Prolactin and the small intestine*

Effect of hyperprolactinaemia on mucosal structure in the rat

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SUMMARY To study the mechanism for the adaptive mucosal hyperplasia which occurs independent of luminal nutrition and pancreatico-biliary secretions in isolated Thiry-Vella segments of intestine from lactating rats, and to examine the effects of prolactin on small bowel mucosal structure in the rat, we used two models of experimental hyperprolactinaemia and compared quantitative histology and several markers of mucosal mass in jejunum and ileum from control rats and from test and lactating animals. Hyperprolactinaemia, induced by perphenazine injections (5 mg/kg/day for two or seven weeks) or transplantation of four pituitary glands from donor animals to beneath the renal capsule in the recipient, was confirmed by radioimmunoassay. Proof of its biological activity was obtained by weighing the mammary pads and by demonstrating true breast hyperplasia on histological section. Median serum prolactin levels increased from 50 ng/ml in the controls to 570 ng/ml in the perphenazine treated animals and to 600 ng/ml in the pituitary transplanted rats—levels comparable with those seen in lactation (870 ng/ml). In the lactating rats, there was striking mucosal hyperplasia of both jejunum and ileum but, despite the hyperprolactinaemia, there were no such changes in villus height, crypt depth, or in mucosal wet weight, protein, or DNA/unit length intestine in the perphenazine-injected or pituitary-transplanted animals. We conclude that prolactin is not trophic to the intestine in rats and that hyperprolactinaemia cannot explain the intestinal adaptive changes of lactation.

Recent studies of intestinal adaptation have attempted to identify trophic factors responsible for stimulating small bowel mucosal growth. Such studies are important because of the close relationship between small bowel structure and absorptive function and because, in theory, such trophic factors could play a therapeutic role in the management of patients with malabsorption, secondary to small bowel resection or mucosal damage. Of the factors so far identified, it is known that luminal nutrition, pancreatico-biliary secretions, and hormonal factors can all stimulate mucosal hyperplasia.^{1,2} Of the hormones, candidates for the role of enterotrophin include enteroglucagon,^{3,4} gastrin,⁵ cholecystokinin,

and secretin,⁶ the anterior pituitary hormones⁷ and prolactin.^{8–10}

Further studies on the effect of prolactin on the intestine were of interest for several reasons. First, we showed recently that the marked adaptive mucosal hyperplasia and hyperfunction seen in lactating rats is independent of luminal nutrition and pancreatico-biliary secretions, as the degree of adaptive change was the same in isolated, Thiry-Vella by-passed loops as in intact intestine.⁹ This suggested that neuro-vascular or, more likely, hormonal factors must have reached the excluded intestine to stimulate mucosal growth. Secondly, Bates *et al.*¹¹ showed that, when rats are injected with large doses of prolactin, gut length (from pylorus to anus) and empty gut weight increase when compared with controls, but there was no information about the effect of these injections on the small bowel (independent of the colon) nor about the changes induced in the intestinal mucosa as opposed to in its muscle coats.¹¹ Thirdly, prolactin is trophic to organs other than the gut, including the mammary gland¹² and the liver.¹³

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To study further the effects of prolactin on small bowel mucosal growth, we used two animal models of hyperprolactinaemia—perphenazine injections¹⁴ and transplantation of pituitary glands from donor animals to beneath the renal capsule of recipients.^{15–16} Having confirmed the presence of hyperprolactinaemia, we compared resultant changes in mucosal mass with those found in lactating animals. This paper reports our findings.

Methods

ANIMALS STUDIED

Adult female rats were used throughout. For the 2- and 7-week perphenazine injection rats and their appropriate controls (see below) we used COBD—Wistar virgin rats (Charles River UK Ltd, Margate) with a mean initial body weight of 235 ($\pm$ SEM) 4 g. For the pituitary transplantation experiments, however, to ensure histocompatibility we had to use an inbred Wistar strain of rats (f. AGUS f/Lac; Bantin and Kingman Ltd, Hull, UK), with an initial body weight of 191 ($\pm$ 2.0) g.

The lactating rats were also from the COBD Wistar strain, and, to avoid the possibility of changes in intestinal structure persisting from a previous pregnancy, we mated nulliparous female rats when they weighed 185 $\pm$ 2.0 g. However, because of the marked changes in food intake and body weight known to occur during pregnancy and lactation, the mean final body weight of the lactating rats (295 $\pm$ 6 g) was greater than that in the perphenazine injection and pituitary transplantation groups.

EXPERIMENTAL DESIGN

The study design is summarised in Table 1.

PERPHENAZINE INJECTION STUDIES

The principal study was carried out in six rats treated with perphenazine for 14 days. This time was chosen

because it is known that the mucosal hyperplasia of lactation has fully developed by two weeks.¹⁷ To see if long-term hyperprolactinaemia had differing effects on the small bowel mucosa from those seen after two weeks' treatment, a less complete study was made in another group of six rats treated with perphenazine for 50 days.

Apart from the stress of the daily injections, treatment with this phenothiazine drug induced drowsiness with a resultant reduction in food intake. As this, of itself, could have affected gut structure and function, we included two control groups: (1) a pair-fed group of six rats in which food intake was restricted to match, 24 hours later, the reduced food intake of the perphenazine injection group. These animals also had daily injections but with the solvent alone. (2) Another group of six weight-matched, non-injected rats fed *ad libitum*.

PITUITARY TRANSPLANTATION STUDIES

The seven pituitary transplantation rats were also studied 14 days after transplantation—again with the aim of simulating the serum prolactin response to lactation. With this experimental model, hyperprolactinaemia becomes fully established one week after grafting.¹⁵ Pituitary transplantation rats grow quicker than normal¹⁵ and to exclude the possibility that this is due to hyperphagia (again with secondary effects on the gut), we limited the food intake of these rats to match that of the seven adipose tissue-grafted controls. Again, there were seven normally-fed, weight-matched, non-operated controls.

LACTATION

Each of the six lactating rats had delivered and suckled five to nine offspring until the time of death, 14 days post-partum. This group was included to provide a definitive model of hyperprolactinaemia¹⁸ and intestinal mucosal hyperplasia¹⁷ (for comparison with the two major test groups) and to establish that the radioimmunoassay used in the present studies could, indeed, measure raised serum prolactin levels.

TECHNIQUES

Perphenazine studies

Perphenazine BP (Fentazin, Allen and Hanburys Ltd, London) was supplied in sterile ampoules containing 5 mg perphenazine/ml solvent (see below). Five milligrams/kg/day was given at 9.15 am by subcutaneous injection near the root of the tail. The perphenazine solvent was made up from a formula supplied by the manufacturer (citric acid 1.75 g, sodium hydroxide 0.67 g, and sodium metabisulphite 0.16 g in 80 ml distilled water) and, in the pair-fed, solvent-injected controls, the volume (0.5 ml) and

Table 1 Summary of animal groups studied and experimental design

Group	Experiment		Lactation (Wistar rats)
	Perphenazine injected (Female Wistar rats)	Pituitary transplantation (Female in-bred Wistar rats)	
Test	Perphenazine injected (PI)	Pituitary isografts pair-fed with adipose tissue grafted controls	Lactating rats suckling 5–9 offspring
Controls	1. Solvent injected and pair-fed with PI group	1. Adipose tissue grafted, fed <i>ad libitum</i>	—
	2. Non-injected, weight-matched fed <i>ad libitum</i>	2. Non-operated, weight-matched fed <i>ad libitum</i>	

timing of the solvent injections were the same as those used in the perphenazine injection rats.

Pituitary transplantation studies

Pituitary is transplantation was carried out as described by Welsch *et al.*¹⁶ The pituitaries were harvested from mature female donor rats and there were four transplants per recipient—two beneath each kidney capsule.

The sham operated controls underwent an identical operation except that a slice of subcutaneous adipose tissue from the abdominal wall of donor rats was placed beneath the capsules of both kidneys.

Although the presence of hyperprolactinaemia *per se* (see below) strongly suggested that the pituitary transplants were viable, in two animals we also obtained histological confirmation that the grafted glands had 'taken' satisfactorily. By light microscopy, the transplants were all well vascularised, the endocrine cells appeared healthy, and there was no morphological evidence of either inflammation or rejection.

EVIDENCE OF HYPERPROLACTINAEMIA

1 Timing of blood samples

Although the effect of suckling on rat serum prolactin levels throughout the day is well established¹⁹ the duration of hyperprolactinaemia after perphenazine injections was not known. Therefore, in pilot studies on four groups of animals given perphenazine at 09.00 hours, we measured serum prolactin levels by radioimmunoassay at 10.30 (n=5), 14.30 (n=5), 18.30 (n=6), and at 08.30 the following morning (n=4). The median serum prolactin values at these times were 1320, 640, 530, and 90 ng/ml respectively, thus establishing that hyperprolactinaemia persists for at least nine hours after perphenazine injection.

During lactation, serum prolactin levels fluctuate markedly throughout the day¹⁹ mainly because of intermittent suckling which provides a potent stimulus to prolactin release. Because of this, we standardised the timing of blood samples in the lactating rats as follows. First, to ensure that they were hungry, the pups were separated from their mothers for a four-hour period at 0900 hours. Then they were allowed to suckle for one hour after which the mothers were promptly killed. In the case of the pituitary transplantation rats, it was assumed that the model would provide a constant release of prolactin with no diurnal variations in serum levels. This too was tested in seven rats by measuring serum prolactin levels in blood samples taken at varying times between 0900 and 1700 hours. The results of these studies showed that there were no major changes in serum prolactin levels throughout the day; they ranged from 420–850

ng/ml. There was no significant difference between the mean serum prolactin levels in the various control groups (the normally-fed, weight-matched, and the solvent-injected rats from the perphenazine injection study and the weight-matched and adipose tissue grafted animals from the pituitary transplantation studies). Therefore, the results from all these animals (n=38) were pooled to provide control data for comparison with the two major experimental groups and with the lactating rats.

2 Radioimmunoassay

To avoid the transient, although modest, increases in serum prolactin levels known to occur after stress or after ether anaesthesia,¹⁹ the rats were stunned by a blow on the head, decapitated, and the blood samples collected from the neck vessels. The samples were maintained at 2–4°C over ice and allowed to clot for one hour before centrifugation to obtain serum samples which were then stored at –20°C until analysed for their prolactin content within one month of the animal being killed.

The prolactin concentrations were measured with a rat prolactin radioimmunoassay (RIA) provided by A F Parlow, the Pituitary Hormone Distribution Program, The National Institutes of Arthritis, Metabolism and Digestive Diseases, the National Institutes of Health, Bethesda, Md, USA. The reference preparation was rat prolactin (NIAMD-rat-prolactin-RP-1) and for iodination with ¹³¹I (Radiochemical Centre, Amersham, UK), purified rat prolactin (NIAMD-rat-prolactin 1-2) was used. In our experience, the standard curve obtained by plotting percentage of radioactivity bound against prolactin concentration gave reliable results in the 1.0–50.0 ng prolactin range. To ensure that the unknown samples were within this range, duplicate sera were diluted 1:2, 1:4, 1:8, 1:16, and 1:32 before assay.

3 Evidence that immunoreactive serum prolactin was biologically active

It is well known that hyperprolactinaemia induced by perphenazine injection¹⁴ and by pituitary transplantation¹⁶ causes gynaecomastia in rats. To confirm that the immunoreactive hyperprolactinaemia found in the present study was indeed biologically active, we removed and weighed the right inguinal mammary pad (which contains the three posterior mammary glands with associated adipose tissue), and processed the tissue for light microscopy.

INTESTINAL STRUCTURE

Immediately after the animal was killed, the abdomen was opened and the macroscopic appearance of the small bowel noted. The small intestine was then

removed, stripped of its mesentery and its length measured against a vertical scale using a 5 g stretch.

1 Quantitative histology

Two-centimetre lengths of jejunum, starting 3 cm distal to the ligament of Treitz and of ileum, ending 3 cm proximal to the ileocaecal valve, were removed, split open, pinned flat on cork, fixed in formol saline, and sectioned (5 μ m) parallel to the long axis of the intestine for histological measurements of villus height and crypt depth as previously described.⁹ Care was taken to measure only well-orientated parts of the tissue sections.

2 Mucosal mass

Mucosal wet weight and the protein and DNA content of the mucosa were measured in mucosal scrapes from 10 cm lengths of jejunum and ileum taken immediately distal and proximal to the segments removed for quantitative histology (see above) as previously described.⁹ DNA results were not obtained in the lactating rats or in 7-week perphenazine injection animals.

For the protein and DNA assays, the tared mucosal scrapes were suspended in 0.15 M saline and stored at -20°C for a maximum period of one month. At the time of assay, the samples were thawed, homogenised, and sonicated (3×15 s at an amplitude of 18 microns) in an MSE Mark II ultrasonic disintegrator. Protein was estimated by the Lowry method²⁰ using bovine serum albumin (Sigma Chemical Company, St. Louis, Mo., USA) as standard. DNA was measured in aliquots of the same mucosal broth by the ethidium-bromide method²¹ after inactivation of RNA by bovine ribonuclease (Sigma). In this assay, calf thymus DNA (Koch-Light Laboratories, Colnbrook, Bucks, UK) was used for the standard curve.

STATISTICAL METHODS

To assess the significance of differences between the results for the indices of mucosal mass and for the mammary gland wet weight, the null hypothesis, that any differences between the three subgroups in the perphenazine injection and in the pituitary transplantation experiments reflected only variations in a common parent population, was advanced and tested by a Gaussian one-way analysis of variance for three independent samples.

The non-parametric Wilcoxon rank sum test for two independent samples was used to test for significance of difference in mean serum prolactin levels.

Results

FOOD INTAKE AND BODY WEIGHT

In all major experimental groups, the animals

remained well throughout the study. However, in the first 24 to 48 hours after operation, the adipose tissue-grafted and pituitary transplantation rats ate less than their weight-matched controls. Thereafter, the fat-grafted animals fed *ad libitum* ate the same amount of food as the weight-matched controls while the transplantation animals, which were pair-fed with fat-grafted group, invariably consumed their daily allowance (mean 17 g/day) of food completely. Although both sets of operated rats lost a little weight in the immediate postoperative period, there was no significant difference in the final body weights in the three subgroups from the pituitary transplantation study (207 ± 3 g in the pituitary grafted group; 204 ± 3 g and 205 ± 3 g in the corresponding controls).

As expected, perphenazine injection induced marked drowsiness with an associated reduction in food intake over the first three days of the study. Thereafter, even though the lethargy persisted, the mean food intake increased to 95% of the control value (20 g/day) by two weeks and, by virtue of choosing heavier rats for the perphenazine treatment than for the solvent injections, there was no significant difference between the final body weights in the three subgroups (245 ± 7 g in the treated group; 248 ± 7 g and 247 ± 7 g in the corresponding controls). The final body weights in the seven weeks' perphenazine experiment were 265 ± 7 g in the treated group and 262 ± 7 g in both controls groups. The lactating rats showed marked hyperphagia with a mean food intake of 58 g/day.

EVIDENCE OF HYPERPROLACTINAEMIA

1 Immunoreactive serum prolactin levels

The serum concentrations of immunoreactive prolactin in the controls, the two major experimental groups, and the lactating rats are shown in Fig. 1.

In the 38 controls, the median serum prolactin level was 50 ng/ml (range 8-360) but the results were not normally distributed, as high values in five rats skewed the data upwards (Fig. 1). Despite this, with the exception of one rat in the perphenazine injection group, there was no overlap between the serum prolactin levels in the perphenazine injection (median 670 ng/ml), pituitary transplantation (500 ng/ml), and lactating rats (870 ng/ml), the results in all three groups being significantly greater than in the controls ($P < 0.001$). However, there was no significant difference in serum prolactin levels between the perphenazine injection and the pituitary transplantation rats or between either of these groups and the lactating rats.

2 Evidence for bioactivity of immunoreactive serum prolactin

The results of mammary pad wet weight are shown in Fig. 2.

Fig. 1 Immunoreactive serum prolactin levels in control rats, in the perphenazine-injected and in the pituitary-transplanted models of hyperprolactinaemia and in lactating rats (14th day post-partum).

The immunoreactive hyperprolactinaemia was associated with marked and highly significant increases in the weight of the mammary pad, the mean values in the perphenazine injection (3.53 ± 0.17) and pituitary transplantation rats (3.36 ± 0.13) being 110 and 100% greater respectively than that in the controls, both these differences being significant at the 0.1% level.

Histological sections showed that these increases in mammary pad wet weight were almost exclusively due to hyperplasia of the glandular tissue with development of alveoli and proliferation of ducts. Conversely, most of the pad weight in the virgin controls was accounted for by adipose tissue.

Despite the enlargement of the mammary glands in the two major test groups (apparent even on naked eye examination), the changes were much more marked during lactation where the pad weight of 6.3 ± 0.47 g was again significantly greater ($p < 0.001$) than that in both experimental groups.

INTESTINAL STRUCTURE

1 Macroscopic

When the abdomen was opened at the end of the study, there was marked enlargement of the entire small bowel in the lactating rats but there was no obvious change in the appearance of either jejunum or ileum in the two test groups when compared with their corresponding controls. There was no difference in the length of the small bowel from the two experimental hyperprolactinaemic groups and for their appropriate controls, the mean values ranging from 113–119 (± 4 –5) cm. However, there was an obvious and significant increase in small bowel length in the lactating animals (145 ± 7 cm; $p < 0.001$).

2 Quantitative histology

The results for the histological measurements of villus height and crypt depth in both jejunum and ileum are given in Fig. 3.

Fig. 2 Mammary pad wet weight in the different experimental groups (see legend Fig. 1). The bars represent mean values and closed circles the individual data points.

Fig. 3 Quantitative histological measurements of villus height and crypt depth in the jejunum (upper panel) and ileum (lower panel) of rats in the perphenazine-injected (2 weeks) and pituitary-transplanted groups, together with their corresponding controls, compared with lactating rats (14th day post-partum). The bars represent mean values and the closed circles the individual data points.

Despite the immunoreactive and bioreactive hyperprolactinaemia, there were no significant differences for the results of either villus height or crypt depth between the perphenazine injection rats and their two control groups, the pituitary transplantation rats and their controls or between the two experimental test groups.

A similar pattern of results was seen with 'long-term' hyperprolactinaemia induced by seven weeks' perphenazine injection: there was no significant difference in jejunal or ileal villus height or crypt depth between the test and control groups at this time.

In keeping with the results of many previous studies, there was marked villus hyperplasia in the

lactating rats, the mean villus height in this group being significantly greater than in all the other sub-groups ($P < 0.001$). This was not true for crypt depth, as the mean value in the lactating rats was not significantly different from the other groups with the result that the ratio of mean villus height: crypt depth was 3.3 in the lactating animals compared with values ranging from 2.12 to 2.43 in the other sets of rats.

3 Mucosal mass

The results for the indices of mucosal mass are given in Table 2.

As with the macroscopic findings and the quantitative histology, there was no increase in any of the indices of jejunal or ileal mucosal mass in the rats with

Table 2 Indices of mucosal mass (mucosal wet weight, protein and DNA/cm intestine) in jejunum (upper panel) and ileum (lower panel)

	Perphenazine experiments (2 weeks)			Perphenazine experiments (7 weeks)			Pituitary transplantation experiments			Lactation
	Weight- matched, non- injected controls (n=6)	Pair-fed, solvent- injected controls (n=6)	Perphen- azine injected (PI) (n=6)	Weight- matched, non- injected controls (n=6)	Pair-fed, solvent- injected controls (n=6)	Perphen- azine injected (PI) (n=6)	Weight- matched, non- operated controls (n=7)	Adipose tissue grafted controls (n=7)	Pituitary grafted (PT) (n=7)	(n=6)
Jejunum										
Wet weight (mg/cm)	26.5±2.1	32.6±2.1	30.9±2.1	23.7±1.5	30.1±1.5†	25.4±1.5	23.9±0.6	22.4±0.6	23.3±0.6	53.9±3.8
Protein (mg/cm)	2.61±0.16	3.77±0.16*	3.16±0.16	2.88±0.18	3.29±0.18	2.81±0.18	2.69±0.07	2.47±0.07	2.47±0.07	6.91±0.21
DNA (mg/cm)	0.098± 0.012	0.145± 0.012†	0.098± 0.012	—	—	—	0.127± 0.005§	0.107± 0.005	0.115± 0.005	—
Ileum										
Wet weight (mg/cm)	23.5±1.7	27.3±1.7	25.1±1.7	20.8±1.3	24.9±1.3	24.1±1.3	17.2±0.8	16.6±0.8	19.1±0.8	41.7±3.4
Protein (mg/cm)	2.10±0.19	2.94±0.19‡	2.53±0.19	2.38±0.17	2.64±0.17	2.72±0.17	2.04±0.01	1.78±0.01	1.88±0.10	5.29±0.32
DNA (mg/cm)	0.155± 0.017	0.207± 0.017	0.151± 0.017	—	—	—	0.134± 0.01	0.121± 0.01	0.131± 0.01	—

n: number of animals studied; results are mean values±SEMs.

*Significantly greater than weight-matched, non-injected controls ($p<0.01$).

†Significantly greater than weight-matched, non-injected controls and the PI group ($p<0.05$).

‡Significantly greater than weight-matched, non-injected controls ($p<0.05$).

§Significantly greater than adipose tissue grafted controls ($p<0.05$).

perphenazine injection or pituitary transplantation-induced hyperprolactinaemia when compared with their corresponding controls. There were inconsistent and minor, although significant, differences in some indices of mucosal mass—mainly in the pair-fed, solvent-injected group (Table 2)—but these differences are probably biologically unimportant. There was a similar trend in the control rats injected with solvent for seven weeks, although none of these differences reached statistical significance.

Despite the negative results in the two major test groups, there were again striking increases in the mucosal mass during lactation. The mean jejunal wet weight/cm intestine increased by 203% and protein by 265% when compared with the normally-fed controls used in the two-week perphenazine injection experiments; the corresponding data for the ileum of the lactating rats were 177 and 241%.

Discussion

The results of this paper clearly show that experimental hyperprolactinaemia is *not* associated with changes in jejunal or ileal mucosal structure in the rat. It seems, therefore, that prolactin is not trophic to the small intestine and that hyperprolactinaemia cannot explain the striking adaptive mucosal hyperplasia and hyperfunction seen in isolated segments of jejunum, by-passed from normal continuity as Thiry-Vella fistulae, in lactating rats.⁹ Our studies have confirmed, however, that perphenazine injection and pituitary transplantation are appropriate models for inducing hyperprolactinaemia.

EXPERIMENTAL DESIGN

The conclusion that prolactin is not the enterotrophic hormone of lactation depends on the validity of the models used. For several reasons, we believe that the models were valid.

First, the immunoreactive prolactin levels found in the two experimental models were comparable with those seen during lactation and, secondly, the experimental hyperprolactinaemia seems to have been well maintained throughout the day. We established that the hyperprolactinaemia persisted for at least nine hours after perphenazine injection, while in the pituitary transplantation animals, the circulating prolactin levels were constantly high. Thirdly, we used two completely different models to induce experimental hyperprolactinaemia and neither was associated with changes in gut structure. Finally, we showed that the immunoreactive hyperprolactinaemia was also biologically active—as judged by changes in the wet weight and histological appearance of the mammary gland.

Furthermore, considerable care was taken to include appropriate controls: fat-grafted animals whose food intake provided a standard for the pair-fed pituitary transplantation group, solvent-injected pair-fed rats for the perphenazine injection experiment and weight-matched, non-operated or non-injected *ad libitum*-fed controls. Despite these precautions, the pattern of food intake and the associated changes in luminal nutrition in a sedated rat may well have had different effects on the gut from those produced when a hungry control animal is given a comparable daily quota of food which is consumed over the first few hours.

PREVIOUS STUDIES ON EFFECT OF PROLACTIN IN INTESTINE

There have been few previous reports on the influence of prolactin on the intestinal tract. The only other study on adult rats was by Bates and colleagues¹¹ who noticed an increase in small bowel *plus* colonic weight in animals injected subcutaneously with ovine prolactin for 16 days. Although they found an increase in overall intestinal weight, for the reasons already stated, it is impossible to draw further conclusions from these results. Yeh and Moog²² also studied the rat but, unlike the present study and that reported by Bates *et al.*,¹¹ they used suckling animals and looked at the effect of intraperitoneal ovine prolactin in hypophysectomized animals. They found no effect on small intestinal weight, villus or crypt size, or in the mitotic index in the small bowel mucosa. The only other study of prolactin's effect on the gut was by Campbell and Fell.⁸ They, too, injected ovine prolactin, this time in mice, but found no changes in small bowel weight. However, there was no evidence from their report that the exogenous hormone was indeed biologically active.

If prolactin is not the enterotrophic hormone of lactation, what are the other possibilities? Ignoring the more remote possibility that neurovascular factors could have caused the hyperplasia and hyperfunction in by-passed jejunum from lactating rats, the other hormonal candidates include gastrin⁵ and enteroglucagon.^{3,4} We have already studied intestinal tissue and plasma enteroglucagon levels in three animal models where adaptive mucosal hyperplasia occurs—small bowel resection, hypothermia, and lactation—and in all three situations there were markedly increased enteroglucagon levels. Whether or not enteroglucagon is *responsible* for the adaptive mucosal hyperplasia of lactation, remains unproven.

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