiated thymidine (^{3}HT) into intestinal DNA was measured and used as an indication of cell proliferative activity. Table 1 shows that the specific activity of incorporation of ^{3}HT into DNA of the ileum was significantly higher in the parenterally fed rats. Because the amount of DNA per diploid somatic cell was essentially constant, the observed decrease in DNA content of the intestines from I.V. fed animals reflected a decrease in cell number.

Table 1 reveals changes in the proliferative compartment in the ileum of parenterally fed rats. The general histology shows that the overall thickness of the intestinal wall was less in the parenterally fed animals. This was reflected in a decrease in the height of the intestinal crypts. The number of cells with a radioactive nucleus was fewer per crypt in parenterally fed rats. The data in Table 1 confirm this observation by showing a decrease in the number of labelled cells from 26 per crypt in the rats fed solid food to 15·6 per crypt in the rats fed parenterally; the epithelium of the ileum from I.V. fed rats also appeared to have more goblet cells per unit length of surface.

Changes in surface morphology of ileal enterocytes

Once mucus was removed, the surfaces of enterocytes from villus tips were seen to be heterogeneous in form in ilea from animals fed solid food orally (Fig. 1). At least three arrangements in surface structure were seen: (1) cell apex completely covered with microvilli, (2) cell apex covered with small blebs and/or scattered microvilli, and (3) cell surface totally devoid of blebs or microvilli (Fig. 1). In contrast, only one type of surface architecture (abundant microvilli) was seen on enterocytes situated at the base or traversing the sides of villi from rats fed orally. In parenterally fed animals, this characteristic surface morphology was associated with enterocytes on the tip as well as at the base and sides of ileal villi (Fig. 2). Goblet cell openings were easily identified in parenterally fed rats by the presence of secre-

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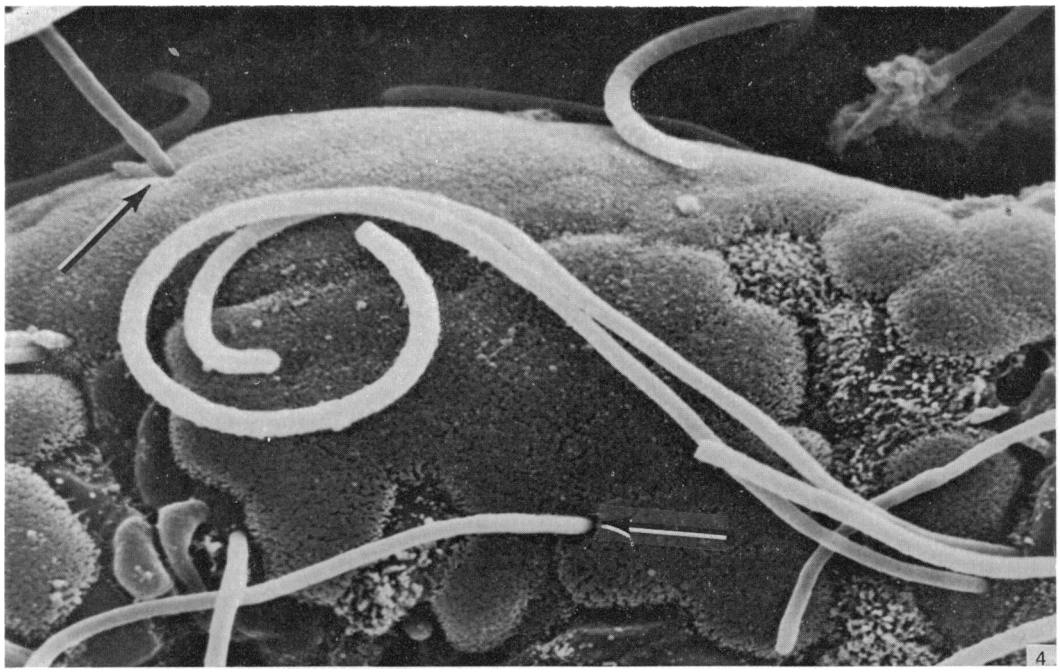
Fig. 1. Apex of villus from ileum of rat fed solid food. Enterocytes are hexagonally shaped and vary in surface morphology from cells covered totally with microvilli to those that are devoid of any surface appendages. Cell borders appear as raised junctions. × 2060.

Fig. 2. Apex of villus from ileum of rat fed parenterally for ten days. All enterocytes covered with abundant microvilli. Goblet cell openings are clearly marked by presence of intact secretory granules. × 2060.

Fig. 3. Brush border of enterocyte from ileum of animal fed parenterally. This blunt dissection shows that microvilli remain closely packed on surface enterocytes in I.V. fed animals. × 10 300.



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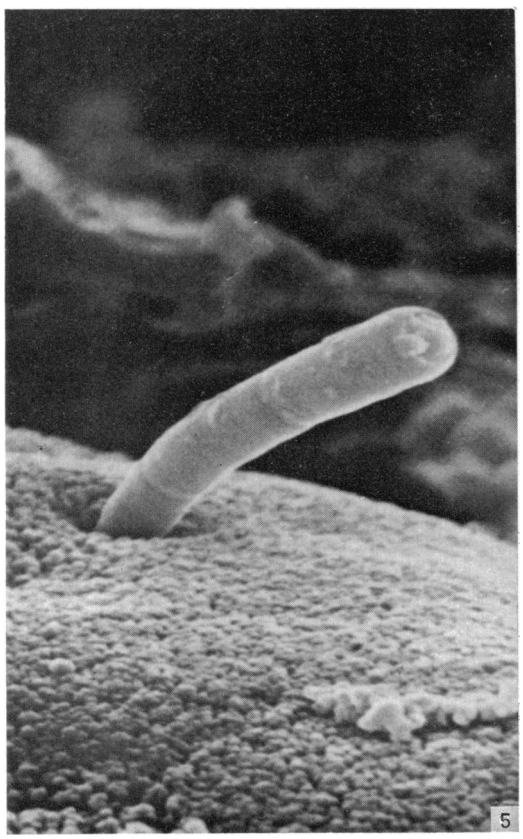

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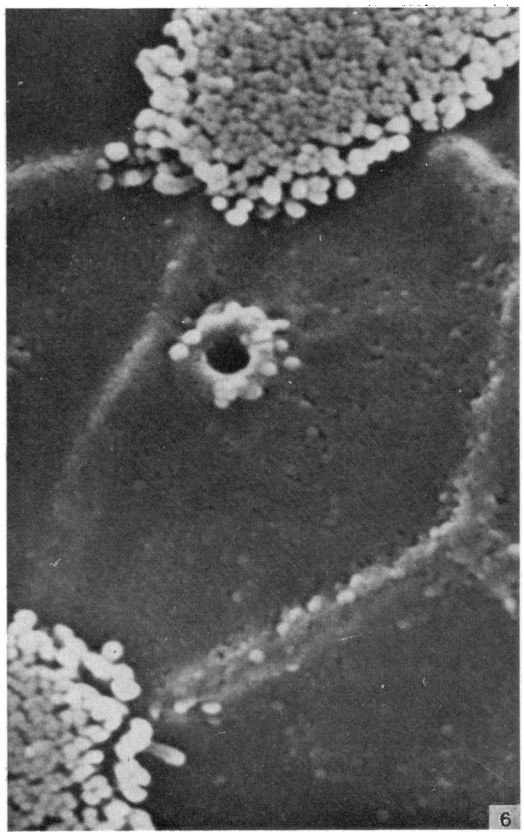

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tory granules at the orifice (Fig. 2), and their surface covering of microvilli of uniform length is demonstrated in Figure 3. This micrograph shows that the surfaces of enterocytes are free of mucus that so commonly obliterates detail in SEM studies of the intestine.

A striking difference between the ileum of rats fed orally and of those fed parenterally was the presence of bacteria on the enteric surface in orally fed animals (Figs. 4–6) and their total absence in I.V. fed animals. Bacteria were most commonly rod-shaped and persisted as long filamentous chains (Fig. 4). Although some chains appeared to be resting loosely on the ileal surface, many were anchored into enterocytes (Figs. 4, 5), forming distinct indentations in the microvillous surface (Fig. 5). Evidence indicating that bacteria were anchored intracellularly is seen in Figure 6 where an enterocyte in the exfoliative zone has lost the characteristic surface microvilli, revealing a hole surrounded by small blebs. The size and position of this hole suggested that it was a remnant of a bacterial attachment site. Although surface holes were common in enterocytes at the tips of villi in ilea of orally fed rats, none was seen in preparations of ilea from rats fed parenterally for ten days.

Marked differences in cell surface morphology were noticeable in the exfoliative zones of ileal villi from rats fed orally (Fig. 7) and from those fed parenterally (Fig. 8). As also shown in Fig. 1, enterocytes in the exfoliative zone of animals fed solid food showed a heterogeneity in surface structure, ranging from cells with bare apices to those with abundant, tightly packed microvilli (Fig. 7). There were also areas where enterocytes were separated laterally from neighbouring cells (Fig. 7) and places where enterocytes appeared to be missing (Fig. 7, arrows). In contrast, the mucosal epithelium of ilea in I.V. fed rats remained intact and most enterocytes retained their microvilli (Fig. 8). Although no cells were seen to be devoid of microvilli in I.V. fed rats, a few enterocytes of the exfoliative zone were seen to protrude into the lumen more than others (Fig. 8). The microvilli on these protruding cells appeared to be thinned out (fewer per unit area) as compared to those on surrounding cells. Enterocytes that protruded from the surface of ileal villi in the exfoliative zone were only occasionally seen in I.V. fed rats whereas cells having no microvilli were seen in the exfoliative zone of all villi from ilea of orally fed rats. Goblet cell openings (Fig. 7, *g*; Fig. 8, inset) were seen in the exfoliative zone of villi from both groups of rats; however, those from I.V. fed rats often had intact secretory granules associated with their openings while those from orally fed rats had none.

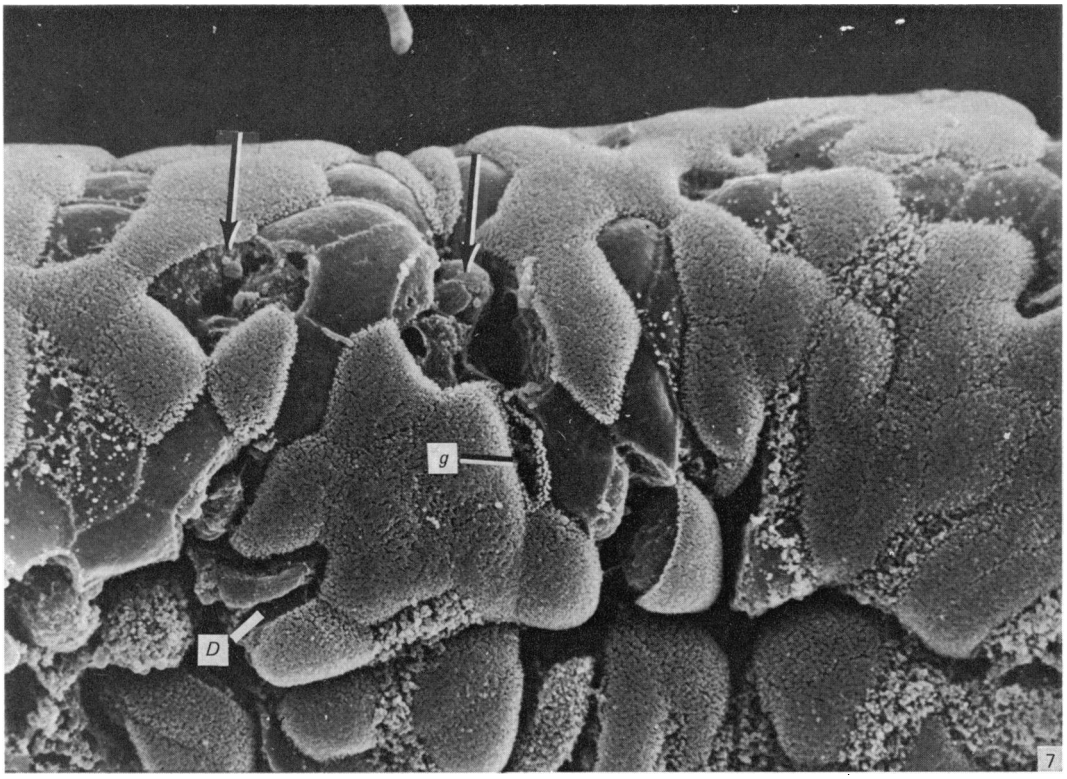
Experiment II

At the beginning of the feeding treatment period, the mean weight of the group to receive parenteral feeding was $414{\cdot}8 \pm 11{\cdot}7$ g, while that of the group to remain on solid food was $410{\cdot}5 \pm 13{\cdot}7$ g. These values are not significantly different. When the experiment was terminated, the parenterally fed rats had gained an average of 6 g.

Fig. 4. Portion of villus from ileum of rat fed solid food. Chains of rod-shaped bacteria are seen on surface of enterocytes. Several chains (arrows) are anchored in enterocytes. × 2300.

Fig. 5. Higher magnification micrograph of rod-shaped bacteria embedded in enterocyte from ileum of orally fed rat. Note depression formed in microvillous surface. Three bacterial cells are discernible. × 9000.

Fig. 6. Hole in surface of enterocyte from ileum of rat fed solid food. The hole is surrounded by small blebs and appears only in ilea of orally fed rats, as do bacteria. It possibly represents former attachment site for bacteria. × 9350.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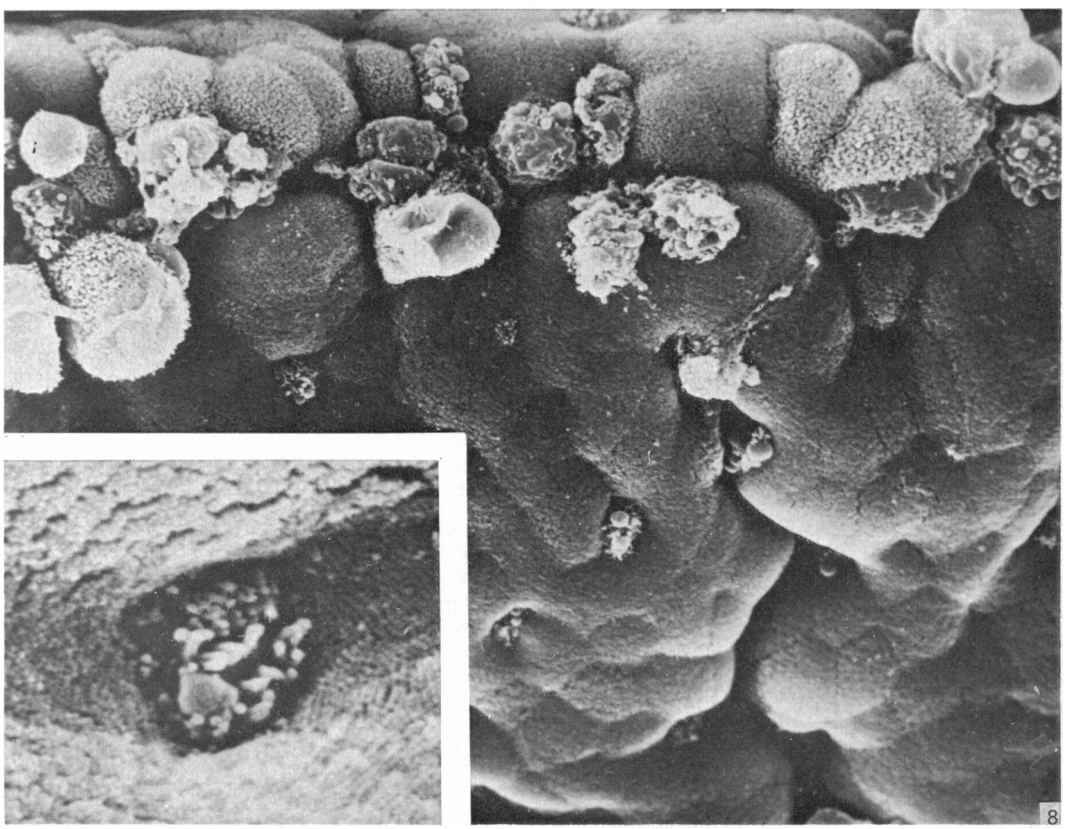
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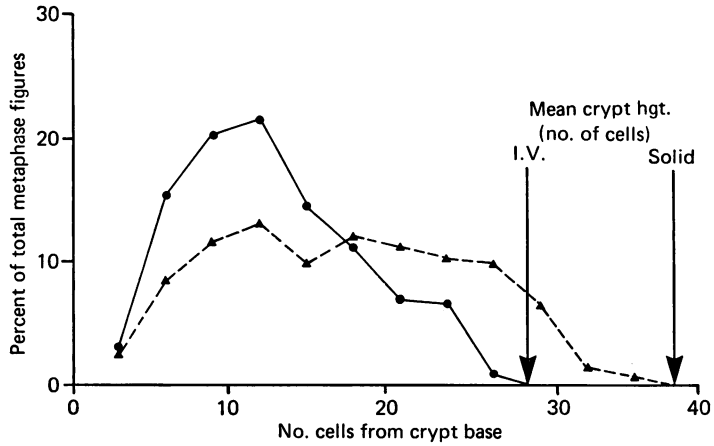

Fig. 9. ●—● Parenterally fed animals. ▲- - -▲ Solid fed animals. Frequency distribution of metaphase figures in ileal crypts from animals in Experiment II. Animals were given colchicine three hours before being killed. The ordinate shows percentage of total metaphase figures and the abscissa shows the number of cells from the crypt base. The mean crypt height (number of cells from the base) is shown to be higher in the solid fed animals ($n = 4$) than in the I.V. fed animals ($n = 6$).

This value is not statistically different from the solid fed rats as determined by Student's t test.

Table 1 shows the responses of the rat ileum to total parenteral feeding observed during this second study. As seen from Table 1, the Student's t test showed significant differences, concerning all parameters analyzed, between parenterally and solid fed animals. Highly significant differences were seen in the number of metaphase figures per crypt and in the number of cells from base to mouth of crypt ($P < 0{\cdot}001$). Total parenteral feeding decreased both the number of metaphase figures per crypt and the number of cells from base to mouth of crypt. The villus height ($0{\cdot}26 \pm 0{\cdot}01$ mm) and the crypt height ($0{\cdot}22 \pm 0{\cdot}002$ mm) in parenterally fed rats were both significantly decreased ($P < 0{\cdot}05$ and $< 0{\cdot}01$, respectively) as compared to solid fed animals.

A further comparison between parenterally fed and orally fed animals can be seen in the frequency distribution of metaphase figures along the crypt side (Fig. 9). This Figure reveals that parenteral feeding decreased the mean crypt height (number of cells) in comparison to the solid feeding. Additionally, metaphase figures were concentrated in the lower half of the crypts in ilea from I.V. fed animals, resulting in a distinct peak in Figure 9. This was not the case in orally fed animals and as shown in the figure the distribution of metaphase figures between the base and top of crypts was platykurtic.

Fig. 7. Exfoliative zone from ileum of rat fed solid food. Some cells have lost microvilli while others possess scattered surface blebs. Cells lacking surface structure appear to be detaching from neighbouring cells (*D*). Arrows indicate sites of recently exfoliated cells. Goblet cell opening (*g*) characterized by circumferential microvilli. ×2000.

Fig. 8. Exfoliative zone of villus from ileum of I.V. fed rat. Certain enterocytes at apex of villus protrude more than surrounding cells and show a thinning of microvilli. Intact secretory granules are seen associated with microvilli of goblet cell openings (see inset). ×2000. Inset ×9700.

DISCUSSION

Effects of total parenteral feeding

In the first experiment the mean body weights of the orally and parenterally fed groups differed by only 3·9 g at the time of killing. This value is not statistically significant and indicates that the parenterally fed group was well nourished throughout the experimental period. Likewise, no significant difference in mean body weight was seen between orally and parenterally fed groups in the second experiment. Cameron *et al.* (1974) have shown that ileal villi are thinner and more closely packed in I.V. versus orally fed rats. It is known that intravenous feeding suppresses upper gastrointestinal tract secretion (Nakajima & Magee, 1970; Hamilton, Davis, Stephenson & Magee, 1971; Towne, Hamilton & Stephenson, 1973) and results in a 50 % reduction of pancreatic acinar cell volume (Pavlat, Rogers & Cameron, 1975). This means that experiments using intravenous feeding must be interpreted cautiously since normal amounts of gastrointestinal secretions are not being synthesized and/or released in the intestine.

That total parenteral nutrition allows one to examine a functional mucosal epithelium has been questioned. Feldman, Dowling, McNaughton & Peters (1976) reported that ilea of I.V. fed dogs failed to adapt functionally following small bowel resection as compared to orally fed animals. This conclusion was based upon an increase in the *in vivo* glucose absorption rate of ilea in orally fed dogs that was not seen in parenterally fed ones; however, the conclusions seem questionable since the absorption rates differed significantly between the two groups prior to the experimental resections.

The data from the second experiment in the present study reveals that total parenteral feeding caused a significant decrease in villus height ($P < 0{\cdot}05$) and crypt height ($P < 0{\cdot}01$). Highly significant decreases in metaphase figures per crypt and number of cells from base to the mouth of the crypt ($P < 0{\cdot}001$) were also seen.

Proliferative activity

The renewal time for ileal enterocytes in orally fed rats falls between two and three days (Altmann & Enesco, 1967; Cameron, 1971). Since the incorporation of ^{3}HT into intestinal DNA is fourfold higher in I.V. fed animals, one might predict that epithelial renewal time is shorter. This is not the case, however, since the number of labelled cells per ileal crypt in I.V. fed animals is only 60 % of that in orally fed rats. Assad & Eastwood (1976) have compared epithelial cell kinetics in rats fed intravenously and in those fed the liquid I.V. diet orally. They found that the oral liquid diet increased epithelial renewal in the ileum compared to the intravenous diet by: (1) increasing the rate of proliferation and possibly by expanding the number of proliferative cells, and (2) accelerating epithelial migration. In the ileum, the total number of cells per crypt was not different between animals fed the oral liquid diet and those fed the intravenous diet. We found that the number of labelled cells and metaphase figures in the proliferative zone of the ileal crypt was significantly higher in animals fed an oral solid diet compared to animals fed an intravenous diet. Additionally, it is well known that small bowel resection leads to hyperplasia in the residual small intestine; however, Levine, Deren & Yezcimir (1976) have shown that hyperplasia does not occur in intravenously alimented resected rats. They concluded that oral intake of food, acting either directly or indirectly upon the intestine, was

required for small intestinal hyperplasia after resection. The decrease we observed in the number of labelled cells per crypt, and the increase in the incorporation of ^{3}HT into DNA (specific activity) in ilea of intravenously alimented rats led us to conclude (1) that there had been a rather large decrease in the number of cells other than cryptal enterocytes in the small intestine, and (2) that the incorporation of ^{3}HT into DNA was increased in I.V. fed animals. Clearly the striking decrease in the surface area (Cameron *et al.* 1974) which was due to a greatly reduced intestinal diameter also indicated a marked decrease in DNA content per cm of small bowel. How, then, was the incorporation of ^{3}HT into DNA increased in I.V. fed rats? If the amount of thymidine in the lumen of the intestine was decreased in I.V. fed rats due to (1) the absence of intraluminal food, and (2) a decrease in the degradation of exfoliated enterocytes (re-utilization of exfoliated enterocyte purines and pyrimidines) it might be anticipated that the concentration of unlabelled thymidine in the lumen was not high enough to compete with systemically delivered ^{3}HT. The nett effect would be increased incorporation of radioactivity per unit of DNA in the I.V. fed animals as compared to the orally fed rats. The quenching effect of high levels of unlabelled thymidine in the intestine has recently been confirmed in our laboratory (Williams, Cameron & Adrian, 1978).

Again it is emphasized that DNA was measured and reported as the amount of DNA per cm of intestine. Expressing the data in this way was necessary because in previous studies we have shown that the weight of the small intestine was highly variable due to experimental treatments, whereas the length of the intestine remained the same (Cameron *et al.* 1974). Some investigators have expressed DNA content per unit tissue wet weight or per unit protein content. Expressing the data in this way can only be done when the tissue wet weight and protein content per cm of ileum have been demonstrated as constant. In the experiments, done in our laboratory, small intestinal length was unchanged by I.V. feeding, whereas the wet weight was significantly changed (Cameron *et al.* 1974).

From our second experiment we conclude that I.V. feeding caused a suppression of cell proliferation in the ileum. This conclusion is based on the fact that the number of colchicine collected metaphase figures per crypt was significantly higher in the orally fed animals ($P < 0{\cdot}001$).

Animals used in the first experiment were 4 weeks younger than those employed in the second experiment. The percentage of cells in DNA synthesis was measured in the younger animals. The number of colchicine collected metaphase figures present in ileal crypts was measured in the second experiment. In both cases I.V. feeding caused a significant decrease in the proliferative index.

Scanning electron microscopy

The radioautographic data suggested that the small intestines from parenterally fed rats were still supporting an epithelial renewal process although at a greatly decreased rate. Scanning electron micrographs of this tissue also led us to believe that the renewal process was proceeding at a slower rate than in rats fed orally. Ito (1965) showed that exfoliating cells in the cat intestine lost their enteric coat. Further, the microvilli on exfoliating cells were reduced to small, scattered blebs. This was reported by others (Potten & Allen, 1977) and was essentially the pattern we saw with scanning electron microscopy in the small intestines of orally fed rats. We did not see this in I.V. fed animals. Several possibilities exist as to why this is so: (1) normal exfoliation requires the presence of intraluminal food or fibre content for

exfoliation, (2) normal exfoliation requires the presence of certain digestion secretions that are suppressed by intravenous feeding, (3) the incidence of exfoliation is reduced because the epithelial turnover time is reduced, and (4) parenteral nutrition maintains a large intracellular store of proteins or is supporting active protein synthesis in enterocytes at the exfoliative zone. Altmann (1975, 1977) has seen premature exfoliation of rat intestinal epithelial cells following a single dose of the protein synthesis inhibitor cyclohexamide.

Additionally, there is evidence that protein synthesis subsides towards the tips of intestinal villi, suggesting that proteins responsible for cell cohesion are no longer produced and exfoliation results (Lipkin & Quastler, 1962; Alpers, 1972; Altmann, 1975; 1977). The use of total parenteral feeding in experiments of the type reported here does not allow one to separate the effects of digestive secretions from those of intraluminal food or fibre on the intestines. The technique does, however, allow one to examine an epithelium, capable of essentially normal amino acid transport (Cameron *et al.* 1974), which is not the case with experiments involving starvation (McManus & Isselbacher, 1970).

SUMMARY

To find out how the ileum adapts to total parenteral feeding, two experiments were performed. In the first experiment rats were given total intravenous feeding for 10 days. The animals were injected with tritiated thymidine (1 μCi/g body weight) 1 hour before being killed. Portions of the ileum were used for (1) radioautography, (2) analysis of the tissue DNA content, (3) specific activity of the DNA, and (4) scanning electron microscopy. The DNA content of ileum was decreased 72 % while the specific activity of DNA was increased 289 % in the I.V. fed rats. In the second experiment rats were given total intravenous feeding for 10 days. The animals were injected with colchicine (1 mg/kg body weight) 3 hours before being killed. The number of labelled cell nuclei per ileal crypt section was significantly decreased by parenteral feeding as was the number of colchicine collected metaphase figures. Light microscopy revealed the crypt and the villus height to be shorter and the number of goblet cells per unit surface area to be increased in parenterally fed rats as compared to those fed solid food orally. Enterocytes of the exfoliative zone from the ileal villi of rats fed solid food showed three distinct types of surface architecture whereas those from I.V. fed rats all possessed abundant microvilli. No bacteria were seen in ilea of I.V. fed animals but many were seen embedded in enterocytes from orally fed rats. Because the amount of DNA per cell is known to be constant, we concluded that the overall number of cells in the ileum decreased about 72 % in the I.V. fed rats and that cell proliferation in the crypts, although significantly decreased, was still supporting an epithelial renewal process.

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