particular reference to the formation of the exocrine pancreas. Pregnant rats were injected from day 14 through day 20 of pregnancy, and sacrificed on day 21 of the 22-day gestation period. Controls were injected with equivalent volumes of saline. Ethionine caused significant decreases in litter size and fetal body weight, and had a selectively disruptive effect on the formation of the fetal pancreas as indicated by measures of pancreatic relative weight and histological examination of fetal pancreatic tissue.

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Effect of Calcium Infusion on Gastric Function in Rats (32890)

K. KOWALEWSKI

Surgical-Medical Research Institute, University of Alberta, Edmonton, Alberta

A number of clinical observations and experimental data seem to indicate the existence of a relationship between parathyroid glands, vitamin D, calcium, and gastric function. Whether morphological and secretory changes of glandular mucosa of stomach represent a direct effect of parathormone or vitamin D, or are due to hypercalcemia, is unknown (1, 2). Many conflicting observations have been reported in man and in animals treated with parathormone, vitamin D, and calcium. The results of these studies appear to depend on the species studied, on the state of gastric innervation, on the rapidity of blood calcium changes, on the methods of stimulation gastric function and on the duration of the experiment (2, 3).

The present short report is concerned with the effect of prolonged infusion of calcium on gastric function in rats bearing permanent gastric fistula.

Gastric secretion was collected in these animals for 24 hours during the infusion. At the end of the experiment, blood and gastric tissue pepsinogen was also determined.

Materials and Methods. Under ether anesthesia, a gastric fistula was produced in adult male Sprague-Dawley rats and a miniature stainless steel cannula of Pavlov's type was inserted (4). Collection experiments started after a postoperative period of 10 days. Prior to infusion studies, rats were conditioned to the procedure by keeping them in Bollman restraining cages (5) for periods of several hours. Rats were fasted for 24 hours prior to the infusion study but water was allowed as desired. On the morning of the experiment, each animal was placed in a Bollman cage and the stopper screw of the cannula was removed. After the initial drainage for 30 min the infusion and collection began. Normal saline or calcium gluconate, 300 mg/100 ml in saline were infused into a femoral vein at the rate of 100 ml/kg of body wt. per 24 hours. Gastric content was collected during the infusion for 24 hours (6,7). Very uniform infusion was obtained using the "Infusion Withdrawal Pump" (Harvard Apparatus Co., U.S.A.). Volume, pH, free and total HCl, and pepsin (8) were determined in the 24-hour gastric secretion.

Urine was collected directly into a beaker placed under the lower abdomen of suspended animals. This is a simple procedure in males. Calcium was determined in the urine (9). Blood was sampled at 0, 1, 3, and 24 hours for calcium (9), at 0 and 24 hours for hematocrit and at 24 hours for pepsinogen (10,11). Blood was taken from the femoral vein at 0, 1, and 3 hours and from the inferior vena cava at the end of the experiment (24-hour samples).

Animals were sacrificed at the termination of 24-hours' infusion. The stomachs were removed, opened along the greater curvature, carefully washed under a stream of running distilled water and homogenized individually in 100 ml of 0.1 *N* HCl in a Waring blender for 10 min. The blender was then washed with an extra 50 ml of the acid, the total suspension was filtered and aliquots were taken for tissue pepsinogen study (8, 11).

Peptic activity was expressed in terms of the amount of tyrosine released by the proteolytic action of a volume of gastric juice, plasma, or homogenized gastric tissue. Free and total HCl were expressed in milliequivalents per liter and per total gastric juice collected in 24 hours. The Wilcoxon Rank Sum Test was used for the statistical analysis of the data (12). Five stomachs of each group were used only for histological study of gastric mucosa. Sections were stained with hematoxylin, eosin, and Masson's stain.

Results. The results of measurements performed in infused rats are summarized in Table I.

TABLE I. Effect of 24-hour Intravenous Infusion of Normal Saline or Calcium Gluconate in Saline on Gastric Function of Male Rats.

Parameters studied	Infusion		*p* Value
	Normal saline	Calcium gluconate	
No. of rats	14	14	
Initial body wt. of rats (gm)	308	316	NS[a]
Loss in body wt. during infusion (%)	7	6	NS
Gastric juice			
Volume (ml/24 hours)	18.3	13.1	NS
pH	2.0	2.2	NS
Free HCl (meq/liter)	60	37	<0.01
Free HCl (meq/24 hours)	1.17	0.51	<0.01
Total HCl (meq/liter)	76	54	<0.01
Total HCl (meq/24 hours)	1.43	0.81	<0.01
Pepsin (mg/ml)	30	24	NS
Pepsin (mg/24 hours)	538	397	NS
Gastric tissue, wt. (gm)	1.41	1.53	NS
Pepsinogen (mg total)	141.3	43.2	<0.001
Blood			
Hematocrit (%) 0 time[b]	50	54	NS
24 hours	69	67	NS
Calcium (mg/100 ml) 0 time	10.2	10.0	NS
1 hour[c]	9.2	13.4	<0.01
3 hours[c]	9.1	15.3	<0.01
24 hours	9.2	14.9	<0.01
Pepsinogen, (μg/ml) 24 hours	121	146	<0.05
Urine (ml/24 hours)	6.5	13.2	<0.01
Calcium (mg/24 hours)	6.8	12.9	<0.01

[a] NS = >0.05.

[b] Time of sampling in relation to infusion period.

[c] Determinations done in eight rats of each group.

Calcium. Hypercalcemia was found in all calcium infused rats and was detected in the early part of the infusion period. It was associated with marked diuresis and increased output of urinary calcium. Fluid balance was not studied, but hematocrit was significantly increased at the end of infusion in both groups of animals.

Gastric juice. A significant reduction in both concentration and content of free and total HCl was found in gastric juice collected for 24 hours in calcium-infused rats, as compared with controls infused with saline. No significant difference was observed between the two groups regarding gastric juice pepsin.

Pepsinogen. Blood pepsinogen was elevated and gastric tissue pepsinogen was reduced in rats infused with calcium, as compared with controls.

Histology. Grossly, the gastric mucosa of both experimental groups appeared normal. Most of the chief cells in gastric glands of calcium-infused animals demonstrated a decrease of paranuclear zymogen granules. Nuclei were generally smaller in these cells than in the chief cells of normal rats. No difference in thickness of glandular mucosa or in parietal cells was seen in either group. No quantitative evaluation of these findings was done. Histological study suggested only a reduction of zymogenic granules in gastric mucosa of calcium-infused rats.

Discussion. The finding of reduced secretion of acid in rats infused with calcium suggests that the parietal cells were not adequately stimulated, or were inhibited in the presence of hypercalcemia. There was a trend to reduced pepsin secretion in hypercalcemic rats, but this was not a significant reduction. An interesting finding is the highly significant reduction of gastric tissue pepsinogen associated with a rise, above control levels, of blood pepsinogen in rats infused with calcium.

It is known that the study of gastric tissue pepsinogen may assist in the evaluation of zymogenic cells' function (11, 13). The data on tissue pepsinogen in the present study seem to suggest that the stores of this enzyme in the gastric wall were reduced after 24-hours' infusion of calcium. Histological data also seem to suggest such a conclusion. Zymogenic cells are known to supply pepsinogen to both blood (endocrine function) and gastric juice (exocrine function). A depletion of gastric tissue pepsinogen found in hypercalcemic rats may be related to the increased output of this enzyme into circulation. However, changes of tissue pepsinogen found in this study were not associated with significant changes in gastric juice pepsin (exocrine function).

In rabbits and rats, treated by others with massive doses of vitamin D or parathormone for a few days, zymogenic cell hyperplasia of gastric mucosa was produced (1) and was considered due to hypercalcemia. However, this study (1) cannot be compared with our work because the methods of production of hypercalcemia were quite different and it was not excluded experimentally (1) that hormone and vitamin do not directly affect gastric mucosa. It was noted previously (2) that it is difficult to distinguish between the separate influences of an endocrine gland, a vitamin, and calcium on gastric function. Acute experimental hypercalcemia produced by calcium infusion was found to stimulate acid and pepsin secretion in man and to reduce these components of juice in some species of animals. However, experimental hypocalcemia also produced gastric hyposecretion in some species of animals (2). The problem therefore appears complex. Under the experimental conditions of the present study, hypercalcemia was associated with inhibition of gastric acid secretion, rise of blood pepsinogen and reduction of gastric tissue pepsinogen. No attempt was made to elucidate the mechanism of the action of hypercalcemia on gastric function. Additional information regarding this mechanism might be obtained by repeating the calcium infusion experiment in the parathyroidectomized rats. Such an experiment will be attempted in the near future in our laboratory.

Summary. Rats having permanent gastric fistulas were infused intravenously for 24 hours with calcium in saline, or with saline alone. Gastric secretion was collected for the period of infusion. In calcium-treated rats significant reduction of gastric acid secretion was found. In these animals blood pepsinogen was increased but gastric tissue pepsinogen was reduced. Reduction of zymogenic granules of chief cells was also found in

glandular mucosa of calcium-infused rats. Hypercalcemia appeared to alter the function of both parietal and chief cells of rat stomach under the conditions of this experiment.

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Essential Fatty Acids, Plasma Protein Bound Iodine, and the Thyroid Gland* (32891)

DAVID GAMBAL AND F. W. QUACKENBUSH (Introduced by E. L. Rongone)
Department of Biochemistry, Creighton University, Omaha, Nebraska, 68131, and Department of Biochemistry, Purdue University, Lafayette, Indiana 47907

One of the earliest signs of an essential fatty acid (EFA) deficiency in the rat is an increased basal metabolic rate (1,2). Although the weight of the thyroid gland in rats is significantly decreased by an EFA-deficiency (3,4,5), histological studies suggest the activity of the thyroid gland is not grossly abnormal and could not account for the large increase in oxygen consumption. The purpose of this investigation was to determine the effects upon the plasma protein bound iodine (PBI) and size of the thyroid gland when the essential fatty acids were restored in the diet of rats which had been fed an EFA-free diet.

Materials and Methods. The basal semi-purified diet containing 3% of hydrogenated coconut oil (6) was fed *ad libitum* to 36 female weanling rats (40–50 gm) of the Wistar strain for 12 weeks. In addition, a positive control group was fed the basal diet containing soybean oil in place of hydrogenated coconut oil. The 36 rats were placed in individual cages and divided into four groups which received the following diets *ad libitum* for 3 weeks: basal; positive control diet; stock colony diet; and basal plus 0.1% thiouracil (Table I). After this period, the rats were weighed and anesthetized. Blood samples were then obtained by heart puncture and the thyroid glands were removed, blotted, and weighed on a torsion balance. Plasma PBI was determined by the procedure described by Highley *et al.* (7).

Results. The body weight and the weight of the thyroid gland of EFA-deficient female rats were less than those of positive controls, and the plasma PBI concentration was higher (Table I). Body weights and plasma PBI concentrations were reestablished at the levels of the positive control group by refeeding soybean oil or the colony stock diet for 3 weeks. The weight of the thyroid gland was increased by feeding the colony diet or soybean oil, however, only the colony diet returned the weight of the gland to normal within 3 weeks.

Feeding thiouracil for 3 weeks did not af-

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