SOME EXPERIMENTS WITH GASTRIN EXTRACTS

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by

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C O N T E N T S

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PART I

INTRODUCTION

This thesis presents observations on several aspects of the action of gastrin extracts on gastric secretion of dogs. The experiments were undertaken in an attempt to gain further information on the mechanism by which this hormone stimulates the fundic glands, and to study the effects of various influences on the response to gastrin. Results of other experiments, chiefly those using histamine as a gastric secretory stimulant are also presented, where comparison seems relevant to interpretation of gastrin experiments, or is important in the formulation of a hypothesis.

The work involved in this thesis was largely done during the tenure of a United States Public Health Services Post-doctoral Research Fellowship, at the Department of Gastroenterology of the Veterans Administration Hospital, Los Angeles, California, and it is a pleasure, at the outset, to acknowledge the stimulus and advice of Dr Morton I. Grossman, Chief of Gastroenterology, from daily discussion in the laboratory.

HISTORICAL BACKGROUND TO GASTRIN

Several excellent reviews have traced the history of the humoral phase of gastric secretion from early hypothesis to more recent conclusive confirmation (Ivy[1], Grossman[2], Ivy, Grossman and Bachrach[3], Babkin[4], Woodward and Dragstedt[5], Peskin and Thompson[6], Grossman[7], Gregory and Tracy[8], Gregory[9]). Only the salient landmarks in this story will be discussed at present. Further historical background to individual aspects of gastrin action will be discussed in the appropriate sections of the thesis.

It was known to Pavlow[10] that the physical presence of food in the stomach, in experiments during which the dogs were asleep and therefore free of any possible psychic stimulation, resulted in the secretion of acid gastric juice. Pavlow[10], however, interpreted this as being purely a nervous reflex, probably mediated by vagal fibres. Although later work showed that gastrin will stimulate the fundic glands to secrete in the absence of all extrinisic innervation, it is interesting that recent work on the role of the vagus in gastrin release and action would seem to suggest that Pavlow's original belief was in part true.

The credit for the conception of a purely chemical phase of gastric secretion is given to Edkins[11], who in 1906 reasoned that a mechanism similar to that of secretin for pancreatic stimulation

(discovered by Bayliss and Starling[12] four years earlier) probably existed in the case of the stomach. He argued that, since the phase of stimulation would be likely to occur before the meal left the stomach, the source of the mechanism must lie proximal to the pylorus. Edkins[11], using anaesthetised cats with vagally denervated total stomach pouches, injected intravenously mucosal extracts of different areas of the stomach of cats and pigs, and found appreciable acid secretion from the fundic glands only in response to extracts prepared from pyloric gland area mucosa. However, a distinct reduction in systemic blood pressure occurred following the injection of each extract, and it is likely that histamine was present in all the preparations. Indeed Sacks et al.[13] isolated crystalline histamine from extracts prepared in a similar manner to Edkins[11] original ones.

A few months after Edkins' original article in 1906, Gross[14] from Pavlow's laboratory, published an account of some experiments in dogs which showed clearly that foods were most effective in evoking a gastric secretory response when selectively brought into contact with the mucosa of the pyloric gland area. He instilled meals of different quality through alternate limbs of an external tube gastro-duodenostomy, the pylorus being occluded. Having found a gastric secretory response only to intra-gastric instillation he further separated the pyloric gland area from the fundus and demonstrated

the response to stimulation of the former with food.

Thus it would appear that the two laboratories, working along similar lines concurrently, should share the credit for the initial evidence suggesting the presence of the chemical phase of gastric secretion.

Edkins[11] believed that the absorption of a specific food substance either alone or in combination with a further product in the gastric mucosa was responsible for the stimulation of fundic gland secretion. He further felt that the fundic gland area, being an actively secreting organ, would show no absorptive capacity, and therefore that the simpler histological structure of the pyloric gland area mucosa was more in keeping with absorption of the digestive products responsible for the initiation of the chemical phase of gastric secretion.

In 1908 Edkins and Tweedy[15], separating the fundic from the pyloric cavity by means of an ingenious balloon, demonstrated (i) that irrigation of the fundus caused no acid response, and (ii) that the following substances, in decreasing order of potency, evoked a fundic acid response when irrigated through the pyloric gland area:- meat extracts, dextrose, dextrin, and (surprisingly) 0.2% hydrochloric acid.

Although a number of workers reported being unable to confirm Edkins' experiments (Babkin[4], Ivy and Whitlow[16], Sacks *et al.*[13]) support for his findings came from two sources, Maydell[17] and Lim[18],

who alone administered their pyloric gland area extracts intravenously as Edkins had done. The others used the subcutaneous route, which, as will be discussed in the review of extraction procedures, appears to have been inadequate with earlier crude preparations. It is of interest that, although he had precisely duplicated Edkins' results, Lim[18], failing to obtain a gastric secretory response from the transfusion of blood from fed dogs, concluded that his experiments did not in fact support Edkins' "hypothesis". He suggested that gastrin activity might be a property of mucus, and that the pyloric gland area was a rich source of "gastrin" simply by reason of its relatively high mucus cell content.

The final proof of a humoral mechanism originating from the pyloric gland area came from a laboratory (Ivy and Whitlow[16]) which had earlier challenged the accuracy of Edkins' observations. Ivy and Farrell[19] in 1925 clearly showed that an autotransplanted fundic pouch secreted hydrochloric acid in response to a meal in the main stomach. As evidence accumulated that a variety of chemical substances, chiefly meat extractives and products of protein digestion, were capable of stimulating the pyloric gland area (Farrell[20]) it became less likely that these substances were acting as secretagogues after absorption. Meat extracts free of histamine (Kim and Ivy[21], Butler, Hands and Ivy[22]) were found to be as effective as cruder preparations, thus ruling out dependence on histamine content.

The finding that a greater acid secretory response followed the local instillation of these extracts into the pyloric gland area than followed intravenous injection (Kim and Ivy[21], Butler, Hands and Ivy[22]) indicated that not all the stimulatory action could be accounted for by direct absorption.

There followed the demonstration by Zeltony and Savich[23] and later by Gregory and Ivy[24] that the stimulating effect of known excitants of the pyloric gland area could be prevented by the local application of cocaine or procaine to the pyloric gland area mucosa. Gregory and Ivy[24] also showed that procaine had no effect on the response to secretagogues introduced into the small intestine, suggesting that these, by contrast to pyloric gland area stimulants, did cause gastric secretion by action after absorption. This finding of blockade of pyloric gland stimulation by local anaesthetics was one of the two main pieces of evidence which led to the acceptance of the "gastrin hypothesis" as a true hormonal mechanism. The other evidence was the clear demonstration by Grossman, Robertson and Ivy[25] that mechanical distention of a pyloric gland area pouch induced acid secretion from a denervated fundic pouch, when either pouch was autotransplanted to a subcutaneous location.

In the meantime the clinical importance of the pyloric gland area as a source of gastric acid hypersecretion was being emphasized by several surgeons. The operations for duodenal ulcer described

by Devine[26], Finsterer and Cunha[27], and McKittrick, Moore and Warren[28] involved exclusion of the pyloric gland area from the pathway of the gastric contents. These procedures were designed to reduce the immediate mortality of duodenal ulcer surgery by avoiding dissection in the region of a large, oedematous, inflamed ulcer with the attendant risk of post-operative leakage or perforation. However, it was soon reported by several workers (Ogilvie[29], Graham[30], Wells[31], McKittrick, Moore and Warren[28]) that any form of "antral exclusion" was followed by a high incidence of recurrent ulcer, often within a very short time interval. Of greater importance was the observation (Ogilvie[29], Graham[30], Wells[31]) that such recurrences could be cured by subsequent excision of the pyloric gland area.

This contribution to the understanding of the gastrin mechanism underlines three important points; firstly, the value of critical clinical observation to the advancement of physiological knowledge; secondly that newly discovered physiological mechanisms must finally be confirmed in man, and thirdly, that a fundamental aim of physiological investigation is the application of the acquired knowledge to the treatment of disease.

In the last 15 years many workers, notably Dragstedt and his associates (Dragstedt *et al.*[32,33,34], Woodward, Bigelow and Dragstedt[35], Oberhelman, Rigler and Dragstedt[36]) have amply confirmed the chemical phase of gastric secretion arising from the pyloric gland

area. Baugh et al [37], who earlier had reported studies on the cell type involved in gastrin production, have recently demonstrated by the ingenious technique of creating mucosal pouches of the pyloric gland area, that gastrin is released from the mucosal cells (Baugh and Gordon[38]).

IMPORTANCE OF VAGUS TO THE GASTRIN PHASE OF GASTRIC SECRETION

The influence of vagal innervation on the regulation and efficiency of the gastrin mechanism has been controversial for many years.

Interdependence between the two principal phases of gastric secretion, vagal and hormonal, was first proposed by Uvnäs[39], who found that excision of the pyloric gland area reduced the response of the fundic glands of anaesthetized cats and dogs to electrical stimulation of the vagus nerves in the neck. He concluded from this and other parallel observations that neither the vagal nor the gastrin mechanism can operate to the full extent in the absence of the other. This view was supported by Thomas[40], and by a recent study of gastric secretion in duodenal ulcer patients by Gillespie et al.[41]. Babkin et al.[42], however, were unable to duplicate Uvnäs' results.

In the consideration of this possible interrelationship several questions arise:

1. Can vagal impulses transmitted to the pyloric gland area cause gastrin release? That this is so seems confirmed by the recent work of Pe Thein and Schofield[43] who were careful to ensure that the pyloric gland area remained alkaline during vagal stimulation, which resulted in slight, but distinct secretion from a denervated fundic pouch.

2. What contribution does direct vagal stimulation of gastrin release make to "physiological" indices of gastric secretion, e.g. the 24-hour output, or the response to a meal?

Indirect evidence relating to this question comes from experiments in dogs with isolated, vagally innervated pouches of the pyloric gland area in addition to Heidenhain type denervated fundic pouches. Under these circumstances the pyloric gland area is not available to direct mechanical or chemical stimulation. The finding of unchanged or increased 24-hour secretion in this type of animal preparation reported by Forrest[44], Oberhelman, Rigler and Dragstedt[36], and Wohlrabe and Kelly[45], suggest that direct vagal release of gastrin is capable of contributing a considerable quantity of acid secretion to the total daily output. This possibility was further supported by the abolition of the hypersecretion after division of the vagal supply to the pyloric gland area noted by Forrest[44]. Oberhelman, Rigler and Dragstedt[36] also reported that the acid secretory response to a meal was greatly increased in this preparation.

However, whether direct vagal release of gastrin is a major contribution to normal digestive or interdigestive processes remains undecided. In these experiments the pyloric gland area mucosa was not exposed to acid gastric juice, and the significance of the elimination of acid inhibition of gastrin release, which affords an alternative explanation for these findings, cannot be assessed.

The role of low pH in the gastrin mechanism will be further discussed in PART III, Chapter 4.

3. Is intact vagal innervation of the pyloric gland area essential for the full efficiency of gastrin release by local mechanical and chemical stimuli?

On this point again there is disagreement among the results of various workers. Forrest[44], reported that the response of a Heidenhain pouch to irrigation of an isolated, innervated pyloric gland area pouch was decreased by section of the seromuscular bridge bearing the vagal branches to the pyloric gland area. He concluded that the vagus potentiated gastrin release. A similar view was expressed by Thaïl et al.[46], to explain the increase in 24-hour secretion of a Heidenhain pouch following tubular fundic resection - a procedure which preserved the vagal trunks coursing along the lesser gastric curvature. Since a similar extent of fundic resection accompanied by division of the lesser curvature, including the vagus, did not lead to hypersecretion of the Heidenhain pouch, they argued that the vagus increased the sensitivity

of the pyloric gland area to stimulation.

On the other hand, no difference in the response to pyloric gland area stimulation before or after vagal denervation was reported by Dragstedt et al.[34], Wohlrabe and Kelly[45], and Nyhus et al.[47].

4. Is intact vagal innervation to the fundic glands essential for the full secretory response to circulating gastrin?

There is suggestive evidence both in man (Stein and Meyer[48]), and in the dog (Orbeli[49]), that vagotomy reduces the acid secretory response to a meal, and this has been interpreted as being due to impaired gastrin release, as discussed in the preceding section. However, vagotomy has been shown to reduce the fasting secretion (Dragstedt et al.[32]) and that evoked by several stimuli, including maximal histamine dosage (Gillespie et al.[41]), and it seems likely that intact vagal innervation is required to enable parietal cells to fully respond to any kind of stimulus. The recent demonstration by Payne and Kay[50] that Mecholyl restores the post-vagotomy maximal histamine response to pre-operative levels emphasizes the permissive role of acetylcholine at post-ganglionic vagal nerve endings in the stomach, with regard to histamine stimulation. It also makes attractive the hypothesis of a similar vagal role in gastrin stimulation. Support for this hypothesis will be advanced in PART III, Chapter 8, which deals with potentiation by acetylcholine of the fundic gland response to injected gastrin extract.

5. Is the presence of the pyloric gland area essential for the full response to direct vagal stimulation of the fundic glands?

The findings of Uvnäs[39], to which reference has already been made (p.8), certainly suggest an affirmative answer. However, it is conceivable that excision of the pyloric gland area reduced the response to direct vagal stimulation by virtue of a large contribution of direct vagal gastrin release to the total secretory response.

The possibility of a certain "tone" of circulating gastrin playing a permissive role in the responsiveness of the fundic glands to histamine has been suggested by the finding that removal of the pyloric gland area in man reduces the maximal histamine response (Gillespie *et al.*[41]). Potentiation of the acid response to histamine by gastrin will be shown in PART III, Chapter 10. Since both acetylcholine and histamine would thus appear to potentiate gastrin, and the removal of the principal source of gastrin reduces the histamine response, it is perhaps likely that the response to acetylcholine, and therefore to vagal impulses, would similarly be reduced after removal of the pyloric gland area.

SUMMARY OF VIEWS ON VAGAL-GASTRIN INTERRELATIONSHIP

1. Gastrin can be released and can stimulate the fundic glands in the absence of all vagal innervation.

2. Vagal impulses transmitted directly to the pyloric gland area

can cause gastrin release. The contribution of this particular mechanism to the response to feeding is not clear.

3. Vagal denervation of the stomach may reduce gastrin release, and probably diminishes the responsiveness of the fundic glands to circulating gastrin.

4. Removal of the pyloric gland area may reduce the response of the fundic glands to direct vagal stimulation by withdrawal of a permissive role of gastrin.

HISTORICAL BACKGROUND TO GASTRIN EXTRACTION PROCEDURES

Edkins[11], in his original study, used several different simple procedures, but found boiling water or 0.4% hydrochloric acid to give the most satisfactory yield of a fundic gland stimulant on intravenous injection into anaesthetized cats.

After the identification of histamine in a variety of tissue extracts (Dale and Laidlaw[51], Barger and Dale[52], Dale and Laidlaw[53]), and the finding of Popielski[54] in 1920 that histamine possessed potent gastric acid stimulant properties, it was generally felt that any secretory response from Edkins' original extracts was solely due to contained histamine. That there almost certainly was histamine present in Edkins' extracts has been already stated (p.2-3), the suggestive evidence being the appreciable reduction in blood pressure following injection of the extract. However, as Grossman[2] has

pointed out, there was no clear parallelism between the fundic acid secretion and the fall in blood pressure, a comparable degree of which occurred also after the injection of extracts of fundic mucosa, without causing acid secretion. This and other indirect evidence suggested that histamine, though present in Edkins' extracts, was not the sole agent responsible for the acid secretory response. This question would seem to have been happily solved by Blair et al.[55] who have demonstrated that extracts of pyloric antral mucosa prepared in the manner used by Edkins are, in fact, rich in gastrin.

The next important progressive development in gastrin extraction came with Komarov's[56] recognition in 1938 that gastrin would probably be protein in nature, and that methods hitherto employed had discarded much or all of the protein fractions of the mucosa.

He used an initial extraction by boiling in 0.15N hydrochloric acid, and described several methods for further purifying and concentrating the gastrin content of this simple extract (Komarov[57,58]). The preparation could be filtered directly after cooling, or filtered after a gradual, partial neutralization to remove some of the inert protein material. Steps described to free the extract of "histamine, choline or other organic crystalloids" included precipitation with 10% trichloracetic acid or saline at either 30% or 10% concentration. Further purification could be effected by (a) fractionation with acetone,

the saline concentration being reduced to below 0.5%, and precipitation with trichloracetic acid, or (b) fractionation with methanol-ether.

The extracts were found to be virtually histamine-free and gave potent stimulation of fundic gland acid secretion in the anaesthetized cat. It is interesting that Komarov found gastrin-like activity present also in extracts prepared by the identical techniques from normal dog duodenum, greater in degree from the proximal segment. This will be discussed further in PART III, Chapter 8.

Uvnäs[59,60,61,62,63] described several further modified extraction processes. His contribution to the gastrin story is two-fold:-

1. The introduction of further steps to remove impurities and concentrate gastrin activity. Such were the following combinations of procedures: (a) precipitation by tannic acid, by 80% alcohol, by adjusting the pH to 8.0, and by trichloracetic acid; (b) precipitation by 10% trichloracetic acid, by 80% alcohol, isoelectric at pH 8.0, and again by 10% trichloracetic acid, with acetone and ether washes; (c) isoelectric precipitation at a range of pH values between 4.0 and 5.5, 5.0 being the optimal for activity, and 1-2 mg/100 ml. copper sulphate to improve the extraction rate.

2. The strong support which all the foregoing steps added to the acceptance of gastrin being protein in nature, since all were designed to precipitate protein.

Uvnäs' work indicated that gastrin had the following characteristics It was a protein of small molecular size, since it could be dialyzed through a cellophane membrane; it was soluble in water but insoluble in ether, acetone, benzene and 80% ethyl alcohol; it was stable in the refrigerator for over one year, and was more stable when slightly acid than when slightly alkaline; it was destroyed by pancreatic juice, to a lesser extent by pepsin, and also by ultra-violet light.

In the anaesthetized cat he repeatedly showed that his gastrin extracts, while strongly stimulating gastric secretion, had no effect on salivary secretion, blood sugar, blood pressure, gastric motility or bile flow.

Unlike Komarov, Uvnäs was unable to detect any gastrin-like activity in extracts of duodenal mucosa of animals, but did demonstrate activity in some specimens of human duodenal mucosa(63). Extract of all other segments of the alimentary tract contained no fundic gland stimulant.

An interesting observation made by Uvnäs was the occasional inhibition of the gastric response to an injection of gastrin extract when followed after a short interval by a second injection, particularly if the second dose was large(62). This phenomenon did not occur if the two injections were separated by a longer time interval. The significance of this observation will be discussed in PART III, Chapter 3. Harper(64) simply used acid alcohol, or 60% alcohol, to extract the

pyloric gland area mucosa, and after evaporating off the alcohol, precipitated the active principle by saturation with sodium chloride or the addition of bile salts and further alcohol extraction of the bile salt precipitate. The extracts displayed satisfactory gastrin activity in cats, and he also reported some acid stimulating activity in extracts prepared in this manner from mucosa of the upper small intestine.

Jorpes, Jalling and Mutt[65] in 1953 claimed to have prepared a more potent extract than previous workers by the following four steps.

1. The mucosa was boiled in 0.1N hydrochloric acid in 95% methanol, in which solution they found gastrin to be soluble.
2. Impurities were removed at pH 5 to 5.5.
3. The active principle was precipitated at pH 7.0.
4. Inorganic salts were removed by dialysis.

The most recent great advance in extraction technique has been the development by Gregory and Tracy[8] of a preparation which is effective in the conscious animal and in the human subject by intravenous, intramuscular or subcutaneous routes. All previous extracts were inactive by the subcutaneous route, which suggests that in these earlier extracts the active principle was almost certainly bound to a protein of larger molecular size, and thus unable to be absorbed from the subcutaneous site. The fact that the gastrin prepared by Gregory and Tracy was effective subcutaneously suggests a higher state of purity. Among the numerous steps introduced into their extraction procedure,

(148) BLAIR, E.L., CLARK, D.G., HARPER, A.A., LAKE, H.J. and SCRATCHERD, T. A gastric phase of pancreatic secretion in cats. J.Physiol. 157, 17-18P, 1960.

(149) WHITE, T.T., LUNDH, G. and MAGEE, D.F. Evidence for the existence of a gastropancreatic reflex. Amer.J.Physiol., 198, 725-728, 1960.

the more important ones, aimed at removing as much inert protein as possible were:

(i) the original extraction of the chopped mucosa with 70 to 80% aqueous acetone containing 4% trichloracetic acid,

(ii) the removal of inert protein at several stages in the process by precipitation at high pH, and

(iii) final purification by passage through a calcium phosphate gel column.

PART II

MATERIALS AND METHODS

GASTRIN EXTRACTION TECHNIQUE

Gastrin extracts were prepared from mucosa of the pyloric gland area of hogs by the following technique, developed jointly by Gregory, Tracy and Grossman as a modification of the method described by Gregory and Tracy[8] in 1961, and briefly discussed at the end of the last section.

The hog pyloric antra were obtained in batches of several hundred, placed in polyethylene bags and packed in ice at the abattoir. The mucosa was separated from the muscular tissue within a few hours of death, cut into small strips and frozen in 200 gram portions until used for extraction. For convenience one kilogram of mucosa was extracted at a time, and the further details apply to this quantity.

One kilogram of frozen mucosa was added to one litre of boiling water. With continued heating and gentle stirring the mucosa was thawed and the mixture brought to boil for one minute. The mixture was transferred to a Waring blendor of one gallon capacity and homogenized at high speed for two minutes. To the resulting suspension four litres of water were added and the mixture was boiled for ten minutes. After cooling to 25°C the suspension was filtered through a wire gauze strainer and the precipitate was discarded. The filtrate was centrifuged for

five minutes at 2000 r.p.m. and, after decanting the supernatant, the precipitate was discarded. The milky supernatant liquid was brought to pH 4.0 with glacial acetic acid and then allowed to stand at 5°C for 16 to 24 hours, during which time a heavy precipitate settled to the bottom of the container. The cloudy supernatant liquid was discarded by aspiration. The infranatant liquid containing the precipitate was centrifuged for 30 minutes at 2000 r.p.m. The opalescent supernatant liquid was discarded by aspiration. The semi-fluid precipitate was transferred to a one litre graduated cylinder and made up to the nearest 100 ml. (usually 600 to 700 ml.) with water. To a volume of acetone equal to three times the volume of diluted precipitate was added 2.7 grams of trichloracetic acid (T:C:A:) per 100 ml. of acetone. While stirring with a motor-driven propeller, the acetone - T.C.A. mixture was added to the precipitate and stirring was continued for one hour.

An eight-inch Buchner filter funnel was prepared with a coarse filter paper moistened with water. Two grams of "Hyflo" (Johns Manville silica filter aid) suspended in 200 ml. water was placed on the paper and gentle suction applied to produce a pad on the filter paper. Five grams of "Hyflo" was added to the extraction mixture, stirred, allowed to settle for two to three minutes, and the mixture was filtered through the Buchner funnel, clearer portion first, sediment last. Minimal suction was used to avoid clogging. Filtration was continued until the filter cake had cracked and no further fluid drained through

the funnel. The precipitate was discarded. To the clear filtrate was added one ml. of concentrated hydrochloric acid for each 100 ml. of filtrate. Two volumes of ether (analytical grade) for each volume of filtrate was added, shaken vigorously and allowed to stand for approximately 15 minutes until a clear interface was apparent between the ethereal and aqueous phases. The ether was aspirated and discarded. The extraction with ether was repeated two more times using two volumes of ether for each volume of aqueous fluid. The aqueous solution was transferred to a large evaporating dish. The pH was adjusted to 3.0 by addition of 50% sodium hydroxide and then 2N sodium hydroxide for the final adjustment. The mixture was heated to 75°C on a steam bath in a hood, with constant stirring to avoid excessive frothing. The solution was cooled to 20°C, transferred to a one litre beaker, and the volume made up to 500 ml. with water. Thirty ml. of an aqueous solution of T.C.A. (100 grams of T.C.A. made up to a final volume of 100 ml. with water) was added dropwise with mechanical stirring. After being allowed to settle for 15 minutes all the precipitate was collected in one 250 ml. centrifuge bottle by repeated centrifugations at 2500 r.p.m. for ten minutes, discarding the clear supernatant liquid by decanting each time. To the precipitate was added 70 ml. 0.15N hydrochloric acid, mechanical stirring being employed until dispersion was complete. The centrifuge bottle was filled with ether, shaken, allowed to settle into two clearly defined phases, and the ether aspirated.

Extraction with ether was repeated two more times. Using water washes the aqueous phase was transferred to an evaporating dish, the pH was adjusted to 8.0 with 2N sodium hydroxide and 1:20 ammonium hydroxide for the final adjustment, and heated to 75°C on a steam bath in a hood. The slightly opalescent solution was made up to 100 ml. with water and stored in a plastic bottle at -20°C.

This procedure gave one ml. of final solution for each 10 grams of mucosa. In all experiments to be presented doses of gastrin extract will be expressed as the equivalent weight in grams of wet pyloric gland area mucosa. After preliminary centrifugation to give water-clear solution, the protein content of the extracts was estimated by measurement of absorption at 280 mμ, using bovine serum albumin as a standard. The extracts contained from 0.9 to 1.3 milligram of protein per gram, wet weight, of mucosa.

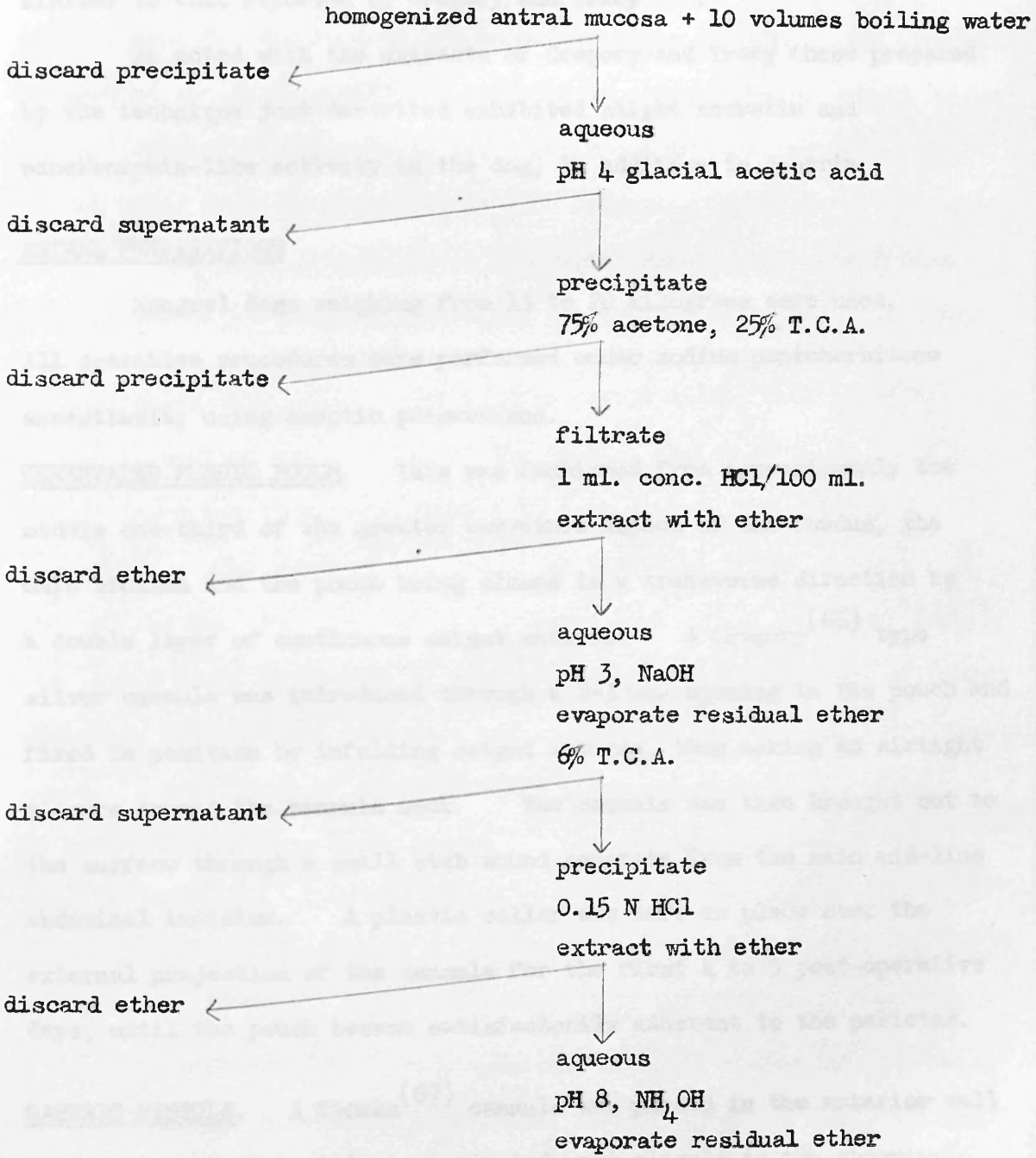
The important steps in this procedure are outlined diagrammatically in Fig.1.

The extracts could be stored frozen for as long as six months without loss of potency. All of more than 40 batches prepared by this method were consistently potent in stimulating acid secretion.

The histamine content of two extracts was assayed on guinea-pig ileum. The apparent histamine content of the extracts was less than 0.001 μg. per gram of mucosa, and even this small amount of activity could not wholly be attributed to histamine because it was not

FIG. 1

OUTLINE OF GASTRIN EXTRACTION



Yield is about 1.1 mg. per gram wet weight mucosa

antagonized by antihistamines. The apparent histamine content was similar to that reported by Gregory and Tracy (8).

As noted with the extracts of Gregory and Tracy those prepared by the technique just described exhibited slight secretin and pancreozymin-like activity in the dog, in addition to gastrin.

ANIMAL PREPARATIONS

Mongrel dogs weighing from 13 to 20 kilograms were used. All operative procedures were performed under sodium pentobarbitone anaesthesia, using aseptic precautions.

DENERVATED FUNDIC POUCH. This was fashioned from approximately the middle one-third of the greater curvature aspect of the fundus, the main stomach and the pouch being closed in a transverse direction by a double layer of continuous catgut sutures. A Gregory(66) type silver cannula was introduced through a 2-3 mm. opening in the pouch and fixed in position by infolding catgut sutures, thus making an airtight closure around the cannula neck. The cannula was then brought out to the surface through a small stab wound separate from the main mid-line abdominal incision. A plastic collar was left in place over the external projection of the cannula for the first 4 to 5 post-operative days, until the pouch became satisfactorily adherent to the parietes.

GASTRIC FISTULA. A Thomas(67) cannula was placed in the anterior wall of the stomach where this approximated most closely to the abdominal

wall, generally 2 to 3 cm. above the fundus-pyloric gland area junction. This allowed the cannula to lie at the most dependent part of the stomach when the dog assumed the normal standing posture. An airtight closure of the gastric wall was obtained by a double layer catgut "purse-string" suture. The cannula was brought out to the surface through a stab wound separate from the mid-line abdominal incision. In dogs with both a gastric fistula and a denervated fundic pouch the Thomas cannula lay to the right of mid-line, the Gregory cannula to the left.

PYLORIC GLAND AREA POUCH. The pyloric region having been freed by gentle dissection and ligation of the minimal number of blood vessels, the duodenum was transected about 2 to 3 mm. beyond the pyloric sphincter. The duodenum was closed by infolding catgut sutures. The stomach was transected at the fundus-pyloric gland area junction, the latter being determined by inspection. A greater extent of lesser curvature than greater curvature was included in the pyloric gland area pouch. Continuity of the alimentary tract was re-established by an end-to-side gastro-duodenostomy. The proximal cut end of the pyloric gland area pouch was closed by a double layer catgut suture. After excision of the distal 2 to 3 mm. of the pouch to ensure removal of all duodenal mucosa, this end of the pouch was brought out through a separate stab wound to form a cutaneous fistula, mucosa being secured to the skin edge by several interrupted silk sutures. This form of opening was preferred to drainage by cannula, in view of the evidence

that mechanical factors are capable of stimulating gastrin release (Grossman, Robertson and Ivy[25]).

POST-OPERATIVE CARE. As a rule during the first two post-operative days the dogs were allowed only one to two ounces of water to drink, 500 ml. of physiological saline being given subcutaneously each day. Thereafter normal kennel diet of a proprietary dog food "Friskies", bone meal, and water ad libitum were given.

No experiment was performed for at least three weeks after operation, except in the case of the portal vein infusion studies described in PART III, Chapter 11.

PROCEDURES.

Continuous intravenous infusions were given by Sigmamotor pump, the rate of flow generally being 20 ml. per hour, and the concentration of gastrin extract or histamine dihydrochloride being adjusted to give the desired dose rate.

Pouch secretion was collected every 15 minutes and the acid concentration was determined by titration with 0.2N sodium hydroxide, using phenol red indicator. The use of a microburette allowed samples as small as 0.2 ml. to be titrated with an acceptable degree of accuracy. The results are generally expressed as microequivalents of acid per 15 minutes.

Pepsin determinations were made by the technique described by Grossman and Marks[68], using radio-iodinated serum albumen as substrate.

PART III

EXPERIMENTS AND OBSERVATIONS

Chapter 1

1. LATENCY OF RESPONSE TO GASTRIN AND TO HISTAMINE

Several of the experiments to be described involved the establishment of a plateau secretory response of the indicator pouch to continuous intravenous infusion of gastrin extracts. It was soon discovered that there was considerable variation from dog to dog with regard both to latency of achieving, and actual level of, this secretory plateau to any given dose of gastrin. This is illustrated in Fig.2 which shows the acid outputs from denervated fundic pouches in three dogs, to which gastrin extract at a rate of 10 grams per hour was given by continuous intravenous injection over a period of 5 to 6 hours. In dog No.43 a plateau was established in $\frac{1}{2}$ to 1 hour, in dog No.47 in 2 hours, and in dog No.44 not until 4 hours after the start of the injection.

These differences in latency might be accounted for by variation in one or more of the following factors:

(i) The circulation time from the site of introduction of the extract (leg vein) to the pouch vessels.

(ii) The rate of equilibration of gastrin concentration in the circulating blood. Since the rate of introduction of gastrin was the same in each instance, the variation would have to occur in the rate of

elimination of the stimulant. Nothing is yet known about the processes involved in gastrin inactivation.

(iii) The sensitivity of the fundic gland cells to gastrin may be subject to spontaneous individual variation, or might have been affected by varying periods of anoxia during clamping at operation, or other surgical influences.

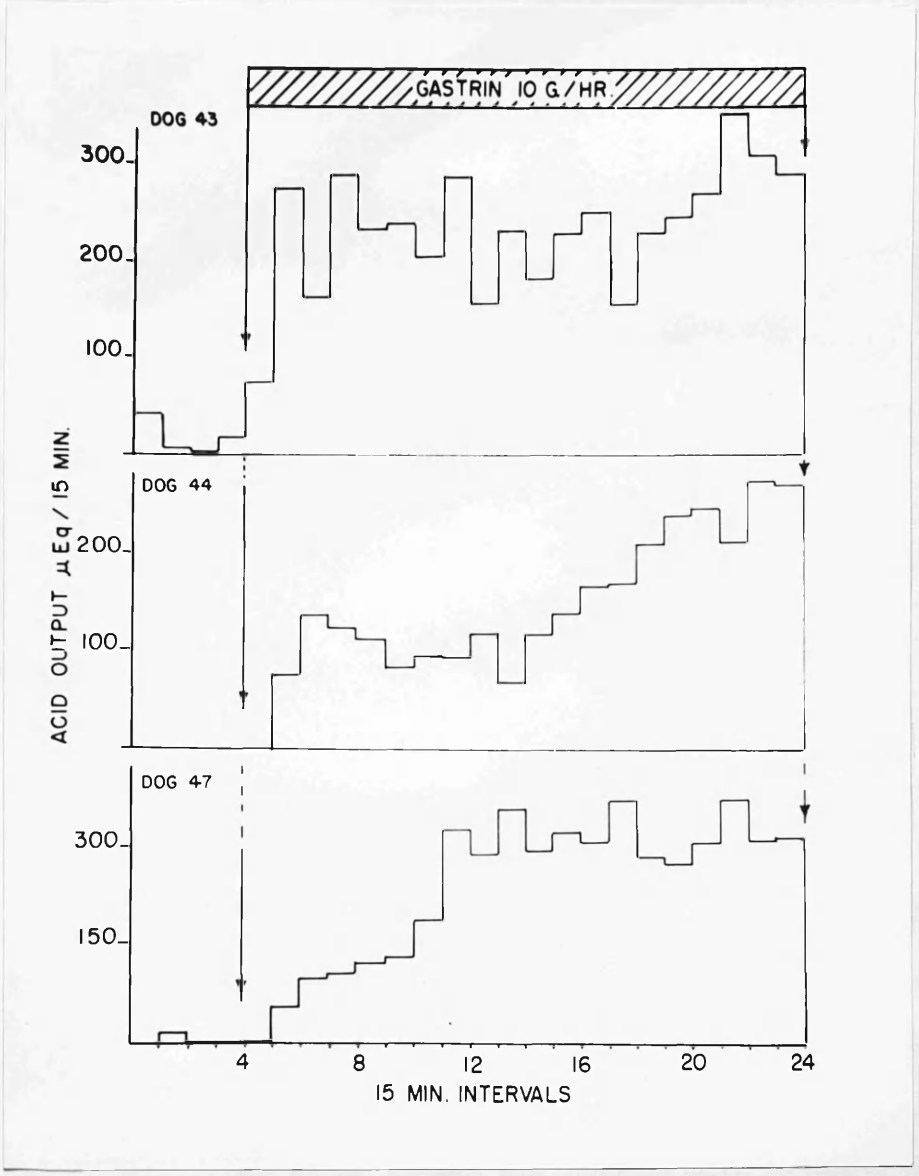
That dogs do vary in their sensitivity to gastrin will be shown in Chapter 2. If this factor were also to explain the differences in latency it might be expected that the latent period would be inversely proportional to the level of the plateau response. Although this was not borne out by the results shown in Fig.2, in which dog No.47, with the highest plateau, had an intermediate latency of achieving it, the results of the three dogs shown in Table I do suggest such a relationship. In these three dogs the mean latent period for the establishment of plateau response correlated with the maximal gastrin response; the shorter the latency the higher the maximal gastrin response.

(iv) The blood supply of the pouches. All pouches were made in similar manner, and judged to be of approximately the same size. However, it is well known that the arrangement of the gastro-epiploic artery and its branches is subject to variation, and the number and calibre of the final leash of vessels supplying the pouches must have varied.

Of these several factors it seems probable that individual

FIG. 2

RESPONSES OF HEIDENHAIN POUCHES TO CONTINUOUS INTRAVENOUS GASTRIN OVER A 5-HOUR PERIOD



Note the marked differences in lengths of the latent periods prior to plateau responses: in dog No.43 - 30 minutes; in dog No.47 - 2 hours; and in dog No.44 - 4 to 5 hours.

TABLE I. COMPARISON OF MEAN LATENCY OF PLATEAU RESPONSE TO GASTRIN AND MAXIMAL GASTRIN RESPONSE

Dog No.	Mean latent period in hours (No. of experiments)	Maximal gastrin response (MEQ per hour)
44	2.5 (14)	2.34
48	2.25 (14)	4.02
53	2.0 (13)	6.61

LEGEND. The latent period was taken as the length of time from the start of the continuous intravenous injection to the first 15 minutes output which did not show a further increase over the preceding one.

Dog No.44 showed the longest mean latent period and the smallest maximal gastrin response; dog No.53 showed the shortest mean latent period and the largest maximal gastrin response; dog No.48 occupied an intermediate position.

sensitivity plays a part in the variable latency, but unlikely that this alone accounts for the large differences observed. The importance of alterations in blood supply to the rapidity of responses to feeding and to injected histamine was stressed by Klein and Arnheim[69], who found prolonged latent periods to these stimuli when using subcutaneous transplanted fundic pouches totally dependent on newly acquired blood supply.

A combination of several local factors probably determines the latency.

In general the latency of response, both to gastrin and to histamine, was greatest with small doses and less with large doses (Table II) but it was sometimes found that the responses to the largest doses of gastrin were also characterized by long latency. With histamine the latency of response tended always to be shorter than with gastrin, and largest doses had, as a rule, the shortest latent periods, in contrast to gastrin. The difference in latency with the highest dose-rates of the two agents suggests the possible presence of an inhibitor influence in the gastrin extracts manifest at high rates of administration, and not apparent in histamine. This question will be investigated further in Chapter 3.

TABLE II. MEAN LATENCY OF PLATEAU RESPONSES TO GRADED DOSES OF GASTRIN AND HISTAMINE

1. GASTRIN	Gastrin extract dose (grams per hour)					
	2.5	5	10	20	40	80
Dog No.	Latent period in hours:					
44	2.25	2.25	2.75	1.75	3.0	3.5
48	2.25	2.75	1.75	3.25	2.75	2.75
53	2.25	2.5	1.5	1.75	1.0	3.0
2. HISTAMINE	Histamine dihydrochloride dose (mg. per hour)					
	0.25	0.5	1	2	4	8
Dog No.	Latent period in hours:					
44	2.5	2.25	2.25	1.75	1.75	1.25
48	2.0	2.0	1.75	2.0	1.5	1.5
53	1.75	1.5	1.5	2.0	1.25	1.0

LEGEND: The latent period represents the length of time from the start of the continuous intravenous injection to the first 15 minute output which did not show a further increase over the preceding one.

With histamine the latent period decreased as the dose rate increased; by contrast, with gastrin the latent period decreased only in the intermediate dose range, increasing again with higher dose rates.

Chapter 2

2. ACID AND PEPSIN DOSE-RESPONSE CURVES TO GASTRIN AND TO HISTAMINE

These experiments were undertaken to investigate the pattern of response of the fundic glands to graded doses of gastrin, and compare it with that to a similar range of histamine doses. The question was studied in two groups of Heidenhain pouch dogs by two slightly different approaches. In all instances a logarithmic scale of dosage was used, each dose being increased over the previous one by a factor of 2. The doses of gastrin ranged from 1.25 to 80 grams per hour in Group A, and 2.5 to 80 grams per hour in Group B., and of histamine dihydrochloride from 0.125 to 8 milligrams per hour in Group A and from 0.25 to 8 milligrams per hour in Group B.

The results of the three dogs in Group A, given in Table III, are plateau levels established after 2 to 3 hours of continuous intravenous infusion at the indicated rate, each single dose rate being administered on a separate day.

In the five dogs of Group B the dose rate was increased every 90 minutes, and the results given in Table IV are measurements of the acid and pepsin outputs during the final 60 minutes of each 90 minute period.

The first point of interest is the marked variation in degree of response to the same range of gastrin and histamine dosage from dog to dog. Taking the acid results of both groups together, maximal

TABLE III. ACID AND PEPSIN OUTPUTS OF HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN AND OF HISTAMINE

(Group A. Dogs No.44, 48 and 53)

(Plateau rates after 2 to 3 hours continuous intravenous infusion - each dose given on separate day)

GASTRIN BY CONTINUOUS INTRAVENOUS INFUSION						
Dose (g/hr)	Acid mEq/hr			Pepsin units/hr		
Dog.No.	44	48	53	44	48	53
1.25	0.67	0.92	0.48	1104	9301	705
2.5	0.81	0.74	1.96	404	644	1810
5	0.83	1.04	2.73	1179	1634	4066
10	0.92	1.45	4.32	849	1425	4863
20	0.73	1.30	4.11	222	1075	3432
40	0.70	1.68	4.84	389	5280	2823
80	0.46	0.57	1.62	335	2890	10603
HISTAMINE DIHYDROCHLORIDE BY CONTINUOUS INTRAVENOUS INFUSION						
Dose (mg/hr)						
0.125	0	0.74	0.25	0	10866	199
0.25	0.62	1.72	0.79	621	14155	3230
0.5	1.18	3.25	2.65	5215	12890	4500
1	1.64	3.37	3.77	4270	2445	3206
2	2.34	3.58	5.02	4100	1951	4057
4	1.44	4.02	5.06	254	861	811
8	1.52	3.81	6.61	319	584	1141

Figures underlined are maximal acid responses.

TABLE IV. ACID AND PEPSIN OUTPUTS OF HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN AND OF HISTAMINE

(Group B. Dogs No.43, 45, 48, 53 and 55)

(Dose increased every 90 minutes - rates estimated from final 60 minutes)

GASTRIN BY CONTINUOUS INTRAVENOUS INFUSION										
Dose (g/hr)	Acid mEq/hr					Pepsin units/hr				
	Dog No.43	45	48	53	55	43	45	48	53	55
2.5	0.09	0.18	0.33	0.52	-	714	653	1015	1038	-
5	0.16	0.19	0.36	1.50	0.49	171	274	615	3308	333
10	0.28	0.50	0.75	2.93	0.62	134	345	454	3415	226
20	0.86	0.95	1.21	3.40	0.69	519	367	1105	5555	2555
40	0.80	1.02	0.92	2.99	0.86	860	790	1119	10638	8371
80	0.74	1.00	0.34	3.18	0.24	1394	644	3796	7634	4165
HISTAMINE DIHYDROCHLORIDE BY CONTINUOUS INTRAVENOUS INFUSION										
Dose(mg/hr)										
0.25	0.53	0.51	0.48	1.14	0.18	1110	2679	3987	764	125
0.5	1.13	1.23	0.87	0.89	0.75	2621	8253	14731	500	5027
1	1.94	2.08	1.93	1.89	1.55	2925	1977	2307	1021	428
2	3.59	3.17	1.92	2.70	3.16	1263	1261	1106	464	502
4	3.83	4.03	1.73	2.90	3.61	575	1132	608	400	354
8	3.46	4.16	1.62	3.19	3.28	83	404	277	455	222

Figures underlined are maximal acid responses.

histamine response was achieved in one instance at 1 mg. per hour, in one at 2 mg. per hour, in three at 4 mg. per hour, and in three at or above 8 mg. per hour. Similarly, the variation in dosage required to achieve maximal acid response was from 10 to 40 grams per hour for gastrin, and from 1 to 8 mg. per hour for histamine dihydrochloride. In dog No.44, Group A, the lowest dose of gastrin used (1.25 grams per hour) produced almost maximal acid response, whereas in dog No.53, Group A, there was a nine to ten-fold increase in response using the same dose range. The other dogs had intermediate rates of increase in response to gastrin.

This variation may well be of importance in the interpretation of experiments in which the secretion of a denervated fundic pouch is evoked by an arbitrarily selected dose of stimulant.

The mean acid and pepsin dose/response curves for Groups A and B are shown in Figs. 3 and 4 respectively. The patterns obtained were basically the same for both groups.

The following features appear noteworthy.

A. Acid Curves

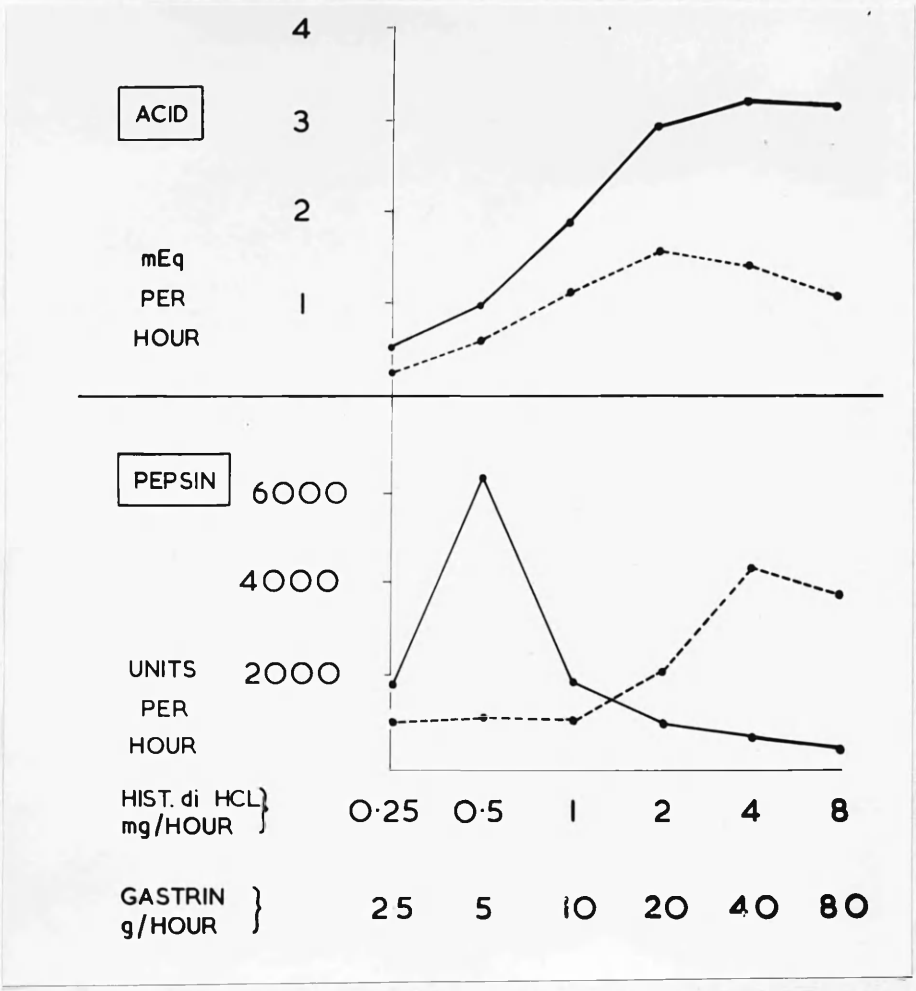
(i) The initial slopes of the two curves were similar.

(ii) The maximal response to gastrin extract was less than the maximal response to histamine, confirming the finding previously reported by Grossman[70]. Among the possible explanations for this difference are the following hypotheses:

FIG. 3

MEAN ACID AND PEPSIN OUTPUTS OF HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND OF HISTAMINE

(GROUP A = Dogs No.44, 48 and 55)



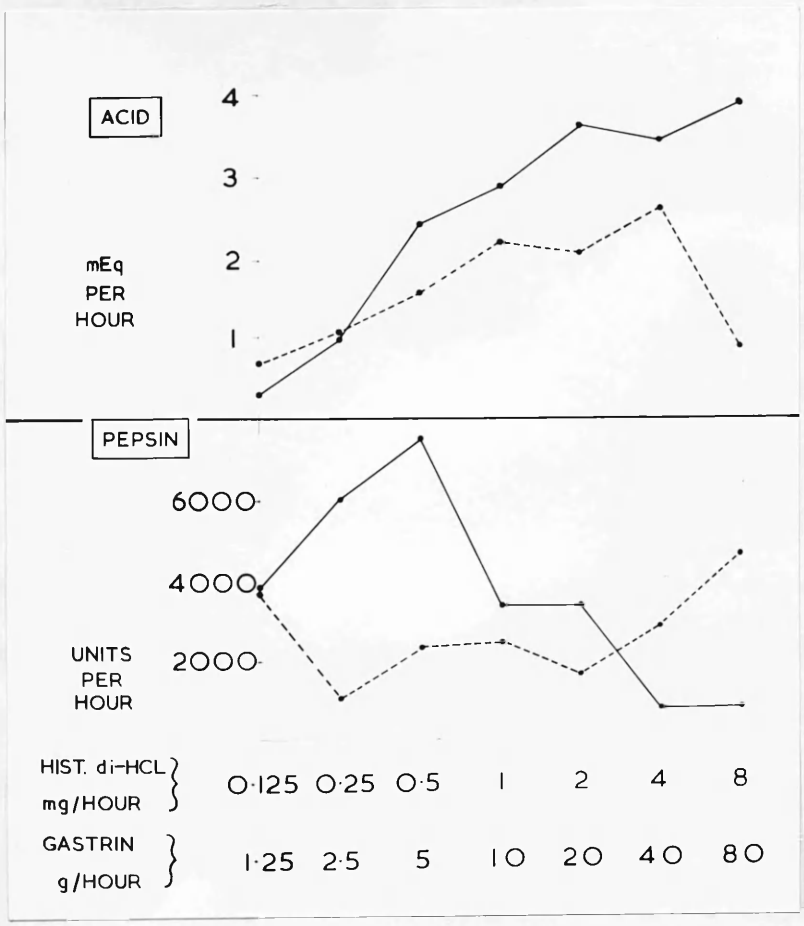
Comments. 1. The acid curves show the maximal response to gastrin to be less than the maximal to histamine.

2. The pepsin curves show an increased output to the smaller doses of histamine, and to the larger doses of gastrin.

FIG. 4

MEAN ACID AND PEPSIN OUTPUTS OF HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND OF HISTAMINE

(GROUP B = Dogs No.43, 45, 48, 53 and 55)



Comments. 1. The acid curves show again that maximal gastrin response was less than maximal histamine response.

2. The pepsin curves show the same pattern as Fig.3 to histamine, viz. increased output to smaller doses only; for gastrin extract there appears to have been increased pepsin output both to smallest and largest doses used.

(a) The parietal cells may be less responsive to gastrin than to histamine.

(b) The present gastrin extracts may still be of insufficient purity for full maximal response to be obtained.

The further investigation of both these questions will have to await the preparation of pure gastrin.

(c) Inhibition of the gastrin response may have been occurring from the resultant acidification of the duodenum. Andersson has shown that instillation of hydrochloric acid into the duodenum will decrease the acid response of a denervated fundic pouch to injected gastrin[71], but not to injected histamine[72]. Thus the flow of acid juice provoked in the main stomach by the injected gastrin might have reduced intraduodenal pH to the range required for the action of this inhibitor mechanism, with consequent depression of the Heidenhain pouch response.

(d) The gastrin extract as prepared may contain an inhibitory, as well as stimulating, property.

This question will be further discussed in Chapter 3.

(e) Maximal responsiveness to gastrin may depend on the presence at intact vagal nerve endings of the stomach of a certain quantity or concentration of acetylcholine, not essential for maximal histamine response. In this respect comparison was made between the maximal gastrin and maximal histamine responses of the vagally innervated and denervated fundic glands in the same animal by using 3 dogs each provided

with a gastric fistula in addition to a denervated fundic pouch. Although the difference was less marked, the maximal gastrin response of the innervated stomach was still less than the maximal histamine response, suggesting that vagal denervation was not the sole explanation for the difference in maximal responses to the two agents seen in Figs. 3 and 4.

(iii) With the largest doses of gastrin extract used there appeared to be a reduction in response from maximal levels - unlike histamine, the largest doses of which continued to give maximal responses. This finding again suggests the presence in the extracts of inhibitor properties.

B. Pepsin Curves

(i) There was a suggestion that the smallest doses of gastrin used stimulated pepsin secretion. Though this is not apparent in Fig.3 as it is in Fig.2, it can be seen from Tables III and IV that in 6 of the 8 experiments the lowest dose used gave greater responses than the subsequent one or two larger dose rates.

(ii) There was a distinct increase in pepsin output to the largest doses of gastrin given, probably indicating true stimulation of pepsin production. Most workers have reported the secretory response to endogenous gastrin, released by feeding or by stimulation of an isolated pyloric gland area pouch, to be low in pepsin (Schofield[73], Grossman, Woolley and Ivy[74], Schofield[75]), and the extracts

prepared by Maydell[17], Komarov[56], Uvnäs[59], and Gregory and Tracy[8], were all reported to be without pepsin stimulating properties. However, it has recently been shown by Dragstedt et al.[76] that the fundic hypersecretion induced by translocation of the pyloric gland area as a diverticulum to the colon is rich in pepsin. It appears that this procedure causes the liberation of unusually large amounts of gastrin, and it may be that only quantities of gastrin above the "physiological" range cause an increase in pepsin production. In the experiments presented it is seen that the increase in pepsin output occurred only when doses capable of evoking maximal acid response were reached, and that further increase in pepsin output was obtained as the acid response decreased. It is interesting to speculate whether the pepsin stimulation might be due to some other fraction in the extract, such as "pepsizymin" (Babkin and Komarov[77]), or "gastrozymin" (Blair, Harper and Lake[78]). If the apparent increased pepsin output with smallest doses of gastrin can be confirmed, the pepsin response to gastrin would be triphasic, - stimulation at lowest and highest dose rates, and depression or lack of stimulation at intermediate rates. Such a finding would make it more likely that the stimulation at highest doses was due to an agent, or agents other than gastrin in the extracts.

(iii) The pepsin response curves to histamine dihydrochloride (Figs. 3 and 4) show similar biphasic patterns, small doses causing increased pepsin output, and larger doses resulting in steadily decreasing

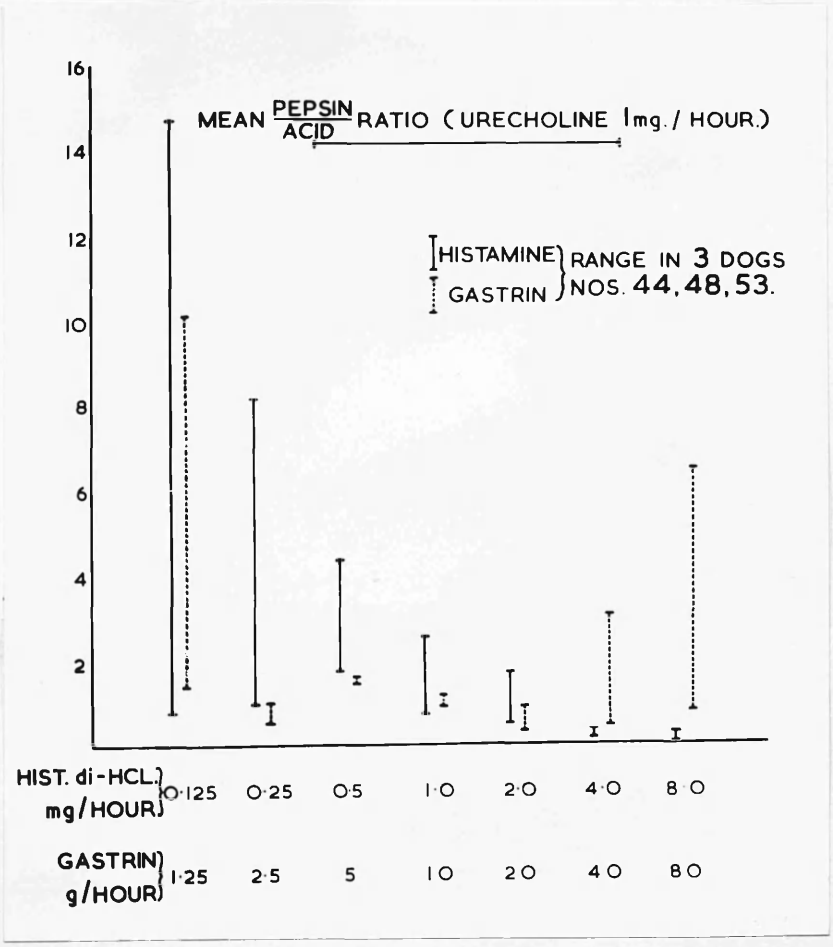
amounts of pepsin.

The controversy regarding whether histamine causes true stimulation of pepsin production or merely causes "wash-out" of preformed pepsin is well known (Polland and Bloomfield[79], Gilman and Cowgill[80], Vineberg and Babkin[81], Toby[82], Ihre[83], Bucher and Ivy[84], Bucher, Grossman and Ivy[85], Ashford, Heller and Smart[86], Hunt[87]). It is felt that the results presented are in favour of true stimulation of pepsin at lower dose rates, from two aspects. Firstly, the peak pepsin output, occurring at a dose rate of 0.5 mg. histamine dihydrochloride per hour, was preceded in both curves by a distinct upward slope. Secondly, since the acid responses to these dose ranges of gastrin, (1.25 to 5 grams per hour), and of histamine dihydrochloride, (0.125 to 0.5 mg. per hour), were similar, there would have been approximately equal opportunity for "wash-out" of preformed pepsin by both agents to occur. The failure of the gastrin pepsin outputs to increase over this range makes the histamine pepsin peak appear more significant. The relative differences between the pepsin responses to the two agents are brought out more clearly by examination of the ratios of pepsin output to acid output shown in Fig.5 (Group A), and Fig.6 (Group B). Each vertical line covers the range of all results in all dogs of the group for the indicated dose rate. The solid lines are histamine results, the interrupted lines, gastrin. Also recorded on Figs. 5 and 6 is the mean pepsin/acid ratio for Urecholine given intravenously at a continuous

FIG. 5

RATIOS OF PEPSIN/ACID OUTPUTS FROM HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND OF HISTAMINE

(GROUP A = Dogs No.44. 48 and 53)



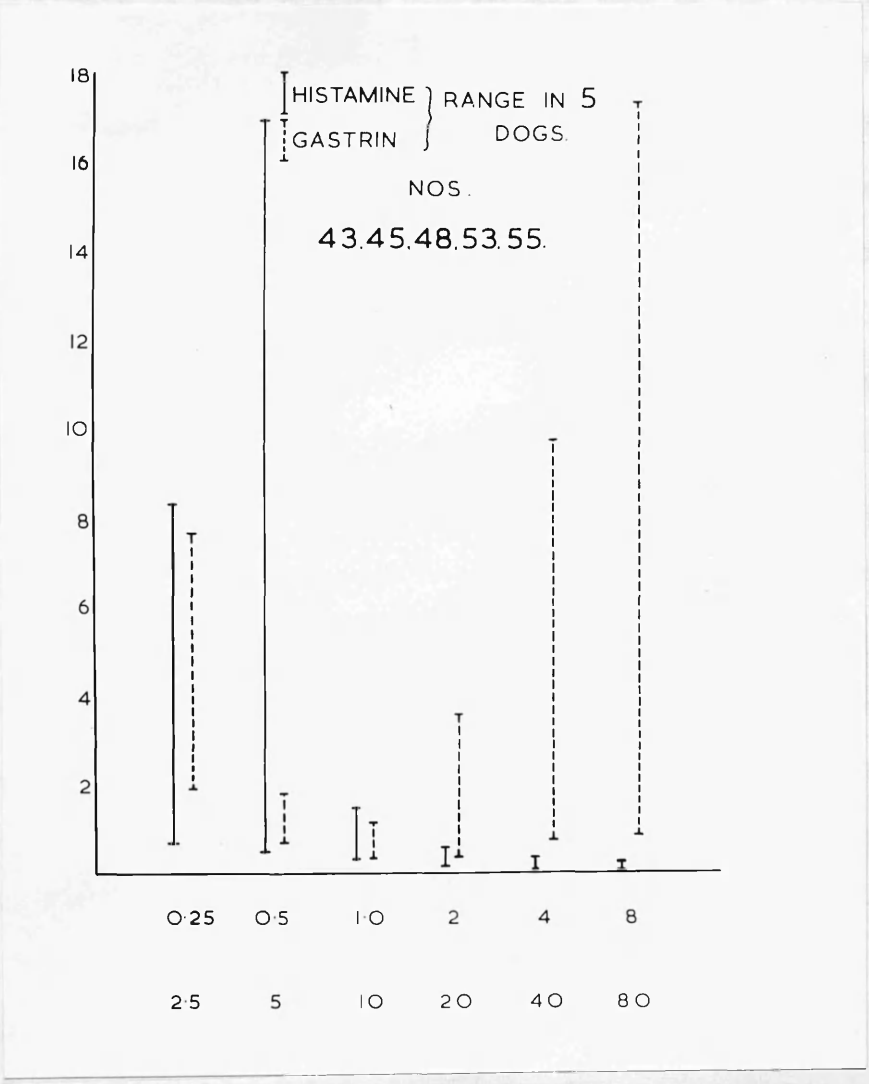
High ratios were encountered with both gastrin extract and histamine in the lowest dose range: in the middle dose range both ratios declined: there was a further rise in ratio with the highest doses of gastrin extract only.

Only with the lowest doses of either gastrin extract or histamine did the ratio approach that produced by Urecholine.

FIG. 6

RATIOS OF PEPSIN/ACID OUTPUTS FROM HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND OF HISTAMINE

(GROUP B = Dogs No.43, 45, 48, 53 and 55)



The same features as shown in Fig.5 are again displayed. The rise in ratio with the highest doses of gastrin extract was more pronounced.

rate of 1 mg. per hour. As is to be expected, this ratio is high, acetylcholine being a powerful pepsin stimulant.

With the lowest doses of both gastrin and histamine the pepsin/acid ratios ranged to high levels, approaching that of the Urecholine. With each increase in histamine dose rate there was a progressive decrease in the ratios, ultimately to very low levels. The gastrin ratios, on the other hand, though showing a similar reduction over the intermediate dose range, displayed a further rise to high values, comparable to Urecholine, with the largest doses.

These patterns would again seem most readily interpreted as showing histamine to stimulate pepsin production at low dose rates only, and the pepsin response to gastrin to be triphasic. It is interesting at this stage to speculate that pepsin responses may reflect acetycholine activity, this substance being the most reliable and potent stimulant of pepsin secretion known. It would then be possible that increasing doses of histamine might depress acetycholine formation or interfere with its action on the pepsin cells. This question will be considered further in Chapter 8, which deals with the interaction of acetylcholine with gastrin and histamine.

As an incidental investigation at this point it seemed that the technique employed by Schofield[88] for the more accurate recovery of small volumes of secretion from pouches was of value in answering this vital question of whether the pepsin response to small doses of

histamine represented true stimulation or simple "wash-out".

A major obstacle to the interpretation of pepsin responses to histamine has been the inability to measure basal pepsin outputs, the volume of basal secretion being so small, and often of a sufficiently high pH to inactivate any pepsin present (Bucher and Ivy[84]). The technique devised by Schofield was to instil into the pouch a small measured volume of weak hydrochloric acid at the beginning of every 15 minutes, and at the end of that time to estimate the acid output of the pouch by subtraction of the mEq. in the introduced acid from the total mEq. in the pouch contents. In 6 experiments in four dogs this was done using 10 ml. instillations of 0.01N hydrochloric acid every 15 minutes, for a period of 2 hours, without any stimulation, and for a second 2-hour period during which histamine dihydrochloride, 0.5 mg. per hour, was given by continuous intravenous infusion. In 4 experiments a third 2-hour period followed, during which the histamine dihydrochloride dose was increased to 2 mg. per hour, to see if such a dose rate produced any reduction of pepsin output, and if so, what relation the resultant output bore to basal levels.

Results are given in Table V, and an illustrative experiment on dog No.53 is shown in Fig.7. It is seen that the mean pepsin output to histamine dihydrochloride 0.5 mg. per hour (2427 units per hour) was approximately 12 times the mean basal output (205 units per hour), and that, though the mean pepsin output to 2 mg. per hour was less than to

TABLE V. ACID AND PEPSIN RESPONSES OF HEIDENHAIN POUCH TO SMALL AND LARGE DOSES OF HISTAMINE BY CONTINUOUS INTRAVENOUS INFUSION

(using Schofield technique - of repeated instillation of 0.01N HCl)

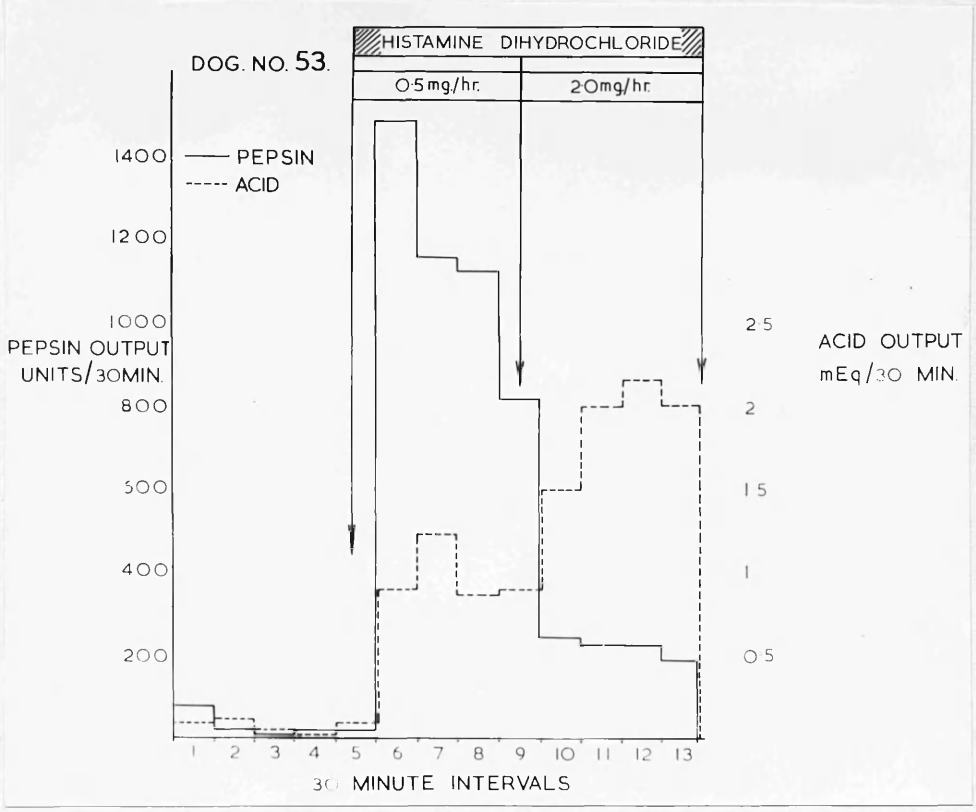
Outputs are expressed as mean hourly rates, each for a 2-hour period

Dog No.	Control		Histamine dihydrochloride			
			0.5 mg/hr		2 mg/hr	
	Acid µEq/hr	Pepsin units/hr	Acid µEq/hr	Pepsin units/hr	Acid µEq/hr	Pepsin units/hr
43	19	353	1482	3260	-	-
45	16	261	1851	2686	6516	3042
48	13	173	2347	1053	3311	1256
53	0	147	1464	1246	-	-
53	157	58	1947	2288	3882	456
53	367	239	2305	4031	6161	1177
Means:	95	205	1899	2427	(4968)	(1483)

FIG. 7

PEPSIN RESPONSE OF HEIDENHAIN POUCH TO SMALL AND LARGE DOSES OF HISTAMINE BY CONTINUOUS INTRAVENOUS INFUSION

(Schofield technique - repeated instillation of 0.01 N HCl)



Note the marked increase in pepsin output during the infusion of 0.5 mg./hr histamine dihydrochloride. When the dose rate was increased to 2.0 mg./hr. there was a reduction of pepsin output towards, but still above, basal levels. The acid outputs showed the expected "step-ladder" response.

0.5 mg. per hour, it was still appreciably higher (1483 units per hour) than basal levels. The acid outputs showed the expected "stepladder" increase with the two dosage increments. The evidence from these results is strongly in support of true pepsin stimulation by the smaller dose of histamine. The response to the higher dose is more in favour of lessening stimulation than active depression of basal pepsin secretion.

Chapter 3

3. INHIBITOR EFFECTS OF GASTRIN EXTRACTS

The studies presented in this section were prompted by the finding, illustrated in the preceding Chapter, that the maximal acid response to gastrin was less than the maximal to histamine. Among the possible explanations for this discrepancy was that the gastrin extracts, as prepared, had an inhibitory as well as a stimulating action on gastric secretion. Since such an inhibitory effect would be more likely to be manifest at dose levels of gastrin extract larger than those required for maximal acid response, it was decided in the first instance to observe the effect of a single rapid intravenous injection of a large dose of gastrin extract on the plateau acid response to the continuous intravenous infusion of a small dose of (a) the same gastrin extract, and of (b) histamine. Plateau acid response was regarded as at least four approximately equal successive 15 minute collections.

Effect of a single large dose of gastrin extract on the response to a stimulatory dose of the same gastrin extract.

This was studied in seven tests in five dogs (Table VI and Fig.8), the constant rate stimulatory dose being 2.5 grams per hour, the single rapid injection after the establishment of a plateau being 50 grams. In each instance there was inhibition of the plateau response, starting in the first 15 minutes after the injection, reaching maximal levels of

TABLE VI. EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HOG GASTRIN EXTRACT, 2.5 grams per hour.

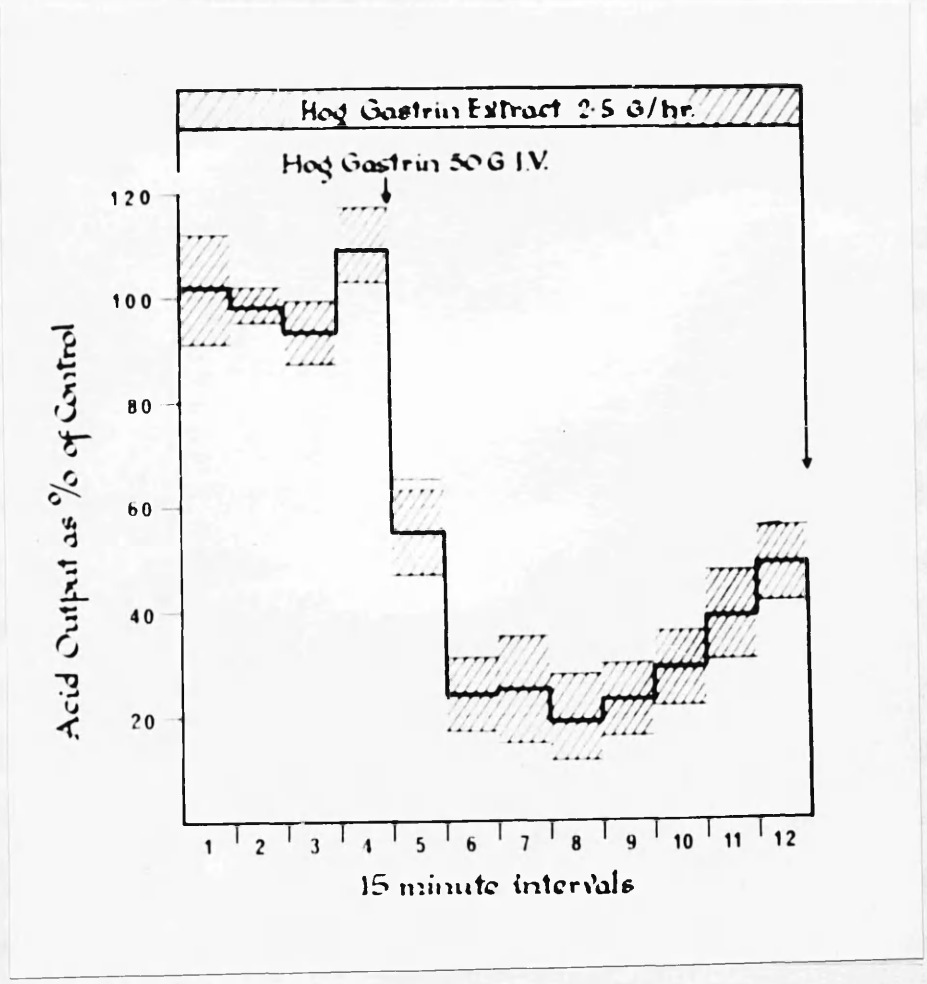
(Control is mean of four 15 min. outputs immediately preceding injection)

Dog No.	Control μEq/15 min	Post-injection 15 min. acid outputs expressed as % of control							
		1	2	3	4	5	6	7	8
44	152	63	17	58	37	41	64	68	68
44	67	75	18	15	0	0	0	15	42
45	37	16	38	35	16	19	16	5	27
48	311	53	5	0	0	9	30	38	55
48	228	61	14	5	0	8	29	49	73
53	391	76	61	63	49	47	47	48	24
55	191	38	17	0	31	37	15	50	52
Means		55**	24**	25**	19**	23**	29**	39**	49**

Asterisks indicate significant difference from control ** P = < 0.01

FIG. 8

EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS HOG GASTRIN EXTRACT, 2.5 GRAMS PER HOUR



Mean and S.E. of 7 experiments in 5 dogs (Table VI)

Profound inhibition followed the single gastrin injection.

inhibition from 30 to 75 minutes after injection, and lessening after this time. Two hours after injection the mean inhibition was still 50%.

Effect of a single large dose of gastrin extract on the response to histamine.

In ten tests on five dogs the effect of a single rapid intravenous injection of 50 grams of gastrin extract on the acid response to the continuous intravenous administration of histamine dihydrochloride at a constant rate of 0.25 mg. per hour was studied (Table VII and Fig.9). In eight of the ten tests inhibition occurred, comparable to that described above with gastrin as the stimulant. However, two experiments on one dog (No.53) showed marked augmentation of the acid response, one following 30 minutes of partial inhibition, the other starting immediately after the single gastrin injection. Because of these two results in this dog, only the first two post-injection 15 minute outputs were significantly less than control. If the results of these two tests had been omitted, the mean output would have reached a low of 8% of control in the third 15 minute period and would have returned to 60% of control by the eighth period. Differences from control would then have been significant ($P = <0.01$) for all eight periods.

It seemed of interest to ascertain whether the inhibitory property of the extract was effective against maximal histamine dosage.

TABLE VII. EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 0.25 mg. per hour

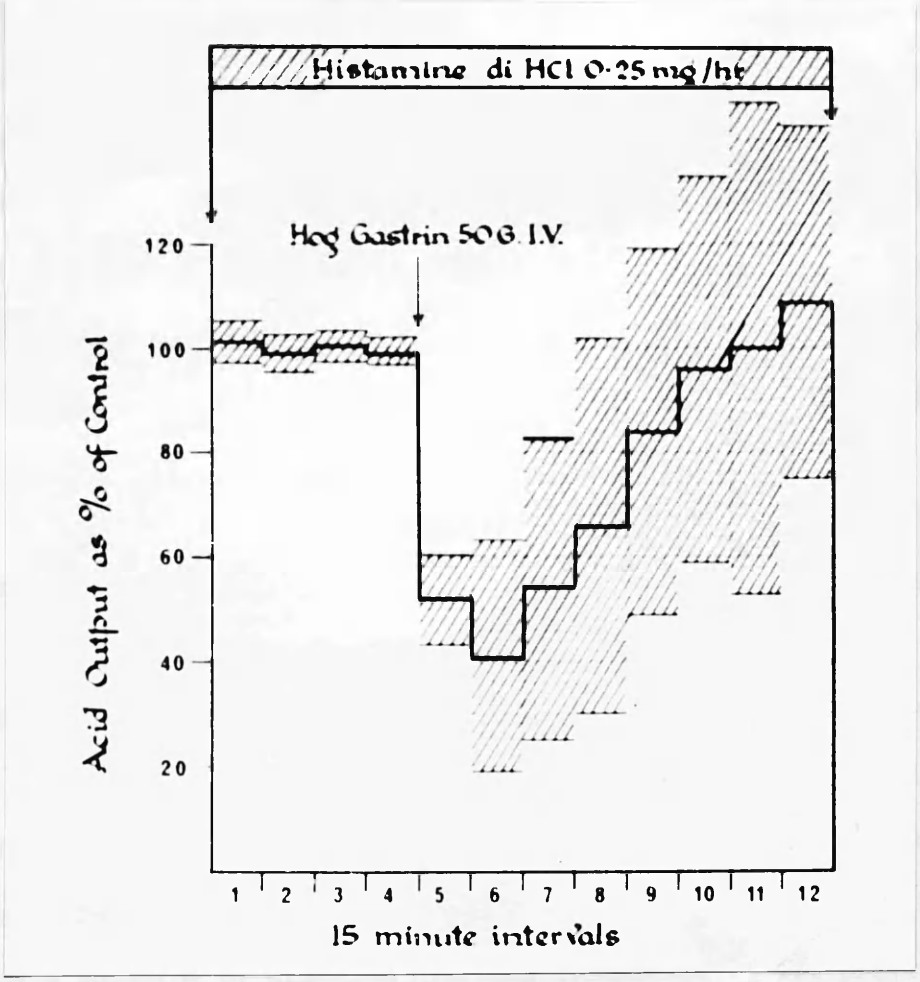
(Control is mean of four 15 min. outputs immediately preceding injection)

Dog No.	Control μEq/15 min	Post-injection 15 min. acid outputs expressed as % of control							
		1	2	3	4	5	6	7	8
43	149	41	5	3	0	12	9	9	19
44	121	91	20	10	5	56	87	86	91
45	509	26	18	4	17	29	39	64	76
45	416	31	2	32	63	82	81	79	85
48	288	52	24	5	5	17	44	33	65
48	370	29	5	2	3	10	18	42	51
48	484	39	1	2	2	17	14	34	38
53	160	106	236	291	366	359	388	394	390
53	351	46	46	50	52	58	77	92	100
53	321	55	55	136	146	203	198	167	173
Means		52*	41*	54	66	84	96	100	109

Asterisks indicate significant difference from control * $P = < 0.05$

FIG. 9

EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE 0.25 mg. PER HOUR



Mean and S.E. of 10 experiments in 5 dogs (Table VII)

Inhibition occurred following the single injection of gastrin, though apparently less profound and less prolonged than that illustrated by Fig.8.

In previous experiments in the same group of dogs (Table III), 2.0 mg. histamine dihydrochloride per hour had been found to elicit acid responses at, or near, maximal levels, and the effect of a single rapid intravenous injection of 50 grams gastrin extract on this dose of histamine was next studied. Results of five experiments in the five dogs are shown in Table VIII and Fig. 10. The mean percentage reductions in acid output, significant for the 75 minutes following the injection, are comparable to those of the previous two groups of experiments. Thus it appeared that the inhibitory property of the gastrin extract was effective against maximal doses of histamine.

Inhibitory action of purified gastrin extract.

As one of the possible explanations for the inhibitory mechanism was the presence in these relatively crude extracts of an inhibitory agent other than gastrin per se, similar tests were done with a purified preparation of gastrin. A batch of extract prepared by the method outlined under "Materials and Methods" was subjected to the Stage III procedure described by Gregory and Tracy[8], in which adsorption on a column of calcium phosphate gel is followed by elution with a solution of disodium hydrogen phosphate. The protein concentration of this purified gastrin, as measured by ultraviolet adsorption, was 0.09 mg. per gram wet weight of mucosa, approximately one-tenth of the corresponding value for the cruder extract. Subcutaneous administration of crude and purified gastrin in equivalent

TABLE VIII. EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 2.0 mg. per hour (Dose that produced maximal or near maximal response)

(Control is mean of four 15 min. outputs immediately preceding injection)

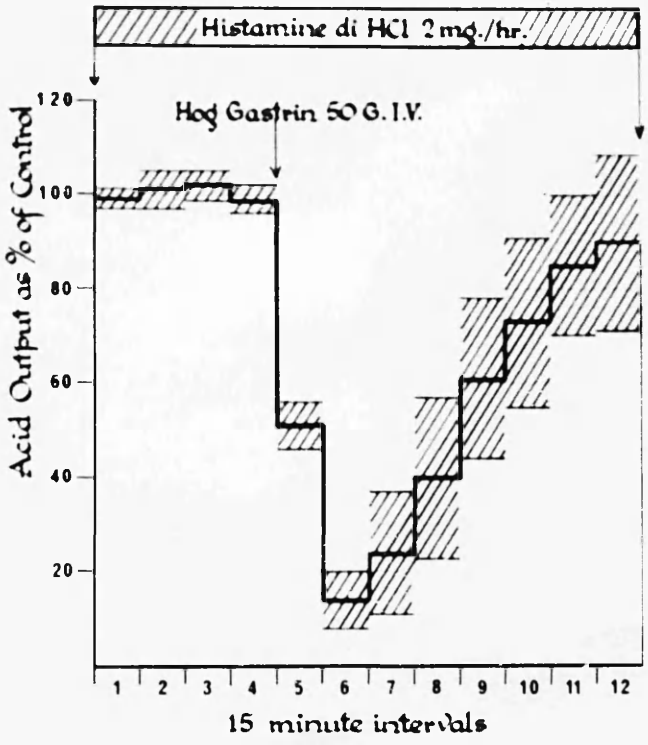
Dog No.	Control µEq/15 min.	Post-injection 15 min. acid outputs expressed as % of control							
		1	2	3	4	5	6	7	8
43	847	56	10	2	1	3	15	48	32
44	515	61	24	67	96	107	103	115	120
45	1349	57	3	1	13	70	108	122	131
48	948	33	2	10	50	80	97	99	109
53	1091	49	32	38	38	43	44	52	57
Means		51**	14**	24**	40*	61	73	85	90

Asterisks indicate significant difference from control **P = < 0.01

*P = < 0.05

FIG. 10

EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 2.0 mg. PER HOUR (Dose that produced maximal or near maximal response)



Mean and S.E. of 5 experiments in 5 dogs.

Profound inhibition followed the single gastrin injection.

doses, based on grams of mucosa from which the extracts were derived, gave acid secretory responses that were not significantly different.

In eight tests on five dogs the intravenous injection of 50 grams of purified gastrin extract (containing 4.5 mg. of protein) produced inhibition of the response to 0.25 mg. per hour histamine dihydrochloride comparable to that seen with the cruder extracts (Table IX and Fig.11). Thus if the inhibition was caused by a constituent of the extracts other than gastrin, it was not removed by approximately tenfold purification.

Inhibitory action of dog gastrin.

To determine whether species difference might account for the depression of acid response, gastrin extract was prepared from the mucosa of the pyloric gland area of dogs by the same method that was used for hog mucosa. In three tests in which only 10 grams of this dog gastrin extract was given by rapid intravenous injection, the inhibition of the response to 0.25 mg. histamine dihydrochloride per hour was as profound and prolonged as with the hog preparation (Table X and Fig.12).

Extracts of other tissues.

To ascertain whether the inhibitory property was confined to mucosa of the pyloric gland area or whether it was shared by other parts of the alimentary tract, extracts were prepared by the same method from the following tissues of normal dogs: pancreas, and mucosa of gastric fundus, duodenum, ileum, and colon. The protein concentration of each extract was measured, and a dose of each equivalent in protein content to

TABLE IX. EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS PURIFIED HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 0.25 mg. per hour

(Control is mean of four 15 min. outputs immediately preceding injection)

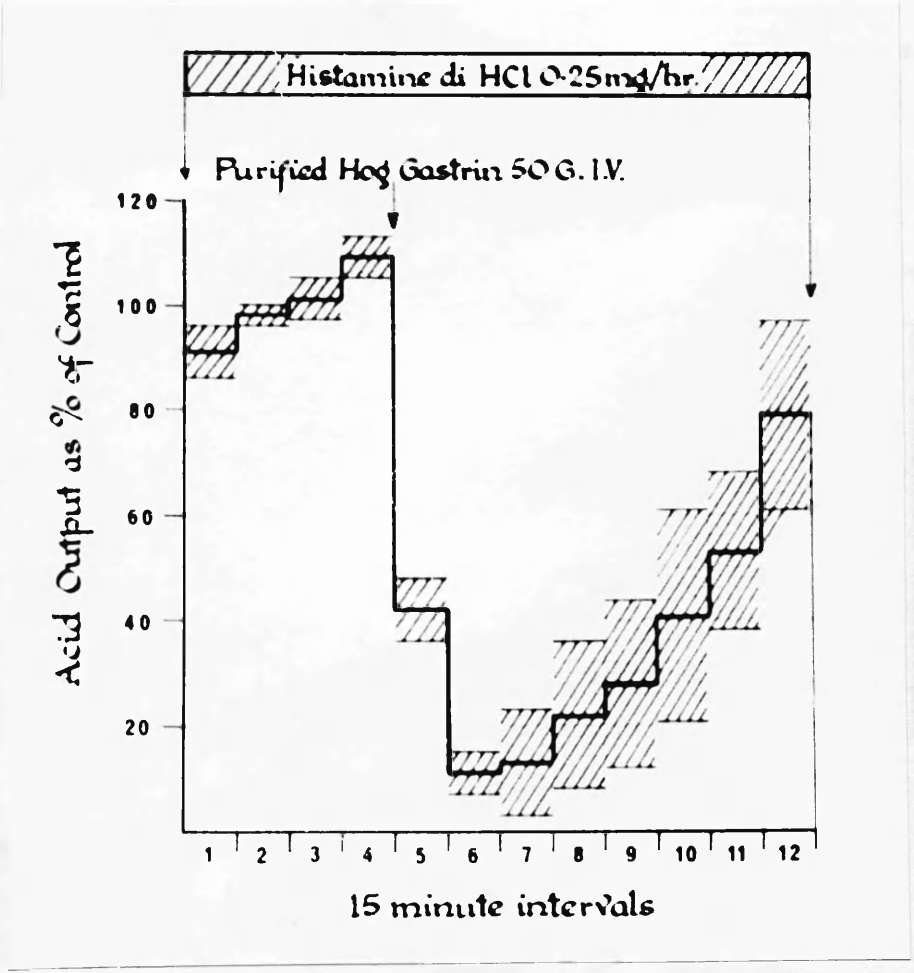
Dog No.	Control μEq/15 min	Post-injection 15 min. acid outputs expressed as % of control							
		1	2	3	4	5	6	7	8
43	161	52	3	2	1	2	5	24	39
45	223	22	9	6	29	13	78	90	165
45	318	30	28	3	0	8	0	30	58
48	636	43	2	6	13	33	37	44	47
48	690	19	0	4	17	20	24	36	52
53	488	65	32	86	118	141	167	141	154
55	208	52	6	0	0	0	0	23	51
55	186	51	11	0	0	3	19	34	64
Means		42**	11**	13**	22**	28**	41*	53*	79

Asterisks indicate significant difference from control ** P = < 0.01

* P = < 0.02

FIG. 11

EFFECT OF RAPID INTRAVENOUS INJECTION OF 50 GRAMS PURIFIED HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 0.25 mg. PER HOUR



Mean and S.E. of 8 experiments in 5 dogs.

Profound inhibition followed the single gastrin injection.

TABLE X. EFFECT OF RAPID INTRAVENOUS INJECTION OF 10 GRAMS DOG GASTRIN EXTRACT ON RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 0.25 mg. per hour

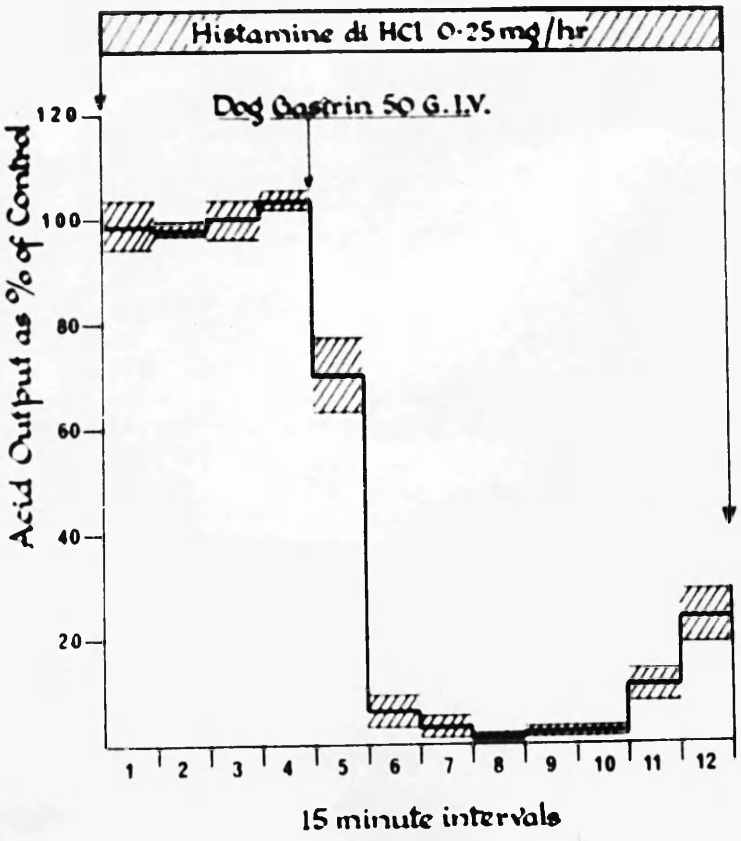
(Control is mean of four 15 min. outputs immediately preceding injection)

Dog No.	Control μEq/15 min	Post-injection 15 min. acid outputs expressed as % of control							
		1	2	3	4	5	6	7	8
43	201	63	3	0	0	2	2	16	34
53	270	84	11	8	2	2	2	11	20
55	360	63	3	2	0	2	3	6	17
Means		70	6**	3**	1**	2**	2**	11**	24**

Asterisks indicate significant difference from control ** P = < 0.01

FIG. 12

EFFECT OF RAPID INTRAVENOUS INJECTION OF 10 GRAMS DOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 0.25 mg.PER HOUR



Mean and S.E. of 3 experiments in 3 dogs

Profound inhibition followed the single gastrin injection.

50 grams of the hog gastrin extract, was given by rapid intravenous injection to dogs secreting in response to (a) 2.5 grams hog gastrin per hour, and to (b) 0.25 mg. histamine dihydrochloride per hour (Table XI). The only extract to produce significant inhibition was that of ileal mucosa, acting against histamine stimulation, and this was much lesser in degree and shorter in duration than that observed with the gastrin (Table XI).

Minimal dose of gastrin extract required to produce inhibition.

In five dogs receiving 0.25 mg. histamine dihydrochloride per hour, doses of gastrin extract ranging from 0.4 grams to 6.25 grams were given by rapid intravenous injection. In all 5 dogs the smaller doses of gastrin extract augmented the acid output over that seen with histamine alone (Fig.13). A dose of 3.2 grams caused marked inhibition in three of the five dogs, and 6.25 grams caused inhibition in all five dogs.

These results suggested that a critical blood level of extract was required to effect inhibition. The question was approached in a different manner in the experiments illustrated in Fig.14. Once a plateau secretory response had been attained to 0.25 mg. histamine dihydrochloride per hour, 50 grams gastrin extract was injected intravenously during measured time intervals of increasing duration, ranging from 1 minute to 64 minutes. A similar degree of inhibition of the histamine response was noted on injecting the 50 gram dose of

TABLE XI. EFFECTS OF SINGLE RAPID INTRAVENOUS INJECTIONS OF EXTRACTS OF VARIOUS TISSUES ON ACID RESPONSE OF HEIDENHAIN POUCHES TO CONTINUOUS INTRAVENOUS
(A) HOG GASTRIN EXTRACT, 2.5 GRAMS PER HOUR, OR
(B) HISTAMINE DIHYDROCHLORIDE, 0.25 MG. PER HOUR

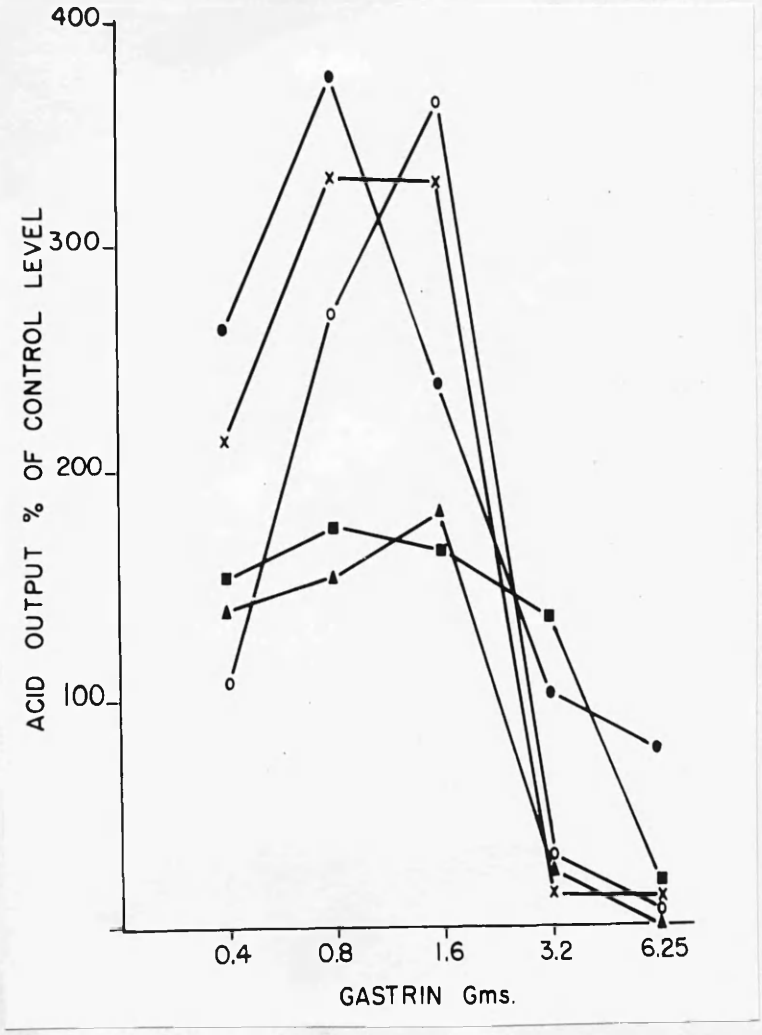
(Dose of each extract equivalent in protein content to 50 grams hog gastrin extract)

(A) GASTRIN Tissue	No. of Tests	Post-injection 15 min. acid outputs, mean (S.E.) % of control			
		1	2	3	4
Gastric fundus	8	93(14)	106(20)	101(25)	113(27)
Duodenum	6	61(15)	79(34)	185(94)	113(57)
Ileum	5	80(10)	151(58)	138(70)	123(20)
Colon	6	109(8)	123(21)	130(21)	107(18)
Pancreas	4	97(22)	51(19)	84(43)	77(53)
(B) HISTAMINE					
Gastric fundus	7	97(3)	93(5)	99(6)	88(4)
Duodenum	7	109(15)	144(35)	137(31)	133(31)
Ileum	7	72(4)*	80(8)*	79(7)*	89(8)
Colon	6	104(10)	94(12)	110(8)	108(18)
Pancreas	11	92(7)	102(7)	89(5)	86(11)

Asterisks indicate significant difference from control * $P = < 0.05$

FIG. 13

EFFECT OF SINGLE RAPID INTRAVENOUS INJECTIONS OF GRADED DOSES OF HOG GASTRIN EXTRACT ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS HISTAMINE DIHYDROCHLORIDE, 0.25 mg. PER HOUR

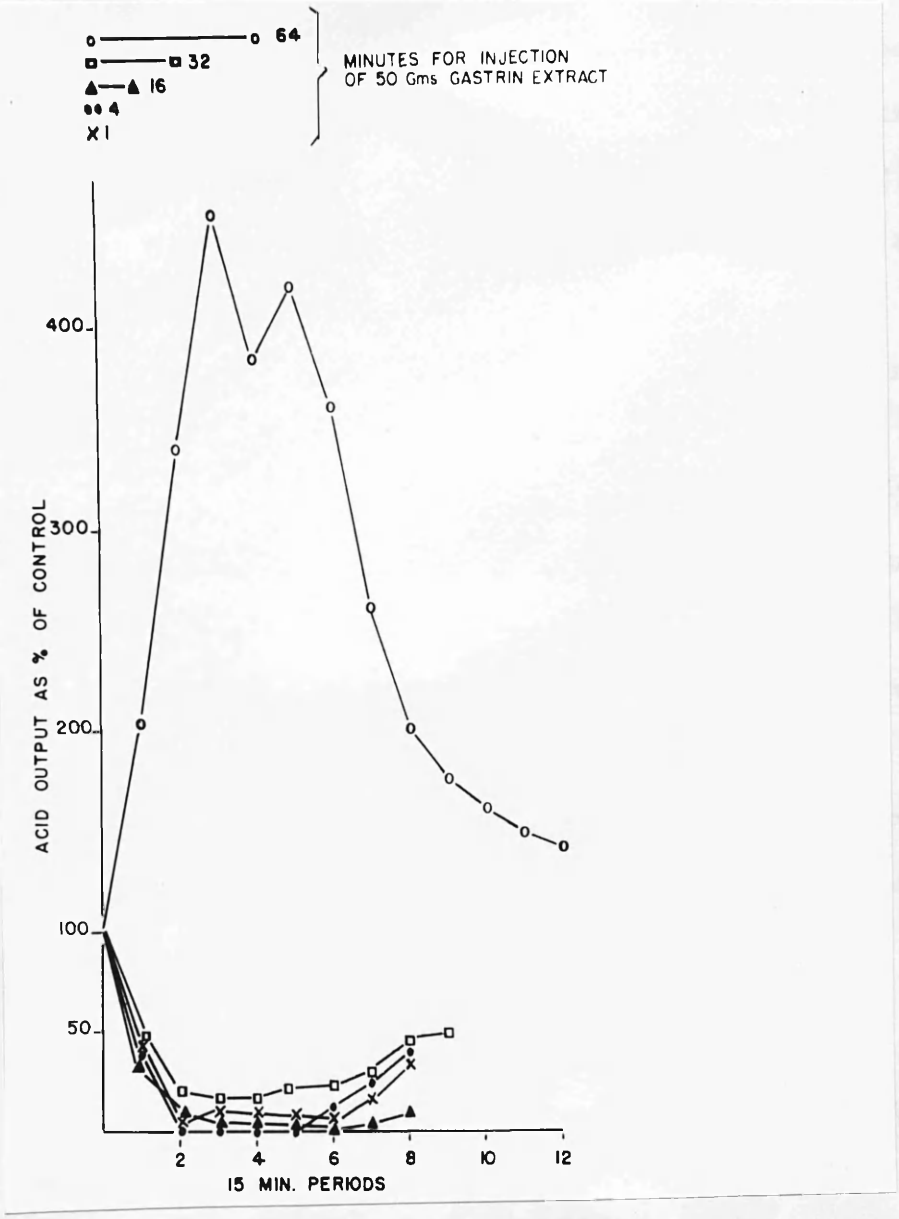


5 Dogs, each represented by a different symbol.

Potentiation of the histamine response occurred from 0.4, 0.8 and 1.6 g. of gastrin extract as single rapid injections; inhibition occurred in 3 of the 5 dogs from 3.2 g, and in all 5 dogs from 6.25 g. gastrin extract.

FIG. 14

EFFECT OF INJECTING 50 GRAMS HOG GASTRIN EXTRACT INTRAVENOUSLY OVER DIFFERENT TIME PERIODS, ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS HISTAMINE DIHYDROCHLORIDE, 0.25 mg. PER HOUR



50 grams gastrin extract produced marked inhibition of the histamine response when given over 1 - 32 minutes, but caused potentiation when given over 64 minutes.

gastrin extract over 1, 4, 16 and 32 minutes. In marked contrast the 64 minute infusion greatly increased the acid output.

The effect on blood pressure of rapid intravenous injection of 50 grams of two of the extracts was studied in a dog anaesthetized with sodium pentobarbitone. In each instance there was a fall of 60 mm. of mercury in systolic pressure beginning within four seconds after the injection and recovering to control levels within 16 seconds. It is unlikely that such transient changes in blood pressure would influence gastric secretion.

In three dogs the rectal temperature was measured hourly before and after the intravenous injection of 50 grams of gastrin extract. The mean maximal rise in rectal temperature was 0.5°C, a rise too small to produce inhibition of gastric secretion on the basis of pyrogenic action (Blickenstaff and Grossman[88a]).

Throughout all experiments the dogs displayed no apparent side-effects, and in particular there were no objective signs of nausea, such as retching, vomiting, salivation, or restlessness.

DISCUSSION OF THE INHIBITOR PROPERTY OF GASTRIN EXTRACTS

The present studies indicate that gastrin extracts prepared in the manner outlined, while stimulating acid secretion from the fundic glands at low dosage rates, are capable of profoundly inhibiting the acid response to gastrin or histamine when given at higher doses. This inhibitory property appears to be confined to extracts of the pyloric gland area and not to depend on species difference. Uvnäs[59] had previously reported that large doses of his gastrin preparations occasionally inhibited the secretory response to histamine, and he attributed this inhibitory effect to a non-specific toxic action of crude tissue extracts. General toxic effects seem an unlikely explanation for the present results in view of the failure of such extracts to affect significantly rectal temperature, systolic arterial pressure, or to cause objective signs of nausea. The experiments suggest that a critical blood concentration of extract is required to effect inhibition, and that this level is relatively slightly in excess of that required to evoke maximal acid response from the fundic glands. The close similarity of all the curves showing inhibition in Fig. 14 supports an "all-or-none" mechanism.

This phenomenon may, at least in part, account for the failure of numerous workers to obtain an acid secretory response from the injection of various extracts of pyloric gland area (Grossman[2]).

When slight or no response to the selected dose of extract had been found, an increased dose was often given in the hope of securing greater stimulation. The increased dose, if displaying marked inhibitory properties similar to those of the present studies, may well have obscured any stimulatory action.

In seeking an explanation for the inhibitory action of gastrin extracts the following two theoretical possibilities may be considered.

(a) The stimulatory and inhibitory actions of gastrin extracts are caused by the same ingredient of the extracts, that is, gastrin itself has both stimulatory and inhibitory actions.

(b) The inhibitory effect is produced by a constituent of the extracts other than that responsible for stimulation, that is, the inhibitor is a separate substance, distinct from gastrin.

The final choice between these two possibilities can only be made when pure gastrin becomes available, and it is determined whether it has inhibitory effects.

Certain findings in the present study support the possibility, but do not establish that gastrin itself is the inhibitor. These include: (i) the inhibitory property appeared to be confined to extracts of the pyloric gland area; (ii) inhibition could not be ascribed to species difference or general toxic effects; (iii) tenfold purification of the extracts led to parallel changes in its stimulatory and inhibitory potency.

The notion that a single agent can, depending on the dose, stimulate or inhibit gastric secretion finds a precedent in studies on choline esters. For example, Gray and Ivy[89] showed that a small dose of Mecholyl greatly augmented the response to histamine, while a large dose profoundly inhibited it.

Turning to the possibility that the extracts contain an inhibitor distinct from gastrin, this might be either (i) a non-specific toxic extract of tissues (rendered unlikely by the failure of other alimentary extracts to cause inhibition), (ii) the factor in gastric juice described by Brunschwig *et al.*[90,91,92], and shown by Hood, Grindlay and Code[93] to be more abundant in the juice secreted by the pyloric glands, or (iii) the hypothetical antral inhibitor hormone released by acidification of the pyloric gland area (Harrison, Lakey and Hyde[94], Jordan and Sand[95], Greenlee *et al.*[96], DuVal, Fagella and Price[97], Thompson, Lerner and Tramontana[98]). The evidence of numerous workers, reviewed by Shapira and State[99], and from experiments recently reported by Gillespie and Grossman[100], to be discussed in Chapter 4, is against the existence of such a hormone.

Chapter 4

4. EFFECT OF ACIDIFICATION OF THE PYLORIC GLAND AREA ON THE ACID RESPONSE OF A HEIDENHAIN POUCH TO INJECTED GASTRIN EXTRACT

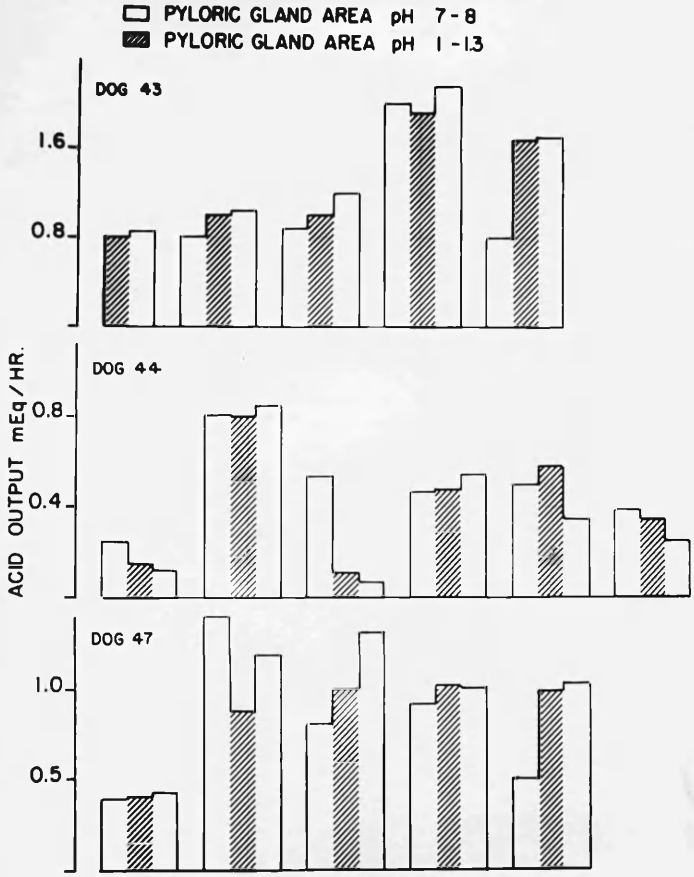
The results of this study which, as noted in the previous section, has been reported (Gillespie and Grossman[100]), illustrated several points worthy of comment. Irrigation of the isolated (and therefore probably vagally denervated) pyloric gland area with solutions of hydrochloric acid of concentration up to the equivalent of near maximal obtainable from the fundic glands (0.15N) failed to demonstrate any significant inhibition of the acid response to stimulatory doses of gastrin extracts given by continuous intravenous infusion. Results of 16 experiments in three dogs using 0.1N hydrochloric acid to irrigate the pyloric gland area are illustrated in Fig.15, and a representative experiment in Fig.16.

The possible release of an inhibitor agent from the pyloric gland area by hypertonic solutions was also investigated, but no evidence of depression of the Heidenhain pouch response to intravenous gastrin extract was obtained on irrigating the pyloric gland area pouch with 50% dextrose.

Although this failure to inhibit the response to a physiological agent was against the production of an inhibitor humoral agent from the pyloric gland area, it must be admitted that the gastrin extracts might have contained an impurity, or impurities, capable of interfering with

FIG. 15

EFFECT OF ACIDIFICATION OF AN ISOLATED PYLORIC GLAND AREA POUCH ON THE ACID RESPONSE OF A HEIDENHAIN POUCH TO INJECTED GASTRIN EXTRACT



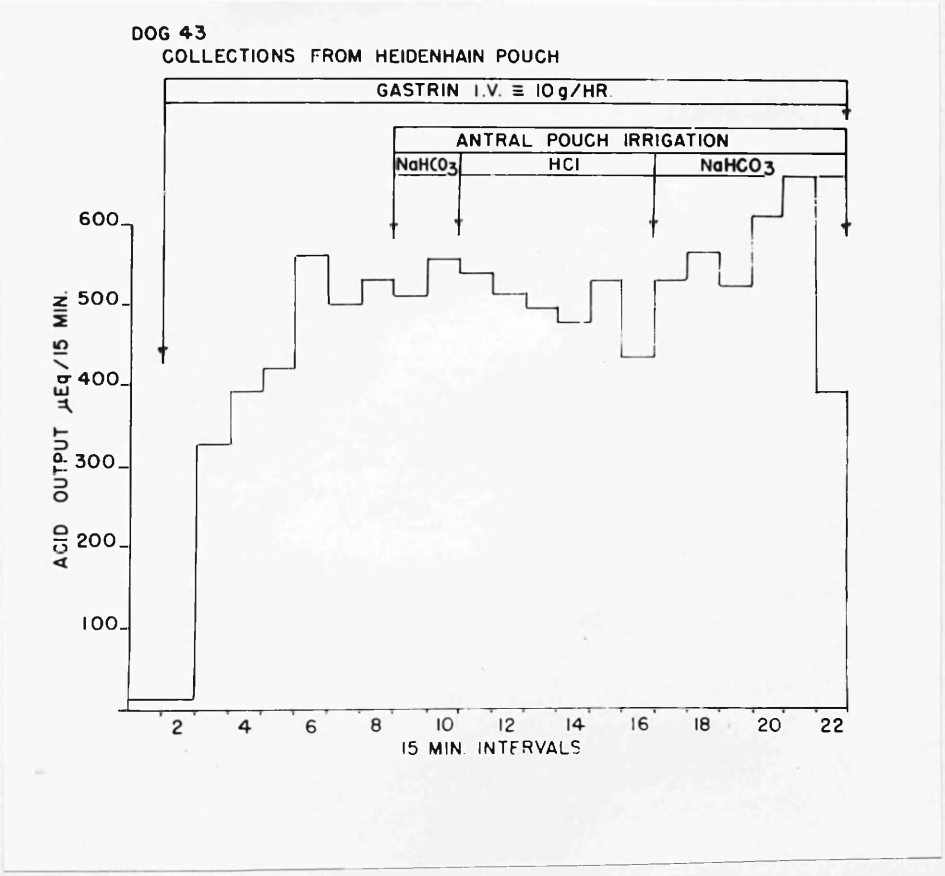
Gastrin was given by continuous intravenous injection throughout each of the 16 experiments. The pyloric gland area pouch was irrigated alternately with 0.1 N $NaHCO_3$ (pH 7-8), and 0.1 N HCl (pH 1-1.3).

Alteration of the pyloric gland area pH from 7 to 1 resulted in no significant change in acid output.

FIG. 16

EFFECT OF ACIDIFICATION OF AN ISOLATED PYLORIC GLAND AREA POUCH ON THE ACID RESPONSE OF A HEIDENHAIN POUCH TO INJECTED GASTRIN EXTRACT

(Illustrative experiment)



During $NaHCO_3$ irrigation pH of effluent from pyloric gland area pouch was 7 to 8; during HCl irrigation pH fell to 1 to 1.3.

No inhibition occurred on acidifying the pyloric gland area pouch.

the interaction of the hypothetical inhibitor agent and pure gastrin. It is also possible that, in addition to the well established prevention of gastrin release by acidification of the pyloric gland area, the liberation of a humoral inhibitor is dependent on intact vagal innervation.

Chapter 5

5. EFFECT OF SECRETIN AND CHOLECYSTOKININ ON THE ACID RESPONSE OF A HEIDENHAIN POUCH TO GASTRIN AND TO HISTAMINE

Numerous workers have reported the inhibitor effects on fundic gland secretion of acid instilled into the duodenum (Sokolov[101], Day and Webster[102], Gregory[103], Jones and Harkins[104], Sircus[105]), and opinion has been divided regarding the mechanism of action of this inhibitory process. Although earlier work by Code and Watkinson[106] had suggested that intact vagal innervation was essential for this form of inhibition, the recent excellent studies by Andersson[71,72,107,108,109] have demonstrated marked inhibitory powers where no attempt was made to preserve vagal fibres. Andersson found that acidification of the duodenum inhibited the response of both innervated and denervated fundic pouches to insulin hypoglycaemia, to injected gastrin, and to a meal, but did not reduce the response to histamine. He concluded that the inhibitor mechanism was humoral.

It seemed reasonable to investigate further the possibility that extracts of duodenal mucosa might contain the humoral agent suggested by the work of Andersson. Recalling the demonstration by Greenlee *et al.*[96] that intravenous secretin inhibited gastric secretion, and before embarking on the lengthy task of preparing extracts from fresh material, it was decided to test secretin and the other principal duodenal extract prepared commercially, cholecystokinin. These substances, extracted by the method

of Jorpes and Mutt[110], were obtained from Vitrum (Stockholm). Dogs with Heidenhain type denervated fundic pouches were used throughout. In all instances the secretin or cholecystokinin (75 clinical units = 0.1 mg. for secretin, 3 mg. for cholecystokinin) was given by a single intravenous injection after a plateau of acid secretion had been established by continuous intravenous gastrin in the first series of experiments, and continuous intravenous histamine in the second series.

The results are presented in Tables XII to XV, and summarised in Table XVI. The output for each successive 15 minute period following the secretin or cholecystokinin injection is expressed as a percentage of the mean 15 minute output during the control period. The gastrin response was inhibited in all experiments (Tables XII and XIII) by both secretin and cholecystokinin, though the pattern of inhibition varied. The inhibition by secretin (Table XII) was of rapid onset, being present in all 10 experiments within the first 15 minutes, and was significant only for the 30 minutes after the injection. The inhibition by cholecystokinin (Table XIII), though significant for 75 minutes following injection, appeared to develop more slowly, being maximal in the third 15 minute period. The degree of inhibition was also greater, the mean maximal inhibition being 78%, as compared to 58% for secretin.

In studying the effects of secretin and cholecystokinin on the acid responses to histamine a dose rate of 0.25 mg. histamine dihydrochloride per hour was selected, as this had been found in the

TABLE XII. EFFECT OF SINGLE INTRAVENOUS INJECTION OF SECRETIN (75 clin.units) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF GASTRIN EXTRACT, 2.5 GRAMS PER HOUR

(Control is mean of four 15 min. outputs immediately preceding injection)

Dog No.	Control µEq/15 min.	Post-injection 15 min. acid outputs expressed as % of control					
		1	2	3	4	5	6
43	106	71	14	61	125	43	67
43	85	62	21	36	92	74	87
44	102	65	120	192	120	98	64
44	151	56	54	47	36	23	23
45	36	53	0	175	153	139	328
45	18	50	44	167	200	217	150
48	281	59	70	79	61	73	106
48	186	40	75	86	65	51	67
53	197	55	13	45	92	74	53
53	213	25	9	42	89	110	109
Means		54**	42**	93	103	90	105

Asterisks indicate significant difference from control ** P = < 0.01

TABLE XIII. EFFECT OF SINGLE INTRAVENOUS INJECTION OF CHOLECYSTOKININ (75 clin. units) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF GASTRIN EXTRACT, 2.5 GRAMS PER HOUR

(Control is mean of four 15 min. outputs immediately preceding injection)

Dog No.	Control μEq/15 min.	Post-injection 15 min. acid outputs expressed as % of control					
		1	2	3	4	5	6
43	30	60	17	27	10	93	280
43	71	37	10	4	42	24	23
44	230	72	27	40	91	104	85
44	62	61	13	18	24	100	116
45	152	84	23	11	63	94	86
45	162	82	28	4	9	37	33
48	417	49	11	10	34	57	51
48	121	36	12	11	11	67	31
53	174	97	71	9	5	39	100
53	242	102	93	86	112	130	100
Means		68**	30**	22**	40**	76**	90

Asterisks indicate significant difference from control ** P = < 0.01

TABLE XIV. EFFECT OF SINGLE INTRAVENOUS INJECTION OF SECRETIN (75 clin. units) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE

(Control is mean of four 15 min. outputs immediately preceding injection)

Dog No.	Control µEq/15 min.	Post-injection 15 min. acid outputs expressed as % of control					
		1	2	3	4	5	6
A. HISTAMINE DIHYDROCHLORIDE 0.25 mg. per hr.							
43	70	110	113	154	133	113	167
43	189	83	123	87	87	80	95
44	163	66	102	104	102	101	111
44	217	67	90	93	103	97	90
45	219	79	58	75	101	60	90
45	268	83	121	188	187	132	152
48	456	109	128	113	108	104	103
48	443	100	91	84	94	97	98
53	498	66	73	84	77	65	71
53	272	72	110	101	88	89	103
Means		83	101	108	108	94	108
B. HISTAMINE DIHYDROCHLORIDE 0.125 mg. per hr.							
43	60	67	127	112	85	45	135
43	103	82	103	98	55	110	75
44	70	103	180	136	114	123	129
44	38	142	187	179	153	116	111
45	31	184	123	55	461	213	216
45	189	61	93	93	110	123	122
48	280	117	94	63	58	64	99
48	280	93	118	120	114	85	98
53	126	94	111	119	138	79	107
53	173	80	53	100	70	70	51
Means		104	119	108	131	103	114

In no instance is post-injection output significantly different from control.

TABLE XV. EFFECT OF SINGLE INTRAVENOUS INJECTION OF CHOLECYSTOKININ (75 clin.units) ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE

(Control is mean of four 15 min. outputs immediately preceding injection)

Dog No.	Control µEq/15 min.	Post-injection 15 min. acid outputs expressed as % of control					
		1	2	3	4	5	6
A. HISTAMINE DIHYDROCHLORIDE 0.25 mg. per hr.							
43	190	75	39	95	58	47	47
43	227	51	96	78	36	66	51
44	170	66	54	74	58	47	69
44	198	57	13	12	28	28	38
45	447	81	42	42	62	69	76
45	314	83	54	53	32	76	85
48	663	60	4	8	23	24	21
48	436	31	2	6	11	20	39
53	315	214	273	310	291	224	248
53	353	138	138	155	160	165	156
Means		86	71	81	76	77	83
B. HISTAMINE DIHYDROCHLORIDE 0.125 mg. per hr.							
43	78	46	72	47	46	35	64
43	87	54	9	3	13	29	37
44	88	51	8	8	7	9	16
44	41	37	0	0	20	44	78
45	80	66	23	21	13	28	41
45	212	52	7	2	3	12	39
48	199	89	10	2	3	3	3
48	266	27	3	0	0	2	6
53	138	183	54	15	37	51	67
53	193	122	58	56	78	88	93
Means		73	24**	15**	22**	30**	44**

Asterisks indicate significant difference from control ** P = < 0.01

TABLE XVI. COMPARISON OF EFFECTS OF SINGLE INTRAVENOUS INJECTIONS OF SECRETIN AND OF CHOLECYSTOKININ ON ACID RESPONSES OF HEIDENHAIN POUCHES TO CONTINUOUS INTRAVENOUS GASTRIN AND HISTAMINE

(Summary of mean post-injection outputs from Tables XII to XV)

	Post-injection 15 min. acid outputs expressed as % of control					
	1	2	3	4	5	6
A. GASTRIN 2.5 g/hr.						
Secretin	54**	42**	93	103	90	105
Cholecystokinin	68**	30**	22**	40**	76**	90
B. HISTAMINE DIHCl. 0.25 mg/hr.						
Secretin	83	101	108	108	94	108
Cholecystokinin	86	71	81	76	77	83
HISTAMINE DIHCl. 0.125 mg/hr.						
Secretin	104	119	108	131	103	114
Cholecystokinin	73	24**	15**	22**	30**	44**

Asterisks indicate significant difference from control ** P = < 0.01

earlier study of dose/response curves (Figs. 3 and 4), to elicit responses of the same order as 2.5 grams per hour of gastrin extract. The first parts of Tables XIV and XV show that neither secretin or cholecystokinin had any constant effect on the secretion induced by this dose of histamine.

At this point it appeared that these two duodenal extracts exhibited the pattern of behaviour described by Andersson as characteristic of acid duodenal inhibition, i.e. inhibition of gastrin but not of histamine. However, on the assumption that histamine might be more resistant to inhibitory influences, and that a smaller dose might be more amenable to inhibition, the experiments were repeated using 0.125 mg. histamine dihydrochloride per hour. The results, given in parts B. of Tables XIV and XV, show that while secretin had no demonstrable effect on the response, significant inhibition followed the injection of cholecystokinin. This was of the same order of intensity and duration as the depression of gastrin response by cholecystokinin.

Comparison of the mean results of all the foregoing experiments (Table XVI) would seem to indicate the following points:

(i) Both secretin and cholecystokinin, as prepared commercially, possess properties inhibitor to gastric secretion.

(ii) Gastrin responses are more easily inhibited than histamine responses.

(iii) Cholecystokinin is a more potent inhibitor than secretin.

(iv) When given at lowest dose rates only, histamine is inhibited by injected cholecystokinin, but not by secretin.

Greenlee et al.[96] reported that intravenous injections of secretin (Lilly) inhibited the response to feeding and to pyloric gland area pouch stimulation, but not to vagal stimulation or histamine, and concluded that the mechanism by which the inhibition was mediated was suppression of gastrin release. The present studies would seem to indicate interference with the action of circulating gastrin by the inhibitory agent.

The question arises as to whether the inhibition is a property of pure secretin and pure cholecystokinin or of some other fraction present in the extracts. Commercially available cholecystokinin is a cruder extract than secretin, and indeed is known to contain small amounts of the latter hormone. This knowledge, plus the finding of greater inhibition from the cholecystokinin extract, supports the belief that the inhibitor agent is an additional factor present in both extracts, not the pure hormones themselves.

Apparently pure secretin has recently been isolated by Jorpes[111] and it will be of great interest to repeat the experiments with the pure compound to ascertain whether inhibition of the response to gastrin is still present.

The solution of the nature of the inhibitor agent is of obvious clinical importance.

Worthy of comment from Tables XII and XV is the fact that in several experiments the inhibition of acid secretion was followed by an increase over control levels. The significance of this occurrence is obscure, but it is interesting to speculate that gastrin-like activity is also present in the duodenum and present in these extracts.

Chapter 6

6. EFFECT OF FEEDING FAT EMULSION ON ACID RESPONSE OF HEIDENHAIN POUCH TO INJECTED GASTRIN AND HISTAMINE

The release by fat in the duodenum or upper small intestine, of a hormone capable of inhibiting gastric acid secretion, was demonstrated by Kosaka and Lim[112], who originated the name "enterogastrone". It has been reported that enterogastrone will inhibit the acid secretory response to histamine (Sircus[105]; Gray, Bradley and Ivy[113]). On reviewing the published evidence Gregory[9] suggested that the inhibition of histamine induced secretion by the introduction of fat into the small intestine or by injection of the various "enterogastrone" extracts prepared by different workers (Kosaka and Lim[112], Gray, Bradley and Ivy[113], Greengard *et al.*[114]) could possibly be explained by resultant nausea, shown by Grossman *et al.*[115] to be a powerful depressant of gastric secretory responses. Gregory stated, however, that enterogastrone did diminish gastrin release from the pyloric gland area (Gregory and Tracy[116]) - a role similar to that postulated for secretin by Greenlee *et al.*[96].

Since it has been shown that the presence of fat in the stomach has no entergastrone-like effect (Shay, Gershon-Cohen and Fels[117], Lim, Ivy and McCarthy[118], Quigley[119]), the oral administration of fat is concluded to be a reliable means of stimulating the enterogastrone

mechanism. In the present studies Heidenhain pouch dogs were given orally 50 ml. of a commercial 66% corn oil emulsion (Lipomul) after a plateau acid response had been established to the continuous intravenous injection of gastrin extract in the first series of experiments, and histamine in the second series. On no occasion was any sign of nausea, such as salivation, restlessness, languor, lip-licking or retching noted. Indeed, all the dogs showed obvious signs of enjoying the emulsion, and consumed the total quantity as soon as proffered. Results of three observations on each of four dogs, using gastrin extracts are presented in Table XVII, and of the same number of observations on the same dogs, using histamine, in Table XVIII. Although the individual patterns showed considerable variation, particularly in the case of gastrin experiments (Table XVII), there was significant inhibition of the secretory responses to both gastrin and histamine after the oral administration of the fat emulsion.

These results suggest that enterogastrone is capable of depressing the response of the fundic glands to circulatory gastrin, but of course do not exclude the possible interference with gastrin release as an additional mechanism. The comparison of the effects of feeding fat on the responses to endogenous and exogenous gastrin in the same animals would be of interest.

TABLE XVII. EFFECT OF FEEDING FAT EMULSION ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF GASTRIN EXTRACT, 5 GRAMS PER HOUR

(Control is mean of four 15 min. outputs immediately preceding oral administration of 50 ml. "Lipomul" fat emulsion)

Dog No.	Control µEq/15 min.	15 min. acid outputs after fat feeding expressed as % of control											
		1	2	3	4	5	6	7	8	9	10	11	12
43	294	51	61	36	17	17	39	45	38	55	81	43	76
43	156	117	60	31	36	49	47	56	69	63	92	84	74
43	219	101	77	119	74	48	116	59	37	106	41	103	105
48	184	54	5	103	61	30	53	60	58	65	76	89	84
48	205	5	34	20	49	26	20	116	31	88	66	77	89
48	136	111	86	64	55	74	58	79	63	110	75	100	108
53	268	101	75	87	67	54	112	89	75	136	103	195	93
53	211	89	67	44	43	40	107	77	53	94	115	115	54
53	481	72	41	29	22	25	24	22	20	23	36	27	29
55	145	136	140	46	52	74	146	74	100	188	138	76	68
55	80	80	110	166	118	156	110	94	91	304	278	401	273
55	258	131	111	82	65	74	105	73	128	156	75	97	126
Means		87	72*	69*	55**	56**	78	70**	64**	116	98	117	98

Asterisks indicate significant difference from control * P = < 0.02
** P = < 0.01

TABLE XVIII. EFFECT OF FEEDING FAT EMULSION ON ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE, 0.25 MG. PER HOUR

(Control is mean of four 15 min. outputs immediately preceding oral administration of 50 ml. "Lipomul" fat emulsion)

Dog No.	Control µEq/15 min.	15 min. acid outputs after fat feeding expressed as % of control											
		1	2	3	4	5	6	7	8	9	10	11	12
43	197	154	100	78	91	75	87	89	71	76	92	64	86
43	287	111	83	90	77	93	53	88	76	70	57	74	62
43	256	121	68	85	82	85	73	63	66	64	61	65	82
48	257	95	85	79	67	60	63	56	47	49	51	51	43
48	473	119	99	74	64	66	57	63	58	48	56	46	50
48	160	123	113	56	61	53	78	64	51	55	38	44	73
53	249	80	58	66	56	64	55	59	58	55	47	45	45
53	437	99	80	70	77	67	74	58	71	70	71	57	66
53	167	97	64	39	47	60	57	64	43	32	40	45	41
55	132	92	106	69	78	60	88	61	62	61	62	53	75
55	174	116	75	92	64	78	86	70	64	78	51	53	59
55	222	91	106	93	78	99	93	91	92	97	105	96	126
Means		92	86*	74**	70**	72**	72**	69**	63**	63**	61**	58**	67**

Asterisks indicate significant difference from control

** P = < 0.01

* P = < 0.025

DISCUSSION OF THE INHIBITORY PATTERNS OUTLINED IN Chapters 3 to 6

The conclusions drawn from the preceding Chapters and from the work of Andersson may be summarised as follows:

(1) Acidification of the pyloric gland area prevents gastrin release, but does not release an inhibitor hormone effective against circulating gastrin.

(2) Acidification of the duodenum inhibits the acid response to injected gastrin, but not to histamine (Andersson).

(3) Secretin and cholecystokinin (Vitrum) exhibit the same pattern of inhibition as noted in (2).

(4) Enterogastrone moderately inhibits the acid responses both to gastrin and to histamine.

(5) The greatest degree of inhibition of both gastrin and histamine responses is seen to follow the injection of gastrin extract at dosage greater than that required to elicit maximal acid response.

If, as it appears, the histamine response is less readily inhibited than the gastrin response, these various results might be interpreted as simply due to different grades of inhibitor potency. It could be postulated that acidification of the pyloric gland area is less potent than duodenal acidification as an inhibitor, that commercial secretin is similarly less potent than commercial cholecystokinin, the

latter being capable of a degree of inhibition comparable to that of duodenal acidification, that enterogastrone is a more potent inhibitor than duodenal acidification, and that the strongest inhibitor agent is contained in extracts of the pyloric gland area, possibly gastrin itself.

Chapter 7

7. EFFECT OF ATROPINE ON THE ACID RESPONSE OF HEIDENHAIN POUCH TO GASTRIN AND TO HISTAMINE

The previous Chapters dealt with possible physiological mechanisms by which the response of the fundic glands to various stimuli might be inhibited. The present Chaper considers the pharmacological inhibitory effects of atropine.

Although it was demonstrated by Atkinson and Ivy[120] in 1939 that atropine inhibited the gastric acid response to histamine, this fact has only recently been confirmed and generally accepted. The variation in the results of different workers (Atkinson and Ivy[120], Janowitz and Hollander[121], Code, Hightower and Hallenbeck[122], Gray[123], Benjamin, Rosiere and Grossman[124], Oberhelman and Dragstedt[125]) seemed possible of explanation in that a wide range of histamine doses had been employed.

Although there was general agreement that atropine depressed gastrin release from the pyloric gland area (Gregory and Ivy[24], Woodward et al.[126]) the few reports of studies on the effect of atropine on the response to injected gastrin extracts revealed widely divergent results. Blair et al.[127], using the anaesthetized cat with a vagally denervated whole stomach, found atropine to have no effect on gastrin induced secretion, agreeing with Komarov's earlier report[58]. In dogs with denervated fundic pouches Grossman[128] concluded that

atropine inhibited the gastrin response to a greater extent than the histamine response.

It seemed important to measure the effect of atropine on a wide dosage range of both gastrin extract and histamine, so that direct comparison could be made between the two agents. The same three dogs used in Chapter 2 (Group A) were studied, an identical series of experiments being performed on each. A different dose of gastrin extract or histamine was given each day, by continuous intravenous injection. Gastrin doses ranged from 1.25 to 20 grams per hour, histamine dihydrochloride from 0.125 to 2.0 mg. per hour. In each experiment, after the secretory plateau was established, atropine sulphate, 0.1 mg. per kilogram body weight, was injected subcutaneously, and collections of secretion from the denervated fundic pouch continued for a further two hours. Inhibition of the acid response occurred in all experiments except those in which the largest doses of gastrin or histamine were given. Inspection of the results showed that in all cases inhibition was most uniform and most complete in the second hour after the atropine injection, and the acid output during this period was compared with the mean hourly acid output for the two hours immediately preceding the injection (Table XIX). Also shown on Table XIX are the mean acid concentrations and mean hourly pepsin outputs for the same time intervals.

The curves of acid output inhibition by atropine for each

TABLE XIX. EFFECT OF ATROPINE ON HEIDENHAIN POUCH RESPONSES TO GRADED DOSES OF GASTRIN AND OF HISTAMINE BY CONTINUOUS INTRAVENOUS INJECTION

(Control is mean hourly output for 2-hour period immediately preceding atropine injection. 'After atropine' results refer to 2nd hour after injection)

Dog No.	A. GASTRIN Gastrin dose (g./hr.)	ACID Concentration (μEq/ml.) Control	ACID Concentration (μEq/ml.) After atropine	ACID Output (μEq/hr.) Control	ACID Output (μEq/hr.) After atropine	ACID Output (μEq/hr.) % Inhibition	PEPSIN OUTPUT Control	PEPSIN OUTPUT After atropine	PEPSIN OUTPUT % Inhibition
44	1.25	141	142	671	19	97	1104	-	-
48	1.25	139	55	917	22	98	9301	199	98
53	1.25	120	114	481	31	94	705	71	90
44	2.5	152	137	814	235	71	404	58	86
48	2.5	139	60	741	44	94	644	74	88
53	2.5	152	140	1957	28	99	1810	14	99
44	5.0	145	133	834	166	80	1179	-	-
48	5.0	143	123	1050	430	59	1634	135	92
53	5.0	153	131	2568	512	80	4066	192	95
44	10.0	150	120	915	133	85	849	-	-
48	10.0	150	143	1452	1183	19	1425	455	68
53	10.0	156	87	4322	563	87	4863	548	88
44	20.0	137	148	732	796	0	222	178	20
48	20.0	144	148	1295	1263	2	1075	463	57
53	20.0	158	101	4112	644	84	3432	594	83
	B. HISTAMINE								
Dog No.	Histamine dihydrochloride dose (mg./hr)								
44	0.125	-	-	-	-	-	-	-	-
48	0.125	124	36	737	32	96	10866	391	96
53	0.125	128	124	249	186	25	199	108	46
44	0.25	134	130	621	377	39	400	122	69
48	0.25	140	136	1717	694	60	14155	360	97
53	0.25	132	123	785	588	25	3230	1056	67
44	0.5	152	153	1177	781	34	5215	191	96
48	0.5	156	154	3246	2382	27	12890	788	94
53	0.5	155	153	2645	2039	23	4500	681	85
44	1.0	156	159	1638	1672	0	4270	774	82
48	1.0	156	163	3373	3312	2	2445	666	73
53	1.0	152	154	3770	3140	17	3206	728	77

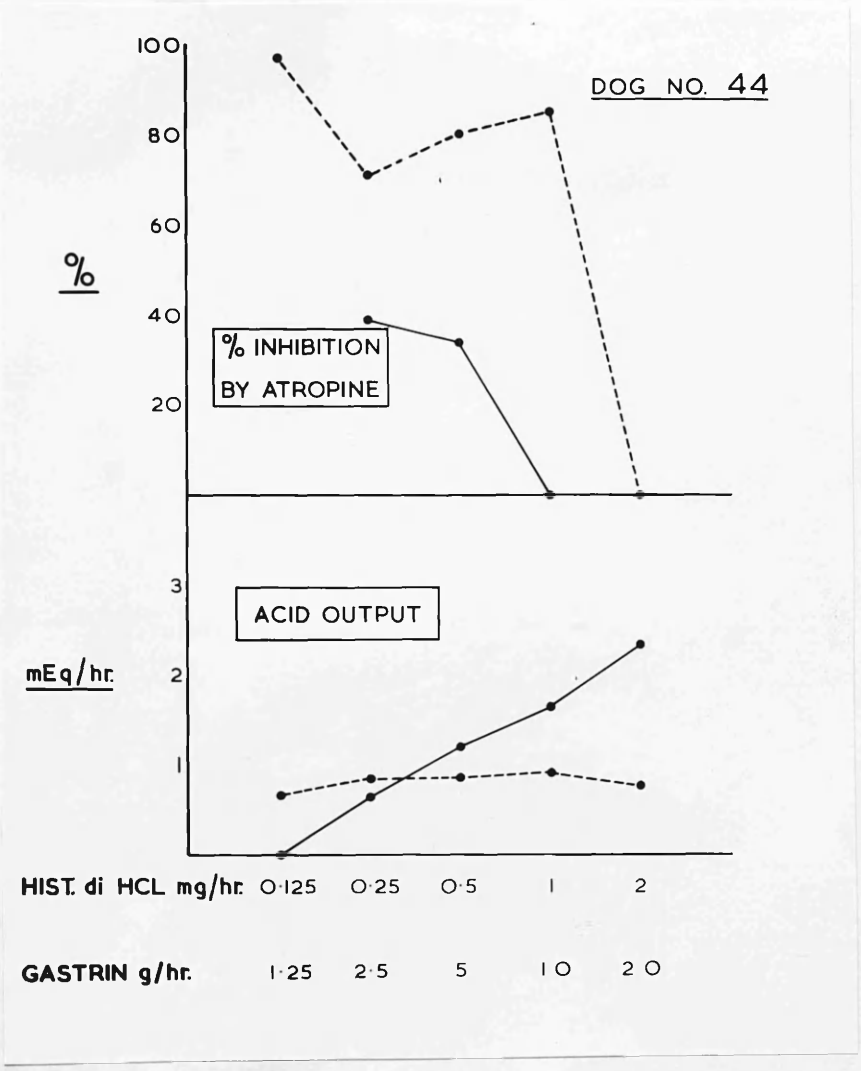
individual dog are shown in Figs.17, 18 and 19. In the lower part of each Figure are the acid output dose/response curves for the same range of doses. The results of dog No.48 (Fig.18) will be considered first, as they demonstrate a wider, and probably more complete range of the effects of atropine. It is seen (a) that atropine inhibited the response to small doses of gastrin or histamine by over 90%, there being little difference in the degree, (b) that in the middle dosage range atropine inhibited the gastrin response to a greater extent than the histamine response, and (c) at highest dose rates neither response was inhibited.

Since dog No.44 (Fig.17) had no acid response to 0.125 mg. histamine dihydrochloride per hour, the effect of atropine on this dose could not be determined. This point apart, the patterns of atropine inhibition are similar to those of dog No.48 (Fig.18).

Although at first sight the results of dog No.53 (Fig.19) appear to differ markedly from those of the other two dogs, they can possibly be interpreted in a similar manner, taking into account the variation in responsiveness to stimulation of acid secretion.
It is noted that in dog No.53 there was no indication that maximal acid response to either gastrin or histamine had been achieved by the largest dose of either agent used, whereas in dog No.48 maximal responses to both gastrin and histamine, and in dog No.44 maximal to gastrin, were obtained. Thus the atropine inhibition curves for

FIG. 17

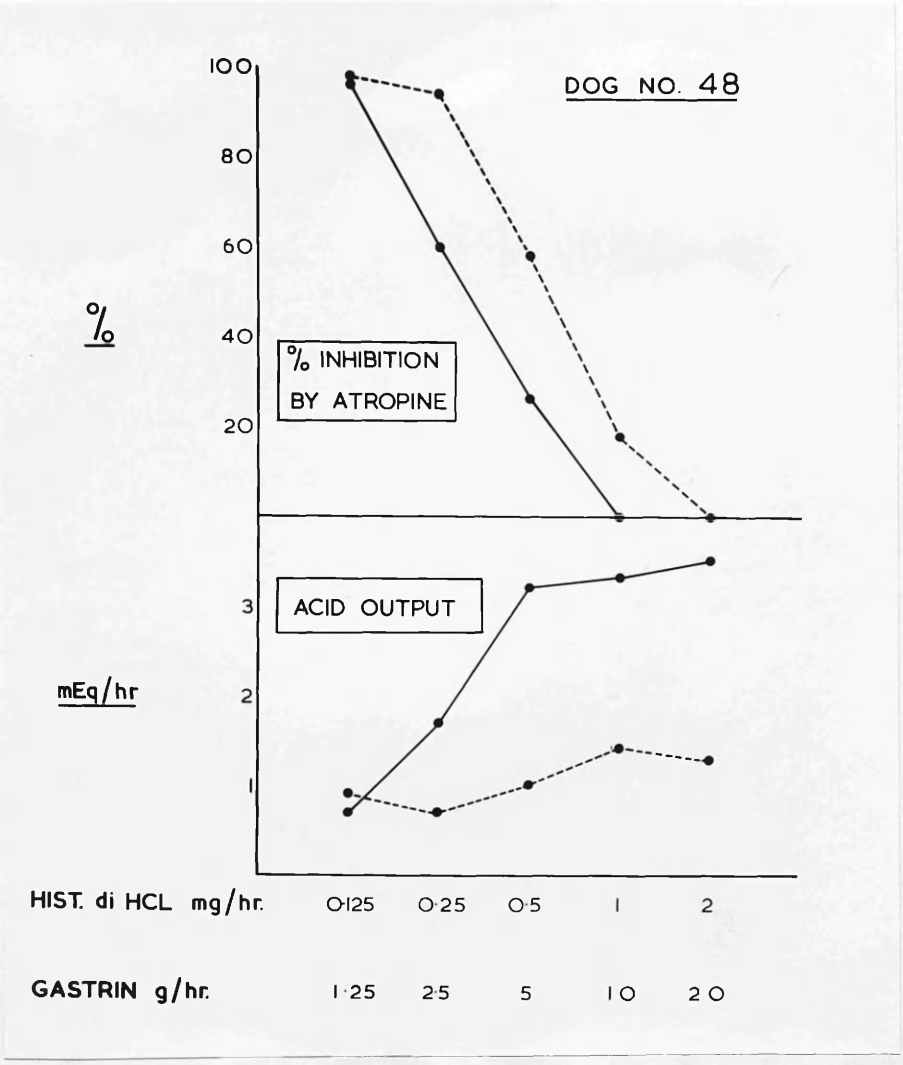
EFFECT OF ATROPINE ON ACID RESPONSES OF HEIDENHAIN POUCH TO GRADED DOSES OF GASTRIN EXTRACT AND OF HISTAMINE BY CONTINUOUS INTRAVENOUS INFUSION



The acid responses to the largest doses of gastrin extract and of histamine were not inhibited by atropine. In the intermediate dose range atropine inhibited the acid response to gastrin extract to a greater extent than the response to histamine.

FIG. 18

EFFECT OF ATROPINE ON ACID RESPONSES OF HEIDENHAIN POUCH TO GRADED DOSES OF GASTRIN EXTRACT AND OF HISTAMINE BY CONTINUOUS INTRAVENOUS INFUSION



Histamine

Gastrin

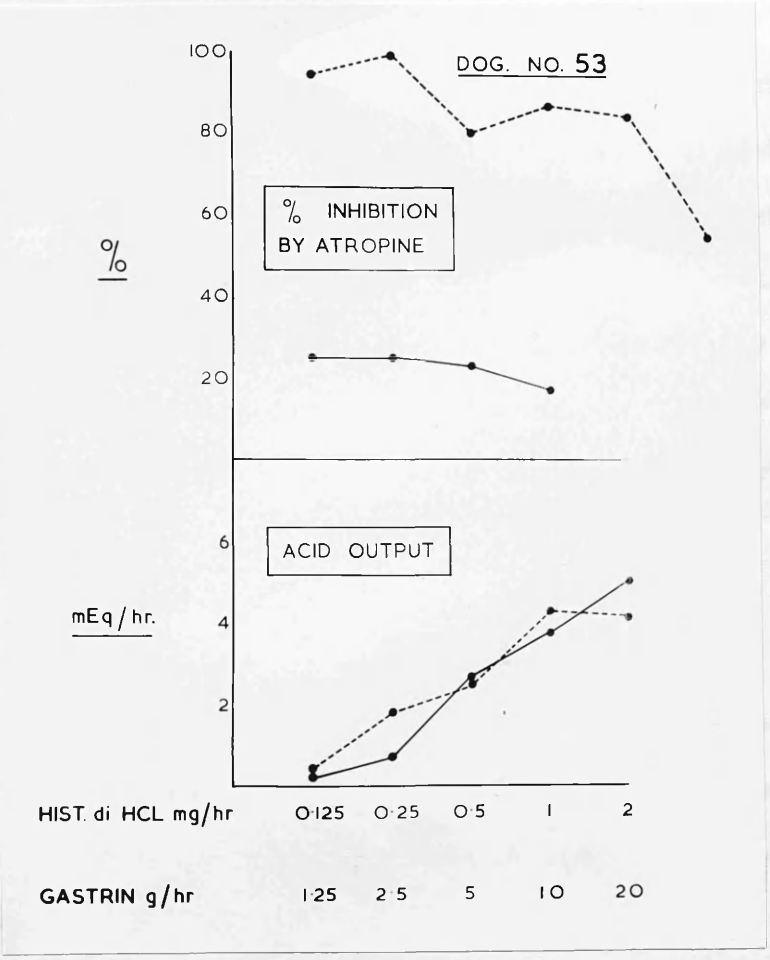
1. At lowest dose rates responses, both to gastrin extract and to histamine were inhibited almost completely by atropine.

2. In the middle dose range atropine inhibited the acid response to gastrin extract to a greater extent than the response to histamine.

3. The acid responses to the largest doses of gastrin extract and of histamine were not inhibited by atropine.

FIG. 19

EFFECT OF ATROPINE ON ACID RESPONSES OF HEIDENHAIN POUCH TO GRADED DOSES OF GASTRIN EXTRACT AND OF HISTAMINE BY CONTINUOUS INTRAVENOUS INFUSION



The acid responses to gastrin extract were more markedly inhibited by atropine than those to histamine. There was reduced inhibition by atropine of the acid output to the largest dose of gastrin extract (20 grams per hour).

dog No.53 (Fig.19) may well be equivalent to the central portions of those of the other two dogs. This variation in the degree of inhibition depending on the dose of acid secretory stimulant may in part explain the discrepancies previously reported.

It is interesting to note from Figs.17 and 18 that although the degree of atropine inhibition steadily decreased as gastrin doses increased there was still moderate inhibition when the first dose to produce maximal acid response was reached, and that several further increases in dose above this level were required before the inhibitory effects of atropine were completely abolished. A similar finding for histamine is suggested in the case of dog No.48, but not in dog No.44.

Possible hypotheses suggested by the features of the atropine inhibition curves discussed include:

(1) Only the smaller doses of gastrin and of histamine require the presence of acetylcholine (perhaps as a potentiating mechanism) for full response, and as the doses increase, so both agents become less dependent on acetylcholine and more capable of direct fundic gland cell stimulation. This being the case gastrin would appear to be more dependent on acetylcholine than histamine since atropine regularly inhibited the gastrin responses to a greater degree than the histamine responses.

(2) Gastrin and histamine may increasingly depress acetylcholine

activity with increasing dosage, continuing this process even after maximal acid stimulatory dosage is reached. This would account for the further decrease in atropine inhibition with dose increases above those required for maximal response.

It has been postulated that the inhibitory effect of atropine on gastric acid secretion is manifest in a reduction of volume only, without any corresponding alteration in concentration, the interpretation being that atropine reduces gastric blood flow. Caution must be used in drawing conclusions from study of concentration alterations, since the final concentration of any particular substance depends upon so many variable factors. However, the fact that, with only one exception, all the experiments in the present study in which inhibition occurred following the injection of atropine, displayed a fall in acid concentration as well as in volume (Table XIX), suggests that atropine has a direct effect on acid stimulatory mechanisms rather than merely a secondary effect as a result of changes in blood flow.

It is seen from Table XIX that pepsin secretion was reduced by atropine in all experiments. The inhibition was of a high order in most instances and did not show a distinct reduction with increase in dose of gastrin or histamine. These findings support the belief that acetylcholine is a major factor in the stimulation of pepsin production.

Chapter 8

8. POTENTIATION BY URECHOLINE OF HEIDENHAIN POUCH RESPONSE TO GASTRIN AND TO HISTAMINE

In this Chapter and the two immediately following attention is turned from factors depressing to those augmenting gastric secretory responses.

The possibility that acetylcholine facilitates the responsiveness of the fundic glands to stimuli, e.g. gastrin and histamine, has been raised in PART I and in Chapter 7. For many years evidence has been accumulating that vagotomy reduces gastric secretion in the fasting state, and that in response to a wide variety of physiological and pharmacological stimuli (Dragstedt *et al.*[32], Stein and Meyer[48], Antia and Ivy[129], Oberhelman and Dragstedt[125]), and the view that the intact vagus, via acetylcholine release, exerts a tonic, synergistic, or augmentatory influence on the response of the fundic glands has been increasingly expressed. Such an opinion was stated as early as 1906 by Orbeli[49].

Gray and Ivy[89] in 1937 showed that at critical dosage Mecholyl would potentiate the response of a vagotomized total stomach pouch to histamine. Robertson and Grossman[130] reported a similar finding using Urecholine. More recently Marks, Komarov and Shay[131] demonstrated that Mecholyl would increase the maximal histamine response of a gastric fistula dog.

Finally, that cholinergic potentiation of fundic gland responses might be of physiological importance was strongly suggested by the work of Grossman[128,132], who showed that distention of a vagally denervated fundic pouch greatly potentiated its response to injected gastrin or histamine, and that such potentiation was abolished by atropine. The distention was accompanied by a minimal rise in pressure, being such as might result from the presence of a meal in the stomach. A distinct secretory response to distention alone, and a greater degree of potentiation of the histamine response were demonstrated in the innervated, antrectomized stomach (Grossman[133]). The former finding illustrated the importance of short vago-vagal reflex arcs, the latter of long vago-vagal arcs. Both emphasized the role of acetylcholine at post-ganglionic nerve endings in the responsiveness of the fundic glands.

Because of these indicators of the probable importance of acetylcholine potentiation, a study was made of the effects of a range of dose rates of Urecholine (the stable choline ester β-methyl-choline chloride) on the responses to a range of dose rates of gastrin extracts, and of histamine.

A further point of interest was to see whether Urecholine would abolish the difference between the levels of maximal acid response to gastrin extract and to histamine, referred to in Chapter 2.

Observations were made on five Heidenhain pouch dogs. On each day Urecholine was given by continuous intravenous injection at

a constant rate, the rates on different days varying from 0.25 to 4 mg. per hour. After two hours of Urecholine alone, gastrin extract or histamine was given by a second continuous intravenous infusion, the dose rate being doubled every 75 minutes. Potentiation, when it occurred, did so within 15 minutes of starting or increasing the gastrin or histamine dose. For this reason the one hour output from 15 to 75 minutes after each alteration in dose rate was taken as a measure of the response to the combination of drugs. With all but the lowest doses of Urecholine side-effects occurred, namely increased salivation, micturition and defaecation. Because of the severity of these side-effects no test was done with a dose of Urecholine greater than 4 mg. per hour. Control runs of Urecholine alone, gastrin extract alone, and histamine alone, over the same dose ranges used in the combined studies were also done on separate days.

The following criteria were taken to signify true potentiation, as opposed to simply additive effects; (i) if the observed response to the combined doses of the two agents exceeded half of the sum of the responses to twice the dosage of each agent given alone; (ii) if the response to the combined agents exceeded the maximal response attainable by either agent alone.

The results presented in the following Figures (Nos. 20 to 26) are mean values for the five dogs. The data from which these means were derived are given in full in Tables XX to XXV at the end of this Chapter.

The acid output results will be considered first.

Urecholine + Gastrin:

It is seen from Fig.20 that the smaller doses of Urecholine (0.25 to 1 mg. per hour) potentiated the responses to all dose rates of gastrin studied. Urecholine at a rate of 2 mg. per hour produced less augmentation of the gastrin responses than 0.5 and 1 mg. per hour, and 4 mg. per hour, the largest dose of Urecholine used, produced outputs from the added gastrin no greater than from that rate of Urecholine alone.

The maximal acid response to the combined administration of Urecholine and gastrin (7.0 mEq. per hour) was greater than the maximal to gastrin alone (2.2 mEq. per hour), and occurred earlier in the increasing dosage scheme (10 grams per hour) than with gastrin alone (20 grams per hour).

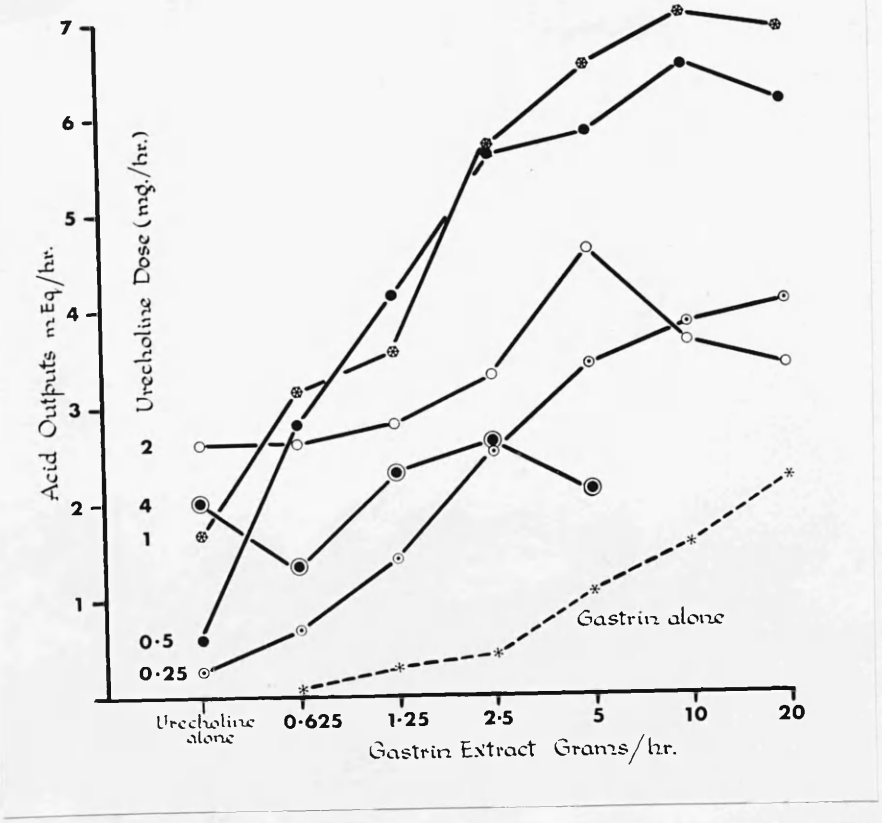
Urecholine + Histamine:

The pattern obtained (Fig.21) was similar in certain respects to that of Urecholine plus gastrin. Clear evidence of potentiation of all histamine responses was found. At a dose of 2 mg. per hour Urecholine did not produce any greater acid response in combination with histamine than did 1 mg. per hour, which would again seem to have been the optimal dose for potentiation - as in the case of gastrin. The maximal acid response to histamine was greatly elevated (from 5.3 mEq. per hour for histamine dihydrochloride alone to 9.3 mEq. per hour for histamine plus Urecholine), confirming the work of Marks, Komarov and Shay[131].

FIG. 20

ACID RESPONSES OF HEIDENHAIN POUCHES TO COMBINED URECHOLINE AND GASTRIN EXTRACT, EACH IN GRADED DOSES

(Mean results of 5 dogs)



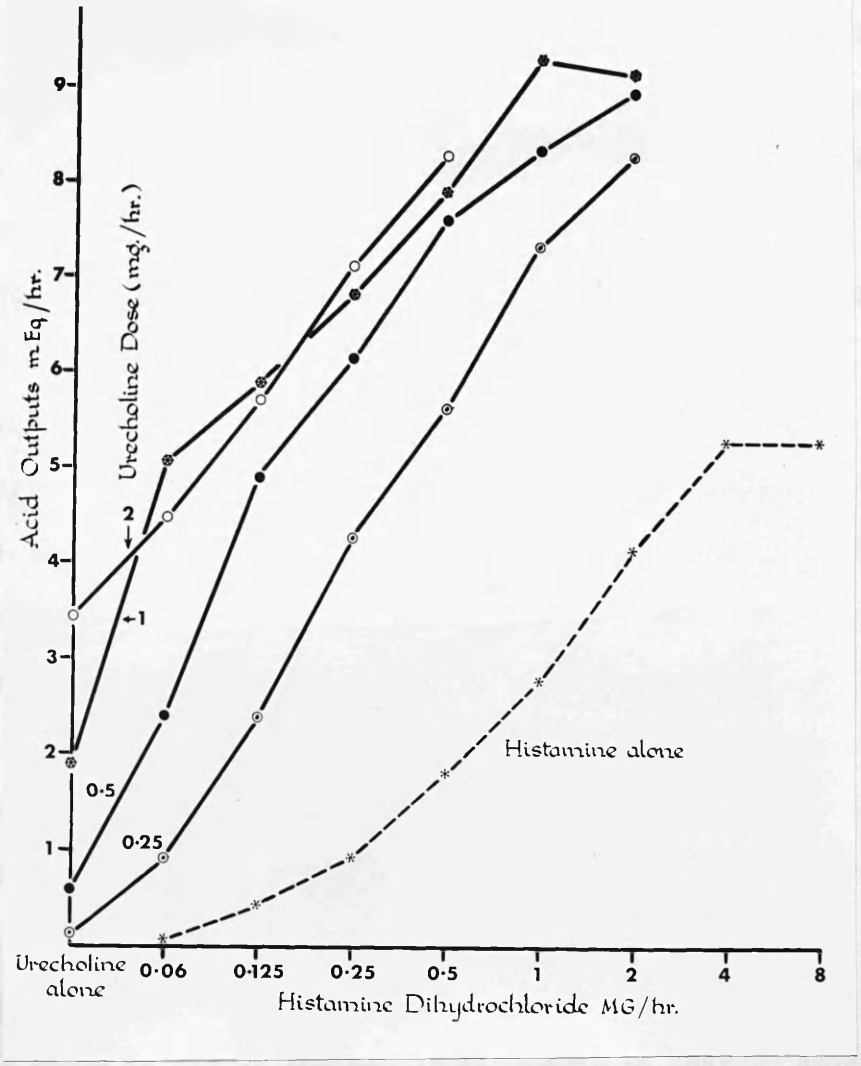
The acid responses to gastrin extract were markedly potentiated by the lower dose rates of Urecholine (0.25 to 1 mg. per hour).

There was less evidence of potentiation when the Urecholine dose was 2 or 4 mg. per hour.

FIG. 21

ACID RESPONSES OF HEIDENHAIN POUCHES TO COMBINED URECHOLINE AND HISTAMINE, EACH IN GRADED DOSES

(Mean results of 5 dogs)



Potentiation of the acid responses occurred between the smaller doses of Urecholine and of histamine. 2 mg. per hour Urecholine did not exhibit any greater potentiation than did 1 mg. per hour.

Again potentiated histamine achieved maximal secretory responses with smaller doses (1 mg. per hour) than did histamine alone (4 mg. per hour).

In no instance was depression of the acid response by Urecholine noted - as had been reported by Gray and Ivy[89] using a large dose of Mecholyl. As has been stated, because of the obvious gross discomfort associated with the largest doses of Urecholine used in the present study, particularly in combination with histamine, it was decided not to investigate the question of whether doses of Urecholine greater than 4 mg. per hour exerted an inhibitory effect on the acid responses to gastrin or to histamine.

Pepsin output results:

Since Urecholine is such a powerful stimulant of pepsin secretion it was not expected that the simultaneous administration of gastrin extract or histamine, both relatively weak pepsin stimulants by themselves, would appreciably increase the pepsin responses to Urecholine. Even the lowest dose rates of Urecholine used resulted in pepsin outputs of the same order as the maximal obtained to gastrin alone or histamine alone. Thus the findings are less well defined than in the case of the acid responses.

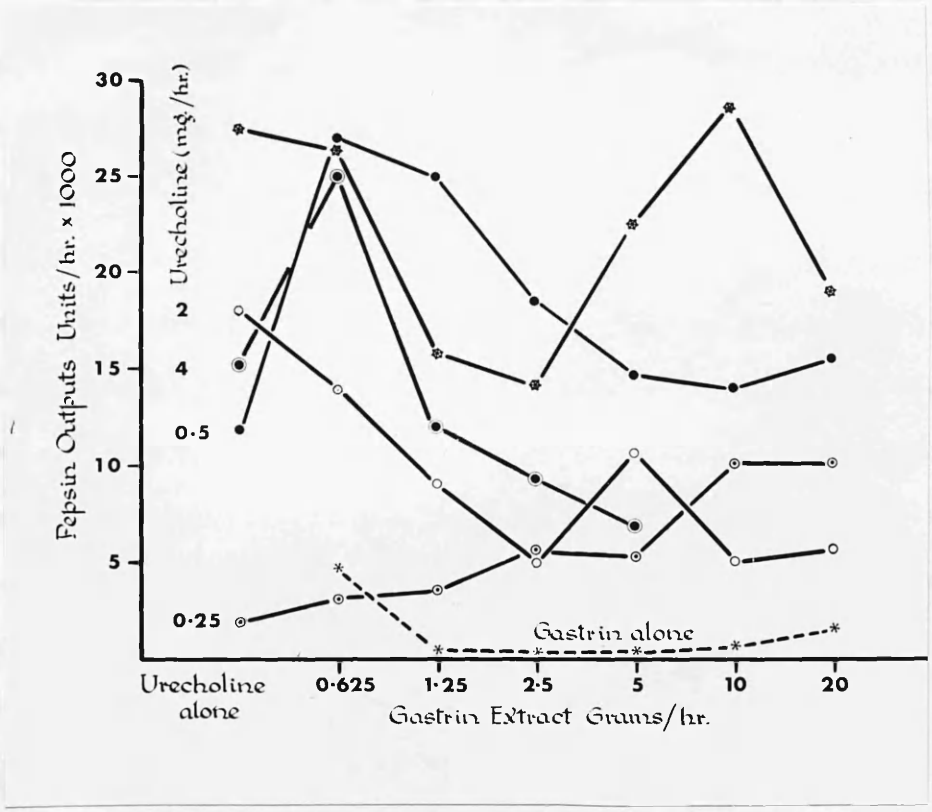
Urecholine + Gastrin:

As shown in Fig.22 the pepsin responses to gastrin alone again suggested the triphasic response referred to in Chapter 2, namely stimulation of pepsin secretion at both smallest and largest dose rates,

FIG. 22

PEPSIN RESPONSES OF HEIDENHAIN POUCHES TO COMBINED URECHOLINE AND GASTRIN EXTRACT, EACH IN GRADED DOSES

(Mean results of 5 dogs)



Only with the combination of the lowest dose of Urecholine (0.25 mg. per hour) and the highest doses of gastrin extract (10 and 20 grams per hour) was there evidence of potentiation. The other dose rates of Urecholine produced, of themselves, high pepsin outputs, which were not materially affected by the addition of gastrin extract.

and failure of stimulation in the middle dose range. Only the response to the lowest dose rate of Urecholine (0.25 mg. per day) was progressively increased by each additional dose of gastrin extract. True potentiation, as judged by the criteria outlined earlier in this Chapter, occurred with only the largest two gastrin doses used, 10 and 20 grams per hour.

The patterns obtained with all the other dose rates of Urecholine suggested triphasic responses, but did not show convincing evidence of true potentiation. It is of interest that in the curves of 1, 2 and 4 mg. Urecholine per hour the addition of doses of gastrin above 0.625 g. per hour appeared to depress pepsin output below the levels obtained from Urecholine alone at the relevant rate. This at first suggested that gastrin or some other component of the extract might be capable of depressing the pepsin response to Urecholine, and therefore to acetylcholine. However, that the response to higher doses of Urecholine alone can decrease over a period of several hours to levels below that at the outset of the experiment (Fig. 25) lessens the significance of the observation.

Urecholine + Histamine:

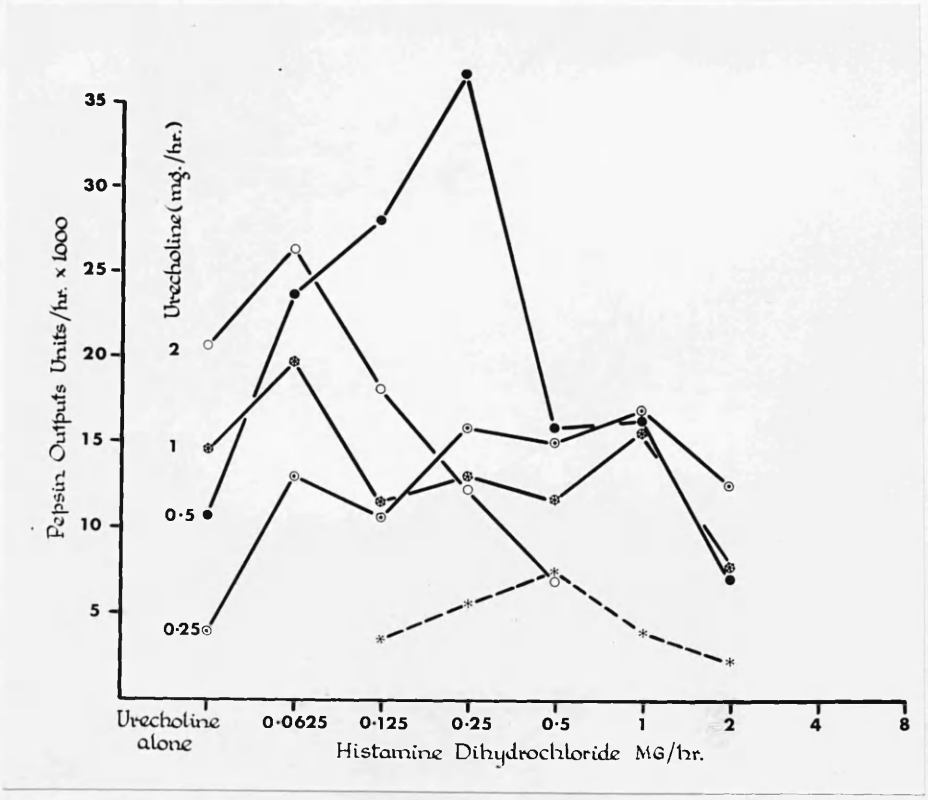
Fig. 23 shows that the pattern of pepsin response to the graded doses of histamine alone was similar to that found in the earlier studies of Chapter 2, namely, apparent increase in output to small doses, and progressively smaller outputs with larger doses.

Potentiation of the histamine responses occurred with 0.25 and

FIG. 23

PEPSIN RESPONSES OF HEIDENHAIN POUCHES TO COMBINED URECHOLINE AND HISTAMINE, EACH IN GRADED DOSES

(Mean results of 5 dogs)



Potentiation occurred between histamine and the lower doses of Urecholine (0.25 mg. and 0.5 mg. per hour). The pepsin responses to 1 mg. and 2 mg. Urecholine per hour appeared to be depressed by doses of histamine dihydrochloride greater than 0.625 mg. per hour.

0.5 mg. per hour Urecholine, being optimal with 0.5 mg. per hour. With 1 mg. per hour, and more particularly with 2 mg. per hour Urecholine, histamine doses in excess of 0.0625 mg. dihydrochloride per hour appeared to depress pepsin output to levels below those obtained to Urecholine alone. The magnitude of the depression of the 2 mg. per hour Urecholine curve was much greater than that observed in the control runs of 2 mg. per hour Urecholine alone over several hours (Fig.25), and strongly suggests interference by histamine with Urecholine or acetylcholine activity.

Urecholine alone

When control observations were run of Urecholine alone at the same rates as those used in the foregoing potentiation studies, the acid output response curves were as shown in Fig.24, and pepsin as in Fig.25, both of which illustrate similar features. The responses to the lowest doses remained reasonably constant after the first two hours of continuous intravenous infusion. When higher dose rates were used the initial large response levels were not maintained, there being a more rapid and more profound decrease in response of both acid and pepsin with 2 mg. per hour than with 1 mg. per hour (Figs.24 and 25).

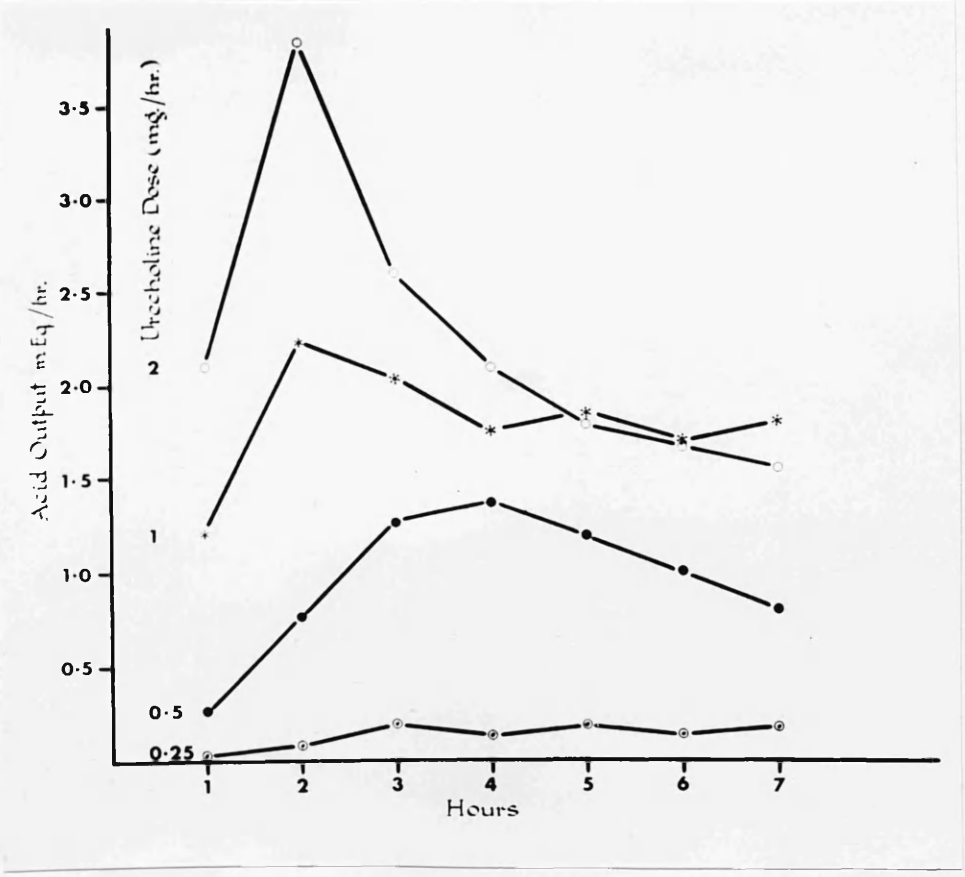
Among the possible explanations for this occurrence are the following:

(i) The initial high outputs were due to "wash-out" of preformed acid and pepsin. However, if this were the case it would be expected that

FIG. 24

ACID RESPONSES OF HEIDENHAIN POUCHES TO CONTINUOUS INTRAVENOUS UROCHOLINE OVER A 7-HOUR PERIOD

(Mean results of 5 dogs)

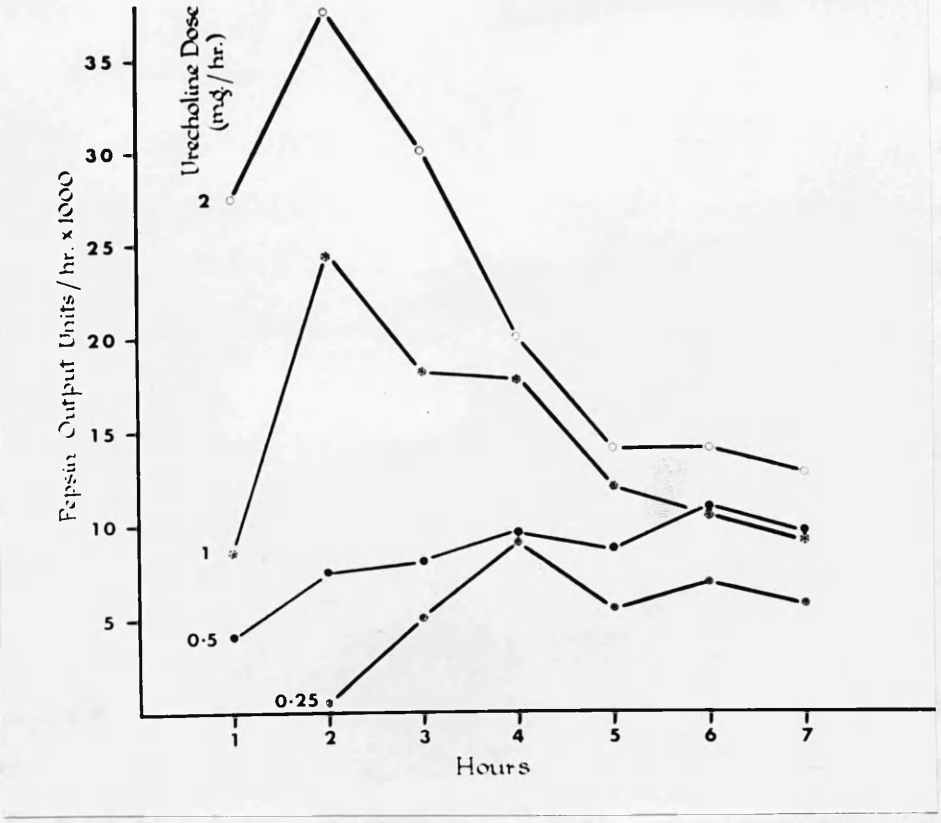


Responses to the higher dose rates appeared not to be sustained after the initial steep increase.

FIG. 25

PEPSIN RESPONSES OF HEIDENHAIN POUCHES TO CONTINUOUS INTRAVENOUS URECHOLINE OVER A 7-HOUR PERIOD

(Mean results of 5 dogs)



Responses to the higher dose rates appeared not to be sustained after the initial steep increase.

the "wash-out" peak would be of shorter duration the higher the dose of Urecholine, responses thereafter becoming relatively stable. Such findings were not borne out by Figs. 24 and 25.

(ii) Both acid and pepsin producing cells became either refractory to, or exhausted by, prolonged cholinergic stimulation at high rates. The close parallelism of both acid and pepsin curves is in support of this hypothesis.

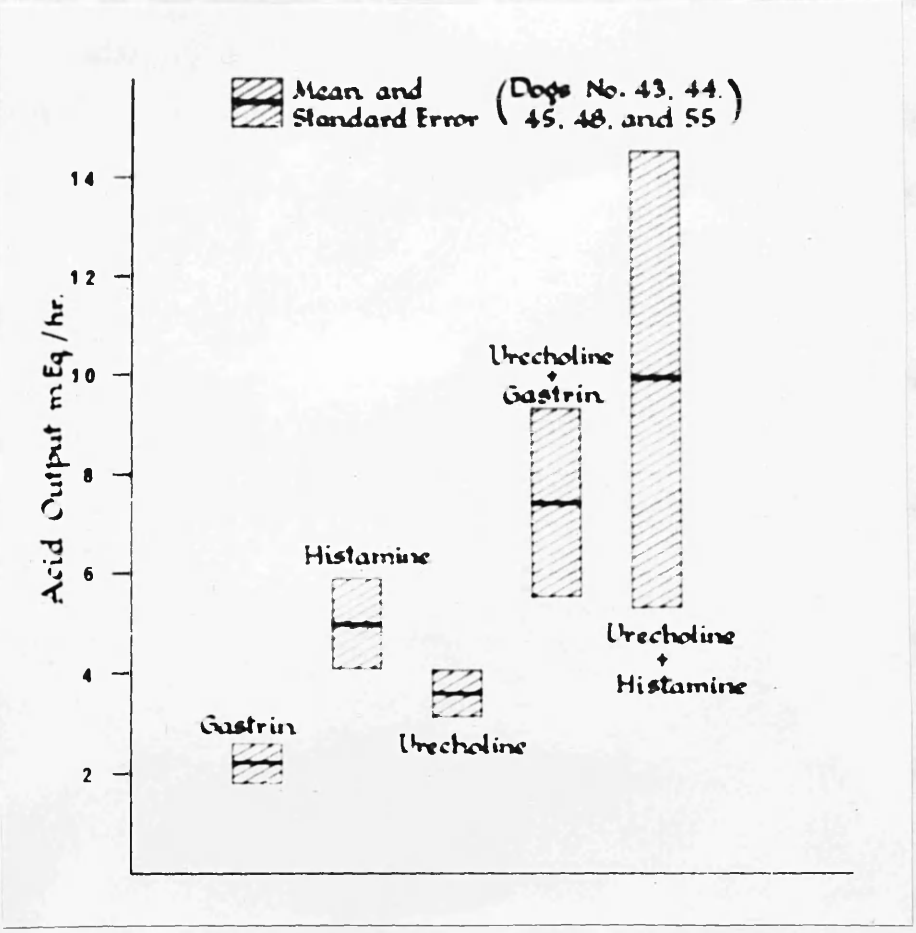
Making allowance for these changes in secretion rate does not alter the interpretation of the potentiated acid responses to the combined administration of Urecholine and gastrin, or Urecholine and histamine, but does materially influence the patterns of pepsin response, as discovered earlier in this Chapter.

Comparison of the maximal acid responses to the combined administration of Urecholine plus gastrin, and Urecholine plus histamine, with those to the three stimulants given separately (Fig. 26) illustrates several points worthy of further discussion.

Firstly, the maximal responses both to gastrin and to histamine were markedly elevated by Urecholine. Thus it would appear that neither agent alone is capable of eliciting a fully maximal response from the parietal cells. It is interesting to speculate whether the potentiated Urecholine - histamine maximal response represents true maximum activity of the fundic glands, or whether it is possible still further to increase

FIG. 26

MAXIMAL ACID RESPONSES OF HEIDENHAIN POUCHES TO GASTRIN EXTRACT, HISTAMINE DIHYDROCHLORIDE, AND URECHOLINE, ALONE AND IN COMBINATION



1. The maximal acid response to Urecholine alone approaches that to histamine alone.

2. While the maximal acid response to Urecholine + histamine is greater than that to Urecholine + gastrin extract, the difference is proportionally less than that between the maximal responses to histamine alone and to gastrin alone.

the maximal acid output by another combination of stimulants, e.g. by the simultaneous infusion of gastrin, histamine and Urecholine. By contrast to the findings of the present study Payne and Kay[50] found no increase in the maximal histamine response of the intact stomach in man by the addition of Mecholyl to the augmented histamine test (Kay[134]). If this finding is confirmed it might reflect a higher acetylcholine "tone" normally present in the stomach of man compared to that of the dog. Other points outlined by Grossman (personal communication) in favour of this hypothesis are the following:

1. Dogs require much greater doses of histamine on a body weight basis than man to elicit comparable acid responses.
2. The latency of acid response to histamine is longer in the dog than in man.
3. There seems to be a greater pepsin response to histamine in man than in the dog.
4. Atropine appears to reduce the histamine response to a greater extent in man than in the dog.
5. Vagotomy appears to reduce the maximal histamine response to a greater extent in man than in the dog.

It is seen that the addition of Urecholine did not abolish the discrepancy between the maximal responses to gastrin and histamine, the potentiated maximal histamine response being again greater than the potentiated maximal gastrin response. However, the percentage

difference between the potentiated mean maximal responses was less, and of lesser significance, the mean Urecholine + histamine maximal being 32% greater than the mean Urecholine + gastrin ($P = < 0.05 > 0.02$) and the mean maximal histamine response being 100% greater than the mean maximal gastrin response ($P = < 0.01$). Noteworthy also is that the maximal acid response to Urecholine alone approached the maximal histamine response.

These findings make it appear unlikely that the reduction in acetylcholine in the gastric wall consequent upon vagal denervation, accounts for the maximal gastrin response of a Heidenhain pouch being less than the maximal histamine response, though it would still be important to examine the acid dose/response curves in the dog provided with an innervated fundic pouch and in addition a vagally denervated one. Preliminary studies in dogs, each with a simple gastric fistula in addition to a Heidenhain pouch suggest, like the present observations, that the difference in maximal responses is still present, though less in degree. It may be that the combination of a number of factors is responsible for the discrepancy.

The present findings suggested that a background stimulation by continuous intravenous infusion of Urecholine might render a Heidenhain pouch more sensitive in the detection of small amounts of gastrin-like activity. There has been increasing interest in the possible presence of such activity in tissues and organs other than the pyloric gland area,

the maximal acid output by another combination of stimulants, e.g. by the simultaneous infusion of gastrin, histamine and Urecholine.

By contrast to the findings of the present study Payne and Kay[50] found no increase in the maximal histamine response of the intact stomach in man by the addition of Mecholyl to the augmented histamine test (Kay[134]). If this finding is confirmed it might reflect a higher acetylcholine "tone" normally present in the stomach of man compared to that of the dog. Other points outlined by Grossman (personal communication) in favour of this hypothesis are the following:

1. Dogs require much greater doses of histamine on a body weight basis than man to elicit comparable acid responses.
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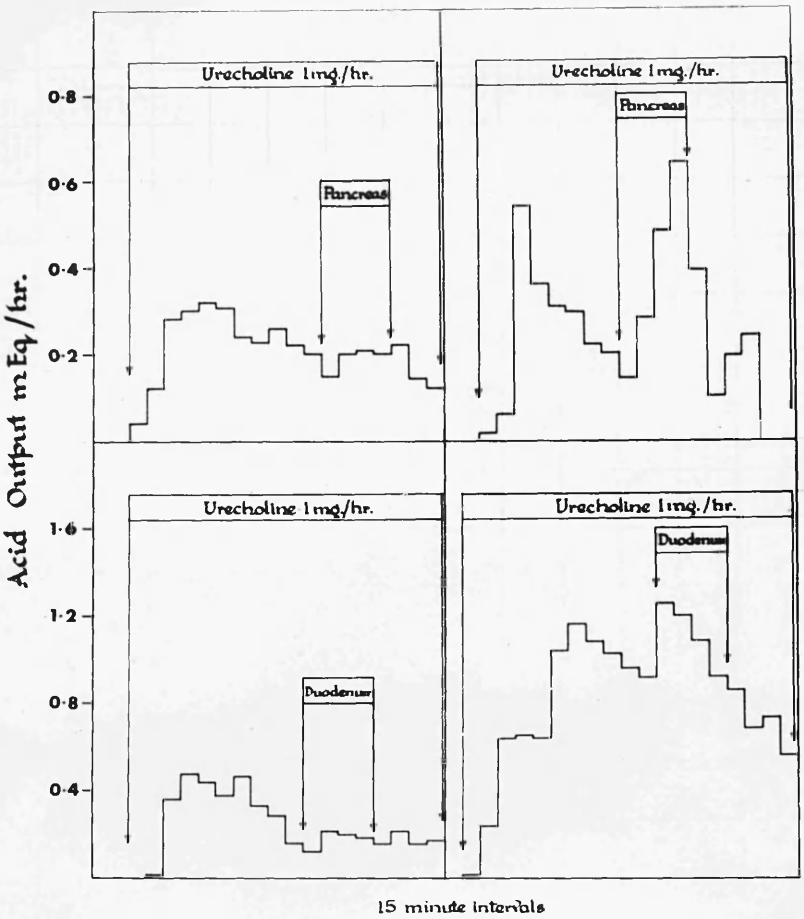
3. There seems to be a greater pepsin response to histamine in man than in the dog.
4. Atropine appears to reduce the histamine response to a greater extent in man than in the dog.
5. Vagotomy appears to reduce the maximal histamine response to a greater extent in man than in the dog.

It is seen that the addition of Urecholine did not abolish the discrepancy between the maximal responses to gastrin and histamine, the potentiated maximal histamine response being again greater than the potentiated maximal gastrin response. However, the percentage

brought to a head by the recent demonstration of a substance with behaviour identical to that of gastrin extracts from the pyloric gland area, in extracts of Zollinger-Ellison tumours, both primary (Gregory et al.[135] and metastatic (Grossman, Tracy and Gregory[136]). The view that the Zollinger-Ellison tumour produces excessive amounts of a humoral agent normally present in the pancreas seemed reasonable. An increased production of such a hypothesized factor seemed also a possible explanation for the acid hypersecretion of the Mann-Williamson preparation, or of the dog with total separation of the pancreas from the duodenum. It seemed also conceivable that the "intestinal" factor of gastric secretion was due to release of a gastrin-like substance from the upper small intestine. However, repeated single subcutaneous injections of extracts, prepared in identical manner to that for pyloric gland area gastrin, from pancreas of normal dogs, Mann-Williamson dogs, and dogs with pancreatic separation, and from duodenal mucosa of the same dogs, failed to demonstrate any gastrin-like activity. When, however, the extracts were given by continuous intravenous injection over a one-hour period against a background of Urecholine, 1 mg. per hour, there was a suggestion of activity in one experiment of two using normal dog pancreatic extract, and one of two experiments using normal dog duodenal mucosa extract (Fig. 27). This would seem to be a promising technique for the further investigation of the question of extra-gastric gastrin activity.

FIG. 27

ASSAY OF PANCREATIC AND DUODENAL MUCOSAL EXTRACTS FOR ACID STIMULATION OF HEIDENHAIN POUCH, AGAINST BACKGROUND OF CONTINUOUS INTRAVENOUS URECHOLINE



In one experiment each with pancreatic and with duodenal mucosal extract there was an increase in acid output suggesting true stimulation.

TABLE XX. ACID RESPONSES OF HEIDENHAIN POUCH TO COMBINED URECHOLINE AND GASTRIN EXTRACT, EACH IN GRADED DOSES

Acid outputs: μEq. per hour

Dog No.	Urecholine dose:mg/hr	Urecholine alone	Gastrin dose, grams per hour					
			0.625	1.25	2.5	5	10	20
43	0	-	79	123	262	513	1590	2785
44	0	-	11	229	667	858	902	1141
45	0	-	12	55	357	244	611	1709
48	0	-	215	809	769	1051	1821	2135
55	0	-	14	196	157	950	1238	3273
	Means	-	66	280	442	723	1232	2209
43	0.25	47	520	1157	2002	2323	2483	3208
44	0.25	305	1163	1361	1629	1950	2149	1916
45	0.25	505	960	2678	4525	4645	4836	4519
48	0.25	35	154	242	1596	2763	2794	2725
55	0.25	430	640	1746	3047	5438	6960	7952
	Means	264	687	1437	2560	3423	3844	4064
43	0.5	263	2343	3667	3788	3955	4018	3617
44	0.5	700	1852	2280	2487	2457	2873	2608
45	0.5	500	2396	5032	7851	9103	11645	11162
48	0.5	589	2511	3799	4852	4853	4432	3969
55	0.5	993	5086	6016	8064	8693	9808	9376
	Means	609	2838	4159	5408	5812	6555	6146
43	1	2051	2191	2902	3748	4892	5374	5762
44	1	1045	2109	2228	2356	2498	2633	241
45	1	2333	3861	4321	8375	11088	13102	13604
48	1	1226	2601	1661	4404	5246	5020	4770
55	1	1692	4801	6640	8418	8918	9106	10086
	Means	1670	3113	3550	5460	6528	7047	6893
43	2	2955	2593	3011	3630	4600	4374	2660
44	2	1777	2960	3026	3067	2066	1239	1016
45	2	-	-	-	-	-	-	-
48	2	2473	1320	1253	1938	4044	1501	2057
55	2	3085	3400	4067	4707	6367	7294	6384
	Means	2573	2586	2839	3336	4579	3602	3029
43	4	663	265	657	962	883	-	-
44	4	1769	1867	2163	2206	1994	-	-
45	4	1978	2262	4347	6040	4141	-	-
48	4	3204	1785	3260	3935	3517	-	-
55	4	2544	550	1131	83	0	-	-
	Means	2032	1346	2312	2645	2107	-	-

TABLE XXI. ACID RESPONSES OF HEIDENHAIN POUCH TO COMBINED URECHOLINE AND HISTAMINE, EACH IN GRADED DOSES

Acid outputs: µEq. per hour

Dog No.	Urecholine dose:mg/hr	Urecholine alone	Histamine diHCl dose, mg./hr					
			0.06	0.125	0.25	0.5	1	2
43	0	-	28	299	1083	1984	3069	4272
44	0	-	10	312	868	1143	1517	2130
45	0	-	-	-	-	2465	4551	6711
48	0	-	220	680	1751	1868	3281	4137
55	0	-	209	617	1423	1790	2742	4853
Means		-	117	477	1281	1782	3032	4421
43	0.25	11	546	1404	2542	2530	3234	4276
44	0.25	157	665	1770	1918	2580	3328	3323
45	0.25	200	2240	3901	7466	9488	12694	14878
48	0.25	183	791	1444	2868	5416	6112	6652
55	0.25	35	599	3370	6600	8048	10990	12303
Means		119	968	2378	4279	5612	7272	8286
43	0.5	415	1208	2143	3903	4570	5770	6127
44	0.5	705	1431	1860	2157	2742	3392	3432
45	0.5	462	4715	8546	9925	12054	15598	17427
48	0.5	556	2496	5046	5815	6469	7450	6837
55	0.5	876	2363	6883	8772	9465	9623	11060
Means		603	2443	4896	6114	7060	8367	8977
43	1	1559	2629	2722	2350	5106	6685	6720
44	1	1237	2466	2607	2860	3240	3897	3793
45	1	3083	10047	10648	13418	15519	18476	18680
48	1	1448	3302	5504	6480	6472	7648	7506
55	1	2476	6656	7904	9056	9076	9936	9258
Means		1961	5020	5877	6833	7883	9328	9191
43	2	2825	3039	3840	4982	5645	-	-
44	2	2708	2755	2795	2907	3270	-	-
45	2	5659	7969	10244	13425	16136	-	-
48	2	1378	2377	3861	4913	6003	-	-
55	2	4692	6182	7944	9600	10432	-	-
Means		3452	4464	5737	7165	8297	-	-

TABLE XXII. PEPSIN RESPONSES OF HEIDENHAIN POUCH TO COMBINED URECHOLINE AND GASTRIN EXTRACT, EACH IN GRADED DOSES

Pepsin outputs: units per hour

Dog No.	Urecholine dose: mg/hr.	Urecholine alone	Gastrin dose, grams per hour					
			0.625	1.25	2.5	5	10	20
43	0	-	-	204	359	367	329	350
44	0	-	-	230	194	420	623	932
45	0	-	-	-	235	154	450	572
48	0	-	4852	1266	567	1037	1357	2270
55	0	-	-	705	212	935	801	4783
	Means	-	4852	601	313	583	712	1781
43	0.25	-	1548	2245	2777	2812	4239	2825
44	0.25	836	2066	2297	3226	2438	4915	2771
45	0.25	3039	4620	4497	4435	3949	6242	4203
48	0.25	-	5126	670	9110	5715	10568	4834
55	0.25	1250	2177	7428	7828	11718	25122	36058
	Means	1708	3107	3427	5475	5327	10219	10138
43	0.5	2828	8966	7634	5486	5796	3885	3417
44	0.5	3416	6350	9970	9052	6339	6566	4900
45	0.5	5807	24084	33291	29752	24739	24880	25247
48	0.5	10640	37066	17607	11025	11659	10281	9535
55	0.5	35940	57393	56532	39116	26151	24220	34957
	Means	11726	26772	25007	18886	14937	13966	15611
43	1	16506	12631	9913	5803	9412	8925	6732
44	1	9680	9473	5986	3805	10418	10415	3880
45	1	35831	29345	22004	34481	47091	71012	46508
48	1	31405	27595	11220	13856	17494	19072	8047
55	1	44073	54390	26308	12318	27627	32990	29231
	Means	27499	26687	15086	14053	22408	28483	18880
43	2	11651	8085	6859	5383	4817	2311	1801
44	2	9253	10632	5686	3314	7021	1771	2471
45	2	-	-	-	-	-	-	-
48	2	15244	17272	14822	13314	14570	3694	4573
55	2	36804	21558	10129	6050	15150	12279	14708
	Means	18238	14387	9374	5215	10390	5014	5888
43	4	6141	29813	12901	5406	2458		
44	4	6732	9950	9410	5313	2702		
45	4	26661	22965	8843	5125	4620		
48	4	14534	16428	14229	21771	17838		
55	4	23584	48578	16471	-	-		
	Means	15530	25367	12371	9404	6905		

TABLE XXIII. PEPSIN RESPONSES OF HEIDENHAIN POUCH TO COMBINED URECHOLINE AND HISTAMINE, EACH IN GRADED DOSES

Pepsin outputs: units per hour

Dog No.	Urecholine dose:mg/hr	Urecholine alone	Histamine diHCl dose, mg/hr.					
			0.06	0.125	0.25	0.5	1	2
43	0	-	-	1310	2243	2777	1276	1008
44	0	-	-	487	2863	1926	2516	3050
45	0	-	-	-	-	16508	3935	1903
48	0	-	4414	6928	4796	12830	6679	3180
55	0	-	5371	5165	13853	7601	5177	6266
	Means	-	4893	3473	5939	8328	3916	3081
43	0.25	-	2032	3976	4063	2456	6309	4837
44	0.25	-	14400	7666	5377	5657	6052	2227
45	0.25	1870	4165	5062	7192	22223	26433	15927
48	0.25	6203	26718	10557	25608	20195	12813	5133
55	0.25	-	18136	26662	37519	23609	32335	33636
	Means	4037	13090	10785	15952	14828	16788	12352
43	0.5	7773	11517	11393	13117	10065	5761	3392
44	0.5	3285	6041	8770	10313	7685	6793	2704
45	0.5	6894	24121	27291	69487	23429	33974	13812
48	0.5	-	38371	47930	32568	8413	2164	514
55	0.5	24897	39830	45378	49920	29977	33668	15874
	Means	10712	23976	28152	35081	15915	16472	7259
43	1	7306	5281	4992	5226	5895	13297	3480
44	1	4572	12437	4927	1918	6596	13826	6093
45	1	20604	34890	24765	28843	27839	37138	18097
48	1	17307	18826	7773	8505	12070	12132	6840
55	1	23477	28000	15326	19494	5623	1845	1617
	Means	14653	19887	11557	12797	11587	15648	7225
43	2	16490	10981	5754	6016	1784	-	-
44	2	7014	6689	6243	5682	2080	-	-
45	2	28876	40600	41141	35598	16891	-	-
48	2	18024	47396	18110	3357	6372	-	-
55	2	32890	26112	19963	10785	7627	-	-
	Means	20659	26356	18242	12288	6951	-	-

TABLE XXIV. ACID RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS URECHOLINE OVER A 7-HOUR PERIOD

Acid output: μEq. per hour

Dog No.	Urecholine dose: mg/hr	Continuous Urecholine - Hour of collection						
		1	2	3	4	5	6	7
43	0.25	7	93	77	79	63	95	103
44	0.25	8	254	605	572	614	338	295
45	0.25	29	24	37	113	121	156	70
48	0.25	0	21	232	117	253	201	341
55	0.25	20	91	120	22	97	215	364
Means		13	97	214	181	230	201	237
43	0.5	155	696	769	789	741	746	858
44	0.5	0	473	434	734	420	454	549
45	0.5	802	2219	3461	3798	3836	3120	2173
48	0.5	416	529	1049	1251	470	403	238
55	0.5	31	65	725	287	455	355	375
Means		281	776	1288	1370	1184	1016	839
43	1	340	2112	1783	1207	1658	1462	1151
44	1	540	1592	1613	1365	1054	1036	1257
45	1	4482	5760	4869	4279	3913	4056	4407
48	1	444	1039	951	912	872	843	921
55	1	204	723	968	995	1566	1411	1183
Means		1202	2245	2037	1752	1813	1716	1784
43	2	662	4056	2522	1856	2375	1647	1413
44	2	931	2650	1773	1378	1197	1574	1410
45	2	4710	6009	3602	2623	2487	2258	2479
48	2	2549	2475	1400	1254	1349	1198	1294
55	2	1683	4092	3771	3330	1558	1741	1192
Means		2107	3856	2614	2088	1793	1684	1558

TABLE XXV. PEPSIN RESPONSE OF HEIDENHAIN POUCH TO CONTINUOUS INTRAVENOUS URECHOLINE OVER A 7-HOUR PERIOD

Pepsin outputs: units per hour

Dog No.	Urecholine dose:mg/hr	Continuous Urecholine - Hour of collection						
		1	2	3	4	5	6	7
43	0.25	-	-	-	-	-	-	-
44	0.25	-	-	3519	6188	3275	3522	2540
45	0.25	-	-	6272	10680	7533	9646	8100
48	0.25	-	-	-	10279	-	12302	-
55	0.25	-	680	-	-	-	2029	1922
	Means	-	680	4896	9049	5404	6875	4187
43	0.5	2226	4243	3912	3146	3733	5402	4118
44	0.5	-	1900	1762	2613	2749	2366	3720
45	0.5	11944	14446	16737	13475	19256	16341	20288
48	0.5	2012	10496	13091	14682	11021	19131	11688
55	0.5	792	-	5672	11308	7816	-	-
	Means	4244	7771	8235	9045	8915	10810	9954
43	1	3849	6908	6026	6433	4224	3097	2151
44	1	2717	6323	4814	5294	4447	5897	5642
45	1	21622	38358	25195	26514	13639	12708	15368
48	1	7425	28370	19773	17814	15222	13046	9065
55	1	7245	43012	36164	32840	21565	16738	15237
	Means	8572	24594	18394	17779	11819	10297	9493
43	2	16262	12486	16260	18805	15205	4497	4528
44	2	8434	9250	6545	6836	7905	14549	7031
45	2	42428	62455	48607	28846	11312	27477	26277
48	2	29599	34389	30100	16639	14974	12265	10302
55	2	41070	71244	49994	30048	22034	12828	16651
	Means	27559	37965	30301	20235	14286	14323	12958

Chapter 9

9. EFFECT OF FUNDIC ACIDIFICATION ON THE POTENTIATION BY DISTENTION OF THE ACID RESPONSE TO INJECTED HISTAMINE

It has been clearly shown that acidification of the pyloric gland area interferes with the ability of local irrigation of acetylcholine to cause gastrin release (Kim[137], and Fig.15). One of the possible explanations for this occurrence is that the acid acts as an anticholinergic, being similar in behaviour to atropine, which also blocks local acetylcholine release of gastrin (Gregory and Ivy[24], Woodward et al.[126]). Since the potentiation by distention of the acid responses to gastrin extract and to histamine has been shown by Grossman[128, 132] to be a cholinergic mechanism, it seemed possible that acid in contact with the fundic mucosa might inhibit the potentiation caused by distention.

Two Heidenhain pouch dogs were studied. Since distention potentiation was of comparable degree with both gastrin extract and histamine, the latter being more readily available, was given by continuous intravenous infusion throughout each experiment at a constant rate of 0.2 mg. of the acid phosphate per hour. After 2 hours of histamine injection alone, by which time plateau levels of acid response were obtained, the Heidenhain pouch was distended with saline for a second period of 2 hours, and with 0.1N hydrochloric acid for a third 2-hour period. The distention was accomplished by introducing every

15 minutes, 100 ml. of the saline or hydrochloric acid into a glass reservoir connected by tubing to the Gregory type Heidenhain pouch cannula. The reservoir was placed only a few centimetres above the level of the gastric pouch, so that minimal rise in hydrostatic pressure should occur. In most instances only about 50 ml. of the distending liquid could be introduced into the pouch and reservoir at first, but gradual accommodation to the full 100 ml. took place generally within 15 to 30 minutes.

Results of six experiments on the two dogs are given in Table XXVI ~~and a representative experiment (No.5) is illustrated in Fig.28~~. In the first three experiments 0.4 mg. per hour histamine acid phosphate was used as the background stimulation. The magnitude of potentiation by distention was not great, and in one experiment (No.2) an apparent abolition of the potentiation occurred on acidifying the pouch (Table XXVI). In the next three experiments the histamine acid phosphate dose was reduced to 0.2 mg. per hour in order to obtain a greater increment of acid output due to the distention, and thus perhaps a better measure of any inhibitory effects acting primarily on this cholinergic mechanism. It is seen from Table XXVI that in these experiments (Nos.4,5 and 6), although there was a general tendency for slight reduction in acid output during the acid distention, the levels were still grossly greater than control. Means of the six experiments, taken as a group, bore this out. One experiment in which the order of

TABLE XXVI. EFFECT OF ACIDIFICATION ON THE POTENTIATION BY DISTENTION OF THE HEIDENHAIN POUCH ACID RESPONSE TO HISTAMINE

Acid outputs, μEq. per hour.

Control is output during second hour of histamine alone.

Expt. No.	Dog No.	Histamine prepn. and dose (mg/hr)	Control (Hist. alone)	Distention			
				Saline		0.1 N HCl	
				1st hr.	2nd hr.	1st hr.	2nd hr.
1	48	Acid Phosph. 0.4	1758	2455	2165	2915	4660
2	48	" " 0.4	2787	3984	3415	1684	2418
3	48	" " 0.4	2415	5086	5729	5997	4580
4	48	" " 0.2	503	3636	4183	2833	2905
5	53	" " 0.2	1875	3788	3835	2986	3198
6	53	" " 0.2	651	2196	2073	1296	1393
		Means	1665	3524	3567	2952	3192
				0.1 N HCl		Saline	
				1st hr.	2nd hr.	1st hr.	2nd hr.
7	48	DiHCl. 0.25	1265	3089	4818	4512	4280

the distending liquids was reversed (No.7) again showed no real difference in output, both being 200% to 300% greater than control output.

It was concluded that the cholinergic potentiation of distention of the gastric fundus is not abolished by local acidification of the fundic mucosa.

Chapter 10

10. POTENTIATION BETWEEN GASTRIN EXTRACT AND HISTAMINE IN THE STIMULATION OF ACID RESPONSE FROM A HEIDENHAIN POUCH

The possibility of potentiation between gastrin and histamine was raised in Chapter 3. As seen in Fig.13, when a Heidenhain pouch dog secreting in response to the continuous intravenous infusion of a small dose of histamine was given a small dose of gastrin extract as a single rapid intravenous injection, the acid output was markedly increased.

A group of five Heidenhain pouch dogs was studied. The acid responses were measured to gastrin extract alone in doses ranging from 2.5 to 80 grams per hour, and on separate occasions to histamine dihydrochloride alone in doses from 0.25 to 8 mg. per hour. The first increase in dose rate was made after two hours initial collection, subsequent changes being made every 90 minutes. In a further group of experiments histamine dihydrochloride at a rate of 0.25 mg. per hour was given by continuous intravenous infusion throughout, and after an initial two hours, an additional continuous intravenous injection administering gastrin extract was started. Doses of gastrin extract ranged from 5 to 80 grams per hour.

Acid outputs are given in Table XXVII, pepsin in Table XXVIII, and the mean values of acid output, pepsin output and pepsin/acid ratio are shown graphically in Figs. 28,29 and 30 respectively.

TABLE XXVII. COMPARISON OF ACID OUTPUTS IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND HISTAMINE, SEPARATELY AND IN COMBINATION

Acid outputs - μEq. per hour.

H = Histamine alone G = Gastrin alone

H + G = Histamine (0.25mg.diHCl/hr) + Gastrin

Hist.diHCl mg/hr		0.25	0.5	1	2	4	8
Gastrin Ext.g/hr		2.5	5	10	20	40	80
Dog No.43	H	1064	2254	3876	7184	7668	6920
	G	186	316	564	1726	1594	1488
	H + G	-	2880	3812	624	92	68
Dog No.45	H	1014	2466	4154	6340	8062	8312
	G	354	376	1006	1906	2042	1996
	H + G	-	6084	7648	6806	5598	4852
Dog No.48	H	954	1734	3850	3834	3456	3236
	G	662	718	1490	2426	1846	672
	H + G	-	4028	5974	4376	2184	1218
Dog No.53	H	2280	1786	3786	5394	5808	6384
	G	1046	2990	5856	6802	5970	6364
	H + G	-	8698	8442	7296	7300	3466
Dog No.55	H	352	1494	3100	6320	7212	6550
	G	-	972	1234	1384	1720	480
	H + G	-	6214	7968	4412	400	318
Means	H	1132	1946	3754	5814	6442	6280
	G	562	1074	2030	2848	2638	2200
	H + G	-	5580	6768	4702	3114	1984

TABLE XXVIII. COMPARISON OF PEPSIN OUTPUTS IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND HISTAMINE, SEPARATELY AND IN COMBINATION

Pepsin outputs - Units per hour.

H = Histamine alone G = Gastrin alone

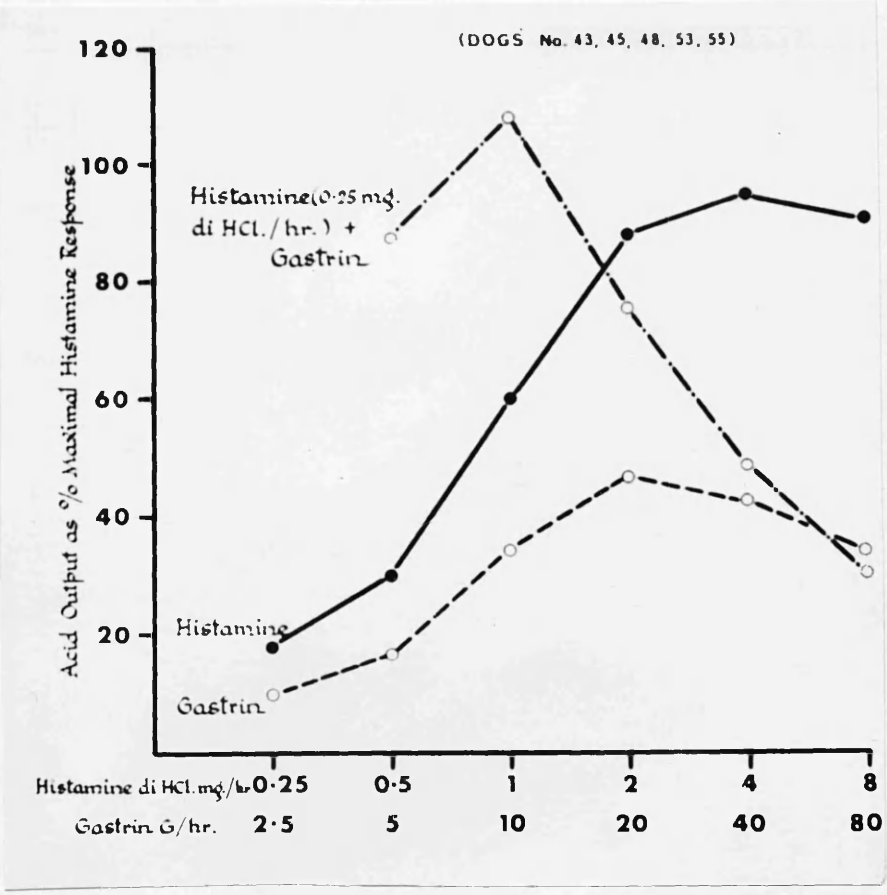
H + G = Histamine (0.25 mg.diHCl/hr) + Gastrin

Hist.diHCl mg/hr		0.25	0.5	1	2	4	8
Gastrin Ext.g/hr		2.5	5	10	20	40	80
Dog No.43	H	2220	3242	5850	2526	1150	166
	G	1428	342	268	1038	1720	2788
	H + G	-	556	1074	1270	86	538
Dog No.45	H	5358	16506	3954	2522	2264	808
	G	1306	548	690	734	1580	1288
	H + G	-	1076	2920	9464	7834	8048
Dog No.48	H	7974	29462	4614	2212	1216	554
	G	2030	1230	908	2210	2238	7592
	H + G	-	1636	5130	8064	5366	13012
Dog No.53	H	1528	1000	2042	928	800	910
	G	2076	6616	6830	11110	21276	15268
	H + G	-	10976	10156	11046	11808	26452
Dog No.55	H	250	10054	856	1004	708	444
	G	-	666	452	5110	16742	8330
	H + G	-	508	3944	13368	3210	4044
Means	H	3466	12452	3464	1838	1228	576
	G	1710	1880	1830	4040	8712	7054
	H + G	-	2950	4644	8642	5660	10418

FIG. 28

COMPARISON OF ACID OUTPUTS OF HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND HISTAMINE, SEPARATELY AND IN COMBINATION

(Mean results of 5 dogs)

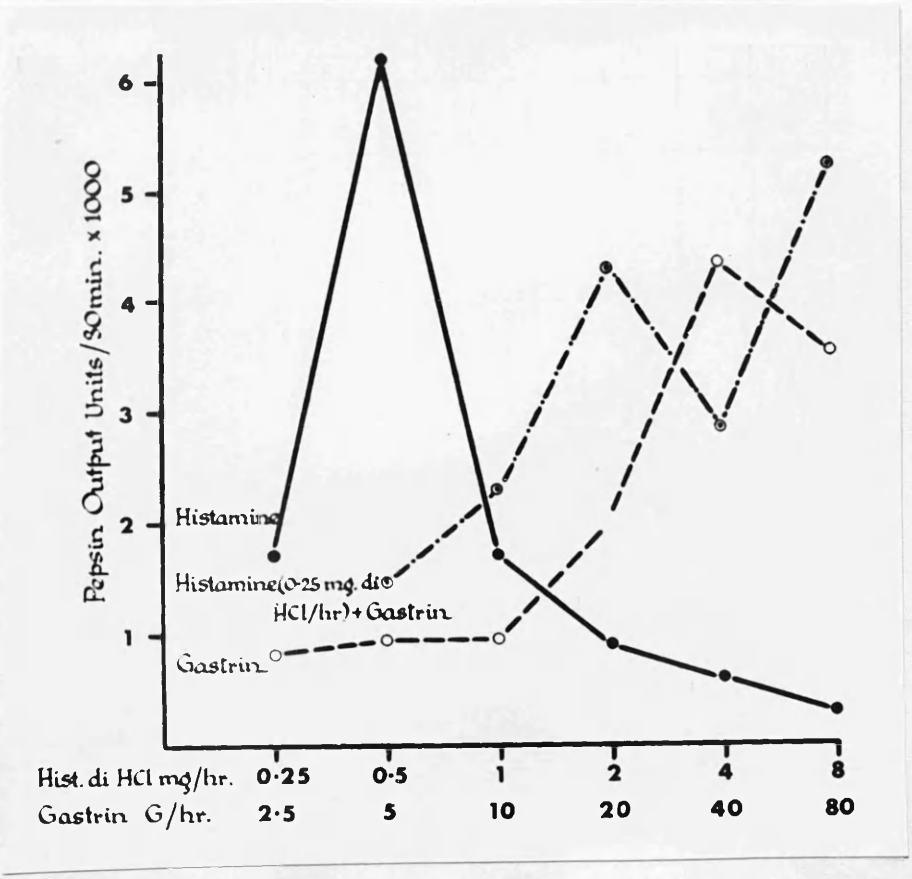


Potentiation occurred between 0.25 mg. histamine dihydrochloride per hour and gastrin extract at 5, 10 and 20 grams per hour. The responses to 40 and 80 grams gastrin extract per hour were not affected by the addition of histamine.

FIG. 29

COMPARISON OF PEPSIN OUTPUTS OF HEIDENHAIN POUCHES IN RESPONSE TO GRADED DOSES OF GASTRIN EXTRACT AND HISTAMINE, SEPARATELY AND IN COMBINATION

(Mean results in 5 dogs)

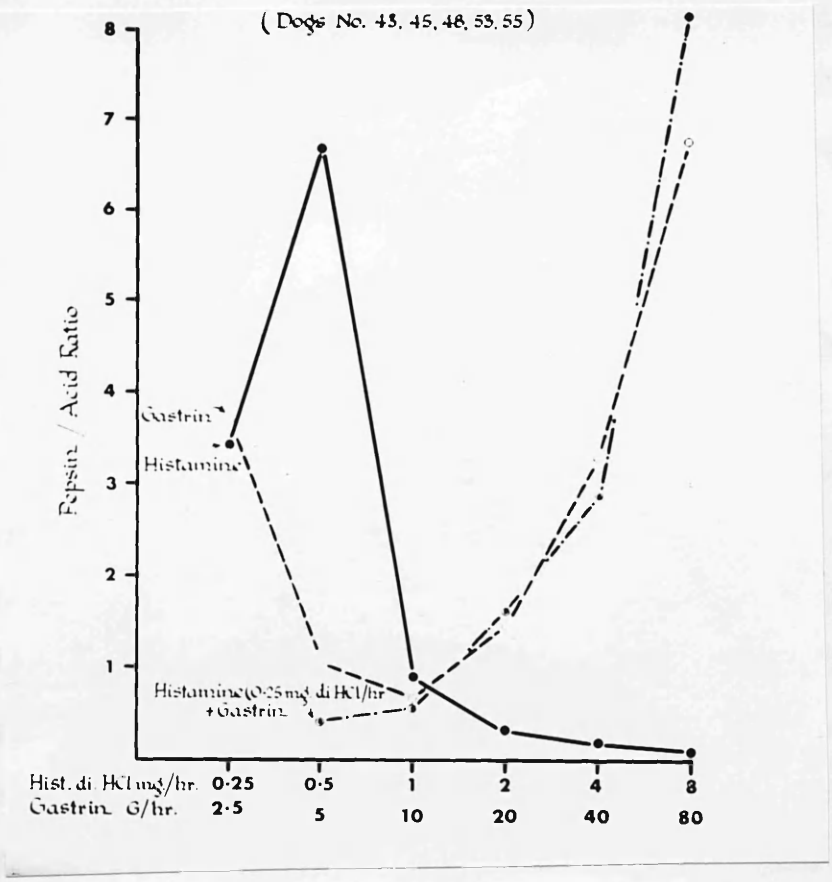


The largest pepsin response to histamine alone occurred at low dose rates, and to gastrin extract alone, at high dose rates. Responses to the combined gastrin extract + histamine resembled those to gastrin extract alone.

FIG. 30

COMPARISON OF PEPSIN/ACID RATIOS OF HEIDENHAIN POUCH RESPONSES TO GRADED DOSES OF GASTRIN EXTRACT AND HISTAMINE, SEPARATELY AND IN COMBINATION

(Mean results of 5 dogs)



The ratios for gastrin extract alone suggest the triphasic pattern of increased pepsin response at both lowest and highest dose rates, with intermediate failure of stimulation.

The similarity of the histamine + gastrin, and gastrin alone ratios for the higher dose rates is striking.

Adopting the criteria for potentiation discussed in Chapter 8, it can be concluded from the acid output results shown in Fig.28 that potentiated responses were obtained from the combination of 0.25 mg. histamine dihydrochloride per hour and the smaller doses of gastrin extract (5,10 and 20 grams per hour). The acid output to 40 and 80 grams per hour was not significantly altered by the concomitant administration of histamine at this particular dose rate. There is even the suggestion that histamine depressed the response to 80 grams gastrin per hour, a finding which invites comparison with the inhibitor effects of large doses of Mecholyl on the histamine response, reported by Gray and Ivy[89]. Further studies employing a wider range of doses of both gastrin and histamine would be of great interest.

The pepsin output results shown in Fig.29 again revealed the divergent response patterns previously encountered in Chapter 2, when gastrin and histamine were given on separate occasions. The largest pepsin output to histamine resulted from low dosage, and to gastrin from high dosage. It is seen that the combined histamine plus gastrin pepsin responses closely resembled those of gastrin alone, suggesting that the pepsin stimulant in gastrin extract is a potent one, and that it is not interfered with by a small dose of histamine. However, it would be of interest to perform similar studies using a larger dose of histamine.

The close parallelism in the pepsin responses to gastrin with and

without histamine is brought even more clearly by examination of the pepsin output/acid output ratios (Fig.30). The high ratio for the lowest gastrin doses once more suggests a triphasic pepsin response to gastrin extracts, discussed previously in Chapter 2.

Discussion of the potentiation experiments.

From the results of Chapters 8,9 and 10 it appears that the combined administration of any two of the three gastric secretory stimulants, gastrin, histamine and acetylcholine can result in potentiated responses from a Heidenhain pouch. It is noteworthy that the general pattern for each combination is much the same, in that the greatest potentiation results from the simultaneous injection of small doses, lesser degrees occurring with larger doses.

It is interesting that all three agents occur naturally in the stomach, gastrin confined to the pyloric gland area, histamine in all areas of the stomach, but in greater concentration in the fundic area (Feldberg and Harris[138], Code[139]), and acetylcholine at post-ganglionic vagal nerve endings. Although physiological roles have been clearly established for gastrin and acetylcholine in the control of gastric secretion, it has not been conclusively demonstrated that histamine, a substance so widespread in a variety of tissues throughout the body, plays a normal part in the gastric response to physiological stimuli. However, a possible pathological stimulation of gastric secretion involving histamine mediation is suggested by one of the features of the following Chapter.

Chapter 11

11. THE GASTRIC SECRETION OF ACID IN RESPONSE TO PORTAL AND SYSTEMIC VENOUS INJECTION OF GASTRIN EXTRACT

This study has recently been reported (Gillespie and Grossman[140]). The results are discussed herein because they illustrate another marked difference between the behaviour of gastrin extracts and of histamine, and also because, as stated in the last Chapter, they raise the question of a pathological role for histamine in the stimulation of gastric secretion.

The stimulating effect of histamine on the gastric parietal cells has been shown to be markedly reduced by passage through the liver (Silen and Eiseman[141], Irvine et al.[142]). The view that gastrin is similarly affected has been stated by several workers who found that the response of a denervated fundic pouch to stimulation of the pyloric gland area was increased following portacaval anastomosis (Irvine[143]; Castaneda et al.[144]).

This question was investigated in three dogs with previously formed denervated fundic pouches, by introducing a fine (2.5 mm. outer diameter) polyvinyl cannula directly into the portal vein, bringing it out to the surface through a long subcutaneous track and observing over a number of days the effects on acid secretion from the pouch of injecting gastrin extract alternately into the portal vein and into a systemic (leg) vein. The results of 10 experiments in the three dogs using gastrin extract, and

for comparison 13 experiments using histamine, are shown in Table XXIX. One gastrin and one histamine experiment are illustrated in Fig.31. The expected greatly reduced response to histamine by the portal vein route was observed, while the two routes of administration of gastrin gave virtually identical responses.

It was concluded, therefore, that the potency of gastrin as a stimulant of the parietal cells was unchanged by selective passage through the liver. This finding is not surprising in view of the fact that gastrin would normally be released directly into the portal venous system from the pyloric gland area. The behaviour of gastrin in this respect is similar to that of secretin, the only other gastrointestinal hormone studied in similar manner (Hart and Clarke[145]).

On reviewing the experiments which demonstrated an increased acid response to pyloric gland area stimulation after portacaval anastomosis, it now seems that an alternative interpretation is possible, namely that the response of the parietal cells to all forms of stimulation is enhanced following portacaval anastomosis. In support of this hypothesis is the evidence that the responses to a meal, to sham feeding, and to histamine are all increased after portal vein ligation (Gregory[146]).

Inspection of Table XXIX shows that in all dogs there was a tendency for the responses both to gastrin and to histamine to fall on successive days. It was noted that basal secretion, normally zero, was present on the days on which the highest acid responses were obtained.

TABLE XXIX. EFFECT ON ACID RESPONSE OF HEIDENHAIN POUCH OF GASTRIN EXTRACT OR HISTAMINE INJECTED ALTERNATELY INTO THE PORTAL AND A SYSTEMIC VEIN

Part I. GASTRIN EXTRACT

Dog	Date	Dose of Gastrin (g./hr)	Acid output from Heidenhain pouch (mEq/hr.) Systemic (S_1)	Portal	Systemic (S_2)
A	27 Sept.	10	3.62	3.54	3.64
A	28 Sept.	10	4.12	3.29	3.69
A	30 Sept.	10	2.52	1.44	1.73
B	11 Oct.	10	7.04	6.52	6.70
B	12 Oct.	5	1.07	2.15	3.08
B	14 Oct.	10	7.76	5.92	6.33
B	16 Oct.	10	5.74	6.30	5.97
C	8 Nov.	5	2.65	2.11	3.27
C	10 Nov.	5	1.03	1.58	1.35
C	14 Nov.	10	2.25	2.94	2.51
		Means	3.78	3.58	3.83

Statistical analysis. Mean difference between S_1 and S_2, 0.047 mEq/hr. (S.E.0.290) not statistically significant ($t = 0.16$, $P = > 0.5$). Also mean difference between the mean of S_1 and S_2 and the portal infusion, 0.227 mEq/hr.(S.E.0.187) not statistically significant ($t = 1.21$, $P = > 0.2$)

Part II. HISTAMINE

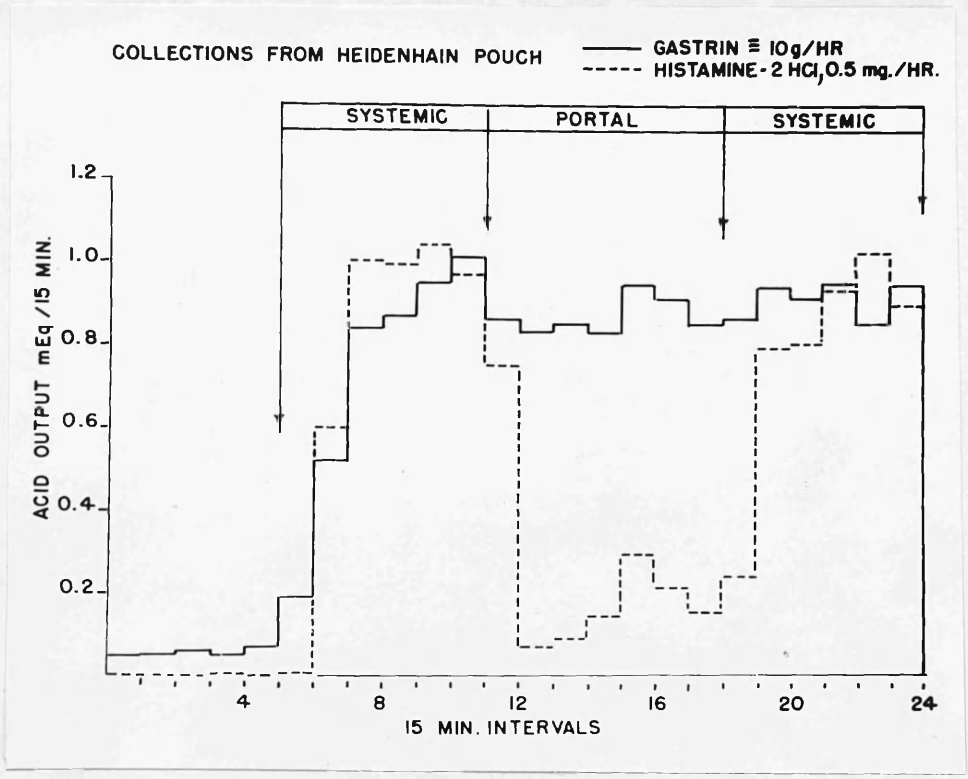
Dog	Date	Dose of Histamine diHCl (mg/hr)	Acid output from Heidenhain Pouch (mEq/hr.) Systemic (S_1)	Portal	Systemic (S_2)
A	26 Sept.	0.75	5.82	3.63	6.78
A	29 Sept.	0.5	3.96	0.80	3.66
B	10 Oct.	0.5	2.00	0.16	1.81
B	13 Oct.	1.0	6.68	0.12	4.44
B	15 Oct.	1.0	5.29	0.78	4.54
B	17 Oct.	1.0	4.47	0.72	3.77
B	23 Oct.	1.0	3.79	0.47	3.31
C	7 Nov.	0.5	7.09	1.11	6.27
C	11 Nov.	0.5	6.67	0.94	5.37
C	16 Nov.	0.25	1.13	0.84	1.17
C	17 Nov.	0.5	3.22	2.48	3.70
C	20 Nov.	0.5	3.97	1.13	3.60
C	28 Nov.	0.5	1.41	0.28	1.30
		Means	4.27	1.04	3.82

Statistical analysis. Mean difference between S_1 and S_2, 0.445 mEq/hr. (S.E. 0.220) not statistically significant ($t = 2.02$, $P = > 0.05$). Mean difference between the mean of S_1 and S_2, and the portal infusion, 3.013 mEq/hr. (S.E. 0.475), highly significant ($t = 6.34$, $P = < 0.001$).

FIG. 31

ACID RESPONSE OF HEIDENHAIN POUCH TO GASTRIN EXTRACT INJECTED ALTERNATELY INTO THE PORTAL AND A SYSTEMIC VEIN

(Similar experiment using histamine for comparison)



Route of injection made no difference in the acid response of the pouch to gastrin extract; by contrast histamine showed the well-known failure to evoke the full acid response on being injected into the portal vein.

In all three dogs a local subcutaneous infection occurred along the track of the portal vein cannula. The inflammatory response was greatest around the second to fourth days, and thereafter gradually subsided. It is interesting to speculate that the local release of a gastric secretory stimulant, e.g. histamine, may have been responsible for the basal secretion and high responses to the injected gastrin and histamine.

Chapter 12

12. SECRETION OF PANCREATIC JUICE IN RESPONSE TO IRRIGATION OF THE ISOLATED PYLORIC GLAND AREA WITH ACETYLCHOLINE

It was noted that the gastrin extracts prepared by Gregory and Tracy[8] and those used in the present studies evoked a small secretory response from the pancreas, and that the pattern of response exhibited features of both secretin and pancreozymin activity. It seemed of interest to determine whether similar pancreatic responses resulted from stimulation of the pyloric gland area.

Two dogs were prepared with a subcutaneous transplant of the uncinate process of the pancreas to the mammary region, the cut surface bearing the duct being brought out through the excised nipple area after the manner outlined by Wang and Grossman[147]. In addition each was provided with an isolated pouch of the pyloric gland area, as described under "Materials and Methods".

Since the animals were always completely fasted for at least 18 hours before testing, the volume of basal secretion was minute or zero in every case. In order to obtain a background of secretion against which to measure the effects of pyloric gland area stimulation, a continuous intravenous infusion of secretin (Vitrum), 30 units per hour, was given throughout each experiment.

Because the local application to the mucosa of the pyloric gland area of an alkaline solution of acetylcholine was well known to cause

gastrin release, a 1% solution, adjusted to a pH of greater than 7.0 was used to irrigate the isolated pyloric gland area pouch. Pancreatic secretion was collected simply by being allowed to drop into a graduated conical glass centrifuge tube loosely supported below the transplant. Every 15 minutes the volume and the protein content were measured, the latter by ultra-violet light absorption, using a stock solution of bovine serum albumen as standard. The total protein content was taken as an estimate of exocrine enzyme output, these being protein in nature. After an initial 2-hour period of intravenous secretin administration alone, the pyloric gland area pouch was irrigated for a second period of 2 hours with 1% acetylcholine (pH > 7.0). At the end of this second period the pyloric gland area pouch was gently but thoroughly washed out with 0.9% saline, and collections from the pancreatic transplant continued for a third 2-hour period, during which once more only secretin was being given.

The volumes and protein outputs of pancreatic secretion during acetylcholine stimulation of the pyloric gland area are compared with those of control periods in Table XXX. Increases in both volume (approximately 2 to 3-fold) and total protein output (approximately 4-fold) were observed on irrigating the pyloric gland area pouch. On stopping the irrigation there was a gradual reduction in both parameters, the volume reaching control levels in 2 hours, the protein output still being approximately 100% greater than control by that time.

TABLE XXX. EFFECT OF ACETYLCHOLINE IRRIGATION OF THE ISOLATED PYLORIC GLAND AREA ON SECRETION FROM A PANCREATIC TRANSPLANT

Protein outputs - mg. per hour

Secretin (Vitrum) 30 units per hour given by continuous intravenous infusion throughout.

Dog No.	Secretin alone		1% Acetylcholine (pH > 7.0) to pyloric pouch		Secretin alone	
	1st hr.	2nd hr.	1st hr.	2nd hr.	1st hr.	2nd hr.
49	49	24	66	27	23	18
49	261	163	475	537	-	105
49	89	59	114	105	115	104
51	21	16	43	48	34	25
51	14	9	35	14	-	-
51	70	201	1316	1519	715	-
51	-	231	677	206	152	120
51	0	0	35	18	3	10
Means	72	88	345	309	174	64
	Volume ml. per hour					
49	1.5	1.4	2.1	2.0	1.6	1.4
49	0.9	1.5	3.2	4.0	-	1.9
49	1.1	0.9	1.2	0.9	1.3	1.2
51	2.3	3.5	6.2	11.8	10.2	9.6
51	1.2	1.4	6.3	4.5	-	-
51	1.2	1.0	2.8	7.0	2.4	-
51	-	2.7	4.0	2.6	2.2	1.6
51	0	0	1.3	2.1	0.6	0.6
Means	1.2	1.6	3.4	4.4	3.1	2.7

The present study supports the view of Blair et al.[148] that a humoral phase of pancreatic secretion originates from the pyloric gland area. Accepting that an increased volume response is characteristic of secretin-like action and an increased enzyme output typical of pancreozymin-like activity, the results suggest the occurrence of both types of pancreatic secretion stimulant in pyloric gland area extracts.

It seemed of interest to see whether acidification of the acetylcholine used to irrigate the pyloric gland area pouch altered the apparent pancreatic response, in view of the well known failure of acetylcholine to cause gastrin release at low pH (Kim[137]). In 6 experiments in the same 2 dogs the pyloric gland area pouch was irrigated with 1% acetylcholine at pH 1.3 for 2 hours, and subsequently at pH 7 for 2 hours. Continuous intravenous secretin (Vitrum), 30 units per hour was again given throughout each experiment, starting two hours before the onset of acetylcholine irrigation.

The results given in Table XXXI suggest that acidification of the acetylcholine locally applied to the pyloric gland area may, in fact, suppress its stimulating effect on pancreatic secretion. It is seen that during irrigation of the pyloric gland area with acetylcholine at pH 1.3, both the volume and the protein output were virtually unchanged from control levels with secretin alone. On changing the pH of the acetylcholine to 7.0 the protein output rose to the highest levels within the first hour, whereas the volume took longer to increase, being greater in the second

TABLE XXXI. INFLUENCE OF THE pH OF ACETYLCHOLINE USED TO IRRIGATE THE PYLORIC GLAND AREA ON THE PANCREATIC RESPONSE

Protein outputs - mg. per hour

Secretin (Vitrum) 30 units per hour given by continuous intravenous infusion throughout.

Dog No.	Secretin alone		1% Acetylcholine to pyloric pouch pH 1.3		pH 7+	
	1st hr.	2nd hr.	1st hr.	2nd hr.	1st hr.	2nd hr.
49	0	0	0	0	84	36
49	0	65	174	98	146	299
49	12	131	58	71	45	93
51	231	377	500	338	529	403
51	71	135	183	0	179	121
51	60	170	131	134	247	230
Means	62	146	174	107	205	197
	Volume - ml. per hour					
49	0.7	0.7	0	0.1	1.0	0.7
49	0	0.5	1.5	1.0	1.6	2.7
49	0.3	0.6	0.7	0.6	0.4	0.5
51	0.6	1.1	1.4	0.9	2.6	4.4
51	1.2	1.1	1.5	0.4	1.2	2.5
51	0.6	1.5	1.3	0.8	1.6	4.5
Means	0.6	0.9	1.1	0.6	1.4	2.6

hour. This might represent a more prolonged depressant effect of acidification of the pyloric gland area on secretin-like activity than on pancreozymin-like activity.

It seems likely that the mechanisms involved in this present study are, in fact, humoral ones, since the pancreatic transplant has had all its attachments to the original anatomical location severed, apart from a single artery and a single vein arising from the tip of the uncinate process. However, it is conceivable that a few autonomic nerve fibres may pass to the pancreas along the outer coats of these blood vessels. The results presented by White, Lundh and Magee[149], have been interpreted as supporting a gastro-pancreatic neural reflex mechanism, and it would thus seem important to repeat the experiments described in this Chapter after division of the small vascular pedicle to the tip of the transplant.

Further investigation will be required to clarify the several points raised. It is interesting however, to speculate on the possible distribution of the various upper gastro-intestinal hormones.

The gastrin extracts of Gregory and Tracy[8], and those used in the present study, gave evidence of secretin and pancreozymin-like activity. The present section has presented evidence suggestive of the release of humoral agents with such properties from the pyloric gland area. The crude gastrin extracts prepared by Uvnäs (Munch-

Petersen and Uvnäs[61]), stimulated an outflow of bile, suggesting the possible presence of cholecystokinin. There was a slight suggestion of stimulation of gastric secretion from the injection of extracts of duodenal mucosa and of pancreas when a background of continuous Urecholine administration was used (Chapter 8). Under the pathological condition of the Zollinger-Ellison tumour pancreatic tissue can produce a gastrin-like substance, sometimes in very large amounts (Gregory et al.[135], Grossman, Tracy and Gregory[136]). The commercial duodenal mucosa extracts, secretin and cholecystokinin possess inhibitor properties against gastrin-induced, and to a lesser extent against histamine-induced gastric secretion (Chapter 5).

There is thus at least suggestive evidence that the three areas, pyloric gland area, duodenum and pancreas might be capable of yielding a variety of stimulatory and inhibitory influences. It is conceivable that gastrin, secretin, pancreozymin, cholecystokinin, "acid inhibitor substance", and possibly other fractions, might all be present in the pyloric gland area,in the duodenum,in the upper small intestine and in the pancreas, and that the principal difference is in the proportion of each present in any particular area. In the pyloric gland area gastrin would predominate,in the duodenum secretin, pancreozymin and possibly "acid inhibitor substance", in the small intestine secretin, pancreozymin and possibly gastrin (responsible for the intestinal phase of gastric secretion), and in the pancreas the main influence on gastric secretory responses would occur under pathological conditions.

SUMMARY AND CONCLUSIONS

The recent major advances in extraction techniques, notably those introduced by Gregory and Tracy[8], have made available consistently reliable preparations of gastrin, of a high order of potency. Although it is freely admitted that such extracts are still far from pure, and almost certainly contain several fractions in addition to gastrin, some of recognizable physiological effect, the use of such extracts has permitted the study of several aspects of the mechanism of action of gastrin on gastric secretion. It may be that some of the effects noted in the present experiments, for example certain inhibitory properties, will be found to be attributable to the non-gastrin fractions of these extracts, when repeat experiments are made with pure gastrin. Such findings would, of course, be of equal, or even greater interest.

The major conclusions and hypotheses drawn from the studies presented are as follows:

1. A wide variation in latency of the acid response of Heidenhain pouches was noted when gastrin extract was given by continuous intravenous infusion. The possible explanations for this finding were discussed.
2. Dose/response curves of the acid and pepsin outputs to gastrin and histamine were compared, using two slightly different approaches. The principal feature of the acid output curves was the maximal

gastrin response being regularly less than the maximal histamine response. Several tentative explanations for this discrepancy were advanced.

The pepsin output curves indicated a biphasic response to histamine and a triphasic response to gastrin. The increased pepsin outputs to small doses of either agent are believed to represent true stimulation of pepsin secretion, and evidence was presented in support of this belief in the case of histamine. The possibility of the increased pepsin response to large doses of gastrin extract being due to "gastrozymin", or "pepsizymin" was discussed.

3. Among the hypotheses considered to explain the difference between maximal gastrin and maximal histamine acid responses was the possible presence of an inhibitor substance in the gastrin extract. Evidence was presented that doses of gastrin extract greater than those required to stimulate gastric secretion were capable of profoundly inhibiting the acid response to stimulatory doses of gastrin or of histamine. The inhibitor property was specific to the pyloric gland area and did not appear to be due to species difference or non-specific toxic reactions. The evidence suggested that the inhibition demonstrated is a property of gastrin itself, but other possibilities were considered.

4. One of the other possibilities considered to account for the inhibition observed in 3. was the presence in the extracts of the

"antral inhibitor hormone", believed by some workers to be released by acidification of the pyloric gland area. No support for the existence of such a humoral substance was obtained from a study of the effect of pyloric gland area pH on the response of a Heidenhain pouch to continuous intravenous gastrin extract.

5. On the other hand there seems more convincing evidence that acidification of the duodenal mucosa causes the release of an inhibitor hormone, and the possible presence of such a substance in commercially available secretin and cholecystokinin was raised by the patterns of inhibition of the secretory responses to gastrin and to histamine effected by these two humoral preparations extracted from the duodenum.
6. The finding that the oral administration of fat emulsion inhibited the acid response of a Heidenhain pouch to continuous intravenous infusion of either gastrin extract or of histamine, suggested that enterogastrone counteracted circulating gastrin and histamine. An additional mechanism of interference with gastrin release was not excluded, and was discussed.
7. The concept of all the inhibitor patterns outlined in the foregoing sections differing from each other primarily in a quantitative manner was discussed.
8. Studies of the effect of atropine on the secretory responses to a wide range of gastrin extract and histamine doses revealed marked

inhibition only in the case of the smallest doses of the two agents, and decreasing inhibition with each increase in gastrin or histamine dose. In the intermediate dose ranges gastrin responses were inhibited to a greater degree than the histamine ones.

Possible interrelationships between acetylcholine and gastrin, and acetycholine and histamine were raised.

9. Cholinergic potentiation of the secretory responses to gastrin and to histamine was substantiated by experiments using Urecholine. Quantitative studies demonstrated appreciable increases in the maximal acid responses of Heidenhain pouches to gastrin and to histamine. The potentiated maximal gastrin response was still less than the potentiated maximal histamine response, though the difference was proportionally smaller than that between the two unpotentiated maximal responses.

   The possible use of a background of cholinergic potentiation in the detection of small amounts of gastrin activity was raised.

10. Acidification of a Heidenhain pouch was found to be without effect on the cholinergic potentiation of the response to histamine brought about by distension. This finding argued against the view that acid in contact with the gastric mucosa acted as an anticholinergic.

11. Potentiation in the stimulation of an acid secretory response was demonstrated between histamine and gastrin extract.

    The possible significance of potentiation between acetylcholine,

gastrin and histamine, all three normally present in the gastric wall, was mentioned.

12. Evidence was presented that gastrin is not selectively inactivated by passage through the liver, in marked contrast to the behaviour of histamine in this respect.

13. Stimulation of the isolated pyloric gland area by irrigation with acetylcholine at an alkaline pH resulted in a pancreatic volume and enzyme response, similar to the effect of combined secretin and pancreozymin stimulation. There was a suggestion of decrease in response when the pyloric gland area was acidified.

14. Evidence of a widespread distribution of the various upper alimentary tract hormones was briefly reviewed.

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