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The Role of Food Intake on Gastric Mucosal Growth and Gastrin Receptors during Pregnancy and Lactation[1] (42603)

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Abstract. We examined the effects of pregnancy and lactation on mucosal growth and the numbers and affinity of gastrin receptors in the oxyntic gland mucosa in rats and compared these with changes in serum gastrin levels and food consumption. Gastric mucosal DNA, RNA, and protein contents were significantly increased during lactation. These changes were not observed in either pregnant or nonlactating rats which had given birth at the same time as the lactating animals. The gastrin-binding capacity of a membrane fraction of the oxyntic mucosa was also increased at the corresponding periods in lactating rats (Days 7, 15, 20). Scatchard plot analysis revealed that the number of gastrin receptors was significantly increased without any change in affinity. Food consumption and levels of serum gastrin remained unaltered in pregnant and nonlactating rats compared to virgin controls, but were significantly increased in lactating rats. Increased serum gastrin levels and gastrin binding capacities in lactating rats (Day 15) were abolished by preventing increased food consumption by means of pair feeding. The results demonstrate that the number of gastrin receptors in the oxyntic mucosa increases during lactation in rats. This increase is probably due to hypergastrinemia caused by increased food intake. The increased number of gastrin receptors may be involved in the mechanism of hypertrophic responses of the gastric mucosa in lactating rats.

Lactation is associated with hyperplasia and hypertrophy of gastrointestinal mucosa in various species including rats (1–5). The weight and nitrogen content of the stomach of lactating rats increase progressively from the time of delivery throughout lactation until the pups are weaned. The numbers of parietal and chief cells in the oxyntic gland mucosa increase dramatically during lactation, correlating with the increased secretory capacity of the stomach (3, 4). Although a number of possibilities such as hyperphagia, gastrin, prolactin, or other hormones have been suggested (1–5) as explanations for these trophic effects, the mechanism(s) which stimulates gut growth during lactation remains unknown.

On the other hand, recent studies have shown that the binding capacity of the gastrin receptor in a membrane fraction of oxyntic gland mucosa is directly dependent on the levels of serum gastrin (6, 7). Since Lichtenberger and Trier (5) reported that serum gastrin levels increased in lactating rats immediately after delivery, it may be assumed that the increased serum gastrin results in changes in gastrin receptor number, and that these combined effects could induce cell proliferation in the gastric mucosa during lactation.

In the present study, we have examined the influences of pregnancy and lactation on mucosal growth and gastrin receptors in the oxyntic gland mucosa, serum gastrin levels, and food intake in female rats.

Materials and Methods. *Animals.* Female Sprague–Dawley rats (180–200 g) were used). A vaginal smear was made in the afternoon, and two rats in proestrus were placed in a cage with one male (300–350 g). The next morning another vaginal smear was made, and the animals in which sperm was found were considered pregnant (Day 0 of pregnancy). Virgin and pregnant rats were housed individually in cages with a solid bottom containing sawdust as bedding material. Purina Chow was provided *ad libitum*, and the amount consumed was measured by weighing the food provided and the spilled food during the experimental period. One day after birth, the pups were redistributed so that there were either 10 suckling rats or no suckling rats per mother.

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General protocols. Under ether anesthesia, groups of eight rats were killed on Days 7 and 15 of gestation and on Days 1, 7, 15, and 21 of lactation. Virgin rats were killed on days corresponding to the dates the pregnant rats gave birth and Day 7 of lactation. All animals were fed *ad libitum* to the time of sacrifice which was at 9:00 AM on each experimental day. Blood was obtained by cardiac puncture, allowed to clot in the cold, and centrifuged. Serum was stored at −20°C until the measurement of gastrin levels. The stomachs were removed and rinsed with cold saline. A portion of antral tissue was dissected out, weighed, homogenized in 5 ml of distilled cold water, and boiled in a water bath for 20 min. The supernatant after centrifugation was stored at −20°C until the determination of gastrin. The oxyntic gland mucosa was scraped off separately from the two halves of the stomach opened along the greater curvature. One half was weighed and its contents of DNA, RNA, and protein were determined. The other half was pooled with those from two to three rats from the same group and processed for the measurement of gastrin binding as described previously (8).

Determination of DNA, RNA, and protein content. Extraction and determination of nucleic acids and protein were performed according to a previous paper (9). Briefly, the scraped mucosa was incubated for 90 min at 37°C with 0.3 *N* KOH for extraction of RNA. DNA was extracted with perchloric acid (60%) for another 20 min at 70°C, and protein was solubilized by adding 1 ml of 1 *M* NaOH. The concentration of RNA was measured with the orcinol reaction (10), DNA with the diphenylamine method (11), and protein according to Lowry *et al.* (12). Results were expressed as micrograms per 100 mg of wet tissue for RNA and DNA and as milligrams per 100 mg of wet tissue for protein.

Determination of gastrin. The concentrations of serum and antral gastrin were measured by radioimmunoassay as described by Yalow and Berson (13) and modified by Dockray and Walsh (14). Antibody 1296 was used and full details of its cross-reactivity and sensitivity have been published (14). Results were expressed as picograms per milliliters of serum of micrograms per grams of antral tissue.

Gastrin binding. Rat oxyntic gland mucosal membranes were prepared and gastrin binding was measured according to methods described in a previous study (8). Briefly, the mucosal scrapings from two to three rats were combined and washed in a cold bicarbonate buffer. After centrifugation, the pellet was homogenized in sucrose buffer in a Kontes glass homogenizer, and centrifuged at 270*g* for 10 min. The supernatant was recentrifuged at 30,000*g* for 60 min. The final pellet was resuspended in 10 m*M* Tris buffer to a protein concentration of 7.5–10 mg/ml as measured with the Bradford assay (15). Synthetic 15-Leu gastrin-17-I was obtained from Research Plus (Denville, NJ) and iodinated using the chloramine-T procedure. The specific activity of the monoiodinated gastrin collected in peak II was 2000 cpm/fmole (8). This peak was used for all assays. Labeled gastrin was added in final concentrations from 25 to 700 p*M*. The membrane sample (20 μl) was incubated for 30 min at 30°C with different concentrations of ^{125}I-gastrin-17 in a total volume of 80 μl. At each concentration of labeled gastrin nonspecific binding was measured in the presence of a 1000-fold excess of unlabeled gastrin. Separation of bound and free gastrin was accomplished by rapid vacuum filtration through a Gelman GA-6 filter at 0°C. The filters were dried and counted in a Searle analytic gamma counter having an efficiency of 80%. Specific binding was obtained by subtracting nonspecific binding from total binding. Each assay was done in duplicate, and the binding capacity was expressed as femtomoles per milligrams of protein.

Statistics. All data are presented as means ± SE from eight rats, unless otherwise specified. Means were compared using the unpaired Student *t* test, and values of $P < 0.05$ were regarded as significant. The binding data were analyzed according to the method of Scatchard (16).

Results. *DNA, RNA, and protein content during pregnancy and lactation.* The amounts of both DNA and RNA in the oxyntic gland mucosa were significantly increased from a week after delivery and remained elevated during lactation (Fig. 1). Protein content was also significantly increased in the corresponding periods, although the ratio of DNA/protein or RNA/DNA remained similar throughout

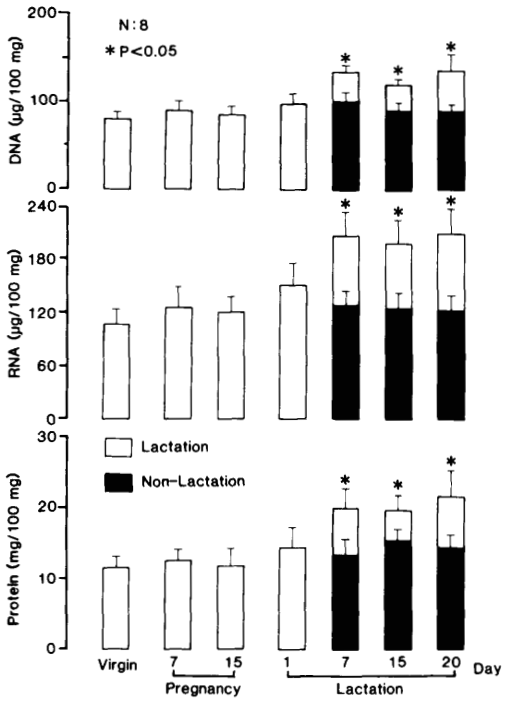

FIG. 1. Changes in DNA, RNA, and protein content in oxyntic gland mucosa of female rats during pregnancy and lactation. Data are presented as means ± SE from eight rats. *Statistically significant from virgin rats, at $P < 0.05$.

pregnancy and lactation (not shown). Oxyntic gland mucosal DNA, RNA, and protein contents in postpartum nonlactating rats were not significantly different from those of nonpregnant rats. They were significantly lower compared to the lactating rats.

Serum and antral gastrin levels. Both serum and antral gastrin concentrations increased during lactation (Days 7, 15, 20), whereas gastrin levels in postpartum nonlactating rats were much the same as seen in nonpregnant rats (Fig. 2). Pregnancy did not appreciably influence the levels of serum or antral gastrin compared to nonpregnant control rats.

Gastrin receptors. The number of gastrin receptors in the oxyntic gland mucosa did not significantly change during pregnancy but increased significantly by Day 7 in lactating animals and continued significantly higher on Days 15 and 20 (Fig. 3). As in the cases of nucleic acid content and gastrin levels, the number of gastrin receptors in postpartum nonlactating rats was not significantly different from that in virgin (nonpregnant) rats, and was significantly lower when compared to lactating rats (Fig. 4). Scatchard plot analysis of the binding data revealed no change in the affinity of the receptor for gastrin during pregnancy and lactation. The mean K_d value for all groups of rats was $2.5–3.2 \times 10^{-10}$ M.

Food consumption. Food consumption did not increase significantly during pregnancy (Table I). However, food intake increased significantly on Days 7, 15, and 20 of the lactation period. In nonlactating rats, no increases were observed at any stage of the corresponding period.

Effects of pair feeding on gastrin levels and gastrin receptors during lactation. During lactation a significant increase in food intake was observed concomitantly with the increases in gastrin receptors and serum gastrin levels. To investigate whether the increased food consumption might account for the above changes, serum gastrin levels and the number

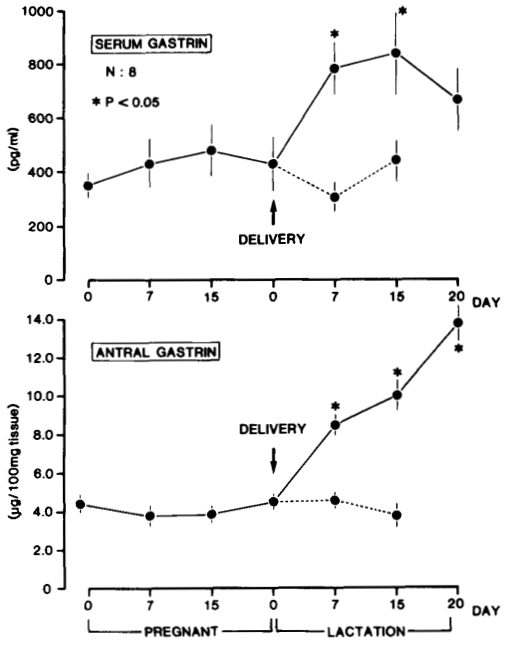

FIG. 2. Serum and antral gastrin levels in female rats during pregnancy and lactation. Data are presented as means ± SE from eight rats. Solid and dashed lines after delivery indicate the values observed in lactating and nonlactating animals, respectively. *Statistically significant from virgin rats, at $P < 0.05$.

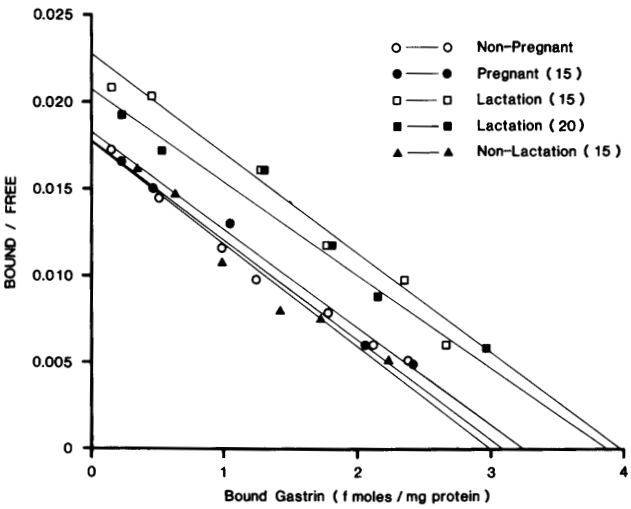

FIG. 3. Scatchard analysis of gastrin specific binding to oxyntic gland mucosa in female rats during pregnancy and lactation. Each line represents data obtained in a pooled sample from three rats. Numbers in parentheses refer to number of days animals had been pregnant, lactating, or, in the case of nonlactating rats, number of days following birth of pups.

of gastrin receptors were examined in pair-fed, lactating animals. As shown in Fig. 5, no significant difference was found in serum gastrin levels between control and pair-fed lactating rats. The binding capacity of gastrin to the oxyntic mucosal membrane preparation obtained from pair-fed lactating rats was 1.7 ± 0.1 fmol/mg protein, which was not significantly different from that of control (1.6 ± 0.2 fmol/mg protein).

Discussion. Hyperplasia and hypertrophy of gastrointestinal mucosa during pregnancy and

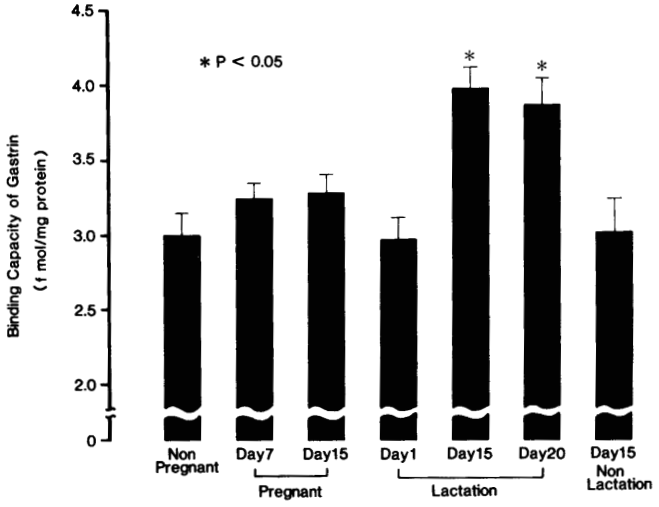

FIG. 4. Changes in gastrin-binding capacity in oxyntic gland mucosa of female rats during pregnancy and lactation. Data are presented as means ± SE from three Scatchard plot analyses. *Statistically significant from virgin rats, at $P < 0.05$.

TABLE I. CHANGES IN FOOD INTAKE IN FEMALE RATS DURING PREGNANCY AND LACTATION

Groups	Day	No. of rats	Amount of food consumed (g/day/rat)	Changes (%)
Virgin	—	16	22 ± 3	—
Pregnancy	7	16	24 ± 4	9.1
	15	16	31 ± 5	40.9
	1	8	30 ± 7	36.4
Lactation	7	8	47 ± 7*	113.6
	15	8	54 ± 8*	145.5
	20	8	54 ± 9*	145.5
Nonlactation	7	8	22 ± 3	0
	15	8	21 ± 2	−4.5

Note. Data are presented as means ± SE.
* Statistically significant from virgin rats, at $P < 0.05$.

lactation have been documented by a number of investigators (1–5). Crean and Rumsey found hyperplasia of the gastric mucosa during the late stages of pregnancy and the duration of lactation. The present study is consistent with previous work (4, 5) showing that both mucosal protein and nucleic acid contents significantly increase during lactation but not during pregnancy. The increases in gastric mucosal size observed in our study were due primarily to increased cell number, since the protein/DNA and RNA/DNA ratios were not changed significantly.

As shown in the present study, lactating rats are hyperphagic. Actually, hyperphagia is the mechanism most commonly invoked to explain the mucosal growth which occurs during lactation (2, 4, 5). Furthermore, due to the well-known relationship between food intake and gastrin levels (17), several investigators have examined the possibility that gastrin mediates the growth changes in lactating rats. Campbell and Fell (2) restricted the diets of lactating rats to those of virgin rats and found no increases in mucosal growth. Harding and Cairnie (18) found mucosal hyperplasia of the intestine of lactating rats placed on restricted diets, although the changes were not equal to those in animals fed *ad libitum*. Lichtenberger and Trier (5) demonstrated that duodenal

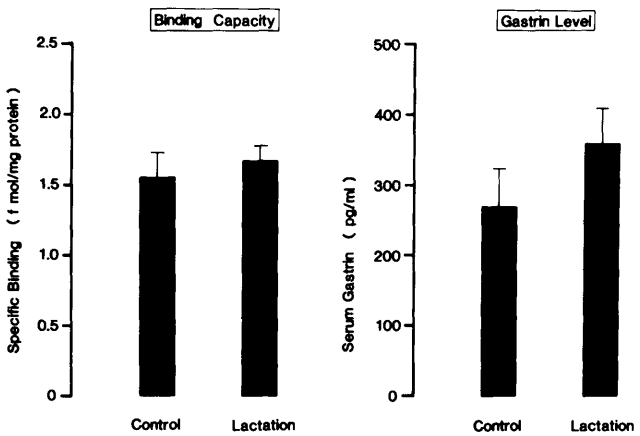

FIG. 5. Effects of pair feeding on binding capacity of gastrin and serum gastrin levels in female rats during lactation. Each bar represents the mean ± one SE from three different Scatchard analyses of gastrin-binding studies or from eight different determinations of gastrin levels.

mucosal growth during lactation was more highly correlated with food intake than with serum gastrin levels. They further examined the relationship between gastrin levels and mucosal growth and found that hyperplasia of duodenal mucosa in lactating rats occurred to the same extent in antrectomized animals as it did in sham-operated lactating controls (5). This study meant that the trophic effect of gastrin (9) could not account for the hyperplasia of the duodenal mucosa during lactation. They concluded that the increased duodenal mucosal growth might be due to direct effects of increased food in the gut.

In the current study increased oxyntic gland mucosal growth was correlated with both increased serum gastrin and increased gastrin receptors in lactating rats. Up-regulation of receptors is a characteristic of the members of the gastrin family of peptides. Both gastrin (6, 7) and cholecystokinin (19) receptors have been shown to increase in number as they are exposed to increased levels of both endogenous and exogenous hormone. As was the case with the increase in mucosal growth, the gastrin-binding capacity did not increase in postpartum nonlactating or pregnant rats. These results suggest that estrogen and progesterone are not responsible for the changes seen during lactation. We have shown, however, that both ovarian and adrenal estrogens are important in determining the level of serum gastrin and gastrin receptors in female rats (20). Normal female rats have about 75% of the number of gastrin receptors present in males. This difference is abolished by ovariectomy of the females but not altered by castration of the males. Pair feeding of ovariectomized rats reduces gastrin-binding capacity, but not to the level of normal intact females. If the pair-fed, ovariectomized rats are also adrenalectomized, gastrin receptors decrease to normal female levels (20). Therefore, the differences in gastrin receptor and serum gastrin levels between normal male and female rats are due to inhibition by estrogens in the females and the increased food consumption of the males.

In the current study pair-feeding totally abolished the differences in both serum gastrin and gastrin-binding capacities seen in lactating and nonlactating rats. This finding emphasizes the role of food intake in triggering these changes during lactation. Although we did not measure prolactin levels, it appears that prolactin did not play a significant role in increasing gastrin levels and receptor numbers, since the pair-fed lactating animals received a suckling stimulus similar to that of the *ad libitum* fed lactating rats. Lichtenberger and Bartke (21) have shown that prolactin was not responsible for the intestinal hyperplasia found in Snell dwarf mice, which have a hereditary deficiency of prolactin, growth hormone, and TSH.

Lilija and Svensson (22) and Takeuchi and Okabe (4) reported significant increases in gastric acid secretion during pregnancy and lactation in rats. The acid hypersecretion during pregnancy was associated with increased vagal stimulation and histamine levels. During lactation, hypersecretion was associated with vagal stimulation, hypergastrinemia, and hyperplasia of the gastric mucosa. The observation that the weight and DNA content of the rat pancreas increase significantly during lactation and then decrease after weaning (2, 3) implies a hormonal or neural mechanism, since the pancreas is not exposed to luminal contents.

These findings plus those of the current study indicate that the acid secretory and trophic effects of gastrin might account for some of the changes observed in the gastric mucosa during lactation. These physiological actions of gastrin would be magnified by both higher than normal serum levels of the hormone and increased numbers of gastrin receptors.

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