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# CONTINUOUS RECORDING OF ACID GASTRIC SECRETION IN THE RAT

BY

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A method is described for the continuous recording of acid gastric secretion in the rat. The stomach of the rat anaesthetized with urethane is perfused with a dilute sodium hydroxide solution by way of the oesophagus and the *p*H of the fluid emerging from a cannula in the pylorus is registered graphically. In passing through the stomach, the perfusate collects sufficient buffer to act as an approximately linear buffer system over the relevant range, so that the change in *p*H becomes a measure of acid secretion. The preparation is suitable for the bioassay of secretory stimulants. Ten or more drug doses can be administered in succession in one preparation, so that each animal serves as a self-contained assay unit. Intravenous doses of histamine, methacholine, carbachol and acetylcholine produce a graded and reversible stimulation of acid secretion. The secretory effect of histamine is markedly and specifically potentiated by antihistaminases.

The effect of drugs on gastric secretion in the rat has usually been tested in the pylorus-ligated preparation (Roe and Dyer, 1939; Komarov, Shay, Rayport, and Fels, 1944; Shay, Komarov, Fels, Meranze, Gruenstein and Siplet, 1945). In this method only one dose of drug is administered to each animal so that large numbers of rats are required for a quantitative assay. In the present paper a continuous recording method is described which is sufficiently sensitive to allow several doses to be administered in succession to the same preparation so that each animal serves as a self-contained assay unit or sub-unit.

Methods for the continuous recording of the *p*H of the gastric contents have previously been described (Flexner and Kniazuk, 1940; Rovelstad, Owen, and Magath, 1952), but they provided no information on the amount of acid secreted. In order to obtain this information it is necessary to use a perfusate which changes its *p*H when acid is secreted. In the present work we used a solution of dilute sodium hydroxide, the *p*H of which changes, when perfused through the stomach of the rat, by up to three units, according to the amount of acid secreted.

As single intravenous injections of drugs produce a measurable secretion of acid in this preparation, it can be used for the bioassay of secretory stimulants. The stimulant effects of histamine and choline esters, and the potentiating effects of antihistaminases, will be described.

A brief description of the continuous recording method has already been published (Ghosh and Schild, 1955).

## METHODS

Two uniform breeds of rats were used, male and female albino rats and hooded rats. Their weights ranged from 150 to 400 g., with an average of 190 g. The animals were not starved, and were anaesthetized by a single intramuscular injection of urethane. The dose of urethane in terms of body weight required for different rats varied slightly; the sensitivity to urethane appeared to depend on seasonal influences. We generally used doses of 0.5 to 0.7 ml./100 g. of 25% solution of urethane; when the first dose failed to anaesthetize in 30 to 40 min., an additional intramuscular dose was given. These doses caused no interference with respiration. After the administration of the anaesthetic, the body temperature fell. In most experiments it was artificially stabilized at 30° by means of a rectal contact thermometer which controlled the heating of the operating table through an electronic relay. The operating table was tilted and was heated from below by a 25 watt electric lamp, and from above by two 40 watt strip lights (tube lamps) fitted with reflectors, one on each side of the table. When the body temperature was checked by a thermometer introduced into the vagina it varied by less than $\pm 0.25°$.

*Operative Technique.*—The trachea was exposed and cannulated. A polythene tube of 11 cm. length and 2 mm. external diameter was passed into the lower

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oesophagus and tied into the oesophagus at the neck excluding the vagus. The jugular veins were then exposed and cannulated with polythene tubes of 1 mm. diameter bevelled at the tip. The abdomen was opened through a midline incision, the pyloro-duodenal junction exposed and a small glass cannula introduced through a cut in the duodenum into the stomach and secured firmly by tying a ligature round the pylorus, care being taken not to include blood vessels within the ligature. The whole stomach was then brought forward and a longitudinal incision about 1¼ in. long was made with an electro-cautery knife along the middle of the anterior surface beginning as near the fundus as possible. Food particles were scooped out carefully with moist cotton-wool, special attention being paid to the fundal region and crevices between the mucous folds. The whole interior of the stomach was washed with cotton-wool soaked in warm saline and the mouth of the cannula was freed of food debris before closing up. The cut edges were then united and secured firmly by means of a continuous suture through the whole thickness of the wall so that no leakage of fluid occurred. Finally, the structures were returned to their proper places and the abdominal wound closed by two or three interrupted sutures with the free end of the cannula projecting. The whole operation lasted about 30 min.

*Continuous Recording of Acid Secretion.*—The stomach was perfused continuously with a dilute solution of NaOH, the fluid emerging from the pylorus passing over a glass electrode which recorded *p*H continuously. By the time the fluid had traversed the stomach it had collected sufficient buffer to act as an approximately linear buffer system over the *p*H range 6.5 to 4.5 when titrated with 0.1 N-HCl. There was thus an approximately linear relation between *p*H and acidity. The shape of the titration curve is presumably due to buffers of various kinds secreted by or diffusing from the mucosa of the stomach.

In most experiments N/4000-NaOH was perfused through the stomach at a rate of about 1 ml./min. When this solution was collected after having passed through the unstimulated stomach it gave an initial *p*H of the order of 6 to 6.5.

*Gastric Perfusion System.*—This is shown in Fig. 1. The solution of NaOH was kept in a Mariotte stock bottle which was fitted with a soda-lime tower and suspended above the preparation. The perfusion rate was controlled by a length of capillary resistance tubing connected to the stock bottle. This in turn was connected to a small jacketed warming coil (kept at 30° by a circulating pump) which was joined directly to the oesophageal tube. The capillary was chosen to give a flow of 1 ml./min. with a pressure of 200 cm., and the flow was maintained constant within 0.1 ml./min. The pyloric cannula was connected to a recording glass electrode through a short polythene tube. The glass electrode was placed 15 cm. below the level of the animal; a slight negative pressure was thus exerted, and prevented distension of the stomach.

Optimal conditions for an assay obtained when the perfusate emerging from the pylorus had an initial *p*H of about 6.5 which would decrease to about *p*H 4.0 by the action of stimulant drugs. An initial *p*H of 6.5 could generally be achieved by adjusting the perfusion rate within the limits of 1 to 2 ml./min. through variation in perfusion pressure, without changing the concentration of NaOH from the standard molarity of N/4000. In some experiments N/2000 or N/8000-NaOH was used.

The recording glass electrode was made of a lithium glass membrane; it had a diameter of 5 mm. and was fitted into a U-shaped glass container with a capillary lumen. One limb of the U-tube was slightly dilated to hold the glass electrode, and at this point the porous plug of the reference electrode was attached. The dead space within the glass container was 0.2 ml., and the total dead space from the gastric cannula to the bulb 1.3 ml. The whole assembly was held by clamps and a stand in such a way that the reference electrode was at a level 12 in. higher than the glass electrode. As a result of this pressure gradient, the KCl solution diffused slowly and steadily

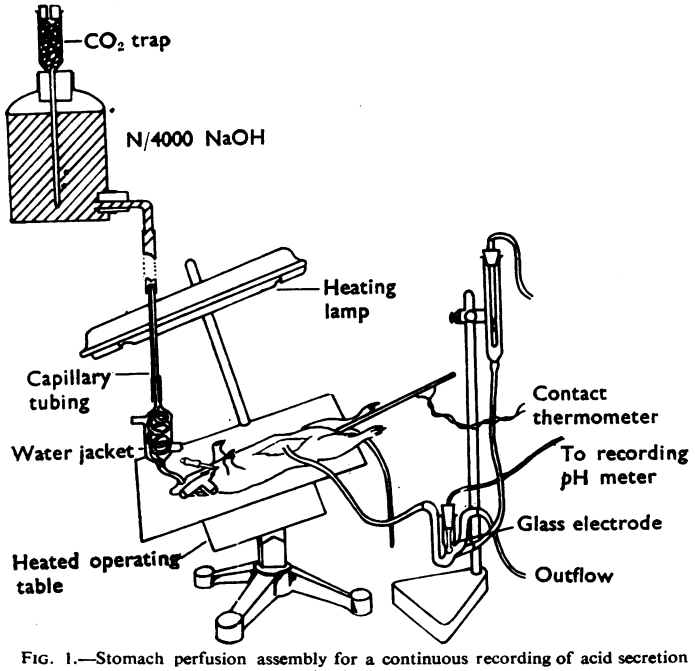


FIG. 1.—Stomach perfusion assembly for a continuous recording of acid secretion in the rat.

through the porous plug serving as an efficient salt-bridge connexion.

The electrodes were connected to a direct reading *p*H meter (Electronic Instruments Ltd., Model 23A) and thence to a circular ink recorder (Fielden's servograph recorder). There was no appreciable drift or lag in the apparatus and the stability of the recorder was within 0.1 *p*H unit. In the illustrations the record has been transformed to rectilinear co-ordinates. Drug responses have been expressed in terms of the maximum deflexion of the *p*H record from the base line.

All drugs were administered intravenously. The first injection was usually made 10 min. after completing the operation. Drugs were injected in a volume of 0.1 to 0.4 ml. followed by a washing injection of 0.1 ml. saline. The concentration of histamine acid phosphate is expressed in terms of the base, and that of the choline esters in terms of the hydrochlorides.

## Results

### *The Effect of Histamine*

A typical effect of histamine on acid secretion is shown in Fig. 2. Although the drug was administered by a rapid intravenous injection, it produced a delayed and gradually increasing effect. The *p*H began to fall 4 min. after the injection and reached the lowest point 13 min. later. Only a small fraction of this delay was attributable to mechanical lag in the perfusion system. A rough estimate of this lag could be obtained by injecting acid into the oesophageal tube ; when a dose of

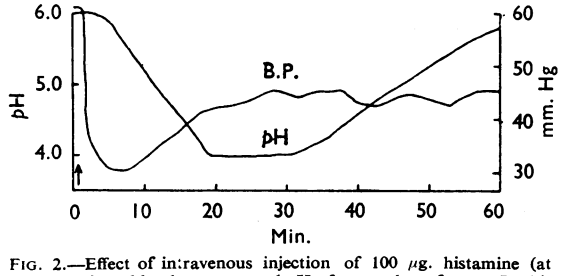


FIG. 2.—Effect of intravenous injection of 100 μg. histamine (at arrow) on blood pressure and *p*H of stomach perfusate. In this and the following illustrations rats anaesthetized with urethane and kept at 30° were used. Ordinates: *p*H of stomach perfusate on the left and blood pressure on the right.

0.1 ml. 0.1 N-HCl was so given, it produced a sharp fall of *p*H at the recording electrode within 40 sec., followed by 90% recovery in 2 min. A record of blood pressure taken simultaneously with the *p*H record in Fig. 2 shows that the two responses have a different time course. The blood pressure fell almost immediately after the intravenous injection and started recovering long before the secretory effect had reached its maximum.

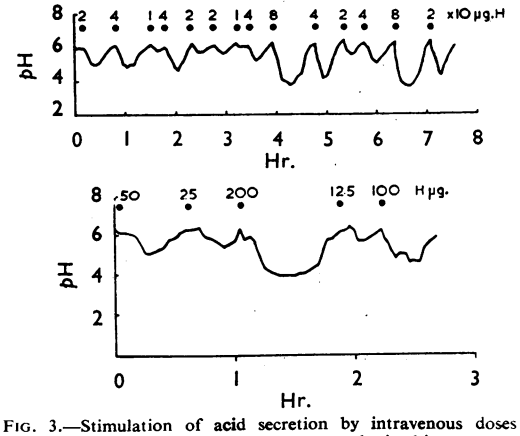


FIG. 3.—Stimulation of acid secretion by intravenous doses of histamine (H). Graded responses were obtained in one preparation with doses of 10 to 80 μg. and in the other with 12·5 to 200 μg. histamine.

Since the effect of an intravenous dose of histamine lasted only 40 to 60 min. several successive doses could be given in the course of an experiment lasting several hours. In the experiments illustrated in Fig. 3, doses over an 8-fold and 16-fold range given in random succession produced graded effects. Repeated administrations of the same dose usually produced constant effects as shown in Fig. 4.

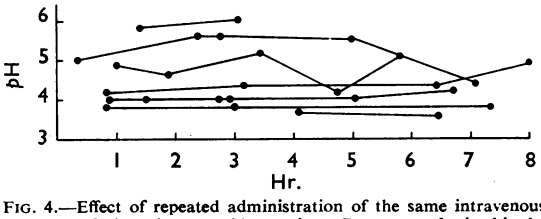


FIG. 4.—Effect of repeated administration of the same intravenous dose of histamine on acid secretion. Responses obtained in the same preparation are joined. The response to the first dose was omitted in each case.

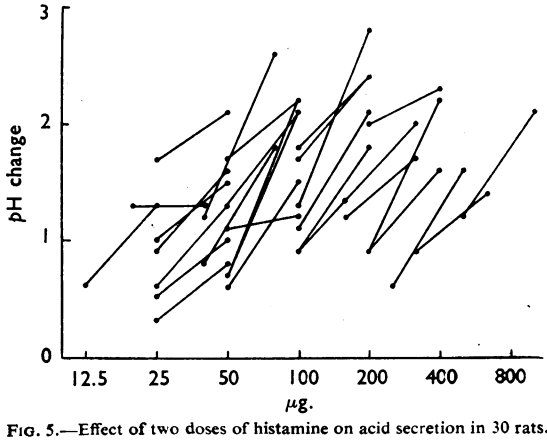


FIG. 5.—Effect of two doses of histamine on acid secretion in 30 rats.

There was a great deal of variation in the sensitivity of the rats. In some, 25 μg. histamine produced an average secretory response ; in others as much as 500 μg. was required, and about 10% failed to respond to doses of 1 mg. Fig. 5 shows the responses obtained with two graded doses of histamine in 30 rats. The average sensitivity of the preparations varied about 20-fold, but in each (except one) the effect increased with dose.

*Effect of Body Weight.*—Fig. 6 shows the histamine dose/100 g. of body weight required for a standard secretory effect when plotted against body weight. There is some evidence of a possible correlation between the two variables, suggesting that small rats are rather more sensitive to the secretory stimulus of histamine than large rats.

*Effect of Body Temperature.*—When the body temperature was changed by altering the external heat supply, the histamine response was consistently decreased by warming and increased by cooling. In one experiment, histamine produced a large effect at 30°, progressively smaller effects at 36°, and another large effect at 30°. In another experiment two large responses occurred at 30°, followed by a small response at 34°. Three further experiments are summarized in Fig. 7. In each, the magnitude of secretory response changed in a direction opposite to the body temperature, and this effect was reversible.

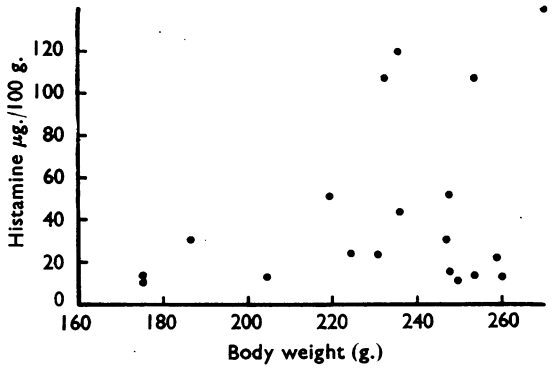


FIG. 6.—The dose of histamine/100 g. of body weight required to produce a standard secretory effect plotted against the body weight.

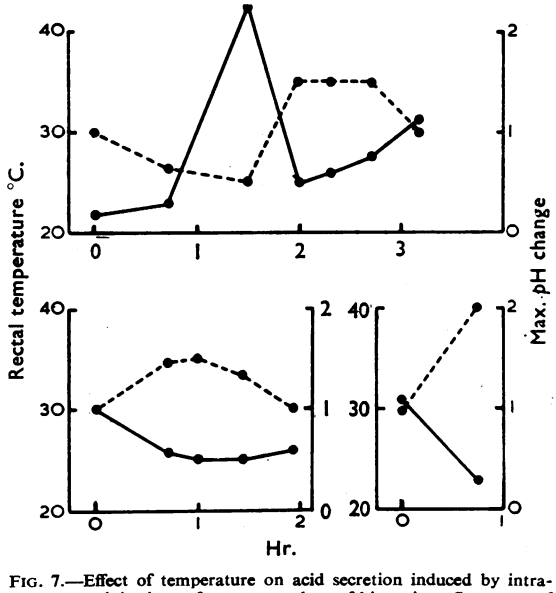


FIG. 7.—Effect of temperature on acid secretion induced by intravenous injections of a constant dose of histamine. Summary of three experiments. Temperature, broken line; *p*H, solid line.

### *Choline Esters*

Acid secretion could be induced by intravenous injection of carbachol, methacholine, and acetylcholine. Acetylcholine was relatively inactive and tended to produce toxic side reactions culminating in respiratory arrest. It was nevertheless possible to record stimulation of secretion by acetylcholine several times. The effect of acetylcholine was potentiated by neostigmine. The degree of potentiation was related to the dose of neostigmine and the potentiation persisted for some time.

Methacholine was more active than acetylcholine. Graded responses were obtained within the range of 0.1 to 20 μg. The sensitivity to methacholine often increased in the course of an experiment. In one experiment the sensitivity increased nearly 100-fold in the course of 4 hours.

Carbachol proved a very powerful stimulant of secretion. In doses of 0.02 to 2 μg., it produced graded effects, the intensity and duration of which

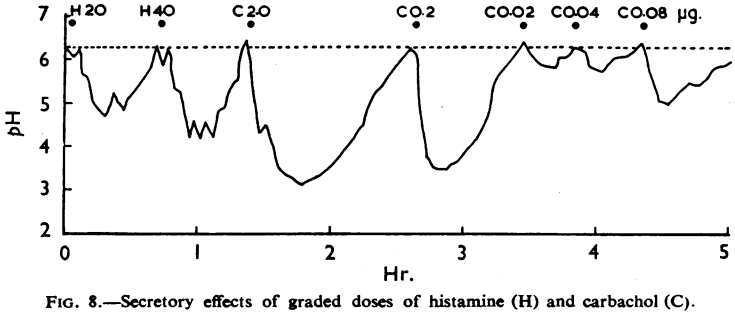


FIG. 8.—Secretory effects of graded doses of histamine (H) and carbachol (C).

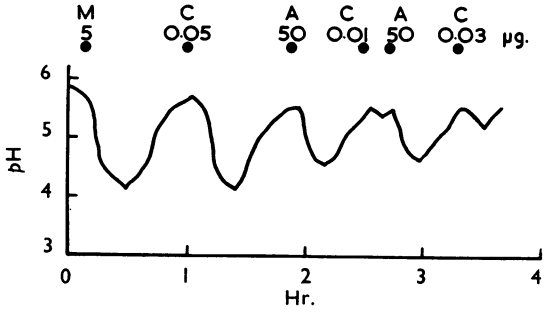


FIG. 9.—Comparison of secretory effects of methacholine (M), carbachol (C), and acetylcholine (A).

increased with the dose as shown in Fig. 8. Weight for weight, carbachol was considerably more active than histamine.

A direct comparison of the secretory effects of these three choline esters is shown in Fig. 9. In this experiment carbachol was 100 times as active as methacholine and about 1,000 times as active as acetylcholine. Table I summarizes the activity ratios obtained in six assays. Although the activity ratios varied in different preparations carbachol was obviously the most active, and acetylcholine the least active of the three compounds. The activity ratios for acetylcholine hold only for small

TABLE I

ACTIVITY RATIOS OF ACETYLCHOLINE, METHACHOLINE AND CARBACHOL

| Drug Ratios | Exp. No. | Estimated Activity Ratios | | |
|---|---|---|---|---|
| Methacholine / Acetylcholine | 168 | >10 | | <20 |
| | 179 | >10 | | <40 |
| | 183 | =200 | | >50 |
| | 191 | | >10 | |
| | 139 | | >25 | |
| | | | >1,000 | |
| Carbachol / Acetylcholine | 168 | >10 | | <100 |
| | 179 | | >500 | |
| | 183 | >1,000 | | <1,600 |
| | 184 | >250 | | =500 |

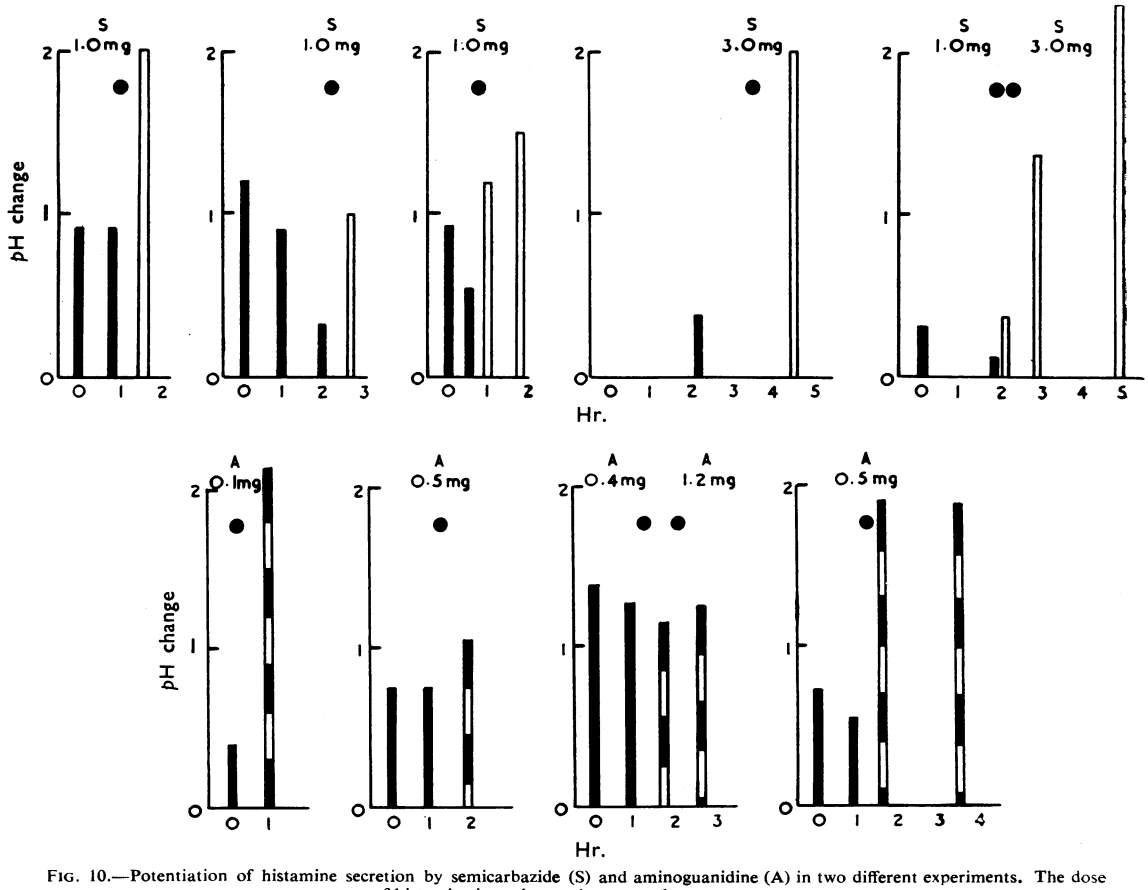


FIG. 10.—Potentiation of histamine secretion by semicarbazide (S) and aminoguanidine (A) in two different experiments. The dose of histamine in each experiment was kept constant.

or medium secretory effects, since doses of acetylcholine producing large effects were not administered owing to their toxicity.

*The Effect of Antihistaminases*

Two inhibitors of histaminase, aminoguanidine (Schuler, 1952) and semicarbazide (Zeller, 1942), were used. The antihistaminases were administered intravenously shortly before or together with the test drug, in doses in which they produced no secretion on their own.

Both aminoguanidine and semicarbazide produced a strong potentiation of histamine secretion. Semicarbazide was administered in five experiments in doses of 1 and 3 mg., some experiments involving several injections. The results are summarized diagrammatically in Fig. 10*a*, which shows that each time the effect of histamine was potentiated. Aminoguanidine was administered in five experiments in doses ranging from 0.1 to 1.2 mg. The results with aminoguanidine are summarized in Fig. 10*b*, which shows potentiation of histamine in all experiments except one.

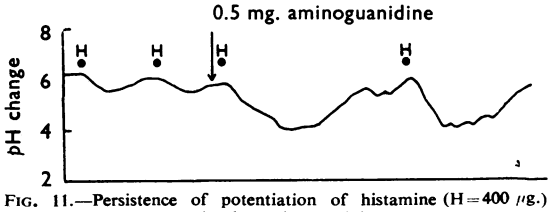


FIG. 11.—Persistence of potentiation of histamine (H = 400 μg.) secretion by aminoguanidine.

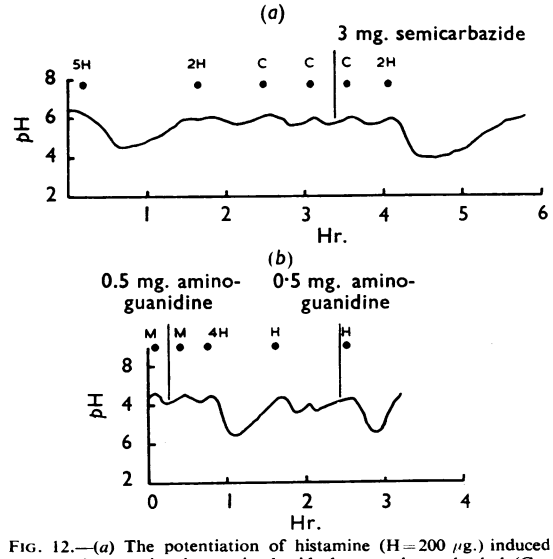


FIG. 12.—(*a*) The potentiation of histamine (H = 200 μg.) induced gastric secretion by semicarbazide but not by carbachol (C = 0·2 μg.) secretion. (*b*) Aminoguanidine potentiation of histamine secretion (H = 12·5 μg.) but not of methacholine (M = 50 μg.) secretion.

Potentiation by a single dose of antihistaminase sometimes persisted during subsequent injections of histamine; in one experiment a second dose of histamine given 1 hr. after the administration of aminoguanidine produced a greater effect than the first dose given with the aminoguanidine. An example of persistent potentiation is shown in Fig. 11. Two consecutive doses of 400 μg. histamine produced only small effects, but the same dose of histamine was still potentiated when given 2 hr. after 500 μg. of aminoguanidine. Neither methacholine- nor carbachol-induced secretion was appreciably increased by antihistaminases. Fig. 12 shows experiments in which antihistaminases were tested with histamine and with choline esters in the same preparation. Fig. 12*a* shows no potentiation of carbachol by semicarbazide, but a strong potentiation of histamine at a later stage. Fig. 12*b* shows no potentiation of methacholine by aminoguanidine, but potentiation of histamine. In two other experiments, antihistaminases produced a slight potentiation of the effects of choline esters; but this was variable and not comparable in intensity with the potentiation of histamine.

## DISCUSSION

The method of continuous recording of *p*H has obvious limitations. It measures total acid secretion, but gives no indication of the volume and acidity of the gastric juice, and the artificial conditions under which the animal is maintained, namely the low body temperature, anaesthesia and alkalinity of the stomach perfusate, may affect both acid and mucus secretion. On the other hand, the method has certain advantages for bioassay work: it is sensitive and follows the time course of secretion closely, so that successive doses of drugs can be administered as soon as the effect of a previous dose subsides.

In the early stages of this work distilled water or saline was used to perfuse the stomach; but it soon became apparent that these fluids were unsuitable since they gave initial *p*H values of 4 to 5 which were little affected by injections of histamine. Perfusion of the stomach with a linear buffer also proved unsuitable. A linear buffer has the theoretical advantage of a more truly linear relationship between acidity and *p*H, but the concentration-action curve becomes flat.

The procedure for recording *p*H continuously, which may be applicable to other species, seemed to depend for its success on the following points: (1) Low body temperature. Anaesthetized rats survived better at 30° than at 37° or even at

34°, and both the circulation and respiration were less liable to fail. (2) Cleaning of stomach. The difficulties of clearing the rat stomach of food residues by fasting are well known. We found that after 24 hr. fasting only about 80 to 90% of animals were free of food residue and that even longer fasting periods were frequently unsuccessful. When the unopened stomachs of these animals were perfused through the oesophagus it was not possible to clear the fundus completely of food residue and the secretory activity appeared very irregular. Opening of the stomach by a wide incision over the major curvature, and scooping out the contents, obviated these difficulties and produced surprisingly little interference with gastric secretion. There was no leakage from the operation wound, and the animals were not devitalized by fasting. (In some recent experiments the unopened stomach was washed out through a thin catheter introduced by way of the pylorus cannula. Satisfactory recordings were obtained by this method.) (3) Urethane anaesthesia. Since anaesthetics affect gastric secretion (Schachter, 1949), it is essential in any bioassay to keep the depth of anaesthesia constant. This cannot be achieved by anaesthetics such as pentobarbitone, which have a short duration of action and must therefore be administered repeatedly. Urethane when given in a single dose produced an apparently unchanged anaesthesia for at least 8 hr.

*The Effect of Histamine*

When an intravenous dose of histamine is given to rats the blood-pressure response is immediate, but the secretory response has a characteristic latent period and reaches a maximum only after about 15 min. This delay, which is surprising since the plasma concentration of histamine must be at its peak immediately after the injection, could be explained in two ways. It might be due to the slow rate at which secreted acid reaches the surface of the stomach. A second dose of histamine would then be expected to produce less delay since the necks of the glands would still be filled with secretion, but, in fact, the response to a second dose is equally delayed. Alternatively, the delay may indicate that the oxyntic cell responds only to a protracted stimulus. It is known that gastric secretion cannot readily be induced in the dog by single intravenous injections of histamine (Popielski, 1920; Rothlin and Gundlach, 1921; Ivy and Javois, 1925; but see Schofield, 1957), but only by slow infusions, and it may be that in rats a single intravenous dose of histamine circulates for some time in the blood stream and acts like a slow infusion. Single intravenous doses of histamine may produce a better gastric secretory response in rats simply because of their high tolerance for histamine, so that it is possible to administer large doses which take a long time to disappear from the blood stream.

*The Effect of Antihistaminases*

Antihistaminases produce a specific potentiation of histamine secretion in the rat. This agrees with their specific potentiating effect in other species and preparations (Arunlakshana, Mongar and Schild, 1954; Westling, 1956) but contrasts with their effect on gastric secretion in dogs. Sircus (1953) and Ivy, Lin, Ivy, and Karvinen (1956) found in dogs that antihistaminases potentiate not only histamine secretion but also secretion induced by carbachol, insulin and feeding. These results were interpreted in terms of a release and subsequent potentiation of histamine from the gastric mucosa by these various stimuli. This would support the theory of histamine as the final common path for gastric secretion (MacIntosh, 1938; Babkin, 1938); but against this interpretation is the fact that the gastric mucosa contains no histaminase to account for the potentiation. In rats, however, the antihistaminases potentiate histamine secretion selectively, and it is thus unnecessary to postulate that they act on the gastric mucosa; they presumably inhibit histaminase elsewhere in the body, for example in the kidney and intestine (Waton, 1956), and, in this way, prolong the circulation time of injected histamine.

*Direct Assays*

Direct assays of secretory stimulants can be performed in this preparation by injecting the drugs intravenously and matching their effects. Carbachol was shown to be 50 times as active as methacholine and 1,000 times as active as acetylcholine. When the choline esters were injected intravenously they exhibited the same sort of latent period and gradually increasing secretory effects as histamine; it would thus seem that the delay in response observed with histamine is not a characteristic of the drug but of the secretory cell. The high secretory activity of carbachol agrees with results obtained in other species; in the rat it is presumably due, partly at least, to stimulation of ganglia since it was found (unpublished observations) to be reduced by hexamethonium. The low secretory activity of acetylcholine in the rat also agrees with results in other species (Necheles, Mortel, Kosse and Neuwelt,

1938 ; Uvnas, 1948 ; Morton and Stavraky, 1949 ; Pevsner and Grossman, 1955) and can be explained by a combination of two factors, firstly the slowness of the response of the oxyntic cell, and secondly the action of cholinesterase. In the presence of neostigmine the secretory effects of acetylcholine were indeed strongly potentiated.

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