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The mechanism for small-bowel adaptation in lactating rats

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Summary

1. To study the relative contributions of luminal nutrition, bile and pancreatic secretions and hormonal factors in intestinal adaptation, lactation hyperphagia was chosen as a model for increased luminal nutrition, either alone (intestinal transection control group) or in combination with (i) exclusion of bile and pancreatic secretions from the jejunum (by transposition of the jejunum above the Ampulla of Vater) or (ii) exclusion of bile, pancreatic secretions and exogenous luminal nutrition from the jejunum (proximal Thiry-Vella by-pass group).

2. The results confirm that in lactation there is mucosal hyperplasia with increases in villus height and crypt depth, and in small-bowel wet and defatted dry-tissue weights per unit length of intestine.

3. There are corresponding changes in absorptive function with increased glucose and water absorption per unit length of intestine.

4. These structural and functional adaptive changes are proportionately greater in ileum than in jejunum.

5. The exclusion of exogenous luminal nutrition, bile and pancreatic secretions from the jejunum did not diminish the degree of intestinal mucosal hyperplasia and functional adaptation seen in lactation.

6. Diversion to the ileum of greater than normal amounts of bile, pancreatic secretions and luminal nutrition did not further increase the degree of mucosal hyperplasia and enhanced absorption seen in the lactating intestinal transection control group.

7. Unlike other models of intestinal adaptation, the changes in small-bowel mucosal structure and function seen in lactation are probably due to hormonal factors.

Key words: absorption, intestine, lactation.

Introduction

After resection or by-pass of the proximal small bowel in the rat, we (Dowling & Booth, 1967; Gleeson, Cullen & Dowling, 1972) and others (Weser & Hernandez, 1971) have shown that the ileum develops mucosal hyperplasia and enhanced absorption. At least three mechanisms have been proposed for these changes. Previous studies from our laboratory have stressed the importance of luminal nutrition, defined as the presence of nutrients in the intestinal lumen; it does not necessarily mean that the nutrients must be absorbed to exert their effect which, theoretically, could also be mediated by secondary changes in pancreatico-biliary secretions, blood flow, motility or by locally acting hormone (Dowling, 1976). However, Altmann & Leblond (1970) have postulated that trophic factors present in bile and in pancreatic secretions stimulate mucosal growth, and Loran & Carbone (1968), on the basis of cross-circulation studies in parabiotic rats, suggested that hormonal factors might be responsible for the mucosal hyperplasia.

To study the relative importance of these three factors, not only after small-bowel resection, but also in other situations where intestinal adaptation

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occurs, we carried out a series of experiments designed to isolate the effects of the individual trophic stimuli. We combined increased luminal nutrition, arising from the hyperphagia which occurs during lactation and which is known to be associated with mucosal hyperplasia (Fell, Smith & Campbell, 1963), with exclusion from the jejunum of either bile and pancreatic secretions alone (by surgical transposition of the duodenum to a distal site in the small intestine), or of bile, pancreatic secretions and also exogenous luminal nutrition, by creating jejunal Thiry–Vella by-pass loops.

We describe the changes in both jejunal and ileal structure and function which occur after these procedures. From our findings, we suggest that the adaptive changes in the intestinal mucosa of lactating rats probably arise from hormonal factors. Lactation thus differs from many other models of intestinal adaptation where the influence of luminal factors predominates (Dowling, 1976).

This paper was presented in part at meetings of the Medical Research Society (1973) and the European Society for Clinical Investigation (1973).

Materials and methods

Animals and diets

Female albino Wistar rats, initially weighing 140–200 g, were starved for 24 h before operation but were allowed free access to water. For 36 h after surgery they were given glucose in sodium chloride solution (154 mmol/l) by mouth; they then resumed a standard pellet diet (Diet 51B, E. Dixon and Sons, Crane Mead Mills, Ware, Herts., U.K.). After a post-operative interval of not less than 4 weeks, rats in all three operated groups (see below) were mated. In the intestinal transection control group, half the rats were mated to provide a lactating control group; the second half served as non-lactating control animals. Tests of intestinal morphology and function were carried out between 14 and 21 days of lactation and at corresponding times in non-lactating control rats.

Surgical procedures

With the animal under methoxyflurane anaesthesia, the abdomen was opened through a mid-line incision. There were three groups of operated rats and the different surgical techniques are illustrated schematically in Fig. 1.

Intestinal transection group (control rats: six non-lactating; eight lactating animals). The jejunum was transected 2–3 cm distal to the ligament of Treitz and the cut ends were brought into anastomosis, with a continuous 5/0 atraumatic black silk suture.

Proximal two-thirds by-pass group (eight lactating animals). The intestine was transected 2–3 cm distal to the ligament of Treitz and two-thirds of the way down the small intestine. The proximal two-thirds of the small bowel with intact mesentery was left in the peritoneal cavity but the cut ends were brought on to the abdominal surface through stab wounds as two muco-cutaneous stomata. Intestinal continuity was restored by end-to-end anastomoses of the jejunal stump and the remaining ileum.

Duodenal transposition group (seven lactating animals). The intestine was transected immediately distal to the pylorus, 2–3 cm distal to the ligament of Treitz and midway along the small intestine. The proximal half of small intestine (jejunum) was then interposed as an isoperistaltic segment between the pylorus and the first part of the duodenum by two end-to-end anastomoses. The distal end of the duodeno-jejunal stump was then brought into anastomosis with the ileal remnant.

Experimental groups. The rats in each of the three above surgical groups were mated after a post-operative interval of 4 weeks and were studied during lactation. Six rats with intestinal transection were not mated and served as a control non-lactating group.

Fig. 1. Schematic illustration of the various surgical techniques used.
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Intestinal structure

Approximately 2 cm segments of intestine were split longitudinally, pinned flat on cork and fixed in 10% (w/v) formalin–sodium chloride solution (formol saline). These segments were taken from the proximal jejunum, 4–5 cm distal to either the proximal anastomosis or stoma, and from the terminal ileum, 4–5 cm proximal to the ileo-caecal junction. Histological sections (4 μm thick) were cut parallel to the long axis of the intestine, stained with Haematoxylin and Eosin and studied under the light-microscope. A calibrated eyepiece graticule was used to measure villus height (from villus tips to crypt mouths) and crypt depth (from base of villus to base of crypt) on the six to ten tallest well-orientated villi in each section and the mean reading (μm) recorded. In the studies of intestinal absorption (see below) the length of the perfused segment of intestine was measured against a vertical scale (5 g stretch). The segment was then weighed after careful blotting (fresh wet tissue weight), and after thorough drying in absolute ethanol (defatted dry tissue weight).

Intestinal function

As indices of absorptive function, water and glucose absorption were measured from a Krebs-Ringer bicarbonate buffer containing glucose (380 mg/100 ml; 21 mmol/l) and polyethylene glycol (mol. wt. 5000, 300 mg/100 ml) as a non-absorbable marker. A modification (Dowling & Booth, 1967) of a recirculation perfusion system in vivo (Sheff & Smyth, 1955) was used to measure absorption from segments of jejunum and ileum simultaneously in each rat. The concentrations of glucose and polyethylene glycol were measured at 10 min intervals between 20 and 70 min and, at the end of the 70 min perfusion, the length of perfused segment of intestine (approx. 30 cm) was measured as described above.

The results of glucose absorption, corrected for fluid transfer, were expressed as mg of glucose absorbed h⁻¹ cm⁻¹ of intestine and those for net water absorption as ml of water absorbed h⁻¹ cm⁻¹ of intestine.

Results

Intestinal structure

The results of the morphological measurements are given for jejunum and ileum in Tables 1 and 2.

Jejunum. The mean villus height increased from 393 (±SEM 24) μm in the non-lactating control jejunum to between 460 and 509 μm in the different groups of lactating rats. The increase of 17% in villus height in the lactating transected control group did not reach statistical significance, whereas the increases of 26% and 30% in the duodenal transposition and proximal by-pass groups were significant, both being significantly greater than in the non-lactating rats (P < 0.025 and P < 0.05 respectively: Table 1). There was no significant difference

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Villus height (μm)</th>
<th>Crypt depth (μm)</th>
<th>Fresh-wet tissue wt. (mg/cm)</th>
<th>Defatted dry tissue wt. (mg/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lactating transected (controls)</td>
<td>393±24</td>
<td>192±39</td>
<td>65±4</td>
<td>9±1</td>
</tr>
<tr>
<td>Lactating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transected (controls) (n = 8)</td>
<td>460±45</td>
<td>288±24</td>
<td>91±12</td>
<td>15±1</td>
</tr>
<tr>
<td>Duodenal transposition (n = 7)</td>
<td>495±33</td>
<td>300±14</td>
<td>108±9</td>
<td>17±2</td>
</tr>
<tr>
<td>Proximal by-pass (n = 7)</td>
<td>509±48</td>
<td>267±20</td>
<td>68±4</td>
<td>12±1</td>
</tr>
</tbody>
</table>

Results are mean values ± SEM; statistical significance refers to difference between lactating rats and the non-lactating control group. n, Number of animals; t, Student's t-test; N.S., not significant.
in villus height between any of the three lactating sub-groups, but the villus hyperplasia produced in jejunum deprived of pancreatico-biliary secretions (duodenal transposition group) and in jejunum excluded from both exogenous luminal nutrition and duodenal secretions (proximal by-pass group) was, if anything, greater than in the lactating transected control rats. This emphasizes that structural adaptation can still occur during lactation in isolated jejunal segments.

Comparable results were seen for crypt depth, which increased by 50, 56 and 39% in the transected (P < 0.005), duodenal transposition (P < 0.001) and by-pass (P < 0.001) groups respectively.

The fresh-wet and defatted dry intestinal weights also increased in the lactating rats but this composite measurement of tissue mass (which includes both serosa and muscle as well as mucosa) showed less-marked changes than the histological measurements of mucosal thickness. The fresh-wet intestinal weight (mg/cm) increased by 40% in the lactating transected control jejunum and by 68% in the duodenal transposition group (P < 0.0025), but there was no change in the weight of mucosa plus muscle and serosa in the by-passed lactating jejunum. A similar pattern was seen for the defatted dry weights (Table 1).

**Intestinal function**

The results of glucose absorption are shown in Fig. 2 and Fig. 3 and of net water absorption in Table 3.

**Jejunum.** The mean glucose absorption from non-lactating control jejunum of 1.98 (± 0.09) mg h⁻¹ cm⁻¹ increased by 58% in lactating transected control rats, 44% in the duodenal transposition
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Fig. 2. Results of jejunal glucose absorption (mean values± SEM) from perfusion of the intestine in vivo in the different experimental groups (see Fig. 1). The difference between the results for all three lactating groups and the non-lactating control group was highly significant ($P<0.001$). There was no significant difference between the results in the three groups of lactating animals.

![Graph of jejunal glucose absorption](image)

Fig. 3. Results of ileal glucose absorption (see also the legend to Fig. 2).

![Graph of ileal glucose absorption](image)

group and 52% in the by-pass group, all these differences being statistically significant ($P<0.001$; Fig. 2) from control values. The magnitude of the increased absorption was similar whether or not pancreatico-biliary secretions had been excluded from the jejenum (by transposition of the ampulla of Vater), or both duodenal secretions and luminal nutrition had been diverted (by Thiry–Vella by-pass). Once again, the observation that functional adaptation can develop in jejunal loops removed from their normal milieu in lactating rats is worthy of emphasis.

The pattern of results for water absorption was similar to that for glucose absorption (Table 3).

**Jejunum**

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Water absorption (ml h$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td></td>
</tr>
<tr>
<td>Transected (controls)</td>
<td>0.18±0.02*</td>
</tr>
<tr>
<td>Duodenal transposition</td>
<td>0.23±0.05*</td>
</tr>
<tr>
<td>Proximal by-pass</td>
<td>0.17±0.05*</td>
</tr>
</tbody>
</table>

**Ileum**

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Water absorption (ml h$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td></td>
</tr>
<tr>
<td>Transected (controls)</td>
<td>0.21±0.04*</td>
</tr>
<tr>
<td>Duodenal transposition</td>
<td>0.20±0.02*</td>
</tr>
<tr>
<td>Proximal by-pass</td>
<td>0.26±0.02*</td>
</tr>
</tbody>
</table>

Discussion

**Phenomena of intestinal adaptation during lactation**

These experiments confirm that marked morphological changes occur in the rat small intestine during lactation.

**Intestinal weights.** The changes in both jejunal and ileal wet weight and defatted dry weight in the lactating rats were less marked than were the increases in the other markers of gut structure and function. However, wet and defatted dry weights are relatively crude indices of intestinal structure and the present results suggest that during lactation, although except that the magnitude of functional adaptation was much greater in ileum than in jejunum. Glucose absorption increased from a mean value of 0.90 (±0.11) mg h$^{-1}$ cm$^{-1}$ in non-lactating control rats, by 212% in the lactating transected group, by 159% after duodenal transposition and by 170% after jejunal by-pass, these differences all being statistically significant ($P<0.001$; Fig. 3). Thus the increased absorption was, if anything, greater in the lactating control rats than in the bypass and transposition groups where the ileum was exposed to greater than normal amounts of luminal nutrition and duodenal secretions. However, differences in glucose absorption between the three subgroups of lactating rats were again not significant.

**Table 3. Jejunal and ileal water absorption in non-lactating rats and in three groups of lactating rats (see text)**

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lactating transected (controls)</td>
<td>0.04±0.06</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>Lactating transected (controls)</td>
<td>0.18±0.02*</td>
<td>0.21±0.04*</td>
</tr>
<tr>
<td>Duodenal transposition</td>
<td>0.23±0.05*</td>
<td>0.20±0.02*</td>
</tr>
<tr>
<td>Proximal by-pass</td>
<td>0.17±0.05*</td>
<td>0.26±0.02*</td>
</tr>
</tbody>
</table>
mucosal adaptation can develop without luminal stimuli, the overall changes in intestinal weight do seem to require the presence of luminal contents.

**Histological measurements.** The increased villus height and crypt depth found in this investigation confirm previous results. Fell et al. (1963) found a 100% increase in the jejunal and ileal villus heights of lactating rats, and Boyne, Fell & Robb (1966) described an increase in intestinal mucosal surface area. Similar morphological changes in the intestine occur during lactation in sheep (Fell, Campbell & Boyne, 1964) and mice (Campbell & Fell, 1964; Barnett, 1971), but there have been no comparable studies of intestinal adaptation during lactation in humans.

**Absorption in vivo.** In addition to the structural changes, the present results have also shown by direct perfusion techniques in vivo that there are corresponding increases in small-bowel glucose and water absorption during lactation. Previous studies of intestinal absorption in lactating animals have also shown increased absorption. For example, Campbell & Fell (1964), using indirect techniques, concluded that there was an absolute increase in overall nitrogen absorption during lactation hyperplasia in the rat. Similarly, Penzes & Simon (1968) measured DL-methionine absorption in vivo and found increased amino acid absorption in lactating rats, and Kostial, Gruden & Durakovic (1969) showed that the transport of both calcium and strontium increased severalfold in everted duodenal sacs prepared from lactating rats. However, Craft (1970) did not find any increase in overall glucose or glycine absorption from closed (15 cm) loops of rat small intestine during lactation, and when he expressed absorption per unit intestinal weight, he found absorption actually to decrease in the lactating animals.

**Mechanism for intestinal adaptive changes**

These present studies suggest that the hyperphagia of lactation is not a suitable model to study the effects of increased food intake per se on intestinal structure and function. However, the results have shed considerable light on the mechanism for intestinal adaptation in lactation. Previously, the mechanism for the adaptive changes seen in lactating animals had been attributed to the accompanying increase in food intake since the greatest changes occurred in the intestine of animals fed ad libitum, and the adaptive increases in small intestinal weight and nitrogen content could be partially prevented by restricting the dietary intake (Campbell & Fell, 1964). Furthermore, collateral evidence from many other models of intestinal adaptation had shown that increased luminal nutrition was almost invariably accompanied by intestinal mucosal hyperplasia (Dowling & Gleeson, 1973).

The mechanism whereby the chyme exerts its trophic effect on the intestinal mucosa is not yet known. Theoretically, the trophic stimulus could be derived directly from the ingested food, indirectly through stimulation of secretion of bile and pancreatic juice, from the luminal contents releasing locally acting trophic hormones, or by the increased exogenous nutrition increasing splanchnic blood flow, with intestinal hyperplasia as a secondary phenomenon.

Unlike other 'models' of intestinal adaptation, our results suggest that in lactation the local effects of increased nutrition in the intestinal lumen do not seem to be the most important factor in stimulating mucosal growth. Exclusion of bile and pancreatic secretions from the jejunum by duodenal transposition, and exclusion of both exogenous luminal nutrition and duodenal secretions from the by-passed jejunal Thiry-Vella segments did not diminish the mucosal hyperplasia and enhanced absorption seen in the transected lactating control group. By inference, therefore, the mucosal hyperplasia in lactation must be due to some systemic factor. This could conceivably have been mediated by the autonomic nervous system, but seems more likely to have been due to a hormonal stimulus.

If endocrine factors are the cause of the intestinal mucosal hyperplasia in lactation, several possible hormones could be responsible. Prolactin is an obvious candidate, and indeed it has been suggested (Turkington, 1972) that the large increase in liver protein synthesis during lactation (Leake, Mayne & Barry, 1968) is due to the secretion of prolactin. Furthermore, Campbell & Fell (1964) were unable to reproduce the mucosal hyperplasia of lactation when they injected newly parous mice with lactogenic hormone. Moreover, recent studies from our laboratory (T. Bates and R. H. Dowling, unpublished work) have shown that when increased prolactin levels were induced in rats by perphenazine, there were no associated changes in intestinal structure and function.

Several lines of evidence suggest that either pan-
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Lactation. First, in a unique patient with high circulating enteroglucagon levels due to an enteroglucagon-secreting tumour of the kidney, there was marked intestinal enlargement with villus hyperplasia. These structural changes were apparently due to the raised plasma enteroglucagon concentrations since, when the tumour was resected, both the plasma enteroglucagon levels and the intestine promptly returned to normal (Gleeson, Bloom, Polak, Henry & Dowling, 1971). Secondly, in cattle (Manns, 1972) and in rats (Jacobs, Bloom, Polak & Dowling, 1976) the plasma enteroglucagon levels increase markedly during lactation. Thirdly, raised circulating levels of glucagon-like immunoreactivity have been found in other 'models' of intestinal adaptation such as partial starvation (Rudo, Lawrence & Rosenberg, 1973) and in experimental diabetes (Rudo, 1973), both of which cause hyperphagia, and we found increased plasma and ileal tissue levels of enteroglucagon in hypothermic hyperphagia (Jacobs, Bloom, Harsoulias & Dowling, 1975) and after jejunal resection in the rat (Jacobs et al., 1976). Fourthly, chronic glucagon administration increases intestinal transport in the rat (Rudo & Rosenberg, 1973). Finally, the adaptive changes seen in the small intestine of partially starved rats can be blunted by the administration of high titre anti-glucagon antisera (Rudo, 1973).

Whether enteroglucagon, prolactin or some other hormone such as gastrin, which has been implicated recently in intestinal adaptation (Johnson, 1976), is responsible for the adaptive changes seen in lactation remains to be determined.

References


