FUNDIC INHIBITION OF ACID SECRETION
AND GASTRIN RELEASE

by

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ABSTRACT

Despite earlier indirect evidence that an antral chalone exists, no such inhibitor has been found in antral extract. Recently, interest in the question of an antral inhibitory mechanism has been revived by studies that showed that for a given rise in serum gastrin caused by antral distension, the response of both the innervated and denervated stomach is greatly enhanced by vagal denervation of the antrum. While this study suggested a neuro-humoral character of the antral inhibitory mechanism, it gave no indication as to the source of the inhibitor. Subsequent studies, however, suggested that neither the antrum nor the CNS was the source for this inhibitor.

The initial aim of this study was to investigate the fundus as a possible source of the inhibitor by studying the effect of proximal gastric vagotomy on the antral inhibitory mechanism initiated by distension. The results gave clear indication that the inhibitor was indeed released from the fundus; indeed, the antral inhibitory mechanism was in reality a fundic one.

Once the fundus was shown to be the source of the inhibitor, it was necessary to establish whether this inhibitor did in fact reside in the fundic mucosa. Four dogs were prepared with a denervated fundic pouch (or Heidenhain pouch, HP), and a fistula of the main, innervated stomach (gastric fistula, GF). The acid secretory responses of both the HP and GF to graded doses of

pentagastrin and histamine was studied. In addition both the secretion of acid and the response of immunoreactive gastrin in the blood in response to a standard meal of 15% liver extract was studied. All these experiments were repeated after excision of the fundic mucosa of the main stomach. The results show that excision of the fundic mucosa reduced the GF acid secretion to the stimuli by 85-100% . By contrast, the maximal HP acid secretion increased by 247% in response to pentagastrin and 200% in response to histamine. The increase in the response to submaximal doses of these exogenous stimuli was even greater. Similarly, the peak 30 minutes HP output in response to feeding increased by 418%. Fundic mucosal excision also resulted in the increase in both basal (from 36±3 to 248±37 pg/ml) and food-stimulated response (from 168±12 preoperatively to 392±49 pg/ml postoperatively). Since the intragastric pH was held constant at 5.5 during the meal tests both before and after the operation, the augmented gastrin response could not be attributed to reduced acid secretion caused by excision of the fundic mucosa.

From these studies it can be concluded that: (1) antral distension releases an inhibitor from the fundus; (2) excision of the fundic mucosa results in increased response of the HP to both submaximal and maximal doses of pentagastrin and histamine indicating that both the sensitivity of the oxyntic cell and parietal cell mass has increased; (3) excision of the fundic mucosa results in increased basal and food-stimulated gastrin response independent of the pH of the meal suggesting removal of an inhibitor of gastrin release.

Further studies are necessary to identify the fundic inhibitor(s). Additional studies we have performed have shown that the increased secretory response caused by fundic mucosal excision could not be reversed by the infusion of exogenous VIP or somatostatin, peptides known to exist in the fundic mucosa.

To investigate further the role of the vagus on the release of the fundic inhibitor, the effect of parenteral atropine and of truncal vagotomy on meal-stimulated gastrin and HP acid response in the postfundusectomy state was studied. These studies show that truncal vagotomy does not increase HP acid secretion further but significantly decreases gastrin response to the meal. Atropine abolishes the postfundusectomy rise in HP acid secretion but has no effect on the postfundusectomy rise in serum gastrin. These results are consistent with the hypothesis that the inhibitory mechanism of gastrin release is mediated by atropine-sensitive vagal fibers which supply the fundus and that the stimulatory action is mediated by atropine resistant vagal fibers which supply the antrum.

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PART I: PYLORO-OXYNTIC NEUROHUMORAL INHIBITORY
         REFLEX OF ACID SECRETION

INTRODUCTION

Distension of the pyloric antrum results in both stimulation and inhibition of gastric acid secretion.

Stimulation of gastric acid secretion after antral distension is mediated both by gastrin release and by a gastrin-independent pyloro-oxyntic reflex.[1]

The inhibitory mechanism of acid secretion by antral distension however, is less clearly understood. Historically, the concept that the antrum may play an inhibitory role was first raised by State and co-workers[2] is 1955, when they showed that resection of the antrum facilitated the production of histamine-induced ulcer. This concept was given support by Harrison and co-workers[3] in 1956. They transplanted half of the antrum onto the colon in dogs with Heidenhain pouches leaving the other half in its normal location. When the portion of the antrum in its normal location was excised, acid secretion from the pouch increased suggesting that acid bathing the antrum in its normal location had released an inhibitor of acid secretion. The hypothesis of an antral chalone was thus born.

During the next decade controversy raged, some investigators showing antral irrigation released an inhibitor,[4,5,6] while others refuted this.[7,8] In 1974 J.C. Thompson[9] reviewed the current status of the antral chalone hypothesis and concluded there was no convincing evidence for it.

Indirect evidence for an antral inhibitory mechanism was supplied by Debas et al[1] when it was unexpectedly noted that after denervation of the antral pouch an increase in response of both the gastric fistula and Heidenhain pouch to a given increment of serum gastrin occurred during antral distension with alkali. The hypothesis was thus made that antral distension released an inhibitor to acid secretion which required vagal tone for its activity. Since both the Heidenhain pouch and the gastric fistula were affected, a humoral as well as a neural mechanism is implicated.

Direct evidence confirming the existence of this antral inhibitory mechanism was supplied when it was shown that acid distension of the antrum significantly inhibited pentagastrin-stimulated gastric secretion from the gastric fistula.[10] Recently, Schoon et al[11] demonstrated the same phenomenon in healthy man and interestingly, a defective inhibition for antral distension in duodenal ulcer patients.

The source of the inhibitor now requires localization. While the antrum could itself be the source, it is equally possible that antral distension initiated a reflex mechanism which releases the inhibitor elsewhere, such as the central nervous system, the fundus of the stomach, the small intestine or pancreas. Neither the antrum nor the CNS are the source since, when vagal communication only between the CNS and antrum is preserved, all other abdominal organs being vagally denervated, antral distension no longer causes inhibition of acid secretion.[10]

This study sought to investigate the effect of vagal denervation of the oxyntic mucosa or proximal gastric vagotomy (PGV) on the inhibition of pentagastrin-stimulated acid secretion caused by antral distension. The results show that PGV completely abolishes the inhibitory effect of antral distension and suggests that the fundus is the source of the inhibitory substance(s) released by antral distension.

PURPOSE

To investigate the effect of vagal denervation of the oxyntic mucosa ie., proximal gastric vagotomy (PGV) on the inhibition of pentagastrin-stimulated acid secretion caused by antral distension.

STAGE I   STAGE II: PGV
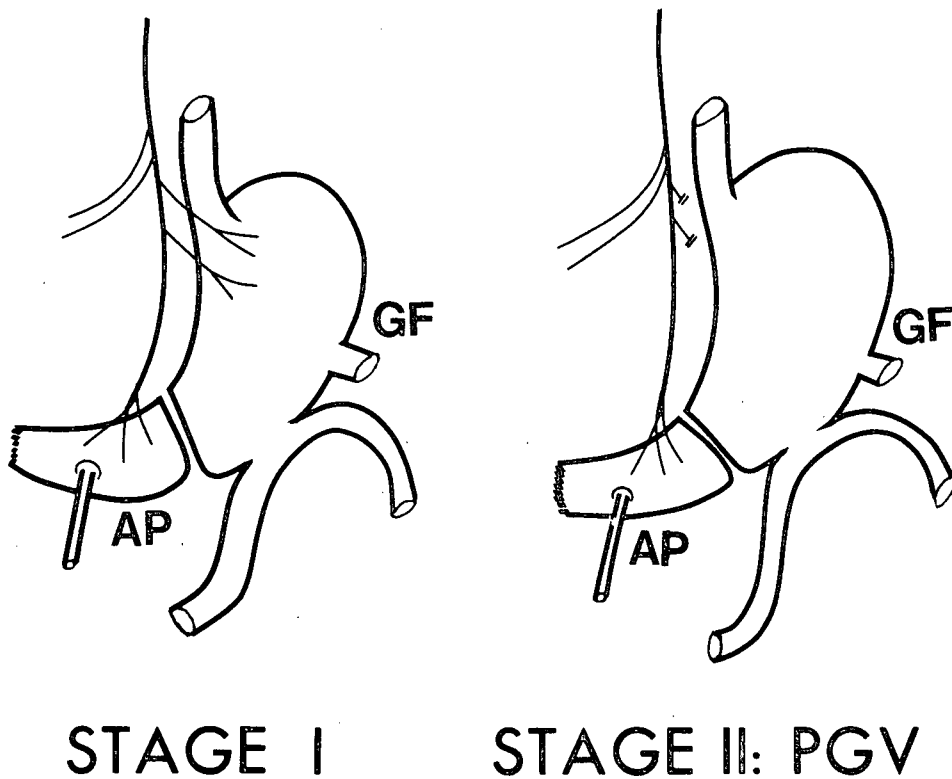
FIGURE 1:   Animal preparation showing antral pouch and gastric
             fistula before and after proximal gastric vagotomy.

MATERIAL AND METHODS

ANIMAL PREPARATION:

Four female mongrel dogs (20-26 kg) were surgically prepared with an innervated pouch of the pyloric antrum (AP). The AP was prepared by dividing the antrum completely from the body of the stomach except for a bridge of 2 cm at the lesser curve where only the mucosa was divided and a double-mucosal septum created. This procedure ensured complete preservation of vagal innervation to the antrum. The pylorus was transected and a Gregory cannula inserted into the body of the AP. Gastrointestinal continuity was restored by anastomosing the body of the stomach to the duodenum. A Thomas cannula was inserted into the body of the stomach proximal to this anastomosis to serve as a gastric fistula (GF). A small amount of acid secreting mucosa was deliberately left with the antral pouch to prevent excessive basal gastrin secretion. (Figure 1, Stage I)

Following recovery, pre-PGV control studies were performed before the animals were subjected to a second operation. In the second operation, PGV was performed by dividing all the neurovascular connections along the lesser curvature from the antrum-body junction to the gastro-esophageal junction leaving intact both the anterior and posterior nerves of Laterjet to the antrum. The last 2 inches of the esophagus was also denuded of all nerve fibers to ensure completeness of PGV. (Figure 1, Stage II)

EXPERIMENTAL DESIGN:

No experiments were performed for at least 3 weeks after each operation. The animals were fasted of food but not of water for 18 hours before each test. No tests were done within 2 days of each other in a given animal.

The following experiments were performed before and after Proximal Gastric Vagotomy:

(1) Histamine Dose-response to characterize GF Response: This was performed by constant intravenous infusion of histamine-dihydrochloride in doses doubling from 5 to 160 μg kg^{-1} hr^{-1}.

(2) Insulin Test For Completeness of Vagotomy: Both GF acid secretion and serum gastrin concentration in response to an IV bolus injection of regular insulin (0.5 U kg^{-1}) was studied.

(3) Control Pentagastrin Plateau of Secretion: Acid secretion in response to a continuous infusion of pentagastrin (4 μg kg^{-1} hr^{-1}) was determined over a 3 hour period. During these control studies, the AP was filled with 0.1 M HCl but distension was prevented by keeping the level of the barostat from which the AP was filled at the level of the pouch.

(4)   Effect of Antral Distension on Pentagastrin Plateau: These
      experiments were similar to the control pentagastrin plateau
      experiments except the AP was distended by lifting the
      barostat to 40 cm during the second hour of the pentagastrin
      infusion.   In these test, the AP was kept filled with 0.1 M
      HCl in the first and third hours of infusion but AP
      distension was prevented by keeping the barostat at the level
      of AP.

DETERMINATIONS

ACID OUTPUT:

GF secretion was collected continuously by gravity and divided into 15 minute samples. Two basal samples were obtained before administration of a stimulus. The volume of each sample was measured to the nearest 0.1 ml and acid concentration determined by titrating 0.5 ml of each sample to pH 7.0 with 0.1 M NaOH on an automatic titrator (Radiometer, Copenhagen). Acid output was calculated by multiplying the volume by the concentration.

SERUM GASTRIN CONCENTRATION:

Venous blood was obtained basally and every 30 minutes after the start of stimulation in all pentagastrin and insulin tests. Serum was separated by cold centrifugation and stored at $-40°C$ until assayed. Serum gastrin concentration was measured by radio-immunoassay using antibody 1296 (the kind gift of Dr. John H. Walsh from the Center for Ulcer Research and Education, Los Angeles). The assay was sensitive to 5 pg/ml and measured the total concentration of immunoreactive gastrin.

STATISTICAL ANALYSIS:

The significance of the difference of the mean acid secretion during the control and distension experiments was analysed using

t-test for paired values. A p-value of less than 0.05 was considered significant.

RESULTS

EFFECT OF PGV ON GF RESPONSES

(1) Histamine Dose-Response:

Figure 2 shows that the effect of PGV on the dose-response to histamine is small. Submaximal but not maximal responses are affected. A significant decrease was only seen at the 20 μg kg^{-1} hr^{-1} dose.

(2) Insulin Test:

PGV completely abolished the GF acid response to insulin hypoglycemia (Figure 3, left panel). An increase in gastrin response to insulin occurred following PGV, but this change was not statistically significant (Figure 3, right panel).

EFFECT OF ANTRAL DISTENSION ON PENTAGASTRIN BEFORE PGV

Antral distension with 0.1 M HCl caused significant inhibition of acid response during the time the distension was applied (Figure 4). The two half hourly responses during the second hour of pentagastrin infusion fell significantly from the control values of 15.2±1.1 and 14.8 mEq to 9.9±1.2 and 8.5±1.4 mEq respectively with distension. Once antral distension was discontinued, there was recovery in acid response.

PGV lowered the plateau response to pentagastrin (4 μg kg^{-1} hr^{-1}) only slightly, the change being statistically non-significant. However, following PGV, inhibition of GF response to pentagastrin, by antral distension, was abolished. (Figure 5)

SERUM GASTRIN RESPONSES:

The effect of antral distension with acid on gastrin responses both before and after PGV are given in Table I. As might be expected, antral distension with acid had no stimulatory effect on gastrin release, and in fact, serum gastrin levels were significantly lower when acid distension was applied. Changes in serum gastrin concentration were of no consequence in the results shown above.
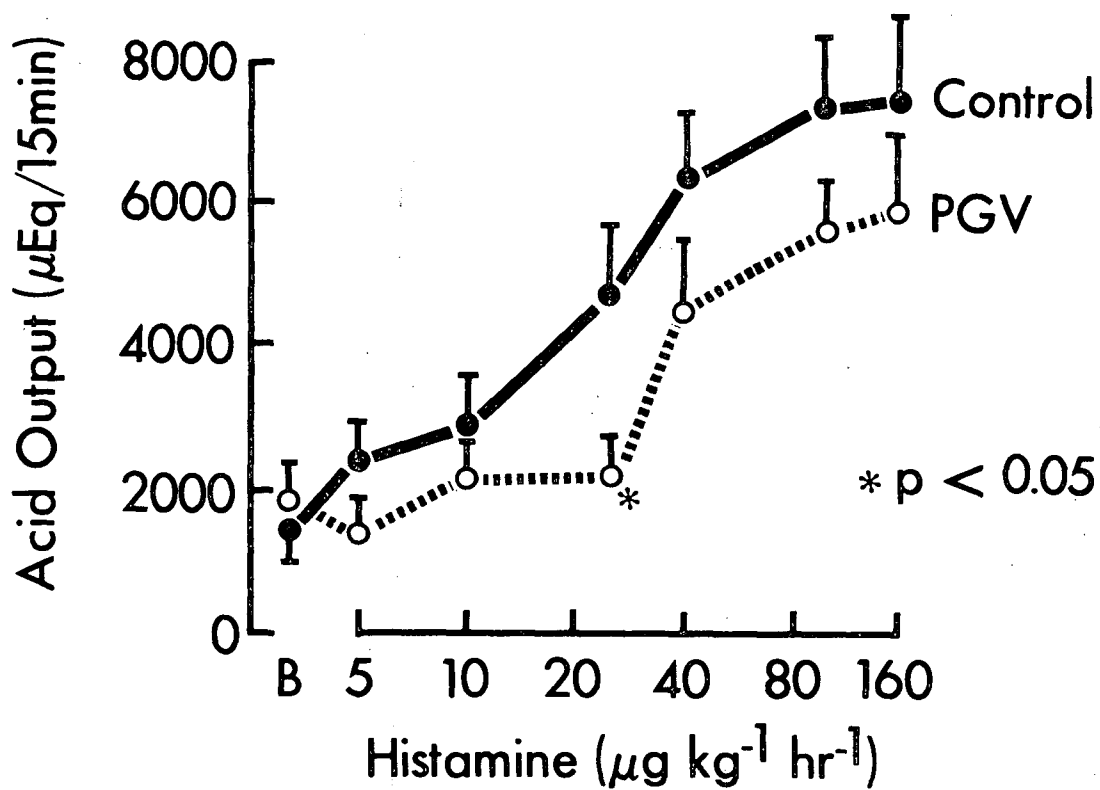
FIGURE 2: The effect of graded doses of histamine dihydrochloride on GF acid secretion prePGV (closed circles) and postPGV (open circles). In this and each of the following figures each point represents the mean (±SE) of two experiments in each of four dogs.
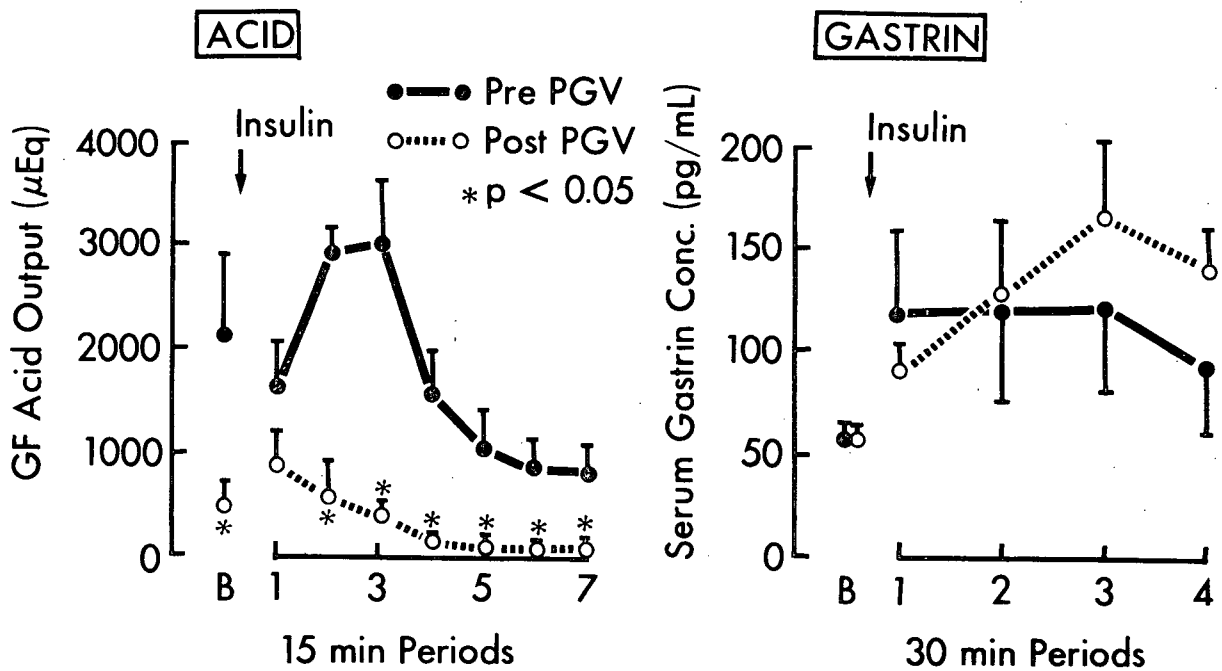
FIGURE 3: The effect of IV bolus injection of regular insulin (0.5 U kg^{-1}) on GF acid output (left panel) and gastrin response (right panel), prePGV (closed circles) and postPGV (open circles).
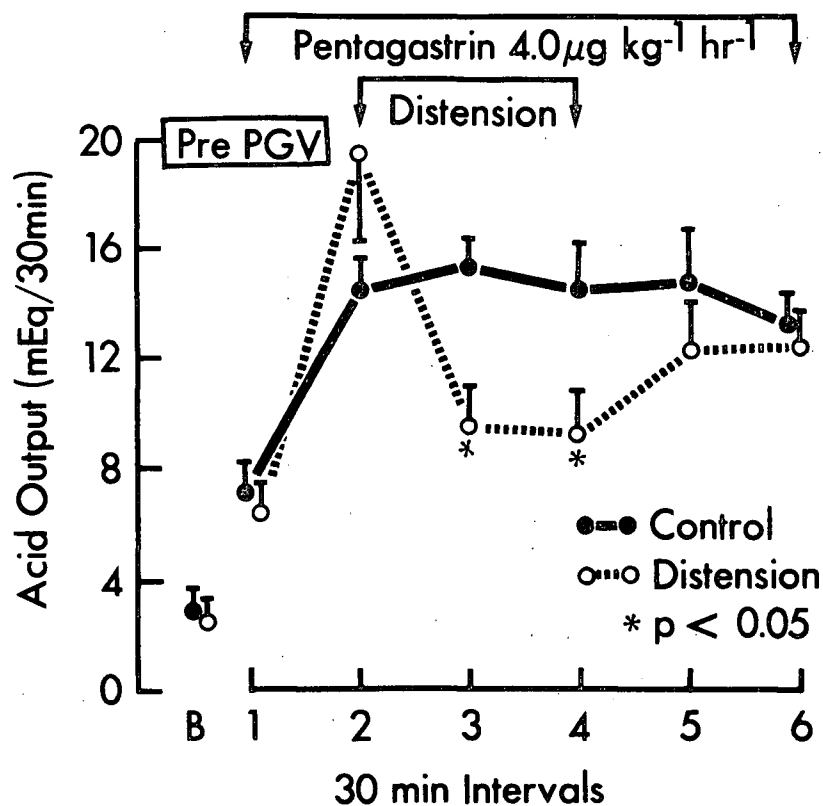
FIGURE 4: GF acid response to constant IV perfusion of pentagastrin (4.0 µg kg^{-1} hr^{-1}) under control condiditions, i.e. no distension (closed circles) and during antral pouch distension with 0.1 M HCl (open circles) in the prePGV stage.
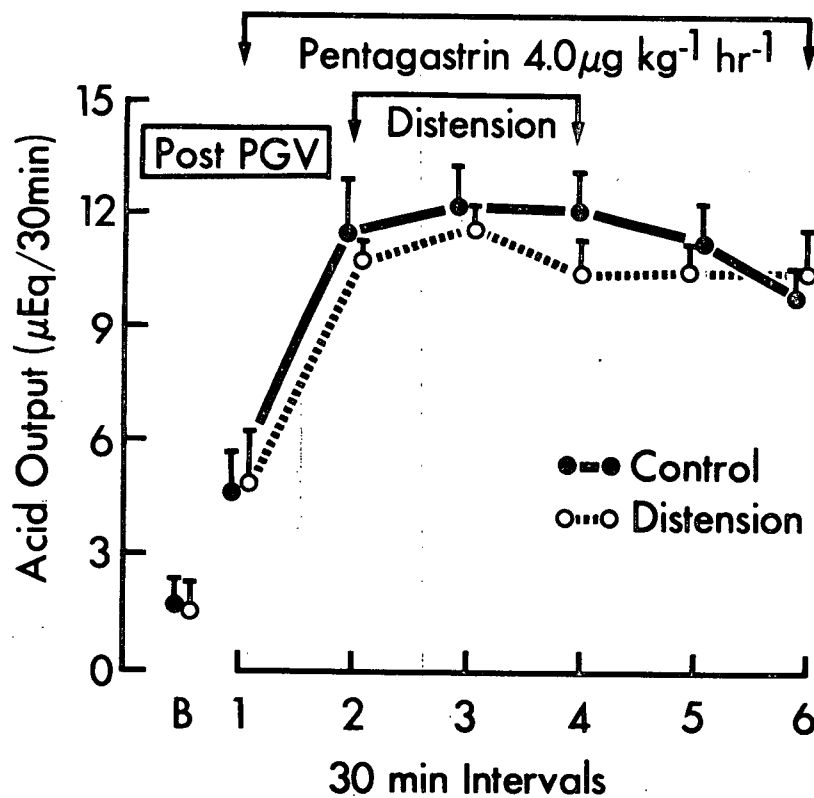
FIGURE 5: GF acid response to constant IV perfusion of pentagastrin (4.0 µg kg⁻¹ hr⁻¹) under control conditions, i.e. no distension (closed circle) and during antral pouch distension with 0.1 M HCl (open circles) in the postPGV stage.

TABLE I: Mean (±SE) Serum Gastrin Concentration (pg/ml) During
           Pentagastrin-Infusion Experiments

EFFECT OF DISTENSION (PRE PGV):

	BASAL	PRE-DIST	DISTENSION	POST-DIST
CONTROL (0 DISTENSION)	71±17	46±9	44±8	54±12
40 cm DISTENSION	47±4	51±8	38±3	32±3*

EFFECT OF VAGOTOMY:

	BASAL	PRE-DIST	DISTENSION	POST-DIST
PRE-PGV (DISTENSION)	47±4	52±8	38±3	32±3
POST-PGV (DISTENSION)	69±13	58±12	46±4	41±5

* $p < 0.05$

DISCUSSION

The impetus for this study came from the unexpected observation that the responsiveness of both the innervated and denervated stomach to a given increment in serum gastrin concentration following antral distension was greatly increased after vagal denervation of the antrum.[1] The implication was that antral distension results in the release not only of gastrin but also of a substance that is an inhibitor to the action of gastrin and that vagal denervation of the antrum abolished the release of the inhibitor but not of gastrin. While it was certain the antrum was the source of the gastrin, that study would not determine whether the inhibitor was released from the antrum or extra-antral sites. These other sites could be the CNS, the oxyntic mucosa, or other vagally-supplied abdominal organs.

A recent study has provided evidence that neither the antrum nor the CNS are the source of the inhibitor,[10] suggesting that the inhibitor was released reflexly from the other vagally-innervated sites. The present study explored the possibility that the oxyntic cell mucosa might be the source of the inhibitor and that the vagal branches to the oxyntic mucosa may be the effector fibers of the neuro-hormonal pathway. Indeed, this study has shown that PGV completely abolished the inhibitory action of antral distension.

The choice to distend the antrum with acid rather than alkali was based on earlier demonstration that acid distension was more

effective than alkali distension. A possible explanation for these differences might be that alkaline distension releases gastrin thus adding to the stimulus of secretion against which the inhibitor has to act. In the present study a dose of pentagastrin was selected that gave near maximal acid response in order to minimize the effect of any decrease in serum gastrin concentration that might result from acid distension of the antrum.

Denervated pouches were not used in the present study for the sake of simplicity and the statement that a humoral agent is released is based on previous studies of this mechanism.[1,10] We now know that various inhibitory peptides are found in the vagal fibers and in endocrine cells within the oxyntic mucosa. Substance P, VIP, and somatostatin have been shown to exist in the vagal nerve endings.[12,13,14,15,19] VIP, somatostatin and glucagon are also found in the endocrine cells of the oxyntic mucosa.[16,17,18] The inhibition caused by antral distension could be mediated by release of these neurocrine and/or endocrine substances. Of course, an entirely different substance than those mentioned above might be involved. The fact that a Heidenhain pouch is inhibited suggests that the inhibitor can be transmitted via the circulation. Since substance P and somatostatin do not appear to have persistence in the circulation, they are weak candidates. Further studies are required to define the nature of the inhibitor(s) involved in this antral neuro-humoral inhibitory mechanism.

The question of whether an antral chalone exists has been the subject of much heated debate since Harrison et al[3] suggested its

existence. Some investigators have been able to show that antral acidification inhibits acid secretion,[4,5,6] others have not.[7,8] It is likely that these discrepant findings were due to failure to control the element of antral distension, so that only those who inadvertently distended the antrum had positive results. The present study along with several others previously published[1,10] indicate that neither antral acidification nor antral distension release an antral chalone, and that the antral inhibitory mechanism is in reality a fundic one.

PART II: FUNDIC INHIBITION OF ACID SECRETION AND GASTRIN RELEASE

INTRODUCTION:

This proof of a pyloro-oxyntic neurohumoral inhibitory reflex strengthens the hypothesis that inhibitory mechanism(s) of acid secretion reside in the fundus.

In addition three types of evidence point to the fundus as a possible source of an inhibitor of acid secretion and/or gastrin release. First, proximal gastrectomy, fundic mucosal stripping[20] and proximal gastric vagotomy[21] all result in a marked increase in 24 hour Heidenhain pouch (HP) acid secretion in dogs. This increase had been attributed to the rise in pH following loss of parietal cell activity. Second, vagal denervation of the oxyntic mucosa results in increases in basal,[22,23,24,25] food-[24,25] and insulin-stimulated[23] gastrin release in man. This postvagotomy hypergastrinaemia occurs independent of change in pH in both man[25] and dog.[26] Third, endocrine cells containing peptides inhibitory to acid secretion and gastrin release have been identified in the mucosa of the proximal stomach.[16,17,18]

This study aimed to define the inhibitory role of the fundus by investigating the effect of fundic (oxyntic cell) mucosal excision on acid secretion and gastrin release in response to endogenous and exogenous stimuli. The results indicate that removal of the fundic mucosa may result in the withdrawal of inhibitory substance(s).

PURPOSE

To define the inhibitory role of the fundus by investigating the effect of fundic (oxyntic cell) mucosa excision on acid secretion and gastrin release in response to endogenous and exogenous stimuli.
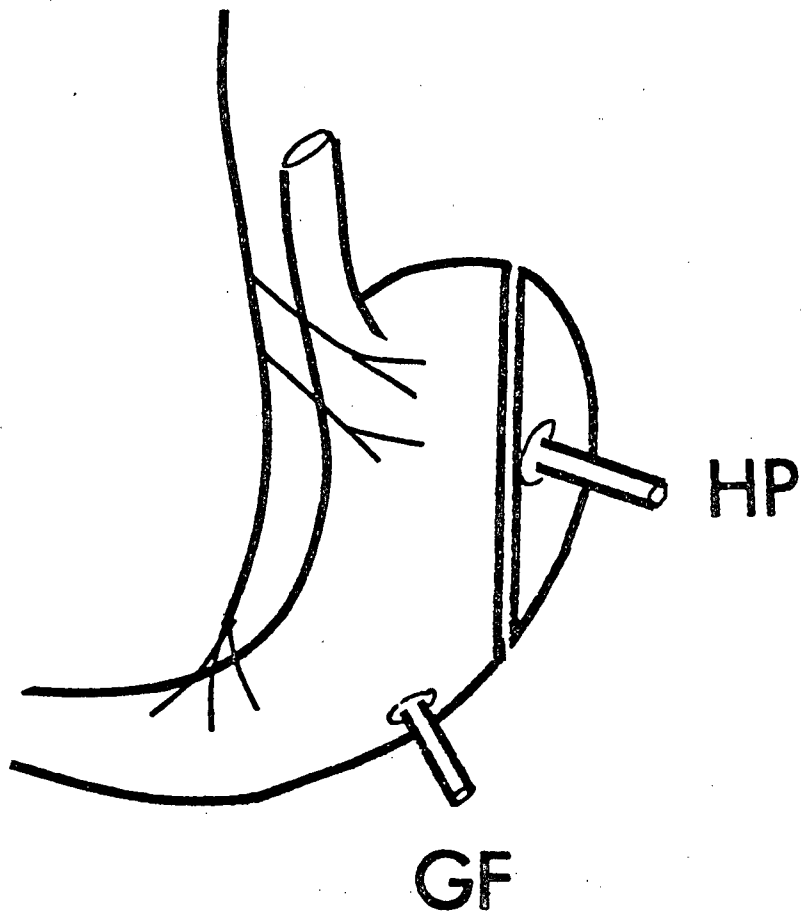
FIGURE 1:   Animal preparation demonstrating the gastric fistula
(GF) of the innervated stomach and a vagally denervated
fundic or Heidenhain pouch (HP).

METHODS AND MATERIAL

ANIMAL PREPARATIONS

Four female dogs (15-20 kg) were provided with a gastric fistula (GF) and a vagally denervated Heidenhain pouch (HP). (Figure 1) Following completion of control experiments the dogs underwent a second operation in which, through an anterior gastrostomy, the oxyntic cell mucosa was excised at a plane superficial to the muscularis mucosa. The entire oxyntic cell mucosa was removed (Figure 2) except for a small rim near the esophageal junction and at the margins of the GF. Figure 3 is a photomicrograph of normal fundic mucosa. Figure 4 shows the specimens of mucosa before and after fundusectomy, demonstrating the extent of mucosal excision.

The dogs tolerated the procedure remarkably well and resumed normal diet within 5-6 days.

EXPERIMENTAL DESIGN

Three weeks were allowed for recovery from the 1st operation, and two weeks from the 2nd. The dogs were fasted for 18 hours before each test. No tests were done within 48 hours of each other. The following tests were done before and after excision of the fundic mucosa:

(1) Meal Test by Intragastric Titration: After removal of the
     cork from the GF, the inside of the stomach was rinsed with

tap water. Two 15 minute collections of basal secretion were obtained from the GF and HP. All collections of HP secretion were made using the technique of irrigation with 50 ml saline each 15 minutes. A meal of 300 ml. of 15% solution of liver extract (w/v) adjusted to pH 5.5 was introduced into the GF which was then connected to an automatic intragastric titration sytem. (Figure 5) The liver extract used was dried water extract of mammalian liver (Reheck Chemical Company, Phoenix, Arizona). The technique of intragastric titration was a modification of the method of Fordtran and Walsh[27] and employed a pH stat assembly (electrode GK3221C, Titrator TTT11, Autoburett ABV13 with 25 ml. burette, Recorder SBR2C, Radiometer, Copenhagen, Denmark), a piston pump (Brewer, Model 60453, Dickinson, Maryland) with the speed adjusted at 10 strokes per minute, and 0.5 M $NaHCO_3$ as titrant. Intragastric titration was performed for two hours with the end-point adjusted to 5.5. Acid output for each 30 minutes was calculated from the titrigraph which recorded the amount of 0.5 M $NaHCO_3$ used to maintain intragastric pH at 5.5.

During each intragastric titration test venous blood was obtained at -15, 0, 30, 60, 90 and 120 minutes. After clotting serum was separated by cold centrifugation and stored at -40°C until assayed for gastrin.

(2) Pentagastrin Dose Response Studies: A continuous intravenous infusion of 0.15 M NaCl is given using a Harvard peristaltic pump at a rate of 28 ml per hour. After two 15 minute basal periods of collection of GF and HP secretion, pentagastrin is added to the infusate starting at 0.5 μg kg^{-1} hr^{-1} and

doubling the dose stepwise every 30 minutes to 16 μ g kg^{-1} hr^{-1}. The lowest dose was infused for 45 minutes. GF collection was done by gravity and HP secretion was collected by an irrigation method as described above.

The volume of each collection was measured to the nearest 0.1 ml, and acid concentration was determined in 0.5 ml and 10 ml samples of GF and HP collections respectively using an automatic titrator (Autoburette, Radiometer, Copenhagen). Acid output was calculated by multiplying the volume by the concentration. The output in the last 15 minutes collection at each dose was taken as the response for that dose of pentagastrin. To minimize differences in HP size, HP output was expressed as % of the maximal response to histamine. HP response to meal were handled similarly.

(3) <u>Histamine Dose-Response Studies</u>: These tests were performed in a manner similar to the pentgastrin tests. Histamine dihydrochloride (Sigma Chemical Co., St. Louis, MO) was employed at doses starting at 5 μ g kg^{-1} hr^{-1} and doubling stepwise every 30 minutes to the highest dose used which was 160 μ g kg^{-1} hr^{-1}. Again the lowest dose was infused for 45 minutes.

DETERMINATION OF SERUM GASTRIN CONCENTRATION:

Serum gastrin concentrations were determined by radioimmunoassay using antibody 1296 which was the kind gift of Dr. J. Walsh from the Center for Ulcer Research and Education (CURE), Los Angeles, California. This antibody cross-reacts with

both the G-17 (little) and the G-34 (large molecular forms of gastrin. The assay was sensitive to 5 pg/ml.

SOMATOSTATIN AND VASOACTIVE INTESTINE POLYPEPTIDE (VIP) INFUSION STUDIES ON MEAL TEST

In addition following excision of fundic mucosa, the effects of infusion of either somatostatin (Peninsula Lab, San Carlos) or VIP (Peninsula Lab, San Carlos) on acid secretion and gastrin release were studied. The meal test by intragastric titration is performed as described above but in addition intravenous infusion of somatostatin (0.5 μg kg^{-1} hr^{-1}) or VIP (1.0 μg kg^{-1} hr^{-1}) is commenced 10 minutes prior to the instillation of the liver extract meal and continued throughout the test. Infusion of each peptide is performed on separate test days.

FIGURE 2a&b: Operative procedure demonstrating excision of the
               oxyntic cell mucosa superficial to the mucularis
               mucosa.

Cut Edge Greater Curvature

Fundic Mucosal Edge

FIGURE 2a

Fundic Mucosa Excised
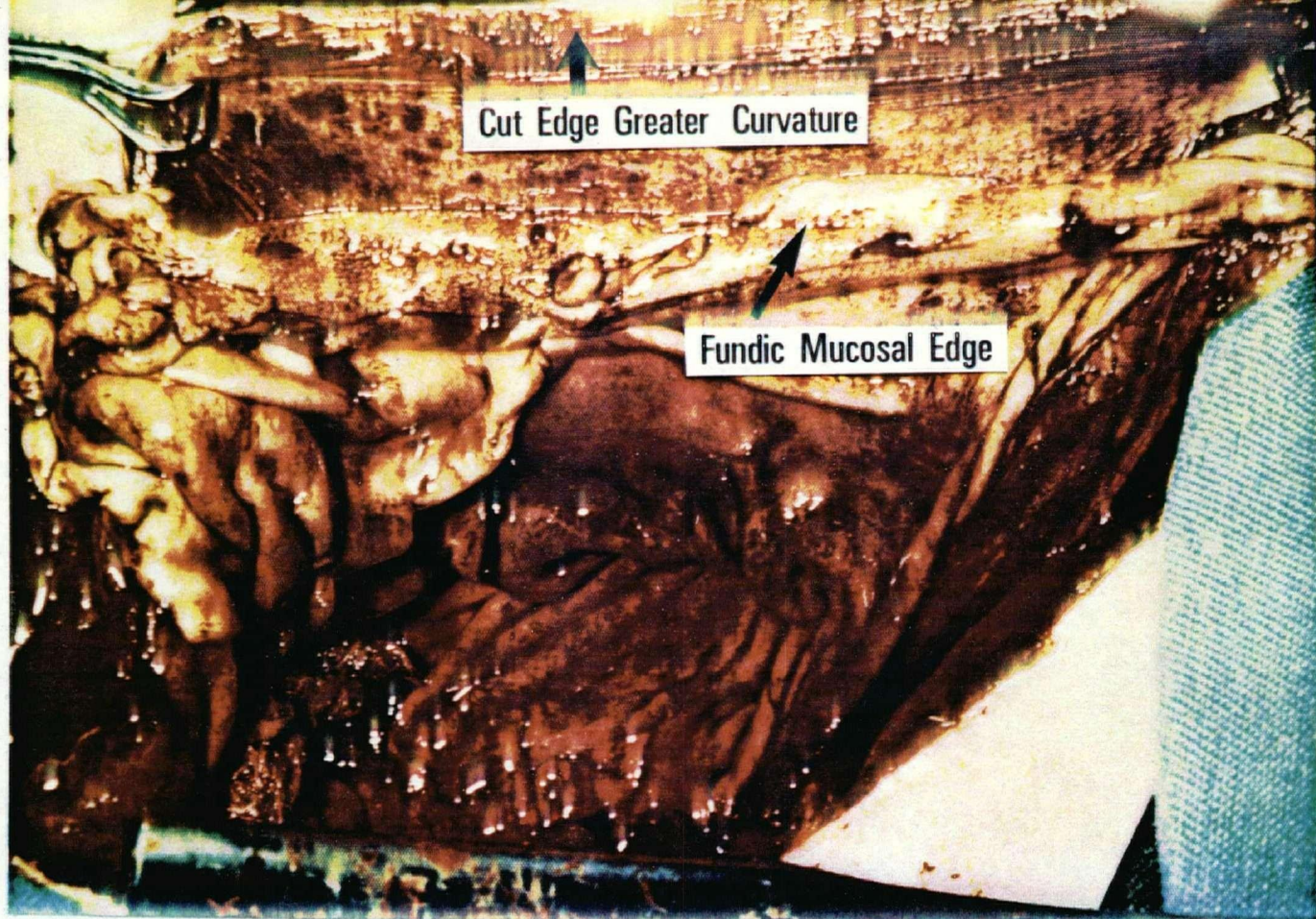
FIGURE 2b

FIGURE 3:   PHOTO MICROGRAPHS TAKEN AT VARIOUS MAGNIFICATIONS OF
              A SECTION OF THE FUNDUS OF THE STOMACH OF THE DOG

    3a: Normal fundic mucosa removed at operation. Note the
            muscularis mucosa and submucosa (11x magnification,
            H + E stain)

    3b: High power view of surface epithelial cells lining
            pit of fundic gland (175x magnification; H + E
            stain)

    3c: High power view of fundic mucosa showing the
            eosinophilic round to pyramidal shaped oxyntic
            (parietal) cell, as well as the more basophilic
            zymogenic cell (175x, H + E)

    3d: High power view of fundic base showing the
            basophilic zymogenic cells. The vacuolated and
            reticular appearance in the cytoplasm representes
            the poor uptake by the secretion granules of the
            H + E stain (175x)

3a

3b

3c

3d
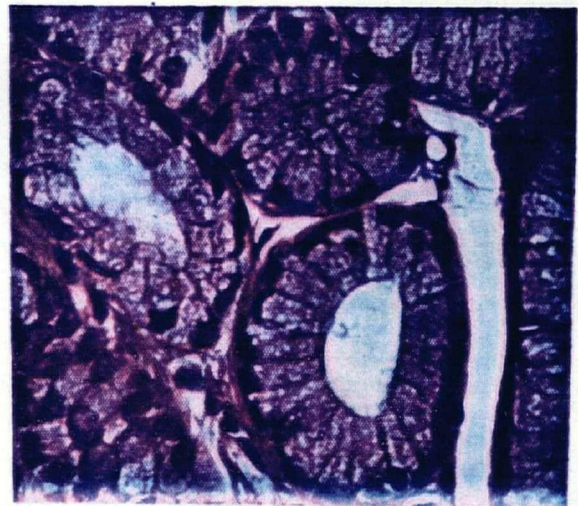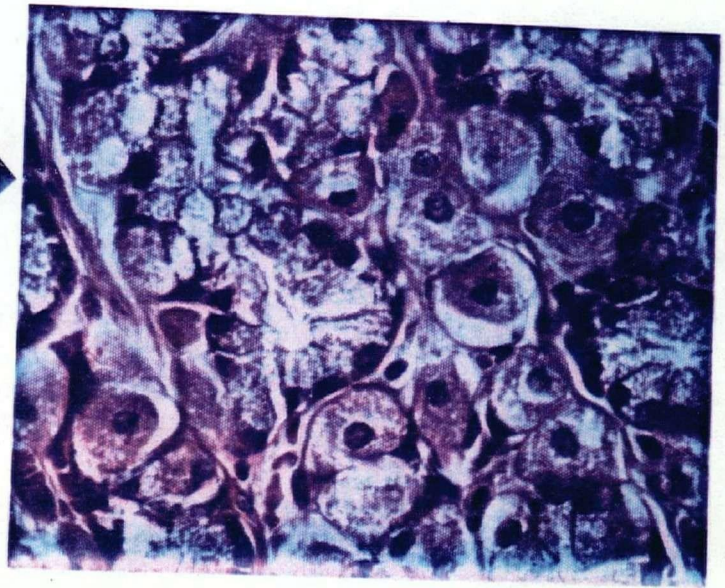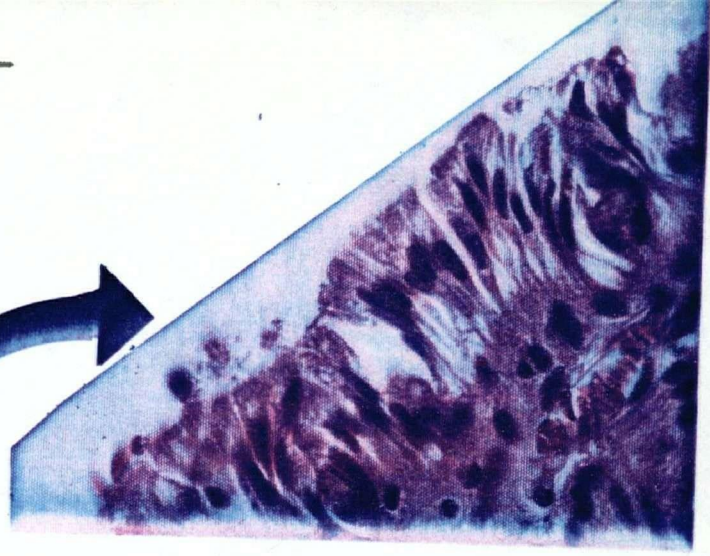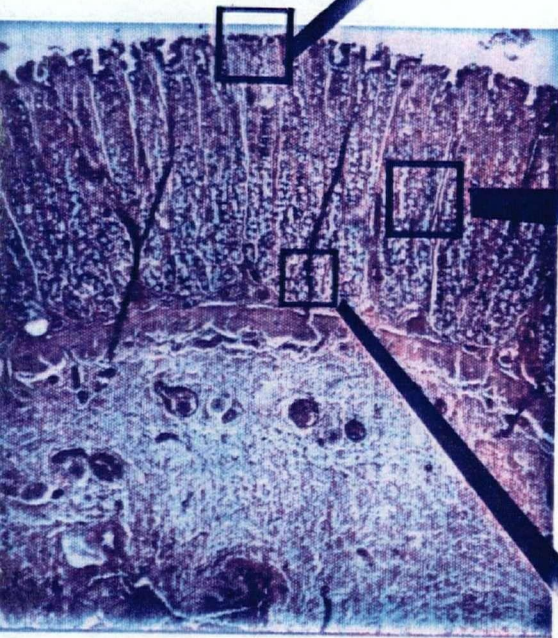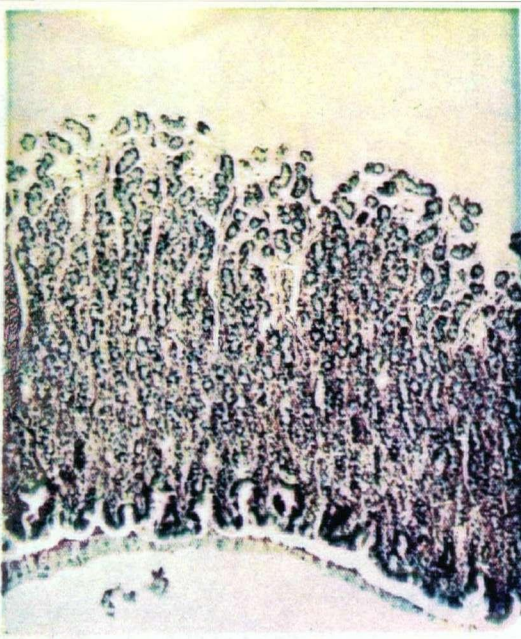
FIGURE 4a: Normal fundic mucosa removed at operation (11x)
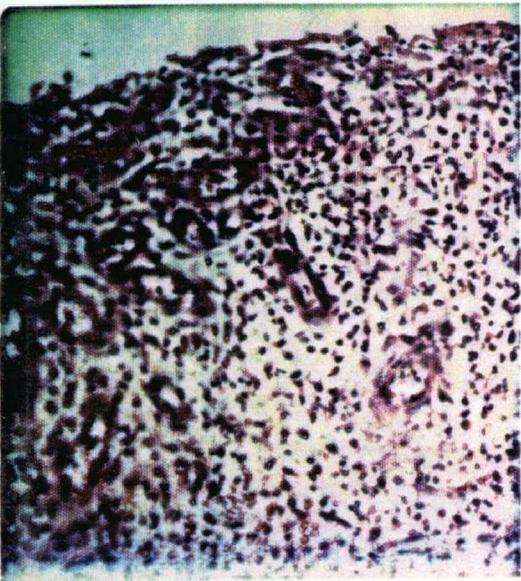
FIGURE 4b: Granulation tissue lining fundus at autopsy. Total absence of parietal cells (11x)
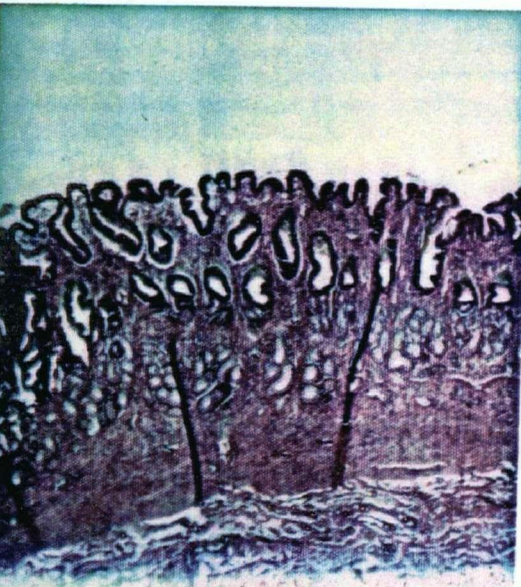
FIGURE 4c: Area of regeneration of fundic mucosa with absence of parietal cells. Note difference in height between mucosa in Figures 4a & 4c (11c)
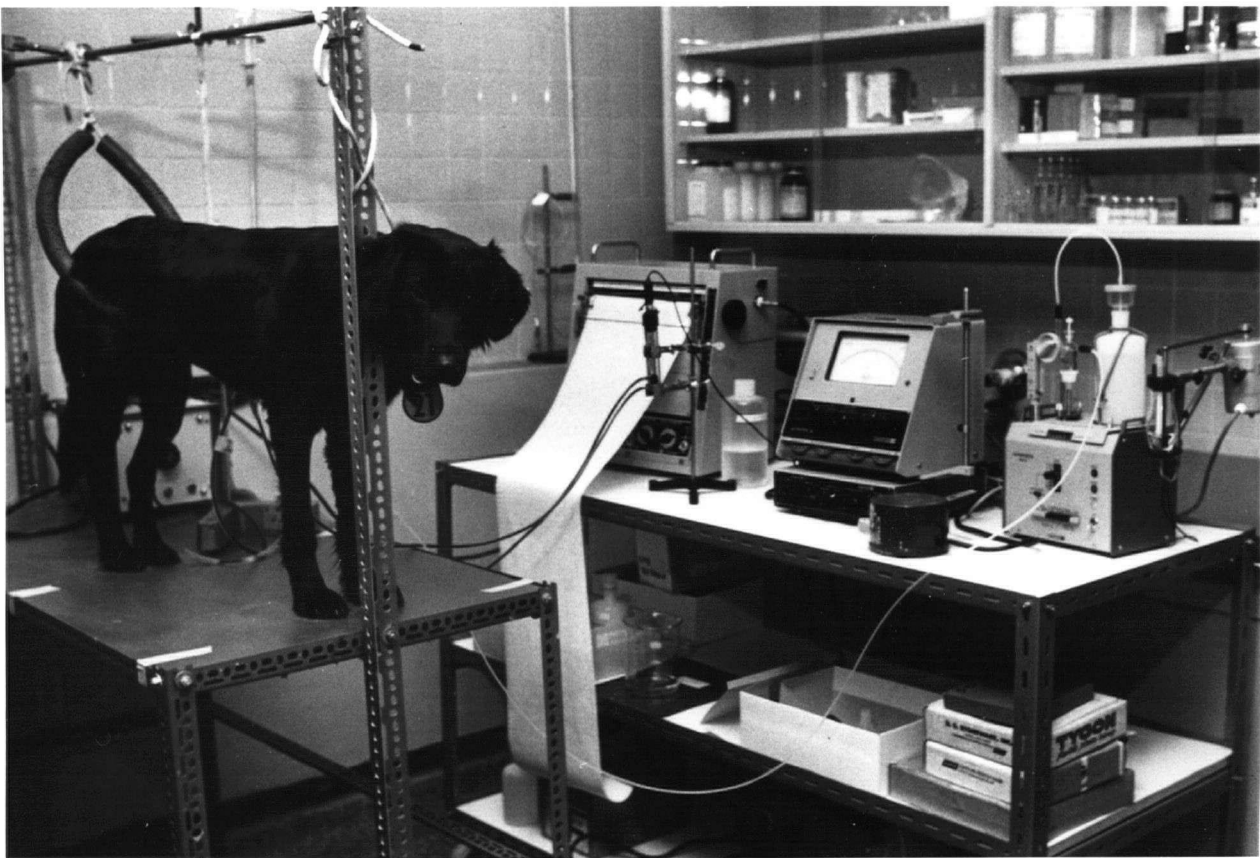
FIGURE 5: Meal test by the method of intragastric titration.

RESULTS

EFFECT OF FUNDIC MUCOSAL EXCISION ON GF RESPONSES:

Excision of the fundic mucosa from the main stomach drained by the GF abolished acid response to meal completely (Figure 6).

The maximal response to pentagastrin was reduced by 83% and to histamine by 84% indicating that some acid secreting mucosa was left behind. (Figure 7)

EFFECT OF FUNDIC MUCOSAL EXCISION ON HP RESPONSES

Basal HP Secretion: Excision of the fundic mucosa resulted in significant increases in basal acid secretion from the HP. Taking the mean of all basal collection taken before each experiment the mean basal 15 minute output increased from $5.5\pm1.6\%$ of the maximal histamine response before excision to $50.1\pm4.6\%$ after excision, an increase of 909%.

Meal Test (Figure 8): Following excision of the fundic mucosa from the main stomach, a significant increase in HP response to the meal occurred. ($p < 0.05$) The peak 30 minute output increased from 38% of maximal histamine output before excision to 159% after. This represents a rise of 418%.

Pentagastrin Dose Response (Figure 9): Similarly, HP response to all doses of pentagastrin significantly increased. ($p < 0.05$) The

increases were more marked with lower doses. The response to the 0.5 µg dose increased by 558% while that to the 16 µg dose increased by only 247%.

Histamine Dose-Response (Figure 10): The exaggeration of the response to histamine was similar to that seen with pentagastrin. The response to all doses was increased, but the response to the lower doses was more markedly elevated compared to the response to the higher doses. Thus, fundic mucosal excision increased the response to the 0.5 µg dose by over 10 times while the response to the 16.0 µg dose was only doubled.

EFFECT OF FUNDIC MUCOSAL EXCISION ON GASTRIN RELEASE:

Basal Gastrin Concentration (Figure 11, left panel): The basal gastrin concentration increased from a mean of 36±3 before fundusectomy to a mean of 248±47 pg/ml after, an increase of 688%. The increase in basal gastrin was evident even with the first experiments done 2 weeks after the operation. Basal gastrin concentration did not appear to rise with time after excision of the fundic mucosa.

Gastrin Response to Meal (Figure 11): The gastrin response to meal was significantly elevated after fundic mucosal excision. The peak response was 168±12 pg/ml before and 392±49 pg/ml after. Since intragastric pH was identical (5.5) in the preoperative and postoperative experiments, this rise could not be attributed to decreased acid response during the meal in the experiments after

fundic mucosal excision. Tests were commenced 14 days after operation and all meal tests completed within 23 days after operation.

Although the absolute serum gastrin response to the meal was higher after fundusectomy, (Figure 11, left panel) the increment of serum gastrin over basal was not changed because of the high basal gastrin concentration after the operation. (Figure 11, right panel).

EFFECT OF SOMATOSTATIN AND VIP INFUSION ON MEAL TEST RESPONSE:

Acid Secretion: Both somatostatin (Figure 12) and VIP (Figure 13) infusion did not revert the meal stimulated HP response to prefundusectomy levels. With both peptides no significant difference was noted from the control (postfundusectomy) levels.

Gastrin Release: Similarly VIP infusion had no effect on gastrin release after a meal test when compared to control (postfundusectomy) levels (Figure 13, right panel). Somatostatin however resulted in significant lowering of the gastrin levels (Figure 12, right panel). Despite this, the gastrin response was still significantly higher than that of the prefundusectomy meal response.
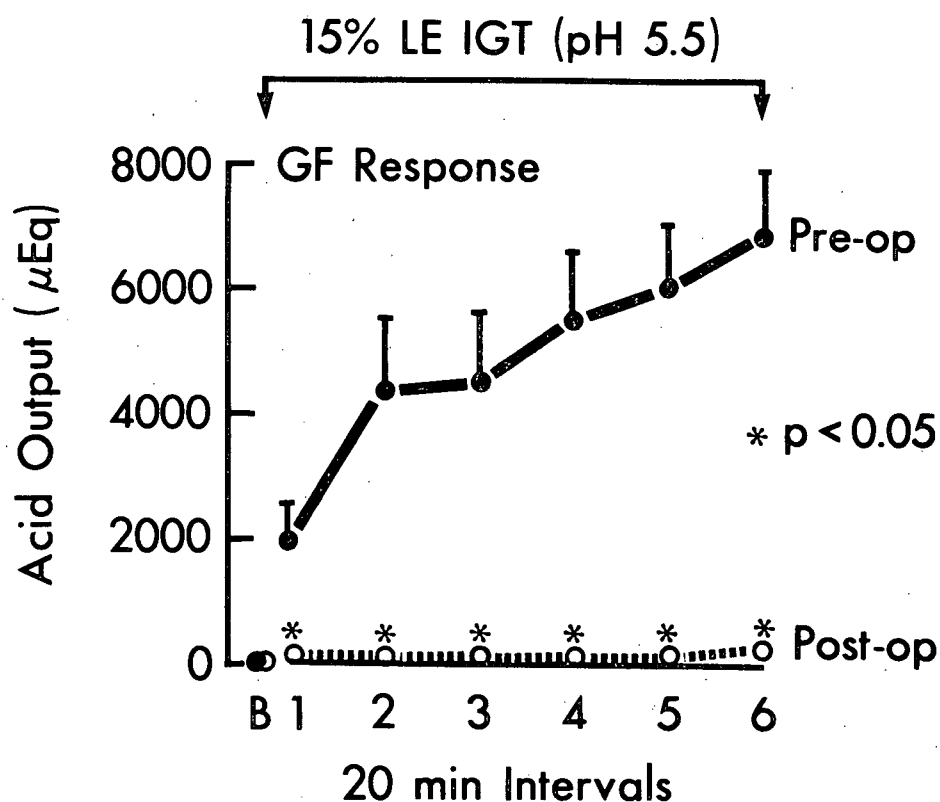
FIGURE 6: GF response to 15% liver extract meal by intragastric titration (pH 5.5) both preoperatively (closed circles) and postfundusectomy (open circles). In this and in each subsequent figure, each point represents the mean (±SE) of two experiments in each of four dogs.
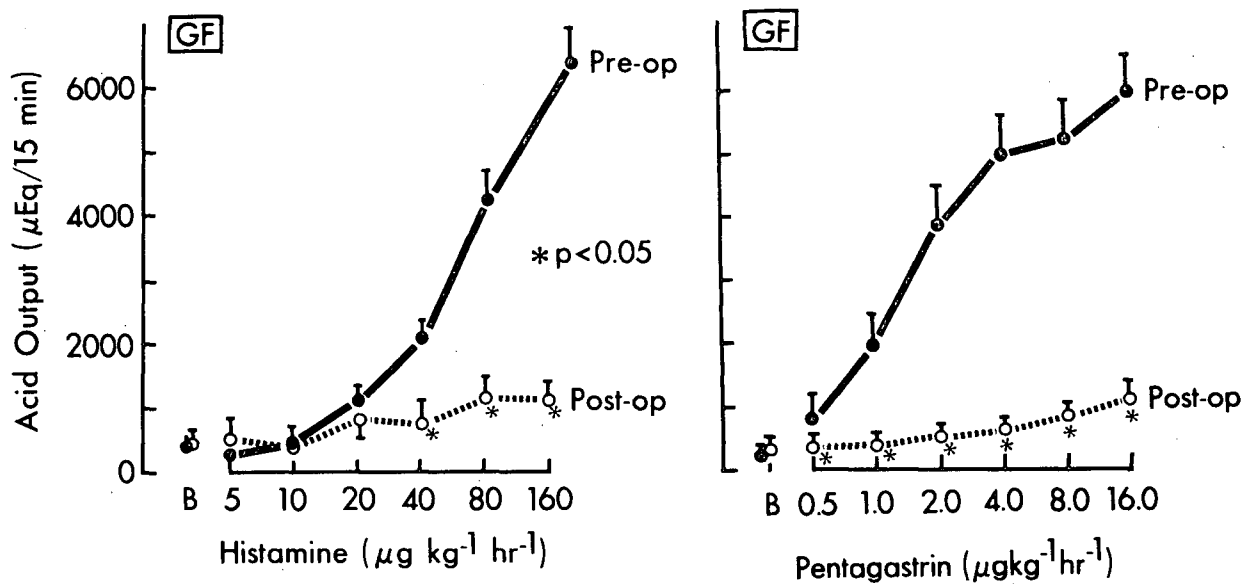
FIGURE 7: The effect of graded doses of histamine (left panel) and pentagastrin (right panel) on GF acid response before (closed circles) and after excision of the oxyntic cell mucosa (open circles).
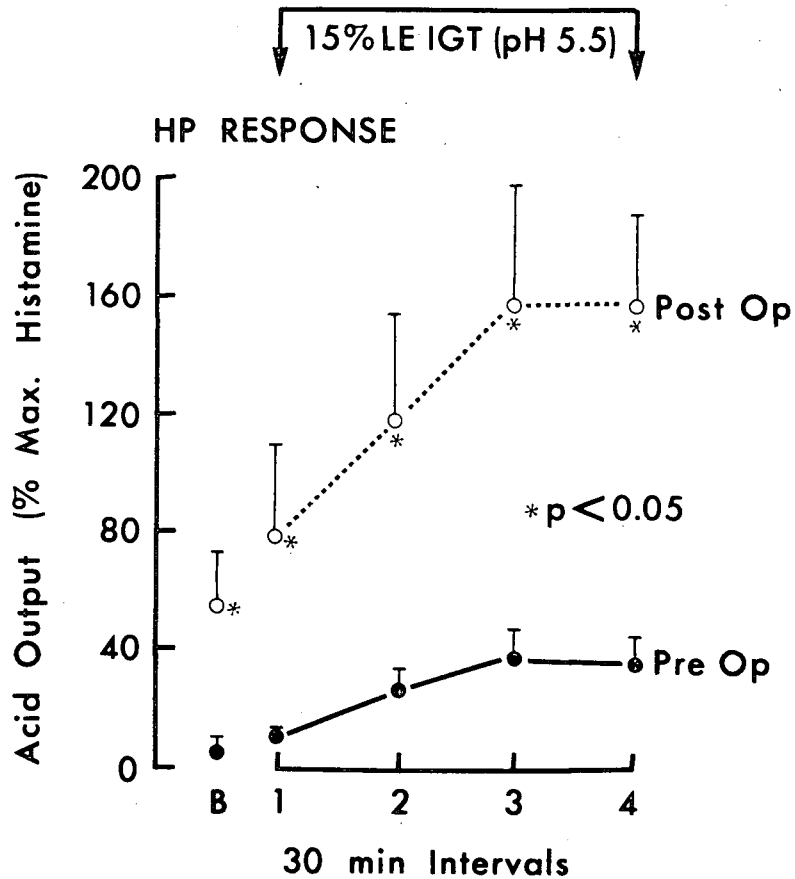
FIGURE 8: Heidenhain pouch response to a 15% liver extract meal. The pH is maintained constant at pH 5.5 throughout the test by intragastric titration. A significant increase in both basal and meal stimulated response is seen in the fundusectomized animals (open circles) as compared to the preoperative controls (closed circles).
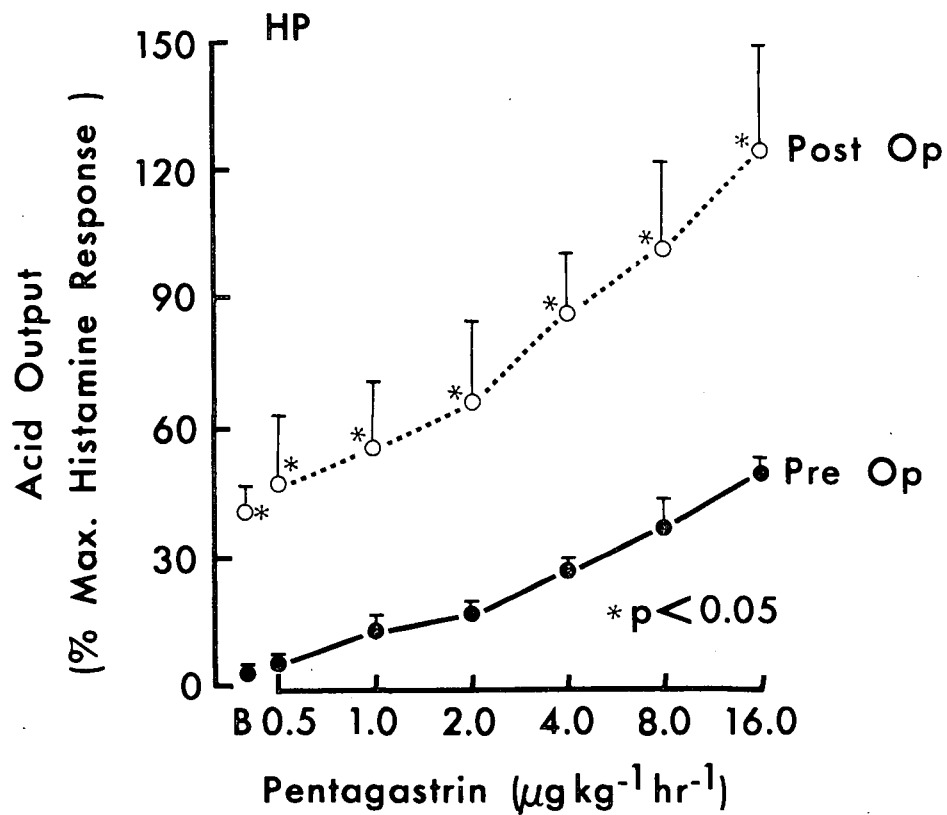
FIGURE 9: Heidenhain pouch response to graded doses of pentagastrin infusion both before (closed circles) and after excision of the oxyntic mucosa (open circles).
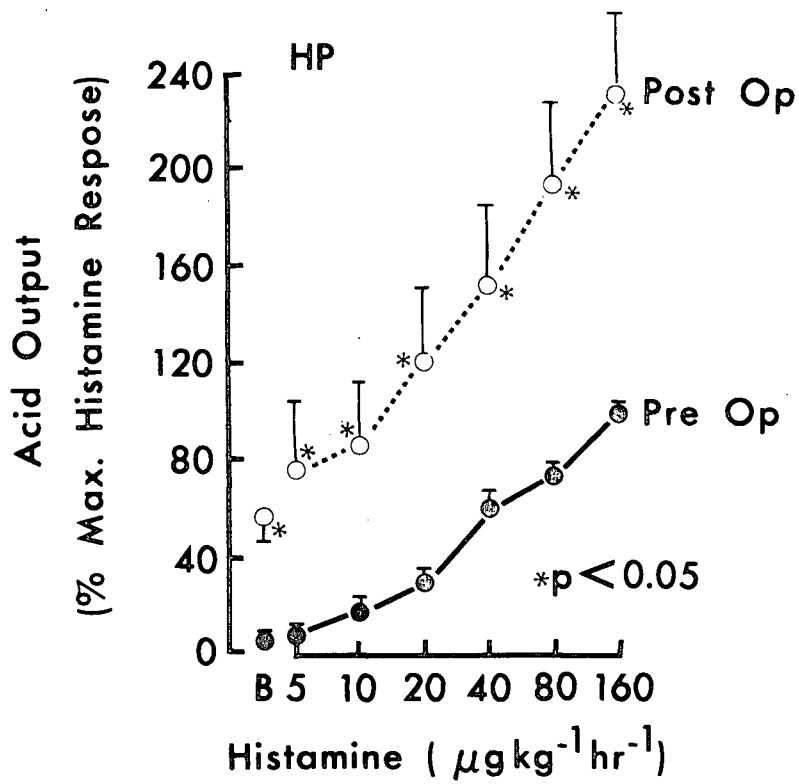
FIGURE 10: HP response to graded doses of histamine
dihydrochloride both preoperatively (closed circles)
and after excision of the oxyntic mucosa (open
circles).
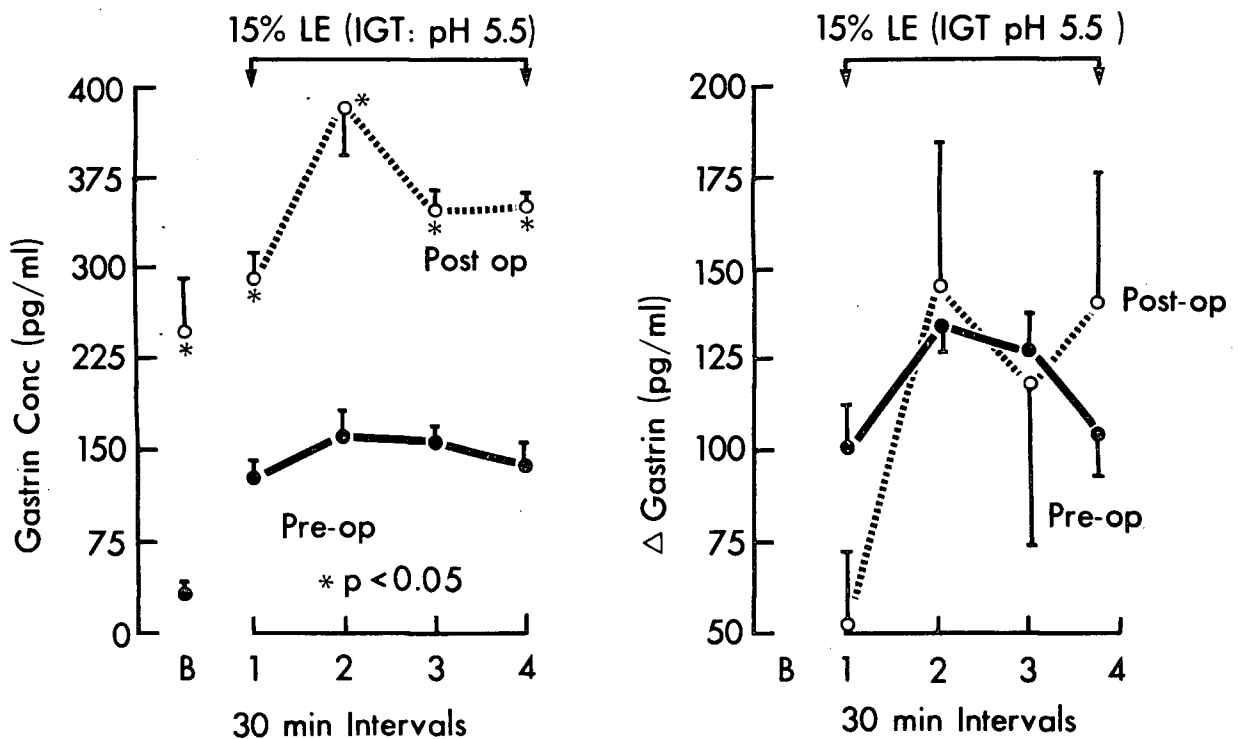
FIGURE 11: <u>Left Panel</u>: Gastrin response to a 15% liver extract meal both preoperatively (closed circles) and following excision of the oxyntic mucosa (open circles). pH was maintained constant (5.5) throughout all meal tests by the method of intragastric titration

<u>Right Panel</u>: Gastrin increment over basal during the 15% liver extract meal preoperatively (closed circles) and postoperatively (open circles).
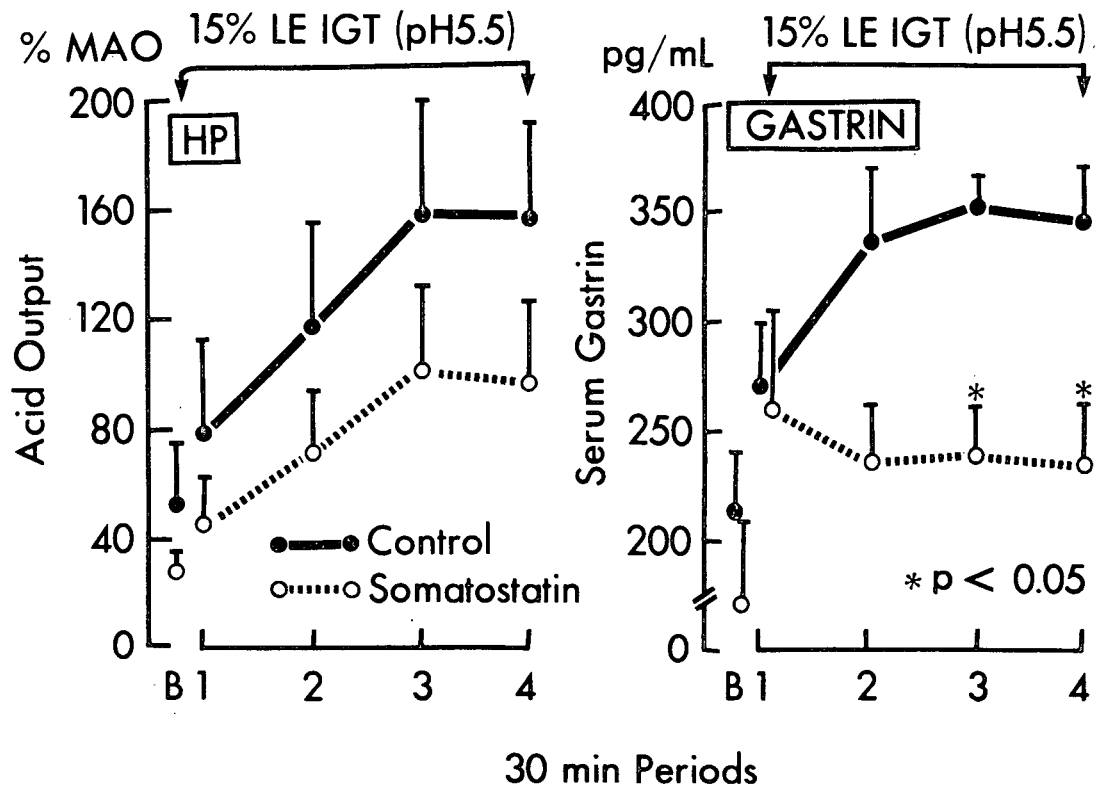
FIGURE 12: Heidenhain pouch (left panel) and serum gastrin
response (right panel) to a 15% liver extract meal in
fundusectomized animals with (open circles) and without
(closed circles) infusion of 0.5 µg kg^{-1} hr^{-1}
somatostatin.
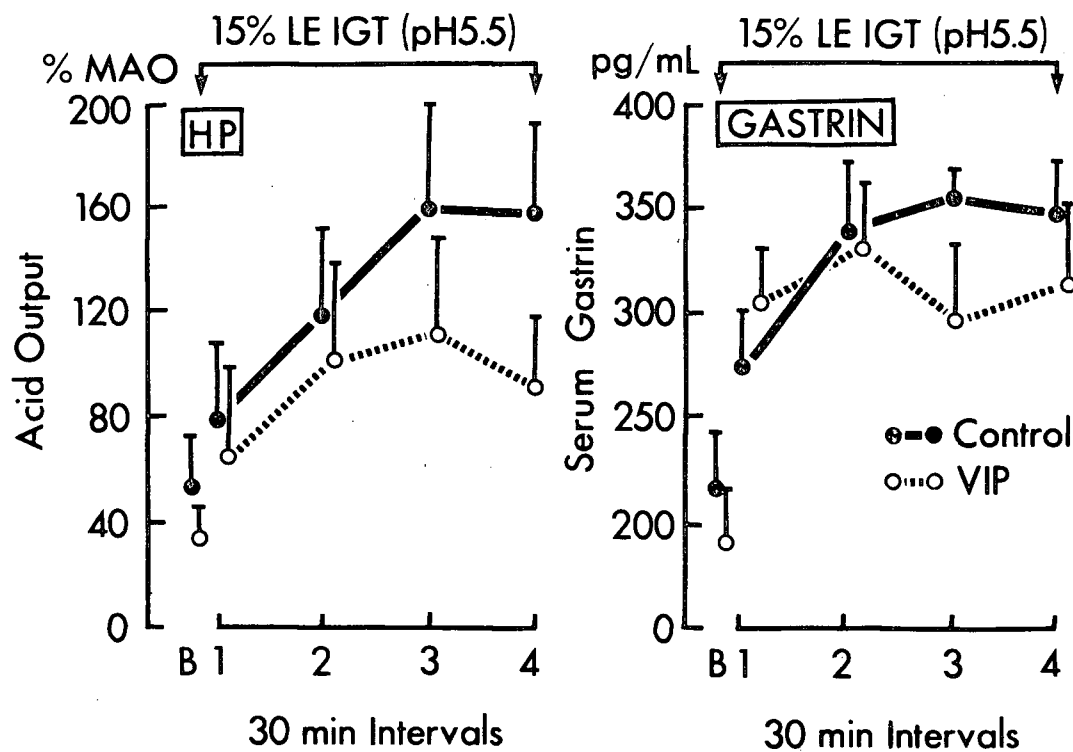
FIGURE 13: Heidenhain pouch (left panel) and serum gastrin
response (right panel) to a 15% liver extract meal in
fundusectomized with (open circles) and without (closed
circles) infusion of 1.0 μg kg^{-1} hr^{-1} VIP.

DISCUSSION:

This study has shown that excision of the oxyntic cell mucosa
of the main stomach causes marked elevations both in basal serum
gastrin and basal HP acid secretion. In addition, there is marked
enhancement in the response to meal, and to pentagastrin and
histamine. These results are consistent with the presence of
tonic and phasic fundic inhibition of acid secretion and/or
gastrin release.

Loss of phasic fundic inhibition following excision of the
oxyntic cell mucosa is a possible explanation for the increase in
HP acid output following a meal. This elevated response cannot be
due to elevations in pH in that the intradigestive pH changes were
eliminated by the method of intragastric titration. Is this
postulated inhibitor acting directly on the parietal cell,
(parietal cell inhibitor) or indirectly, by inhibiting gastrin
release (G-cell inhibitor)? The design of our study does not
allow us to definitively answer this question. However, despite
the significant increase in gastrin concentration following the
meal in the postfundusectomy dogs, we find no significant change
in the increment in gastrin over basal. This suggests that the
elevation in the HP is as a result of loss of inhibition of the
parietal cell directly.

Evidence for removal of a tonic fundic inhibitory
substance(s) of acid secretion and gastrin release is supported by
the significant elevation in basal acid output and basal gastrin

levels noted following excision of the oxyntic cell mucosa. A second explanation for this finding could be that the hypochlorhydria that follows these operations may remove the negative feedback control of gastrin release resulting in basal hypergastrinaemia and subsequently increased basal HP output. Both mechanisms may be involved. However all basal gastrin levels were measured within 23 days of the operation - whether G-cell hyperplasia could occur within this period of time to account for the 688% increase in basal gastrin and 909% increase in basal HP output is unknown. To date there are no studies reported on G-cell turnover in dogs, but Lehy and Williams[28] reported that in mice the G-cell turnover time was 2 to 4 months.

The increased sensitivity of the HP to exogenous pentagastrin and histamine also suggests that fundic inhibition requires tonic activity.

The observation that the HP response to submaximal doses of pentagastrin and histamine is increased, suggests that the sensitivity of the oxyntic cell to these stimuli is increased. While the increased sensitivity to histamine can be explained by the presence of basal hypergastrinaemia following excision of the fundic mucosa, the increased sensitivity to pentagastrin cannot be explained on the same basis. The results suggest that an inhibitor(s) of acid secretion has been removed. Glucagon, somatostatin and VIP are candidate inhibitors that may have been removed by excision of the oxyntic cell mucosa. It appears unlikely that the removal of the latter two peptides can explain

the loss of inhibition since exogenous infusion of these peptides
in high doses did not revert the HP acid output nor the serum
gastrin levels to that of the prefundusectomy state in response to
a meal. While we have not excluded glucagon, the possibility
remains that a completely new hormone, yet to be identified, is
responsible for these changes.

The observation that the maximal histamine response is
increased indicates that the parietal cell mass in the HP has
increased and is compatible with the hypothesis that either a
fundic factor important in the trophic regulation of the parietal
cell has been removed or the resultant hypergastrinaemia has
produced a marked trophic effect on the HP. There is growing
evidence that gastrin has such a trophic action - chronic
administration of pentagastrin to rats results in increased
parietal cell population,[29,30] and in man, patients with the
Zollinger-Ellison syndrome have a higher parietal cell mass.[31]
The design of our study does not allow us to choose between these
factors. Indeed, both factors ma;y be important.

An important question as yet unanswered by this study is the
role of the vagus in the release of this fundic inhibitory
substance. VIP, somatostatin and substance P have been identified
in vagal nerve endings. The question thus arises: Is this
inhibitory substance released by the fundic mucosa (endo or
paracrine), and if so, is vagal activity required for its release
or is this inhibitory substance(s) released directly from the
vagal ending supplying the fundus (neurocrine). With regard to

fundic inhibition of acid secretion, (parietal cell inhibitor) the
fact that the HP is affected suggests that this inhibitor is
endocrine (humoral) rather than para or neurocrine. Our study
however does not enable us to conclude the same for the fundic
inhibitor of gastrin release from the antrum (G-cell inhibitor).

In summary, we have shown that within the fundic mucosa may
reside substance(s) which both tonically and phasically inhibit
acid secretion and gastrin release. Glucagon, VIP and
somatostatin are candidates for these substances. Our study
suggests that neither VIP nor somatostatin subserve this
inhibitory mechanism, but does not exclude the possibility that
glucagon or another as yet unidentified substance(s) may be
involved. In addition to its role as inhibitor of secretion, this
fundic substance(s) may also be trophic to the parietal cell
mucosa.

PART III: THE ANATOMY OF VAGAL INHIBITION OF GASTRIN RELEASE

INTRODUCTION

We have provided evidence that inhibitory substance(s) of acid secretion and gastrin release reside in the fundus. The role of the vagus in the release of these substances requires clearer definition.

The vagus both stimulates and inhibits gastrin release. Evidence for vagal inhibitory fibres was provided by Farooq and Walsh[32] who showed that atropine enhanced the gastrin response to hypoglycaemia in man. Since the pH in the stomach was held constant, this enhancement could not have been promoted by alkalinity. Similarly, atropine enhances gastrin release stimulated by food.[33,34] Further evidence for these inhibitory fibers is provided by the findings that selective cooling of the cervical vagi results in increased gastrin response to insulin hypoglycemia,[35] that sham feeding inhibits pentagastrin-stimulated acid secretion in dogs,[36] and that truncal vagotomy increases basal,[22,23,24,25] food,[24,25] and insulin-stimulated[23] gastrin release. This postvagotomy hypergastrinemia occurs independent of change in pH in both man[25] and dog.[26]

The release of gastrin by vagal stimulation represents therefore a balance between the effects of stimulatory and inhibitory fibers. A clearer definition of both the anatomic distribution and the physiologic role of the vagus with respect to the control of gastrin release is required. This study aims to do this by investigating the effect of atropine and truncal vagotomy

on the secretory patterns in fundusectomized animals. The results of our study suggest that the inhibitory mechanism of gastrin release is mediated by vagal fibers supplying the fundus and that the stimulation of gastrin release is mediated by vagal fibers supplying the antrum. In addition evidence is supplied for the presence of a nongastrin stimulant of acid secretion in dogs.

PURPOSE

To define the role of the vagus in the fundic inhibitory mechanism by investigating the effect of atropine and truncal vagotomy in fundusectomized animals, on acid secretion and gastrin release in response to endogenous and exogenous stimuli.

MATERIAL AND METHODS

ANIMAL PREPARATION:

Three dogs each prepared with a gastric fistula (GF) and Heidenhain pouch (HP) underwent control studies before excision of the oxyntic cell mucosa was performed as described previously. The studies were repeated following recovery from the operation. In addition, the effect of atropine on the postfundusectomy response was studied. Following completion of these studies bilateral transthoracic truncal vagotomy was performed.

EXPERIMENTAL DESIGN:

The dogs were fasted of food but not of water for 18 hours before each test. No tests were done within 48 hours of each other.

A. ### CONTROL STUDIES:

(1) Meal Test by Intragastric Titration: The GF was opened, and the inside of the stomach washed with tap water. Two 15-minute basal collections were obtained from the GF and the HP. The techniques of acid collection and calculation of acid output were the same as previously described. During each intragastric titration test venous blood was obtained at -15, 0, 30, 60, 90 and 120 minutes. After clotting the serum was separated by cold

centrifugation and stored at -40°C until assayed for gastin.

(2) <u>Pentagastrin Dose Response Study</u>: The acid response from the GF and HP to graded doses of pentagastrin was studied as described before.

(3) <u>Histamine Dose Response Study</u>: Similarly the effects of graded doses of histamine dihydrochloride was assessed as described previously.

B. <u>THE EFFECT OF FUNDUSECTOMY</u>:

Following excision of oxyntic cell mucosa, the control studies above were repeated. In addition the effect of atropine on the meal test was assessed.

<u>Effect of Atropine on Meal Test</u>: Two 15-minute basal collections were obtained from the GF and HP. Atropine sulphate (0.2 mg/kg) (Galaxo, Toronto) was then injected subcutaneously. Thirty minutes after atropine injection the meal test was commenced. Acid and blood collections were performed as in the control study.

C. <u>THE EFFECT OF TRUNCAL VATOTOMY</u>:

Following completion of the above studies, truncal vagotomy was performed. Three weeks were allowed for recovery from surgery. The following tests were then performed:

(1)   meal test by intragastric titration

(2)   pentagastrin dose response studies

(3)   histamine dose response studies.

DETERMINATION OF SERUM GASTRIN CONCENTRATION:

This has been described in detail previously.

STATISTICAL SIGNIFICANCE

The significance of the difference of the mean acid output and gastrin responses during the control and postoperative experiments was analysed using a t-test for unpaired values. A p-values of less than 0.05 was considered significant.

RESULTS

A. <u>EFFECT OF FUNDUSECTOMY</u>:

(1) <u>Meal Response</u>:

<u>HP Acid Output</u>: Fundic mucosal excision resulted in a significant increase in both basal and meal stimulated acid secretion from the HP (Figure 1, left panel), as seen in Part II.

<u>Gastrin Response</u>: Similarly a significant increase in basal and meal stimulated gastrin release occurred following the operation (Figure 1, right panel).

(2) <u>Pentagastrin and Histamine Dose Response Studies</u>: The HP acid output in response to exogenous pentagastrin and histamine increased significantly after excision of the oxyntic mucosa (Figures 2 and 3).

B. <u>EFFECT OF ATROPINE SULPHATE ON MEAL RESPONSE AFTER FUNDUSECTOMY</u>:

<u>HP Acid Output</u>:

The HP response in the fundusectomized animals in response to a meal was significantly decreased (74%) after subcutaneous atropine injection. The peak secretion

decreased from an output of 198±51% maximal histamine acid output (MAO) before to 52±21% after atropine injection (Figure 4, left panel). The effect of atropine was to return the meal response of the HP to the prefundusectomy level (peak acid output 46±10% MAO).

Gastrin Release:

The elevated gastrin response to the meal seen in the fundusectomized animals persisted after atropine sulphate injection. No further elevation in gastrin response was noted, the response in the fundusectomized dogs with atropine showing no significant difference from those without atropine (Figure 4, right panel).

C. EFFECT OF TRUNCAL VAGOTOMY AFTER FUNDUSECTOMY

(1) Meal Test by Intragastric Titration:

HP Acid Output: No significant difference was noted between the meal responses of the fundusectomized dogs before and after truncal vagotomy (Figure 5, left panel), the peak response being virtually identical (198±51% maximum histamine acid output compared to 198±48% respectively).

In the fundusectomized animals, the effect of atropine compared to the effect of truncal vagotomy

differed signficantly, the peak response being 42±17%
and 198±49% maximal histamine acid output respectively.

Gastrin Release: After truncal vagotomy in the
fundusectomized dogs the gastrin response was still
significantly elevated when compared to the
prefundusectomy study (Figure 5, right panel). However
this elevation in gastrin release was significantly
lower than in the fundusectomized animals before truncal
vagotomy.

This drop in serum gastrin levels after truncal
vagotomy (Figure 5, right panel) was not accompanied by
a corresponding drop in HP output (Figure 5, left
panel), suggesting the presence of a nongastrin
stimulant of acid secretion.

When compared to the effect of atropine, both HP
acid output and gastrin release to a meal significantly
differed.

(2) Pentagastrin and Histamine Dose Response Studies

Both the pentagastrin and histamine dose response
studies in the fundusectomized animals did not not
change after truncal vagotomy (Figure 2 and Figure 3).
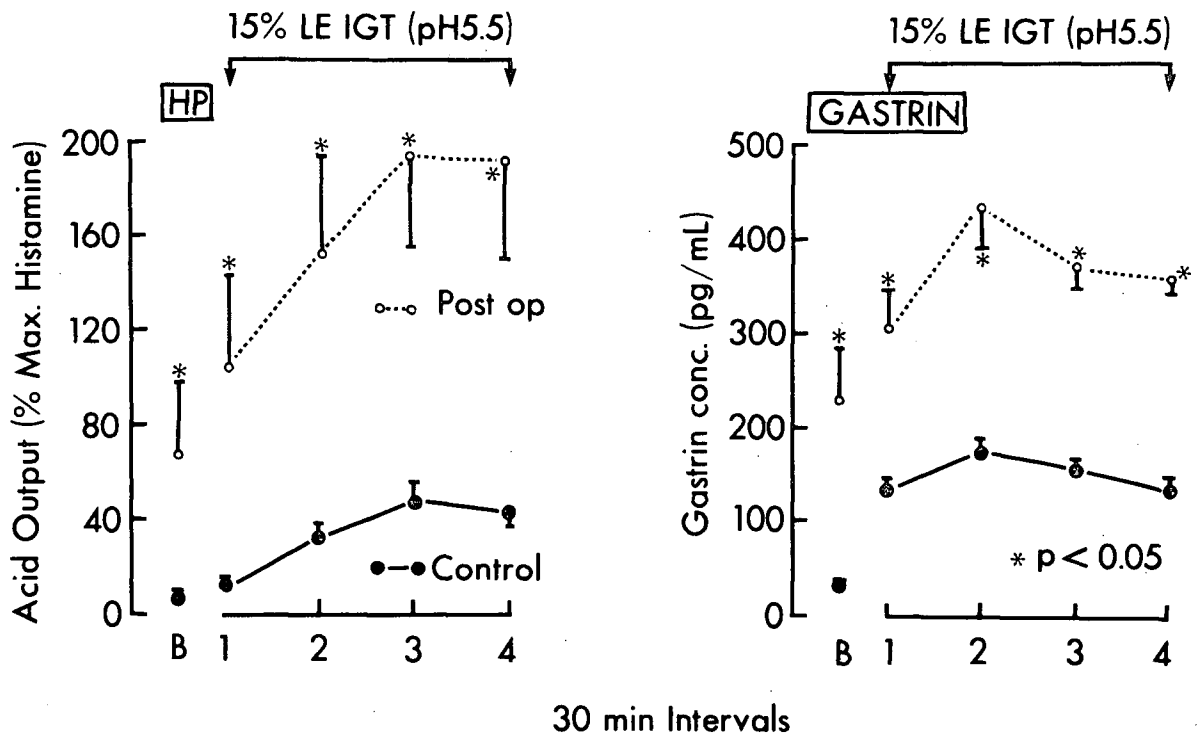
FIGURE 1:   Heidenhain pouch acid output (left panel) and gastrin
             response (right panel) after a 15% liver extract meal
             by intragastric titration. Each point represents the
             mean±SE of two experiments in three dogs before
             (closed circles) and after (open circles) fundic
             mucosal excision.
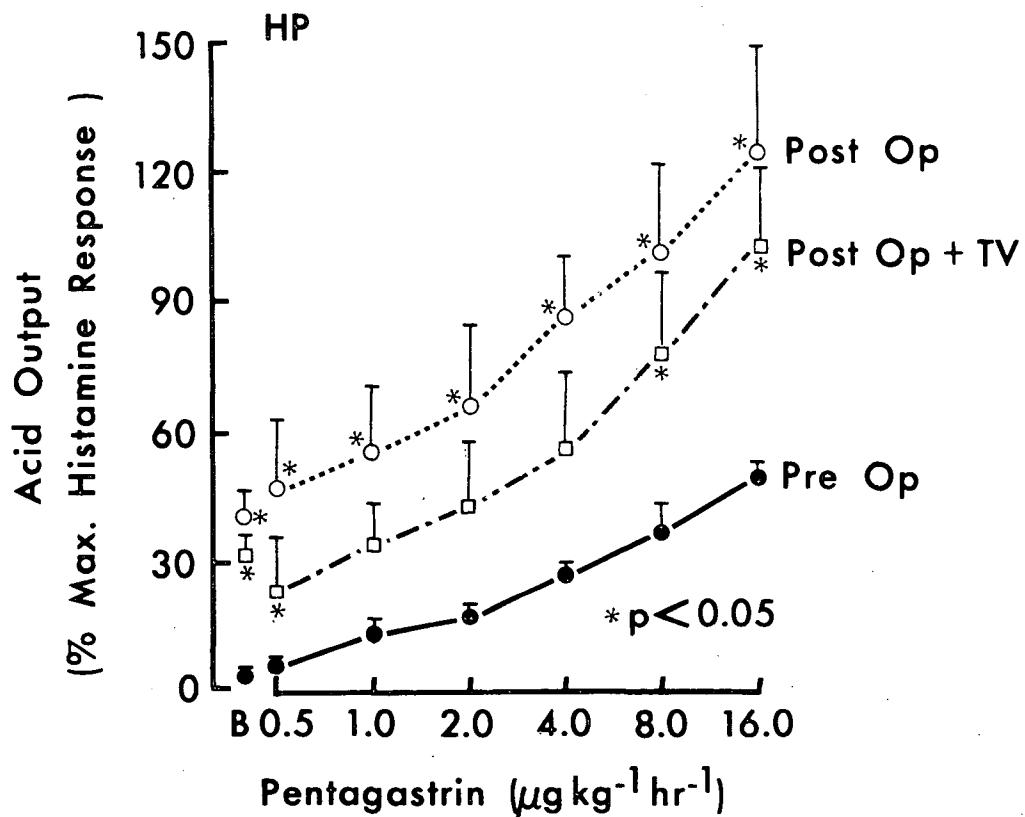
FIGURE 2:   Effect of graded doses of pentagastrin on HP acid
            response in control studies (closed circles), in
            fundusectomized animals (open circles) and following
            truncal vagotomy in fundusectomized animals (open
            squares).
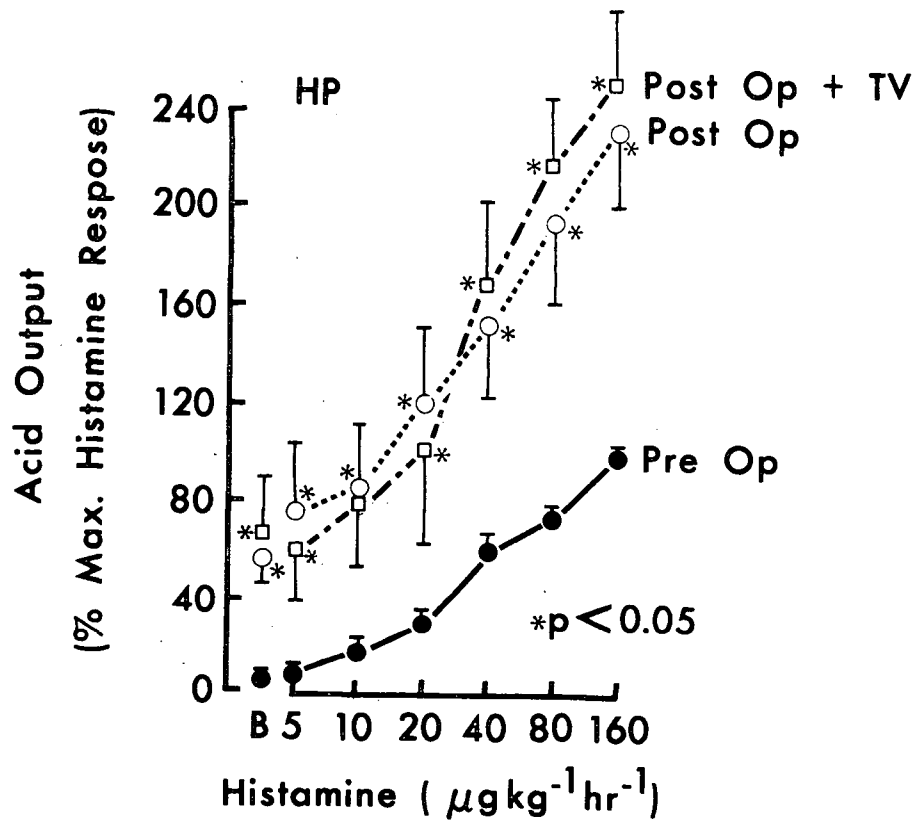
FIGURE 3: Effect of graded doses of histamine dihydrochloride on HP acid response in control studies (closed circles), fundusectomized animals (open circles) and following truncal vagotomy in fundusectomized animals (open squares).

15% LE IGT (pH5.5)

HP

Acid Output (% Max. Histamine)

200

160

120

80

40

0

B   1   2   3   4

*
*
*
*
*

o···o Post op

o···o Post op +
Atropine

•—• Control

15% LE IGT (pH5.5)

GASTRIN

Gastrin conc. (pg/mL)

500

400

300

200

100

0

B   1   2   3   4

*
*
*
*
*
*
*
*
*
*

* p < 0.05

30 min Intervals

FIGURE 4:   Heidenhain pouch acid output (left panel) and gastrin
response (right panel) after a 15% liver extract meal
in control studies (closed circles), following fundic
mucosal excision (open circles) and following atropine
injection in postfundusectomy dogs (open circles).
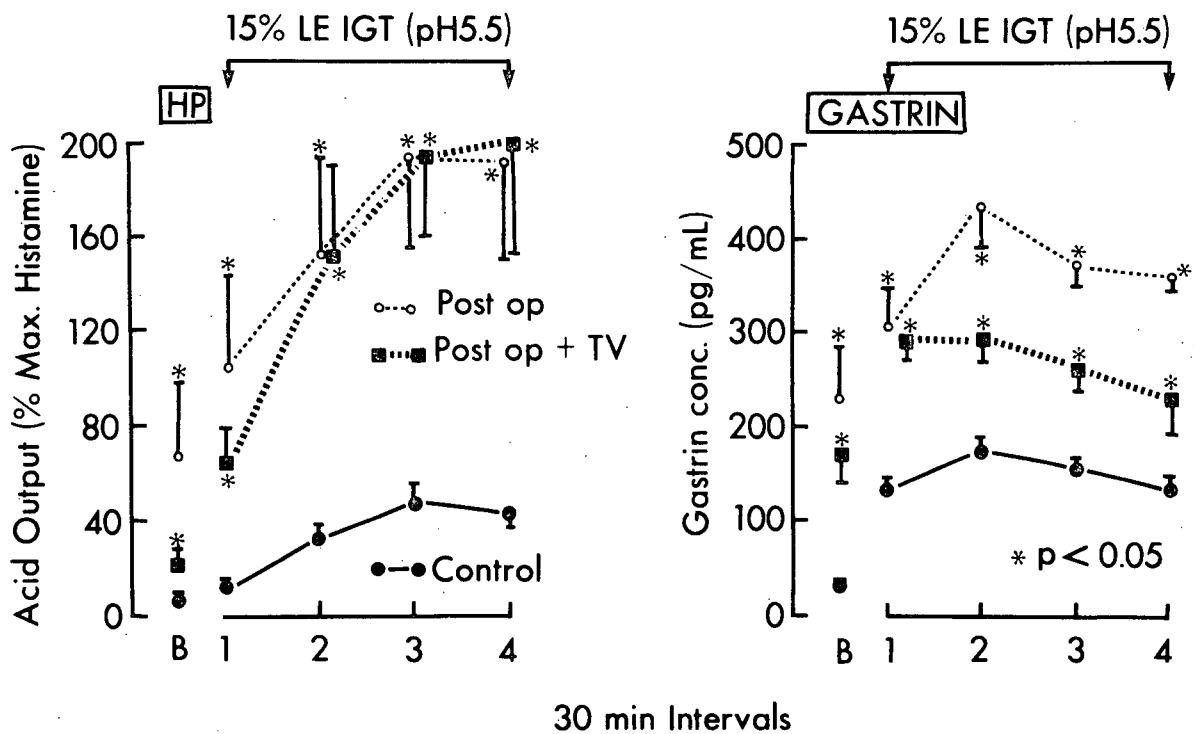
FIGURE 5: Heidenhain pouch (HP) (left panel) and gastrin response (right panel) to a 15% liver extract meal in control studies (closed circles), following fundic mucosal excision (open circles) and following truncal vagotomy after fundusectomy (closed squares).

DISCUSSION

Excision of the oxyntic mucosa resulted in increased Heidenhain pouch response to meal and exogenous pentagastrin and histamine. These results are the same as those seen in Part II. Similarly, an increased gastrin response to the meal occurred. These findings confirm the presence of a fundic inhibitory mechanism of acid secretion and gastrin release.

The enhanced gastrin response to a meal seen after excision of the oxyntic cell mucosa was neither decreased nor increased further by systemic atropine. Th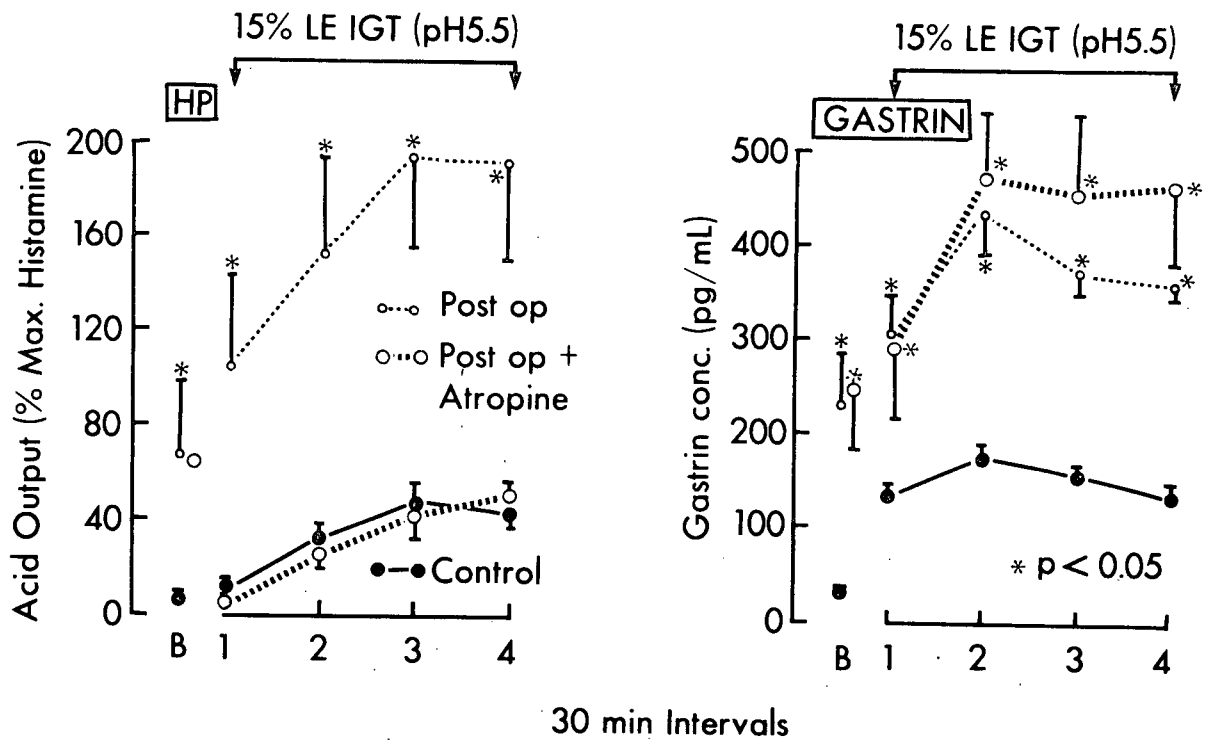is suggests that the enhancing effect of atropine on food stimulated gastrin release noted by previous investigators[33,34] may be the result of loss of fundic inhibitory mechanisms requiring vagal, atropine-sensitive innervation.

Following fundusectomy atropine reverted the HP acid response to a meal to control levels but failed to abolish acid secretion completely. This finding is at variance with previous work in which atropine was found to abolish acid responses of gastric pouches to feeding.[34,40] A possible explanation for this discrepancy is the presence of a nongastrin stimulator of acid secretion, now unmasked by fundusectomy, which is atropine resistant. We cannot exclude the possibility that this meal stimulated HP response after atropine reflects an increased parietal cell mass.

Our studies showed that truncal vagotomy does not change HP response to meal, to pentagastrin or to histamine in fundusectomized dogs. In nonfundusectomized dogs, truncal vagotomy (TV) has been shown to enhance HP acid response. Bearing in mind that fundusectomy has already enhanced HP response to these stimuli, the lack of further effect of TV may indicate that denervation of the fundus may be responsible for the effect of TV in nonfundusectomized dogs. A study of the effect of parietal cell vagotomy on HP response to these stimuli is required to confirm this hypothesis. We have shown that the inhibition of acid secretion from the main stomach caused by antral distension with acid is abolished by parietal cell vagotomy. It is possible, but unproven, that the inhibitory effect of antral distension may be mediated by a reflex release of this inhibitor from the fundic mucosa.

The gastrin response to a meal in the fundusectomized dogs dropped significantly following truncal vagotomy. This implies that cutting the vagus nerves removes not only the inhibitory fibers to the fundus but also stimulatory fibers to the antrum. This supports the findings that in dogs the HP response to 2-deoxy-glucose, which presumably acts the vagal release of gastrin, was greatly decreased after vagal denervation of the antrum.[29] That atropine failed to inhibit the stimulatory fibers in response to food, suggest that the mechanism for gastrin release is noncholinergic. This concurs with findings of Csendes who showed that gastrin release by feeding is resistant to atropine.[34]
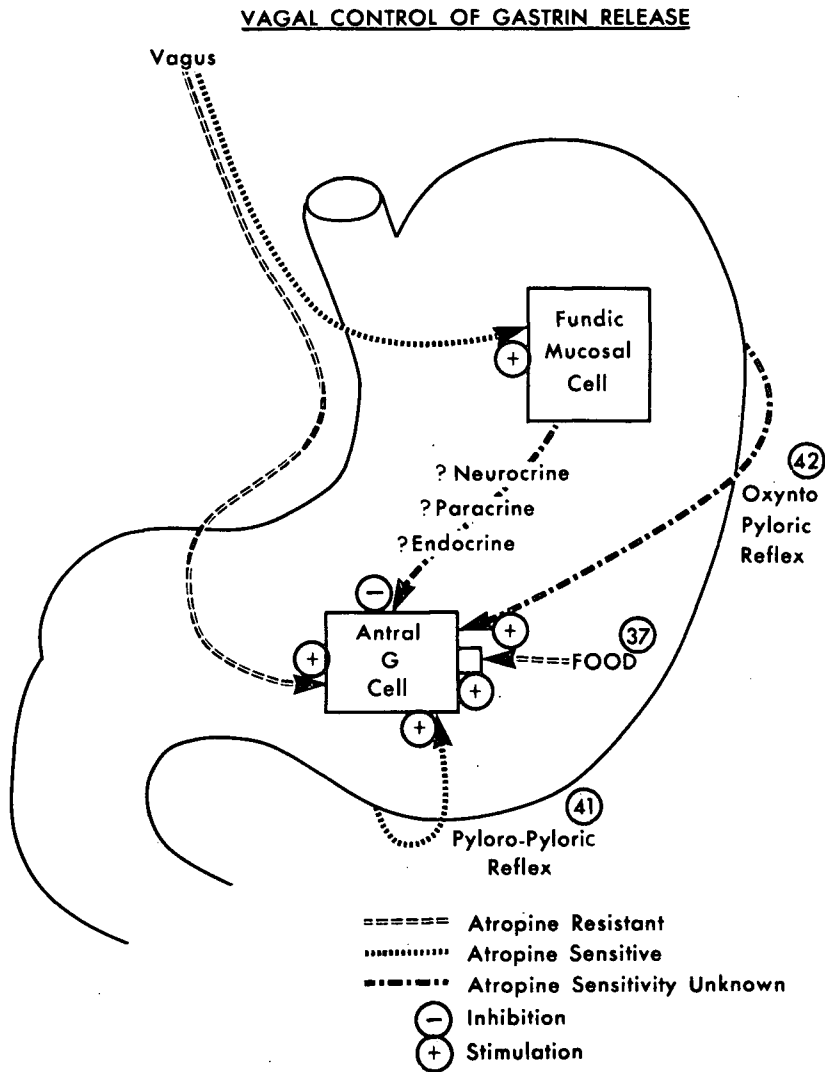
VAGAL CONTROL OF GASTRIN RELEASE

FIGURE 6:   Vagal control of gastrin release.

The amount of gastrin released by any mode of vagal stimulation is substantially smaller than can be released by food or topical acetylcholine in the antrum. This suggests that gastrin release represents a balance between the effect of stimulatory and inhibitory vagal fibers. Gastrin is released by direct vagal stimulation during the cephalic phase[38] and by both long and short neural reflexes,[41,42] as well as by direct chemical action of food on the G-cell durng the gastric phase.[37] Both atropine[33,34] and truncal vagotomy increases food stimulated gastrin release giving support to the presence of vagal inhibitory fibers.

The results of this study suggest that inhibitory mechanism of gastrin release is mediated by vagal fibers which supply the fundus and that the stimulatory action is mediated by vagal fibers which supply the antrum. The former are atropine sensitive, the latter atropine resistant. This hypothesis, in combination with the known mechanisms of gastrin release, allows the description of a model for the mechanism of gastrin release (Figure 6). This model offers an explanation for several findings. Parietal cell vagotomy results in hypergastrinemia in both man[25] and dog[26] which cannot be explained by changes in pH. A possible explanation is that the PCV removes inhibitory fibers which subserve a fundic inhibitory mechanism. Fundic mucosal excision might remove the same inhibitory agent. In man, increase in gastrin release in response to feeding is greater in proximal vagotomy than in selective vagotomy.[24] These findings can be explained by the hypothesis that inhibitory mechanisms of gastrin release mediated

by vagal fiber which supply the fundus and that the stimulatory
fibers of gastrin release supply the antrum.

Truncal vagotomy in the fundusectomized animal causes
significant decrease in meal stimulated gastrin release.
A corresponding fall in HP secretion does not occur. Indeed the
HP acid output is virtually identical before and after truncal
vagotomy. These findings are in keeping with the hypothesis that
removal of the fundic mucosa unmasks a nongastrin mechanism of
stimulation of acid secretion. Alternatively, this observation
might be explained that TV has interrupted extra-gastric vagal
fibers which subserve release of an intestinal inhibitor
(vagogastrone).[44]

In summary, these physiological observations have served to
define the anatomy of the distribution of vagal fibers to the
stomach. The inhibitory action of the vagus is mediated by
atropine-sensitive fibers that go to the fundus, while the
stimulatory action is mediated by atropine resistant fibers that
go to the antrum.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

This study provides evidence for a servo control mechanism between the fundus and the antrum. It supports the hypothesis that the fundus acts as an inhibitory organ.

The results of this study have shown that acid antral distention inhibits acid secretion from the main stomach. This inhibition is abolished by parietal cell vagotomy, suggesting that a pyloro-oxyntic vagal reflex mechanism is involved. Previous studies have shown that antral distension inhibits HP responses suggesting that a neurohumoral mechanism is involved. Thus, the antral inhibitory mechanism is in reality a fundic one.

Excison of the fundic (oxyntic cell) mucosa of the main stomach causes marked elevation both in basal and stimulated gastrin and HP acid responses. These results are consistent with the presence of substance(s) in the oxyntic cell mucosa which both tonically and phasically inhibit acid secretion and/or gastrin release. The fact that HP acid secretion is affected implies that the parietal cell inhibitor is humorally mediated. This study is unable to define whether the G-cell inhibitor is endocrine, paracrine or neurocrine in nature.

An increased HP response to maximal histamine stimulation implies an increased parietal cell mass. Whether this increase is due to hypergastrinemia or to loss of a trophic substance with removal of the fundic mucusa remains to be answered.

The results of the effect of atropine and truncal vagotomy on fundusectomized dogs support the hypothesis that the inhibitory mechanism of gastrin release is mediated by vagal, atropine sensitive fibers which supply the fundus and that the stimulatory action is mediated by vagal, atropine resistant fibers which supply the antrum. In addition, the findings suggests the presence of a nongastrin stimulant of acid secretion.

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