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Increased Sensitivity of Heidenhain Pouches to Exogenous Gastrin After Removal of Main Stomach.* (28207)

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Preliminary tests showed that, when exogenous gastrin is given parenterally to dogs with Heidenhain pouches, gastric secretion does not increase consistently in proportion to the dose of gastrin. When any gastric secretagogue is administered to a dog with a gastric pouch, both the main stomach and the pouch are stimulated. Acid secreted by the main stomach bathes the antrum and enters the duodenum at irregular intervals. It is possible that the responses of Heidenhain pouches to exogenous gastrin are inhibited by effects of acid in the antrum and duodenum. The present study, in which the gastric secretory responses of Heidenhain pouches to exogenous gastrin before and after total gastrectomy are compared, was performed to test this hypothesis and to seek a sensitive preparation for detection of gastrin activity in extracts of tissue.

Materials and methods. Vagally denervated (Heidenhain) gastric pouches were prepared in 4 adult mongrel dogs and studies of gastric secretion were begun 3 weeks postoperatively. The dogs were fasted for 16 hours preceding each test.

Preparation of gastrin. Gastrin was prepared by extraction of hog antral mucosa by use of stage I, with minor modifications, of a method described by Gregory and Tracy(1). Mucosa was dissected from fresh antra and divided into batches of approximately 750 g. Each batch was boiled 5 minutes in water (1 ml/g), homogenized in a large blendor (Waring), and frozen. At a convenient time later, the mixture was thawed, diluted with water (4 ml per gram of mucosa), boiled for 20 minutes, cooled, and strained through nylon chiffon gauze. The cake was discarded; the filtrate was acidified to pH 4.0 with acetic acid and the mixture was kept overnight in a refrigerator. The supernatant fluid was aspirated and discarded, and the remaining suspension was centrifuged. The precipitate was stirred mechanically for 2 hours with 4 volumes of 75% acetone containing trichloroacetic acid (TCA) in concentration of 4 g/100 ml, then the mixture was filtered through coarse filter paper coated with Hyflo.

The filtrate was extracted with ether to remove acetone and TCA, the pH was adjusted to 3.0, and residual ether was removed by evaporation in a stream of air. The mixture was then heated twice to 70°C, first at pH 5.5 and then at pH 8.5. A precipitate was separated by centrifugation and discarded. The supernatant fluid was cooled to 10°C and enough cold TCA solution (100 g/100 ml) was added to make the final concentration of TCA 4%. After standing in a refrigerator overnight, the mixture was centrifuged and the supernatant fluid, which contained

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most of the histamine present, was discarded. The precipitate was washed with 4% TCA solution, dissolved in 0.15 N HCl, and lyophilized.

Histamine activity of gastrin extract. Histamine activity of the gastrin extract used in this study was estimated by bioassay(2) and found to be only 0.4 μg per gram of mucosa. Thus, the larger of 2 dosage rates used (2.5 g per hour) furnished only 1 μg of histamine per hour, which is not nearly enough to stimulate a gastric pouch to secrete.

Testing response to gastrin. For each test, weighed portions of powdered antral extracts were dissolved in physiologic saline to make concentrations equivalent to 0.5 g (solution A) and 2.5 g (solution B) of original antral mucosa per 20 ml. The pH of these solutions was adjusted to 7.5 by titration with 0.1 N NaOH.

Physiologic saline was given intravenously for 30 minutes at a rate of 20 ml per hour controlled by a motor-driven syringe. Subsequently, the saline solution was replaced with 20 ml of solution A and then with 20 ml of solution B. In this way, each dog received gastrin in a dosage equivalent to 0.5 g of antral mucosa per hour during the first hour and 2.5 g per hour during the second hour.

Secretions from the pouches were collected during the tests and volumes were measured for each 30-minute period. One milliliter of each collection was titrated with 0.1 N NaOH using Töpfer's reagent (dimethylaminoazobenzene) as the indicator to determine the free acidity of the juice. Total output of free hydrochloric acid was calculated in milliequivalents for each 30-minute sample. Two tests were performed on each dog both before and after total gastrectomy.

Basal secretion. Each pouch was observed for 2 hours during fasting 3 times before and 3 times after total gastrectomy.

Surgical procedure. The surgical approach for total gastrectomy was through the left thoracic cavity. The end of esophagus was joined to the side of a Roux-Y loop of jejunum after the duodenal stump had been closed. Three weeks elapsed before testing was resumed. The dogs were in excellent condition while tests were being performed after gastrectomy.

TABLE I. Means of 2 Responses of Heidenhain Pouches to Continuous Intravenous Injection of Hog Gastrin in 2 Dosages Before and After Total Gastrectomy.

	Free hydrochloric acid, meq/hr			
	Solution A*		Solution B†	
Dog	Pre-operative	Post-operative	Pre-operative	Post-operative
1	0	.27	.79	1.8
2	0	.22	.10	2.1
3	0	.12	.06	1.0
4	0	.50	0	1.3

* Solution A supplied gastrin in a dose equivalent to 0.5 g of antral mucosa per hr.

† Solution B supplied gastrin in a dose equivalent to 2.5 g of antral mucosa per hr.

Results. Response to gastrin. Before gastrectomy, none of the pouches responded to gastrin given at a rate equivalent to 0.5 g of antral mucosa per hour and only one responded with more than a 0.1 meq of HCl per hour when the dose of gastrin was increased to the equivalent of 2.5 g of mucosa per hour.

After gastrectomy, a modest but definite secretion of free hydrochloric acid occurred in all dogs when the smaller dose of gastrin was given and much greater rates of secretion followed administration of the larger dose of gastrin (Table I).

Basal secretion. No free hydrochloric acid was secreted during fasting either before or after total gastrectomy.

Comment. Heidenhain pouches in gastrectomized dogs have enhanced sensitivity to exogenous gastrin which has proved useful in searching for secretagogues that resemble gastrin in extracts of islet-cell tumors and of normal and atrophic pancreatic tissue. Gastrectomized dogs with Heidenhain pouches are more difficult to maintain in good condition than are ordinary dogs with Heidenhain pouches. Selection of the dogs is a factor of great importance. Vigorous, aggressive animals that eat ravenously usually do better after gastrectomy than timid animals that eat delicately. After the main stomach has been removed, the dogs learn to eat slowly. They also seem to have varying preference for foods and eat better if the diet is changed frequently. Although a few dogs, not used in

the present study, ate so poorly after gastrectomy that they could not maintain weight and health, several have remained in good health after 6 months.

The hypothesis that acid produced by the main stomach when exogenous gastrin is given acts in some way to limit further secretion of acid by pouch and stomach is attractive and is supported by Andersson's observation that acidification of the duodenum inhibits the secretory responses of gastric pouches to gastrin(3). More work is necessary to prove or disprove this hypothesis in this setting and to explain the mechanism involved.

Summary and conclusions. Secretory responses of 4 Heidenhain pouches to gastrin prepared from antral mucosa of hogs were augmented following gastrectomy. The Heidenhain-pouch preparation with gastrectomy is useful for detection of gastrin-like activity in extracts of tissue.

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Clearance of Rb^{86} from Coronary Blood by the Atria of Dogs.* (28208)

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Little is known about blood flow to the atrial wall because conventional methods cannot be applied readily to this portion of the myocardium. The rate at which the ventricular myocardium clears Rb^{86} from arterial plasma closely parallels the amount of coronary blood flow reaching it(1). Recent studies have shown that the relationship of flow and Rb^{86} uptake is the same in the right and left ventricles, and that the rate of flow to the ventricles can be predicted from the rate of myocardial Rb^{86} clearance with a mean error of approximately 6%(2). These results prompted the present measurements of rates of Rb^{86} uptake in the atria.

Materials and methods. Eighteen dogs weighing 13.7 to 24.0 kg were anesthetized with pentobarbital, 30 mg/kg and subjected to right lateral thoracotomy with artificial respiration, 300 ml air/kg/min. After heparinization, Rb^{86} in 0.15 M NaCl was infused at a continuously decreasing rate(3) over a period of 10 minutes, following which the animals were sacrificed. During the infusion of isotope, blood was continuously aspirated for measurement of mean arterial Rb^{86} concentration by scintillation counting. Cardiac output was measured by the dye dilution technique after 5 minutes of isotope administration. Following sacrifice, 5 specimens were taken from the atria and their Rb^{86} content was determined after they had been digested in HNO_3. These were: portions of the left and right atria which were free of gross deposits of fat; the remainder of each atrium; and the entire interauricular septum. Samples were also obtained from each ventricle. Pressure within the right atrium was measured with a saline manometer. The reference level was the superior surface of the exposed heart.

Results and interpretation. An average of 10.9% of the isotope taken up by the heart was present in the atria. Of this amount, 39% was found in the left atrium, 45% in the right atrium, and 16% in the interauricular septum. Fig. 1 shows that atrial clearance of Rb^{86} ranged from a maximum of 16.5% to a minimum of 7.1%. It made up a larger portion of the total myocardial uptake in ani-

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