PROOF OF A HORMONAL MECHANISM FOR GASTRIC SECRETION—THE HUMORAL TRANSMISSION OF THE DISTENTION STIMULUS

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PROOF for the existence of a hormonal mechanism for gastric secretion has, until now, been lacking. Strong but nevertheless inconclusive evidence for the existence of such a hormone has long been available, but evidence that can be considered crucial has not been adduced.

The status of the problem up to the time of the present studies has been clearly and extensively reviewed (1-4). In broad outline it can be summarized as follows.

A humoral mechanism for gastric secretion has been proven to exist (5) by the demonstration that the feeding of a meal produces a secretory response in a subcutaneously transplanted (and thus extrinsically denervated) gastric pouch. This has raised the question of whether the stimulation of the transplanted portion of the stomach by food in the main stomach is due to absorption of certain constituents of the food into the blood stream or to the formation of a hormone by the gastric mucosa under the influence of contact with stimulating chemicals in food.

The further analysis of the humoral mechanism has involved the study of the site of origin of the humoral agent and the nature of this agent. As regards the site of origin, it has been demonstrated that a humoral agent can arise from both the stomach and the intestine (4). In the present studies we are concerned only with the humoral agent arising in the stomach.

Two kinds of stimuli acting in the stomach are effective in evoking gastric secretion; namely, chemical stimuli and mechanical (distention) stimuli. That the chemical stimuli can be humorally transmitted has been well known. In the only previous study in which an attempt was made to determine whether the distention stimulus is humorally transmitted, the results indicated that it was

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1 A preliminary report of this work was read before the XVIIth International Physiological Congress, Oxford, England, July 1947.
not (4). Therefore all previous considerations of the nature of the humoral agent have dealt only with the chemical stimuli and have been primarily concerned with the question of whether the humoral agent is absorbed secretagogues or is a hormone the release of which from the gastric mucosa into the blood stream is caused by the secretagogue.

Inasmuch as no secretagogue is known which will stimulate gastric secretion when placed in the stomach but will not stimulate when injected intravenously, the possibility must be entertained that absorbed secretagogues are the humoral agent. However, it has been well established (5) that secretagogues are stronger secretory stimulants for a transplanted gastric pouch when perfused through the main stomach than when introduced intravenously. This evidence favors the view that not all of the stimulatory action of secretagogues on such a transplanted preparation is due to their absorption into the blood stream and suggests that the secretagogue may also cause the release of a hormone. The other possible explanation of these facts is that the gastric juice acts upon the secretagogue in some way to increase its stimulatory action when it is absorbed. The fact that Kim and Ivy (6) found no augmentation of stimulation by incubation of secretagogue (liver extract) with gastric juice speaks against this latter possibility.

Distention Stimulus for Gastric Secretion

The question of whether distention of the stomach stimulates a secretory response was considered controversial until Lim, Ivy and McCarthy (7) unequivocally demonstrated the existence of such a mechanism. The literature up to 1925 on this subject has been comprehensively reviewed by these authors and we shall only refer here to those studies which have a bearing upon the mechanism of the distention stimulus. The distention stimulus has been shown to occur in the stomach completely deprived of extrinsic nervous connections and even in some instances in the subcutaneously transplanted stomach (8, 9). It has also been shown that distention of the pyloric portion of the stomach will induce secretion of acid in the fundic glands of a pouch even after the extrinsic nerves to this pouch have been severed (7). The interpretation placed upon this observation has varied. Lim, Ivy and McCarthy favored the view that a vasomotor effect transmitted the distention stimulus between these two portions of the stomach unconnected by nerves, whereas Chang and Lim (10) considered it to indicate a hormonal mechanism. However since the classical work of Ivy and Farrell (8), it has generally been recognized that in order to prove that any particular mechanism of gastric secretion is humorally transmitted it is necessary to demonstrate that mechanism by the use of the subcutaneously autotransplanted pouch. Unless the transplantation technique is used the possibility of participation of extrinsic nerves cannot be eliminated with certainty regardless of the thoroughness with which the denervation is done.

One attempt to determine whether distention of the main stomach would evoke secretion in the transplanted fundic pouch has been made by Gregory and Ivy (4). These workers observed no secretory response under the conditions of their experiments. Inasmuch as it had been clearly shown by a number of workers (11, 10, 7) that the distention stimulus could be transmitted between two separated portions of the stomach, these negative results tended to indicate that this transmission depended upon nervous pathways which had not been completely severed in the earlier experiments but which were unquestionably interrupted in the transplantation operation.

In experiments of this type a positive result is crucial whereas a negative result might be due to technical difficulties. Because several possible sources of such technical imperfections were recognized in the studies of Gregory and Ivy, the present work was undertaken to reinvestigate the problem.

METHODS

Several different kinds of animal preparation were used in this study. These will be described individually.

Group I. Subcutaneously transplanted fundic pouch with vagally denervated pouch of the remainder of the stomach. This is essentially the same preparation used by
Gregory and Ivy (4) with the exception that in the animals used in the present studies a much larger portion of the fundic portion of the stomach was transplanted.

In two animals (no. 1 and 2) the operative procedure was the same as that used by Gregory and Ivy. At the first operation the fundic pouch was placed under the skin and its vascular pedicle was permitted to remain intact. From one third to one half of the fundic portion of the stomach was used for making the pouch, so that these pouches were from two to three times the size of those used by Gregory and Ivy. The second operation, performed four to eight weeks after the first operation, consisted of ligation and transection of the pedicle to the subcutaneous pouch and formation of a pouch from the remainder of the stomach. The latter was accomplished by transection at the pyloro-duodenal junction and at the esophago-gastric junction followed by esophago-duodenal anastomosis to restore intestinal continuity. The fundic end of the stomach was closed and the pyloric end was brought out through a stab wound to serve as a stoma. Figure 1 is a sketch of the steps in the operative procedure.

In one animal (no. 3) the operative procedure was reversed in that a pouch of the entire stomach was made at the first operation. At the second operation the stomach
was transected at the level of the incisura angularis and the entire fundic portion was placed in a subcutaneous pocket, allowing the vascular pedicles to remain. Six weeks later a third celiotomy was performed at which the vascular pedicles were ligated and transected. In this animal almost the entire acid-secreting portion of the stomach was incorporated in the transplanted pouch.

In still another animal (no. 4) after performing the first stage as in animals no. 1 and 2, at the second operation the stomach was transected at the level of the incisura angularis and at the pyloric sphincter. The lower portion of the stomach was fashioned into a pouch and the upper end was anastomosed to the duodenum. In this animal the intra-abdominal pouch was formed almost entirely by the pyloric portion of the stomach. Some acid-secreting glands were, however, included in it.

**GROUP II. Subcutaneously transplanted pyloric pouch and gastric fistula.** Two dogs (no. 5 and 6) of this type were prepared. The first stage operation consisted of transection of the stomach at the level of the incisura angularis and again at the pyloric sphincter. The vessels along the greater curvature were not divided and they served as the sole vascular supply to this pyloric pouch which was placed in a subcutaneous pocket in the same manner as the fundic pouches described above. At a
second operation, 3 to 6 weeks later, this vascular pedicle was transected between ligatures and a fistula of the Mann-Bollman type (12) was made into the lower ventral surface of the main stomach utilizing a small segment of jejunum as the fistula path. This operation is sketched in figure 2.

**Basal secretion.** All animals were fasted for at least 12 hours before each experiment. In each experiment the basal secretion was collected, measured and titrated for from one to six hours before distention was performed. This permitted a comparison to be made in order to determine whether the rises in acid output during spontaneous fluctuations in the basal secretion were ever as great as those occurring in response to distention.

**Method of distending.** Condom balloons tied to the ends of pieces of rubber tubing were used to distend the pouches. The size of the balloon was adjusted to the size of the pouch. Similarly, the amount of air introduced into the balloon varied with the various types of pouches. In the animals with subtotal gastric pouches (dogs 1 and 2) approximately 200 cc. of air was used; in the dogs with intra-abdominal pyloric pouches (dogs 3 and 4), about 50 to 100 cc.; and in the animals with subcutaneously transplanted pyloric pouches (dogs 5 and 6), 10 to 20 cc. In each instance the amount of air used induced strong contractions. In most instances the distention period was 30 minutes. Usually the balloon was held in the pouch by hand and a glass hypodermic syringe was kept attached to the rubber tube leading to the balloon. The plunger of the syringe was held in by hand with moderate resistance so that during the height of the contraction wave the plunger was displaced.

**Urecholine.** In the experiments in which urecholine² (carbamyl beta-methyl choline) was used, 1.5 mgm. of the drug was injected intramuscularly in an oil and beeswax vehicle containing 10 mgm. of the drug per cc.

**RESULTS**

**Basal secretion.** All of the animals at times secreted some free HCl during the basal period. This occurred both in the gastric fistula dogs (no. 5, 6) as well as in the animals with transplanted fundic pouches (no. 1–4). However, in all animals free acid was more often absent than present in the specimens collected during the basal period. The data on the basal secretion are included in the tabular summaries (tables 1 and 2).

**Secretion of acid by the pouch which was being distended.** In dogs 1 and 2 the main stomach pouch was distended and the secretion was collected from the fundic transplant. Secretion of acid gastric juice by the distended main stomach pouch regularly occurred but no effort was made to collect and measure this juice. Likewise in animals 3 and 4 in which the intra-abdominal pyloric pouch was the one distended, on the few occasions on which tests were made the secretion from the distended pouch was shown to contain free HCl. These pouches thus obviously contained some acid-secreting mucosa in addition to the entire pyloric portion of the stomach. The pyloric pouches of dogs 5 and 6 were fashioned from the same portion of the stomach as those of dogs 3 and 4 but were subcutaneously transplanted. No acid secretion from these subcutaneously transplanted pyloric pouches was ever observed even

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² The urecholine was kindly supplied by Dr. D. F. Robertson of Merck and Co.
though it is probable that the line of transection was high enough to have included
some acid-secreting cells. The only secretion ever observed from these transplanted
pyloric pouches was a few drops of mucoid fluid.

Secretion by the fundic glands in response to distention of the pyloric portion of the
stomach. In all of these experiments either the portion from which the secretion was

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\text{Table 1}
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<tr>
<td>Totals and average</td>
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\[1\] This animal secreted free acid during the basal period during the course of several tests with
negative response.

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\text{Table 2}
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<tr>
<td>Total</td>
<td>27</td>
<td>38</td>
</tr>
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</table>

collected (Group I, dogs 1-4) or the portion being stimulated by distention (Group II,
dogs 5, 6) was subcutaneously transplanted.

Frequency of positive response. The response to distention was considered to be
positive if the secretion of free HCl during the half-hour period of distention or the
immediately ensuing half-hour period was higher than any of the basal half-hour
periods for that day. On this basis positive responses occurred in 29 of 43 trials in
the dogs in Group I and in 8 out of 24 trials in the dogs in Group II. Table 1 summa-
rizes the results of these experiments and figure 3 is a graphic record of one such
experiment in dog 1.
Latency and duration of responses. Frequently a drop of Töpfer's solution was added to the collection flask during the period of distention in experiments in which there was no free hydrochloric acid during the basal period. In this way the time of onset of free acid secretion could be noted. There was considerable variation in the latent period between the beginning of distention of the pyloric portion and the appearance of free hydrochloric acid in the juice from the fundic glands. The shortest latent period noted was about 10 minutes. However, a few times no free hydrochloric acid appeared during the half-hour distention period and the response to distention occurred only in the half-hour period following the cessation of distention.

The rate of secretion usually had returned to the basal level by the second half hour after cessation of distention. Occasionally the response lasted only during the period of distention whereas on a few occasions the response appeared to persist for as long as one hour after the cessation of distention.

Urecholine. Because in some dogs the frequency with which positive responses occurred was quite low, some method of potentiating the response was sought. The work of Gray and Ivy with mecholyl (13) had suggested that parasympathomimetic drugs potentiate the response to stimuli such as histamine. Our more recent studies (14) have confirmed this finding and have shown that the distention stimulus is also potentiated by these drugs.

A series of experiments was therefore performed in which urecholine was used. Dogs 3, 4, 5 and 6 were used. A control distention without urecholine was first performed. One half hour after this first distention, when the response to it, if any, had subsided, 1.5 or 3.0 mgm. of urecholine in oil and wax was injected intramuscularly. One hour after the urecholine injection a second distention was performed. In a few
of the experiments the preliminary distention before urecholine injection was not carried out. The results are summarized in table 2.

It will be noted that the response to urecholine alone varied from zero to a rather high value. However, even when a secretory response did not occur in response to the urecholine alone the response to distention was enhanced by the urecholine. This is illustrated by the graphic record of an experiment in dog 6 (fig. 4).

Positive responses occurred in 12 out of 27 experiments in which distention was performed before urecholine was administered, whereas 36 out of 38 trials were positive after the drug.

DISCUSSION

When Gregory and Ivy (4) performed experiments essentially similar to our experiments on dogs of Group I, they failed to observe a secretory response in the transplanted fundic pouch when the main stomach pouch was distended. In several such 'two-pouch dogs' other than those which were used in the present studies we too were unable to elicit such a response. A number of factors may have contributed to the failure of these earlier experiments. As already indicated we believe that the most important of these factors is the responsiveness of the transplanted pouch preparation, and this has been enhanced chiefly by making the transplanted pouches larger.

All of the transplanted fundic pouches used in the present study secreted free hydrochloric acid at times during the basal period. This did not occur in Gregory and Ivy's animals nor has it been observed in other transplanted fundic pouches previously studied in our laboratories. Furthermore, the responses of the transplanted pouches used in the present studies is definitely greater than that which has been observed in previous studies.

Another factor which must be taken into consideration is the amount of distention used as a stimulus. If the pouch which is being distended has intact sympathetic innervation, excessive distention may lead to retching and vomiting and we (15) have shown that retching inhibits histamine stimulated gastric secretion even in the transplanted pouch of the fundic portion of the stomach. Chang and Lim (10) studying the effect of distention of the isolated pyloric pouch (sympathetics intact) upon acid secretion by the fundic glands stated that "volumes of air larger than 20 cc. not infrequently precipitated nausea and vomiting, and usually failed to excite secretion from the fundus." The statement of Gregory and Ivy (4) is of interest in this regard: "even when excessive pressures are used (15-30 cm. H2O), causing signs of nausea in the animal (salivation, restlessness, retching), and are maintained for periods up to 2 hours, no production of free acid from the transplant is detected, [italics theirs] despite the fact that the amount of free acid secreted meanwhile by the main pouch may be considerably in excess of that evoked by perfusion with liver extracts, which has been shown to cause a humoral response from the transplant." In the present studies we have attempted to avoid the occurrence of nausea by using only moderate distention.

In the dogs in Group II, the pyloric pouch was subcutaneously transplanted and nausea did not occur even with severe distention.

The mechanical and chemical stimuli for gastric secretion had previously been
shown to be similar in a number of ways: a) both chemical and mechanical stimuli are more effective in stimulating acid secretion by the fundic glands when they act in the pyloric region than when they act in the fundic region, b) both are prevented from acting by the application of procaine to the part of the stomach being stimulated and c) the action of both is blocked by atropine. With the present studies the only apparent discrepancy in the mechanism of action of the two stimuli for the gastric phase of gastric secretion is resolved by demonstrating that the mechanical stimulus, like the chemical stimulus, releases a humoral agent. Inasmuch as the question of absorption of the stimulating agent does not arise in the consideration of the nature of the humoral agent for the distention stimulus, the conclusion that it is hormonal in nature would appear to be justified.

The present studies give no new information about the nature of the hormonal agent.

SUMMARY

Distention of the pyloric portion of the stomach stimulates the secretion of hydrochloric acid by the fundic glands. This effect still occurs when all nervous connections between the stimulated portion of the stomach and the portion responding with secretion have been interrupted. This interruption can be accomplished by subcutaneous transplantation of either the part of the stomach which is to be stimulated, namely the pyloric portion, or the part which responds to the stimulus, the fundic portion. This demonstration of the humoral transmission of the distention stimulus is considered for gastric secretion.

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