Evidence for both luminal and systemic factors in the control of rat intestinal epithelial replacement

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Summary

1. Isolation of a self-emptying sac of rat small intestine in vivo halved the cell production rate in the crypts of Lieberkühn as measured by colchicine blockade.

2. Starvation for 48 h of rats with isolated intestinal sacs caused a further halving of cell production rate in the sac.

3. Infusion of distilled water into isolated intestinal sacs for 72 h caused a similar degree of stimulation of cell production in sacs in fed and starved rats, suggesting that the fall in cell production rate on starving a rat bearing an isolated sac is not due to the non-availability of the substances required for cell synthesis.

4. The possible contribution of innervation of the gut, and of systemic hormonal influences, on intestinal epithelial replacement is discussed.

Key words: mitosis, mucosa, small intestine.

Introduction

The intestinal epithelium is replaced rapidly and the constant appearance of the healthy mucosa implies an efficient control mechanism which balances the rates of cell production in the crypts and cell shedding from the villi (Creamer, 1967). The ultimate role of the control mechanism is presumably to ensure an adequate number of enterocytes capable of meeting the absorptive requirements of the organism, and many investigators have concentrated on measurements which reflect either absorptive capacity or the number of enterocytes.

The functional importance of the replacement of the epithelium was demonstrated by Redgrave & Simmonds (1967), who showed that aminopterin blockade of mitosis in rats resulted in malabsorption of fat, the timing of which was consistent with the idea that a continuous supply of new enterocytes is necessary for adequate intestinal function. The daily requirement for cells constitutes a considerable load on the metabolic resources of the organism and it is difficult to explain the considerable cell production which occurs, unless it is for the supply of new enterocytes.

Cell turnover could be affected either by events within the lumen of the gut ('luminal'), or by 'systemic' stimuli reaching the mucosa through the intestinal wall. 'Intraluminal nutrition' is clearly a possibility, as has been emphasized by Dowling and his colleagues (Dowling, 1970; Gleeson, Cullen & Dowling, 1972). However, intraluminal nutrition is not an adequate explanation for the changes in jejunal Thiry-Vella loops observed by Elias & Dowling (1974), who suggested hormonal stimulation as a mechanism.

If systemic stimuli do have a role to play in the control of epithelial replacement, the isolated intestinal sac may be a useful test object for their investigation. The experiments to be described in this paper show that starvation, like lactation, has an effect on cell production in an isolated sac and an attempt is made to identify the mechanism responsible.

Materials and methods

Conventionally reared, male albino Wistar rats were obtained from the Nottingham University Joint Animal Breeding Unit, Sutton Bonington, Leics., and kept in the Medical School Animal Unit for at
least 2 weeks before use, on diet 41B (Pilsbury, Birmingham) and tap water ad libitum. At the start of the experiment, they were 10–12 weeks old and weighed between 240 and 260 g. They were caged individually.

One group of ten rats had no operation. Two other groups of rats underwent the surgical isolation of a sac of upper small intestine under sodium pentobarbitone anaesthesia. The sac was about 350 mm long (one-third of the small-intestinal length). In one group of fourteen rats, the sac was closed at its upper end, and its lower end opened on to the skin of the abdominal wall (Clarke, 1974a). In the other group of twenty rats, a similar sac was created but a plastic infusion tube was implanted into its upper end, and brought out through the skin so that fluid could be infused into the sac in the conscious unrestrained rat (Clarke, 1974b). On the seventh post-operative day, distilled water was infused through the tube into the sac at a rate of 4 ml/h for 72 h. On the eighth post-operative day, some animals in each of the three groups were deprived of food for 48 h but continued to have access to tap water. Eleven further rats underwent operation and implantation of the plastic tube but had no infusion and were not starved.

Since preliminary studies had shown no difference between animals 10 and 28 days after the construction of an intestinal sac, on the tenth post-operative day all animals, operated and unoperated, starved and fed, were injected intraperitoneally with a solution of colchicine (Colcemid, Ciba), 2.5 mg/kg body weight, and the small intestine was fixed between 30 and 150 min later (Clarke, 1974a). In the unoperated animals, specimens of small intestine were examined from sites 10%, 30%, 50% and 95% of the length of the small intestine from the pylorus. In the operated animals, specimens were examined from sites 5% of the total length of the small intestine below the upper end of the isolated sac, 5% above the lower end, from the mid-point of the sac, and from the intestine-in-continuity 5% above the ileocaecal valve (95% from the pylorus). This last site reflects the level of proliferative activity in the intestine-in-continuity (Clarke, 1974a). Specimens were treated in bulk by the Feulgen technique (Wimber & Lamerton, 1963), and villus height, crypt depth, crypt/villus ratio (number of crypts per villus) and colchicine-metaphase accumulation rate were measured (Clarke, 1974a).

Differences in villus height, crypt depth and crypt/villus ratio were compared by Student's t-test, and in colchicine-metaphase accumulation rates by the variance ratio (F) test (Snedecor & Cochran, 1967).

**Results**

All rats thrived post-operatively, but one batch of four rats died during the combination of starvation and water infusion. Colchicine blockade was achieved in all surviving animals.

**Starvation of unoperated rats for 48 h (Table 1)**

This resulted, at all four sites, in small changes, usually reductions, in villus height and crypt depth,
Control of intestinal epithelial replacement

**Table 2. Mucosal architecture and colchicine-metaphase accumulation rate in fed and starved rats with isolated intestinal sacs**

Results shown are mean values ± SEM except for the colchicine-metaphase accumulation rate figures (slope ± se). Differences between members of pairs are not significant, except for values marked by asterisks: **P<0.01. n = 7.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Treatment</th>
<th>Isolated sac</th>
<th>Intestine-in-continuity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>33%</td>
<td>45%</td>
</tr>
<tr>
<td>Villus height (µm)</td>
<td>Fed</td>
<td>362±23</td>
<td>330±26</td>
</tr>
<tr>
<td></td>
<td>Starved</td>
<td>322±18</td>
<td>302±23</td>
</tr>
<tr>
<td>Crypt depth (µm)</td>
<td>Fed</td>
<td>168±7</td>
<td>162±7</td>
</tr>
<tr>
<td></td>
<td>Starved</td>
<td>148±8</td>
<td>144±6</td>
</tr>
<tr>
<td>Crypt/villus ratio</td>
<td>Fed</td>
<td>20.2±1.2</td>
<td>18.8±0.8</td>
</tr>
<tr>
<td></td>
<td>Starved</td>
<td>20.5±0.6</td>
<td>19.4±0.8</td>
</tr>
<tr>
<td>Colchicine-metaphase</td>
<td>Fed</td>
<td>13.1±2.0</td>
<td>18±1.9</td>
</tr>
<tr>
<td>accumulation rate (cells/h</td>
<td>Starved</td>
<td>6.0±0.8**</td>
<td>11.4±2.5</td>
</tr>
<tr>
<td>per crypt; slope ± se)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Mucosal architecture and colchicine-metaphase accumulation rate in uninfused and distilled water-infused intestine, fed and starved**

Results shown are mean values ± SEM except for the colchicine-metaphase accumulation rate figures (slope ± se). Differences between members of triplets are not significant, except for values indicated in the footnotes. Numbers (n) shown in the top lines apply throughout.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Treatment</th>
<th>Isolated sac</th>
<th>Intestine-in-continuity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>33%</td>
<td>45%</td>
</tr>
<tr>
<td>Villus height (µm)</td>
<td>Fed, uninfused</td>
<td>405±25</td>
<td>360±23</td>
</tr>
<tr>
<td></td>
<td>(n = 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fed, infused</td>
<td>372±34</td>
<td>318±10</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Starved, infused</td>
<td>335±55</td>
<td>333±18</td>
</tr>
<tr>
<td>Crypt depth (µm)</td>
<td>Fed, uninfused</td>
<td>177±6</td>
<td>174±7</td>
</tr>
<tr>
<td></td>
<td>Fed, infused</td>
<td>214±10(3)</td>
<td>147±6**</td>
</tr>
<tr>
<td></td>
<td>Starved, infused</td>
<td>20-9±0.8</td>
<td>15±5±0.7</td>
</tr>
<tr>
<td>Crypt/villus ratio</td>
<td>Fed, uninfused</td>
<td>20-9±0.1</td>
<td>14.6±0.4</td>
</tr>
<tr>
<td></td>
<td>Fed, infused</td>
<td>19.2±1.0</td>
<td>14±3±0.7</td>
</tr>
<tr>
<td></td>
<td>Starved, infused</td>
<td>13.7±3.0</td>
<td>15±0±2.5</td>
</tr>
<tr>
<td>Colchicine-metaphase</td>
<td>Fed, uninfused</td>
<td>13.7±3.0</td>
<td>15±0±2.5</td>
</tr>
<tr>
<td>accumulation rate (cells/h</td>
<td>Fed, infused</td>
<td>29-7±5.2(1)</td>
<td>12±3±3.5</td>
</tr>
<tr>
<td>per crypt; slope ± se)</td>
<td>Starved, infused</td>
<td>25-0±4.5(1)</td>
<td>11±9±2.2</td>
</tr>
</tbody>
</table>

(1) Infused different from uninfused, P<0.05.
(2) Infused different from uninfused, P<0.01.
(3) Starved different from fed, P<0.05.
(4) Starved different from fed, P<0.01.
(5) Starved infused different from fed infused, P<0.05.
significant at only one site, no consistent or significant change in crypt/villus ratio, but a significant reduction in colchicine-metaphase accumulation rate at three of the four sites examined.

Starvation of animals with an isolated intestinal sac (Table 2)

Isolation of a sac of intestine did not significantly change villus height, crypt depth or crypt/villus ratio in the fed animal, although it caused a reduction in colchicine-metaphase accumulation rate in that sac (15.5 ± 1.4 cells/h per crypt for all three sites combined, compared with 28.6 ± 1.8 for the three proximal sites in the fed unoperated animals; \( P < 0.001 \)). Starvation of such animals caused reduction in villus height and crypt depth (only significant for crypt depth at one site), no change in crypt/villus ratio, and a reduction in colchicine-metaphase accumulation rate, both in the isolated sac (significant at two of the three sites), and in the terminal ileum of the intestine-in-continuity (\( P < 0.005 \)). The rate of colchicine-metaphase accumulation was thus reduced by isolation of a sac of intestine, and further reduced by starvation of the animal.

Infusion of distilled water into the isolated sac (Table 3)

Villus height and crypt/villus ratio showed no significant changes in animals infused with water, compared with sham-infused animals. Crypt depth and colchicine-metaphase accumulation rate were both significantly increased in the upper sac near the site of infusion in both fed and starved animals (which did not differ significantly from each other), but at the lower end of the sac and in the intestine-in-continuity, water-infused animals were not different from the sham-infused control animals. At these sites, however, the differences between fed and starved animals were again visible. Thus the infusion of water into the isolated sac caused a local rise in colchicine-metaphase accumulation rate, of comparable size in fed and in starved animals.

Discussion

The findings may be summarized as follows: (i) isolation of a sac of intestine caused a fall in cell production rate in that sac; (ii) starvation of a rat bearing such a sac caused a further fall in cell production; (iii) the infusion of water into such a sac stimulated cell production locally to a similar degree in fed and starved rats.

The alterations in cell production cannot be ascribed to the effects of intestinal section and suture, since they did not occur in sham-operated animals (Clarke, 1974a), nor to the presence of the infusion tube. The reduced cell production in an isolated sac is due to the exclusion of the sac from contact with intestinal contents (Clarke, 1974a), and might be due to the absence of 'luminal nutrition' (Gleeson et al., 1972). But the further fall in cell production which occurred when the sac-bearing rat was starved cannot easily be explained by 'luminal nutrition', since no overt further change in the lumen takes place. We must therefore look to systemic influences on the gut, of which three spring to mind: bloodborne nutrients, blood-borne hormones and nervous activity.

It could be argued that the availability of bloodborne nutrients is reduced in a starved animal, and that cell production might be reduced by lack of materials needed for cell synthesis. But the infusion of water into the sac caused a local stimulation of cell production (Table 3). The infusion of water has been shown to damage enterocytes (Clarke & Kobayashi, 1975), and enterocyte damage could stimulate cell production in the crypts (Eder, 1969), by analogy with coeliac disease (Padykula, Strauss, Ladman & Gardner, 1961), infestation with Nippostrongylus brasiliensis (Symons, 1965), explantation of the intestine to the skin (Loehry & Grace, 1974) or infusion of lactic acid (Riecken, Bloch, Menge, Idelberger, Kramer, Miller & Lorenz-Meyer, 1972). If this is the case, the findings imply that nutrients are certainly available for increased cell production, and that it must be the demand for cell production which is reduced in the starved isolated sac. Nutrient substances pass from the bloodstream to the intestinal lumen in substantial quantities (Nasset & Ju, 1961; Gent & Creamer, 1972), and it is possible that, in the starved animal, reduced passage of nutrients from the bloodstream to the intestinal lumen could lead to reduced concentrations of nutrient in the lumen, and thus to reduced cell production. This possibility could be tested by biochemical analysis of the intestinal contents of fed and starved isolated sacs.

The products of cell degradation might themselves be a stimulus for cell production, but Tutton (1973a)
was unable to demonstrate that an extract of villus cells had any effect on crypt cell proliferation.

The possible role of systemic hormones was suggested by Elias & Dowling (1974). Glucagon concentrations are raised in fasting (Aguilar-Parada, Eisentraut & Ungar, 1969), but Gleeson, Bloom, Polak, Henry & Dowling (1971) observed mucosal hypertrophy, not atrophy, in a patient with a renal tumour which secreted a glucagon-like substance. Growth-hormone concentrations are also raised in fasting, but with one exception experiments on the effect of growth hormone on the intestinal mucosa have not included the control of food intake. Riecken, Menge, Bloch, Lorenz-Meyer, Warm & Ihloff (1974) demonstrated a reduction in crypt depth in hypophysectomized rats compared with pair-fed control animals; the difference was abolished when the hypophysectomized rats were given thyroid and growth-hormone replacement therapy. Raised plasma cortisol concentrations in starved animals does not seem likely to reduce cell production. Vagotomy is said to accelerate enterocyte migration and cell production (Silen, Peloso & Creamer, 1967). Tutton (1973b) also noted that sympathectomy inhibited cell proliferation. Thus increased sympathetic nervous activity in starvation does not appear to reduce cell production. Tutton & Helme (1973) also showed that beta-adrenergic receptor stimulation inhibited cell proliferation, as did stress (Tutton & Helme, 1974), and it is possible that the demonstrated reduction in cell production in the isolated sacs of starved rats was due to inhibition by circulating adrenaline. If this is true, then adrenalectomy or beta-adrenergic receptor blockade should abolish the reduction in cell production.

This investigation confirms the importance of luminal factors in the regulation of epithelial cell production, as shown by the effects of isolation of a sac of intestine, and of infusion of distilled water. The reduction in cell production which occurs when an animal bearing an isolated sac is starved shows, however, that systemic factors cannot be ignored. The case against blood-borne, tissue-specific stimulants of cell production has been presented elsewhere (Clarke, 1974a); as argued above, several other possible hormonal candidates cannot account for the observations, and circulating adrenaline emerges as a possible systemic effector in the control mechanism. Further work will be needed to pursue this possibility, but it is clear that future experiments to elucidate the control mechanisms of cell production should incorporate the control of factors, both luminal and systemic, which might influence cell production.

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References


