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HISTAMINE-STIMULATED HYDROGEN ION SECRETION BY *IN VITRO* PIGLET GASTRIC MUCOSA

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SUMMARY

1. A new preparation of gastric mucosa isolated from new-born piglets is described. The piglet gastric mucosa was easily separated from the serosal muscle layers by a 'blistering' technique which appeared to cause minimal trauma to the tissue and which allowed extended study *in vitro* in a suitable chamber. Normal resting p.d. was approximately −30 mV (mucosal side negative with respect to serosal side), resistance about 100 $\Omega.cm^2$ and H^+ secretion was absent or occurred at very low rates (0–1 μequiv/cm^2.hr).

2. Maximally stimulating doses of histamine ($1–6 \times 10^{-5}$ M) caused H^+ secretion to increase (up to 15 μequiv/cm^2.hr), p.d. to increase and resistance to decrease. A close correlation was observed between the increase in H^+ secretion and decrease in transmucosal resistance. The threshold dose of histamine appeared to be 10^{-8} M; concentrations 10^{-4} M and higher reduced H^+ secretion somewhat.

3. Pentagastrin (10^{-9}–10^{-7} M) and acetylcholine (10^{-7}–10^{-5} M) did not significantly stimulate the piglet gastric mucosa. Pentagastrin concentrations above 4×10^{-6} M reversibly inhibited H^+ secretion of histamine-stimulated mucosa. High concentrations of acetylcholine (above 4×10^{-4} M) did not affect histamine-stimulated H^+ secretion, but a significant reduction in p.d. was observed.

4. This investigation demonstrates the utility of the piglet gastric mucosa for *in vitro* studies of the mechanism H^+ secretion and the action of secretagogues. From a consideration of such factors as the thinness of tissue and ease of preparation it is suggested that neonatal animals may represent a good source of *in vitro* mammalian gastric tissue.

INTRODUCTION

The isolated gastric mucosa has been a most useful preparation for detailed electrophysiological and pharmacological analyses related to the process of HCl secretion (e.g. Rehm, 1972; Forte & Solberg, 1973). Amphibian gastric preparations have most frequently been used since procedures for the isolation and subsequent *in vitro* study of gastric mucosa from mammalian species have met with variable success. Possible reasons for the difficulties encountered in achieving functional mammalian gastric preparations *in vitro* are (*a*) inadequate oxygenation due to thickness of the whole stomach and (*b*) damage to epithelial integrity when the mucosa is separated from muscle coats. Tissue incubation under hyperbaric oxygen tension has been used to overcome the former difficulty (Davenport & Chavré, 1953).

Wright (1962) has shown that stomach preparations from late foetal stages and neonatal rabbits were capable of secreting acid *in vitro*. Since the whole stomach wall is much thinner in the new-born animal than the more aged counterpart, it seemed that the neonate might represent a useful tissue source for a functionally responsive *in vitro* gastric preparation. For the present work we have used the gastric mucosa isolated from new-born piglets up to 5 days old. Such preparations are physiologically stable for several hours *in vitro* and can readily be stimulated by histamine to secrete acid.

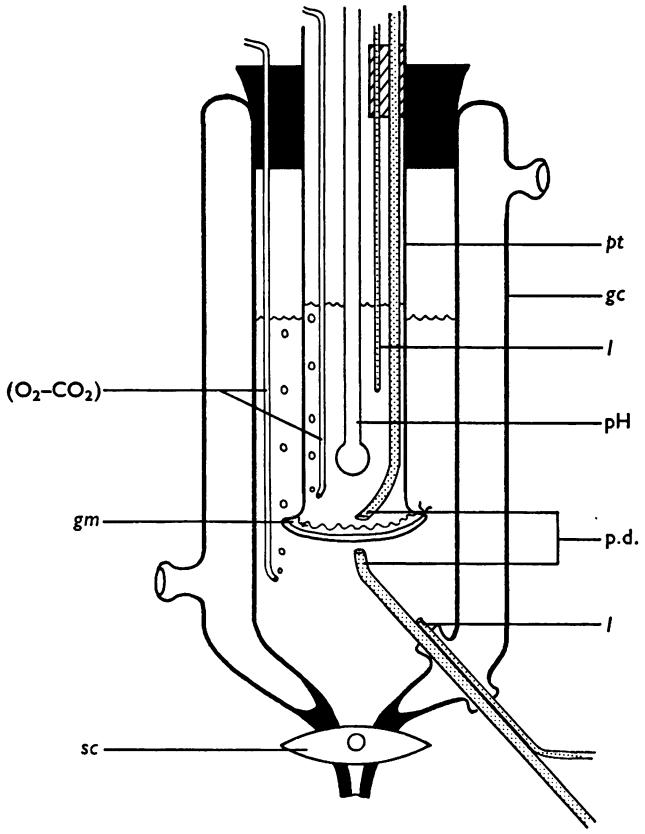
METHODS

Baby pigs (new-born to 5 days of age) were stunned by a blow on the head and rapidly killed by sectioning the cervical region of the spinal cord. The stomach was removed from the animal after clamping off the duodenal and oesophageal ends with haemostats. A bolus of warm, oxygenated Ringer fluid (3–5 ml.) was injected via a hypodermic syringe between the outer muscle coats and the gastric mucosa in the fundic region of the stomach, thus producing a blister of fluid between the two tissue layers. This portion of tissue was then cut from the rest of the stomach around the periphery of the blistered region. The serosal muscle coats were dissected away and the remaining sheet of gastric mucosa was carefully placed and tied on to the end of the mounting tube (1·4 cm^2 area) which was quickly assembled into the apparatus shown in Text-fig. 1. Warmed bathing solutions were introduced into the chamber and gassing with 95 % O_2 and 5 % CO_2 commenced immediately; recordings of electrical parameters (transepithelial potential difference (p.d.) and resistance) were subsequently initiated. With experience the preparative procedure, from the time the animal was killed until the chamber was assembled and operational, took less than 10 min. The bathing solutions were maintained at 37° C for the entire experiment by circulating pre-warmed fluid from a regulated water bath through the double-jacketed chamber (see Text-fig. 1). The Ringer solution bathing the serosal side of the mucosa contained (in mM) 147·0 Na^+, 5·0 K^+, 1·3 Mg^{2+}, 1·3 Ca^{2+}, 129·6 Cl^-, 25·0 HCO_3^-, 1·3 SO_4^{2-} and 11·0 glucose, while the unbuffered mucosal solution contained 147 Na^+, 5·0 K^+, 1·3 Mg^{2+}, 127·0 Cl^-, 1·3 SO_4^{2-} and

25·0 isethionate (hydroxyethylsulphonate). Both solutions were agitated with the 95 % O_2 and 5 % CO_2 gassing mixture.

Acid secretion was followed by the pH-stat method. The unbuffered mucosal solution was titrated (Radiometer AutoBurette, pH meter, and Titrator) to a constant pH 5·0 with 0·1 N-NaOH.

The bridges for measurement of transmucosal electrical potential difference (p.d.) and for the passage of electric current through the mucosa were made of 1 M-NaCl immobilized in 4 % agar. The chamber design was such that the p.d.



Text-fig. 1. Diagrammatic sketch of chamber apparatus used for studying *in vitro* piglet gastric mucosa. The tissue (*gm*) was tied with thread so that the mucosal surface faced the interior of the plastic tube (*pt*). The secured tissue and tube were placed into the double-jacketed glass chamber (*gc*) so that the gastric mucosa separated the mucosal and serosal bathing solutions. The solutions were added, or replaced, via a polyethylene cannula or stopcock (*sc*). Bathing solutions were warmed by 37° C water passing through the jacket and oxygenated through additional polyethylene cannulae (O_2–CO_2). Acid secretion was measured by the glass electrode (pH) and a pH stat; p.d. was measured and current was passed through 1 M-NaCl bridges (p.d. and I) as described in the text.

bridges were within 2–3 mm of the mucosa while current passing bridges were 10–15 mm from the tissue. The p.d. measuring bridges were connected through calomel half cells to a high impedance electrometer (Keithley) which was connected to a recorder. Current was delivered through the saline bridges from a variable d.c. source via Ag–AgCl electrodes. For the measurement of mucosal resistance 100 μA of current was passed through the tissue and the immediate (approximately 1 sec) change in potential was recorded. From this change in potential the equivalent value for chamber resistance was subtracted (i.e., change in potential for 100 μA current through assembled chamber with no tissue present). The corrected value was designated as $\Delta p.d._0$ and was used to calculate mucosal resistance (R_0) from Ohm's Law

$$R_0 = \Delta p.d._0/\Delta I,$$

where ΔI is the pulse of current (100 μA) passed through the tissue.

To determine the sensitivity of the preparation to secretagogues, stock solutions of histamine, acetylcholine, and synthetic pentagastrin (Peptavlon, ICI) were prepared in distilled water, kept refrigerated, and added to the serosal solution (13 ml.) in volumes of 0·2 ml. or less. The stock acetylcholine solutions were kept at pH 4·0 to insure stability. Fresh stock solutions of all the secretagogues were prepared weekly.

When testing the secretagogues, the experimental protocol was first to allow the resting gastric mucosa to come to a steady state for 30 min. Then the drugs were added; a new steady state (i.e. less than 10 % variation during 10 min) was reached before further additions, which were always in steps of increasing concentrations. The quoted values for acid secretion rates, p.d. and resistance were always those measured during the steady state. Because of this experimental design, there often occurred the conflicting circumstances of time-induced decay of the preparation *vs.* stimulatory effect of the drugs. This occasionally made analysis of the effects on p.d., resistance, and acid secretion difficult, and these instances are mentioned specifically in the text.

Epithelia from neonatal to 5-day-old piglets were also prepared for histological examination. Pieces of whole stomach or of 'blistered' gastric mucosa were fixed in Bouin's fluid, imbedded in paraffin and finally sectioned for a light microscopic examination. Sections were stained either with haematoxylin and eosin or by the periodic acid-Schiff reaction.

RESULTS

Visual and microscopic examination of gastric tissue

It was readily apparent by visual examination that the total thickness of the piglet stomach wall increased significantly during the first week of life. Although accurate measurement of stomach wall thickness is difficult in view of possible variations in states of contraction, approximate thickness was obtained from measurements of fixed tissue sections obtained at various ages (Table 1). During the first week of life the increase in fundic tissue thickness was predominantly in the submucosal connective tissue and smooth muscle layers. Variations occurred in glandular mucosal thickness, but these were relatively small compared with the submucosal growth (Table 1) or the continued growth in glandular mucosa which takes place as the animals grow older. For instance, measurements of

glandular mucosal thickness of 4-month old or fully mature pigs ranged from 1·2 to 1·8 mm.

It was consistently observed that the glandular mucosal tissue thickness was greater for sections from the intact stomach than for sections of the same tissue which was fixed immediately after the separation and isolation procedure used for these studies (Table 1). It is very likely that the isolation procedures produced a stretch in the mucosa and hence the somewhat thinner tissue. We noticed that the introduction of the bolus of Ringer fluid to separate the mucosa from the underlying tissue was accomplished with less pressure and produced a more uniform blister in the younger piglet than in animals older than one week. It is possible that the increased growth and investment of connective tissue in the older animals contributes to the greater resistance to blistering.

TABLE 1. Thickness (mm) of various regions of piglet stomach as determined from histological sections

Piglet age (days)	Whole stomach thickness	Glandular mucosa		Submucosal layers
		Intact stomach	Blistered preparation	
0	1·14	0·44	0·30	0·70
2	1·20	0·36	—	0·84
3	1·08	0·22	0·20	0·86
4	1·48	0·39	0·21	1·09
5	1·76	0·39	0·15	1·37
7	2·16	0·48	0·36	1·68

The general histological appearance of the 4-day-old isolated piglet gastric mucosa is shown in Pl. 1. Surface epithelial mucous cells extend into the gastric pits. Numerous mitotic figures were observed throughout the glandular epithelium below the pits. At the level of the light microscope oxyntic cells could sometimes be identified, but frequently they could not be distinguished from epithelial cells of unspecified function. These latter cells had a general undifferentiated appearance, and examination by electron microscopy suggested they would develop into pepsin-secreting (chief) cells and oxyntic cells (T. M. Forte, T. E. Machen & J. G. Forte, in preparation).

Sensitivity to secretagogues

Histamine. The typical response of the piglet gastric mucosa to the addition and removal of histamine is shown in Text-fig. 2. In this experiment the resting mucosa secreted no acid, had a p.d. of -30 mV (mucosal solution negative with respect to serosal solution) and a resistance of

74 $\Omega.cm^2$. Approximately 3 min after adding 10^{-5} M histamine to the serosal solution, acid secretion commenced, the p.d. began to increase (i.e. became more negative) and the resistance began to decrease. The overall effect of 10^{-5} M histamine was to increase acid secretion; in the case shown in Text-fig. 2 the secretory rate increased to about 15 μequiv/$cm^2.hr$. In early attempts to obtain even larger rates of acid secretion, we followed suggestions from the experiments of Knauf (1972) by adding 4·5% albumin to the serosal bathing solution of several preparations. The albumin had no effect.

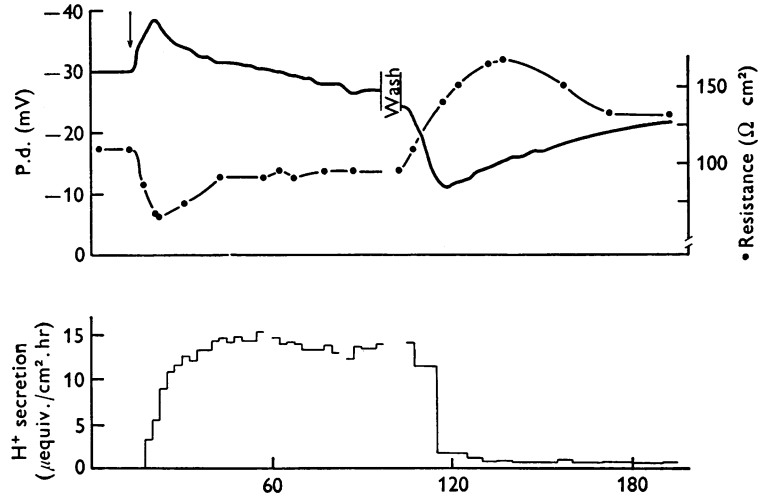
During the first 20 min after histamine addition the p.d. and resistance both transiently increased and decreased, respectively, to a greater extent than the steady-state levels achieved after 25–30 min. We have termed these transient effects 'overshoot' although we realize they may be normal features of doses which effect a most rapid alteration in either of these particular parameters. It was noted that the p.d. had a tendency to decline during the course of an experiment (Text-fig. 2) while acid secretion rates and resistance remained more stable. There was no marked variability, though, in the rate of decline of these parameters from tissue to tissue. The gastric mucosae were stable for at least 6 hr under these conditions.

After histamine was removed from the nutrient solution (Text-fig. 2) acid secretion, p.d., and resistance returned to near-resting levels. Similar to the transients observed upon the addition of histamine, the p.d. and resistance exhibited nearly mirror image 'overshoot' after histamine removal. These transient effects occurred primarily when concentrations of histamine between 10^{-5} and 6×10^{-4} M were used; they were either less apparent or absent with lower concentrations (e.g. 10^{-8} M).

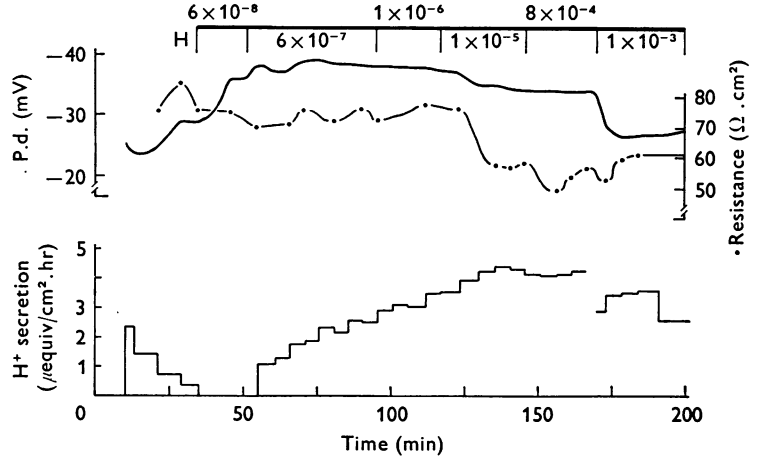
The responsiveness of this mammalian preparation prompted our interest in evaluating the effects of different histamine concentrations. A typical experiment showing the response of the mucosa to different doses of histamine is depicted in Text-fig. 3. During the initial equilibration period the acid secretory rates gradually declined to very low levels. Then, as increasing concentrations of histamine were added to the serosal solution, acid secretion increased, with the maximal rate being achieved at 10^{-5} M. As the concentration was increased further, the secretion rate declined. In the experiment shown, the threshold for stimulating acid production was approximately 10^{-7} M.

Cumulative results of experiments where acid secretion was measured in response to various dose levels of histamine are presented in Text-fig. 4. The curve shows that maximum acid secretion is elicited by histamine concentrations from 1 to 6×10^{-5} M. These maximally stimulating doses gave an average secretion rate of $7{\cdot}6 \pm 1{\cdot}5$ (8) μequiv $H^+/cm^2.hr$ (mean

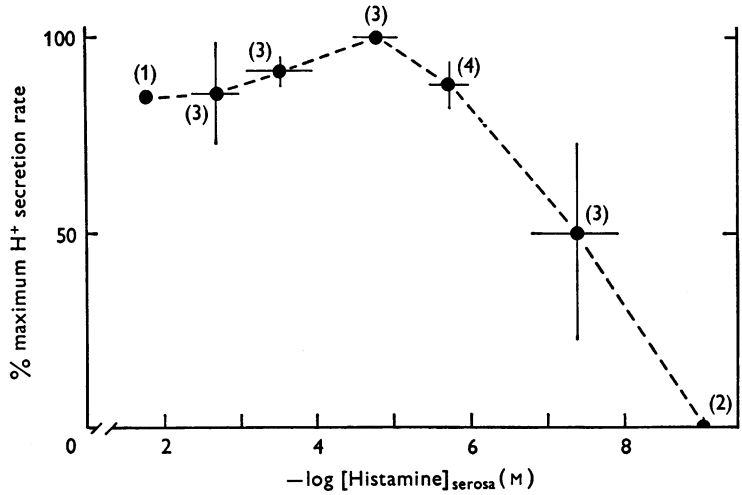
± S.E. of mean, with number in sample given in parentheses). No increase in acid secretory rate above resting level was elicited by 10^{-9} M histamine in two mucosae, and from these cumulative data 10^{-8} M histamine seemed to be the threshold dose. At concentrations 10^{-4} M and higher, acid secretion decreased.



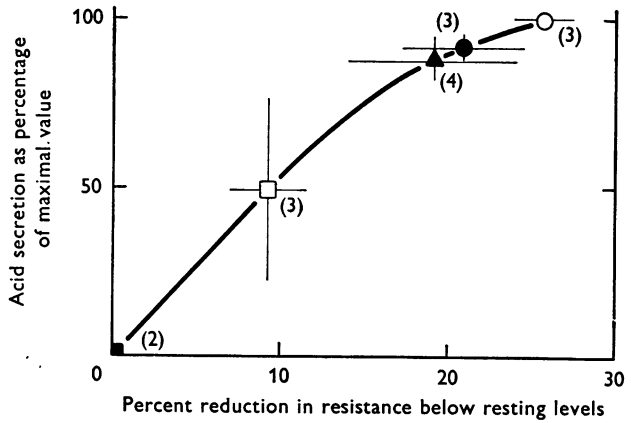
Text-fig. 2. Effects of addition and removal of 6×10^{-5} M histamine to the serosal solution of piglet gastric mucosa. Acid secretion was effectively stimulated by histamine; p.d. (continuous line) and resistance (uninterrupted line) underwent characteristic and reversible changes.



Text-fig. 3. Response of new-born (0 days) piglet gastric mucosa to increasing concentrations of histamine. Acid secretion, p.d. (continuous line) and resistance (interrupted line) were monitored while histamine (H) was added to the serosal side in the increasing doses as indicated.



Text-fig. 4. Dose–response characteristics of piglet gastric mucosa to histamine. Maximum H^+ secretion rate in any one mucosa has arbitrarily been assigned the value 100 %. Data was accumulated from experiments where histamine was added to the serosal solution in increasing doses and are grouped for concentrations as indicated by the width of the bar. The height of the bar is the mean relative secretory response ± 1 S.E. of mean. Numbers of experiments are in parentheses.



Text-fig. 5. Relationship between acid secretion and resistance at various levels of histamine. The % maximum acid secretion rate has been plotted against the % decrease in resistance from resting values. The maximum acid secretion rate and the resting resistance in any one mucosa were arbitrarily assigned values of 100 %. The data were grouped for various concentrations of histamine including: 1×10^{-9} M (■); 1×10^{-8}–5×10^{-7} M (□); 1–6×10^{-6} M (▲); 1–6×10^{-5} M (○); and 1–8×10^{-4} M (●). The height and width of lines through the point indicate ± 1 S.E. of mean. Number of experiments are in parentheses.

As mentioned above, histamine caused transepithelial resistance to decrease and acid secretion to increase (Text-figs. 2 and 3). In fact, these two parameters seemed to be inversely related; when acid secretion was large, the resistance was small.

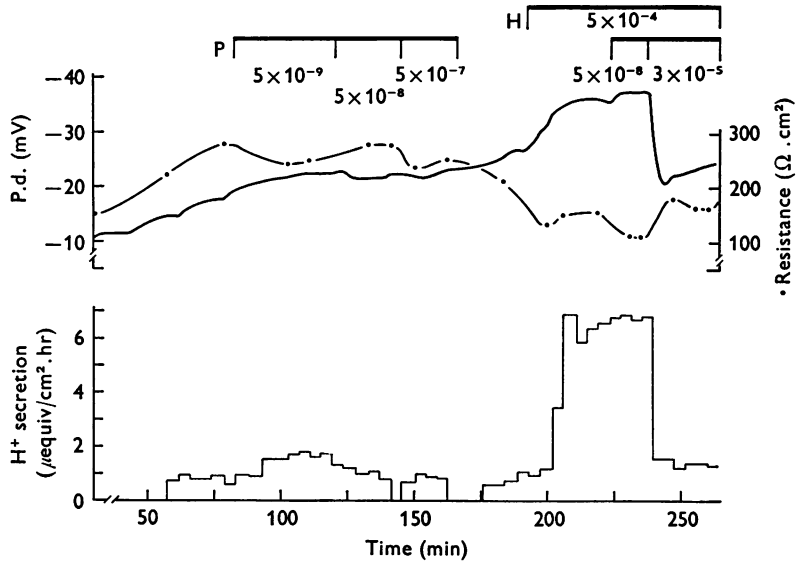
The inverse correlation between the percent decrease in resistance below resting levels and the percent of maximum acid secretion rate is demonstrated in Text-fig. 5. Results from eleven different gastric mucosae have been normalized so that the resting level resistance value and the maximal acid secretion rate achieved in any one mucosa were arbitrarily assigned values of 100 %. At the maximally stimulating dose of $1–6 \times 10^{-5}$ M histamine the resistance decreased by 26 ± 2 %, which represented a decline from the resting resistance of 92 ± 10 to 69 ± 10 $\Omega.\text{cm}^2$.

In addition to the previously mentioned effects, histamine caused the p.d. to increase (Text-figs. 2, 3). As an experiment proceeded, however, the p.d. tended to decrease. Because of this fact and because histamine was always added to the preparations in increasing concentrations, effects on the p.d. often appeared larger at concentrations below 10^{-5} M, the concentration which seemed to elicit the largest acid secretion response. For example, in Fig. 4 it can be seen that 6×10^{-8} M histamine caused the p.d. to increase above resting levels; 6×10^{-7} M caused a further small increase. However, increasing the histamine concentration above these levels had no further effect on the p.d., despite the fact that the typical resistance decrease associated with a maximally stimulating dose (10^{-5} M) did occur. Because of the conflicting occurrences (time-induced decay of the preparation *vs.* histamine stimulation), we have not attempted analysis of the effects of different histamine concentrations on the p.d. It was noted, though, that 10^{-9} M histamine had no effect while 10^{-8} M seemed to be the threshold for stimulation of the p.d. and the concentration at which the maximum change occurred.

Pentagastrin and acetylcholine. In addition to histamine, gastrin and acetylcholine are well known stimulants of acid secretion *in vivo*. We were therefore interested to see the response of the *in vitro* piglet gastric mucosa to these secretagogues.

Synthetic pentagastrin elicited responses like those shown in Text-figs. 6 and 7 where the pentapeptide was added in increasing concentrations. In three experiments no effects on acid secretion, p.d. or resistance were noted below 10^{-9} M. In these three mucosae, concentrations between 10^{-9} and 10^{-7} M pentagastrin caused low-level increases in acid secretion ($0{\cdot}4 \pm 0{\cdot}2–1{\cdot}5 \pm 0{\cdot}3$ μequiv/cm^2.hr, $P < 0{\cdot}1$), while there was no significant change in resistance ($154 \pm 58–135 \pm 56$ $\Omega.\text{cm}^2$, $P > 0{\cdot}4$). As the concentration was increased still further (up to 5×10^{-6} M), no additional effects were seen. That pentagastrin does not stimulate the mucosa to the

same degree as does histamine was further shown by experiments where histamine was added in the presence of low levels of pentagastrin or to preparations where the pentapeptide had been removed (see Text-figs. 6 and 7). Thus the over-all effect of pentagastrin was one of very low-level stimulation of the gastric mucosa compared with the responses elicited by histamine.

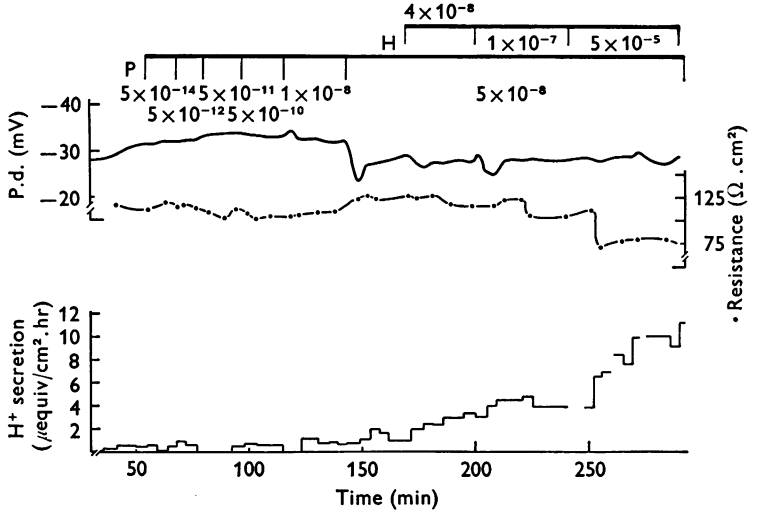


Text-fig. 6. Effects of pentagastrin and histamine. A typical experiment in which a one-day-old piglet gastric mucosa was exposed to increasing pentagastrin concentrations, both before and following histamine stimulation. The particular concentration of pentagastrin (P) or histamine (H) present in the serosal solution is indicated between the bars. 10^{-9} M pentagastrin on the serosal side elicited minimal secretory and electrical responses. These changes were much larger and typical when histamine was used. 5×10^{-8} M pentagastrin did not affect the histamine-induced response, whereas 3×10^{-5} M pentagastrin was inhibitory.

Finally, when pentagastrin was added at high concentrations (8×10^{-6}–8×10^{-4} M) to histamine-stimulated mucosa, acid secretion was reversibly inhibited as demonstrated by Text-fig. 6. In three similar experiments, histamine-stimulated secretion was reduced in every case by large doses of pentagastrin, the mean secretory rate going from $5{\cdot}2 \pm 2{\cdot}0$ to $2{\cdot}3 \pm 1{\cdot}6$ μequiv/cm^2.hr.

In experiments on three mucosae with acetylcholine at concentrations of $1{\cdot}5 \times 10^{-7}$, $7{\cdot}7 \times 10^{-6}$ and 1×10^{-5} M no effects were observed on acid secretion or p.d. compared with resting levels. The data at these different concentrations were therefore grouped together. The average resting acid secretion rate and p.d. were $0{\cdot}9 \pm 0{\cdot}2$ μequiv/cm^2.hr (3) and

$-21{\cdot}8 \pm 4{\cdot}8$ mV (3) while the respective values after acetylcholine addition at the above-mentioned concentrations were $0{\cdot}5 \pm 0{\cdot}2$ μequiv/cm^2.hr (5) and $-24{\cdot}0 \pm 2{\cdot}2$ mV (5). The effects of acetylcholine on resistance were variable; sometimes causing small increases, sometimes small decreases. The averages were not statistically different from each other (resting: 139 ± 21 Ω.cm^2 *vs.* acetylcholine: 154 ± 15 Ω.cm^2).



Text-fig. 7. Effects of pentagastrin and histamine. The piglet gastric mucosa was exposed to different pentagastrin and histamine concentrations. Symbols as in Text-fig. 2.

When high levels (4×10^{-4} M and greater) of acetylcholine were added to mucosae maximally stimulated by histamine, no effect on acid secretion was seen, but there was a substantial reduction in p.d. In five experiments acetylcholine in concentrations between 4×10^{-4} and 4×10^{-3} M was added to histamine-stimulated gastric mucosae; the results were all similar and were therefore grouped. The average histamine-stimulated rate of acid secretion ($4{\cdot}6 \pm 1{\cdot}2$ μequiv/cm^2.hr) was little changed ($4{\cdot}5 \pm 1{\cdot}1$ μequiv/cm^2.hr) upon addition of the acetylcholine. However, in these same experiments (plus two others in which acid secretion was not measured) the high acetylcholine concentrations caused the p.d. to be decreased reversibly from an average of $-22{\cdot}6 \pm 3{\cdot}6$ to $-14{\cdot}0 \pm 2{\cdot}1$ mV. Mean values for resistance were the same before (110 ± 23 Ω.cm^2) and after (110 ± 6 Ω.cm^2) acetylcholine.

DISCUSSION

The gastric mucosa of the new-born piglet (0–5 days old) has been found to be a useful *in vitro* preparation for studying acid secretion and the electrophysiological parameters associated with this process. Several groups of workers using *in vitro* whole stomachs from adult guinea-pigs, rats, monkeys, dogs, cats and humans (Cummins & Vaughan, 1965; Sernka & Hogben, 1969; Shoemaker, Sachs & Hirschowitz, 1966; Kitahara Fox & Hogben, 1969) have experienced only moderate success; acid secretion rates were generally low (1–3 μequiv. $H^+/cm^2.hr$) and could not be increased by secretagogues.

The mean rate of H^+ secretion by isolated piglet gastric mucosa after maximum stimulation (≈ 8 μequiv $H^+/cm^2.hr$) is considerably higher than values typically reported for *in vitro* amphibian preparations. We often observed rates of 10–15 μequiv $H^+/cm^2.hr$ (in one experiment, 19 μequiv $H^+/cm^2.hr$), which are greater than any reports we have been able to find in the literature for *in vitro* preparations.

One of the possible reasons for the difficulties encountered by previous authors in their work with isolated mammalian gastric preparations may have been the result of inadequate oxygenation due to the thickness of the tissue. Certainly this would appear to be the case for isolated whole stomachs where tissue thickness is easily several millimetres thick. (For a discussion of some of the parameters and limitations of oxygen diffusion through thick, unstirred layers in gastric tissue, see Kidder, 1970.) In their very comprehensive studies of acid secretion by isolated mouse stomachs, Davenport & Chavré (1950, 1953) used very high oxygen tensions of 3200 torr to enhance the chemical gradient for diffusion of oxygen into the tissue.

In the case of the neonatal pig gastric mucosa studied here there are two significant features which may have led to improved oxygenation of the tissue. First, the isolated gastric mucosa alone is obviously much thinner than the intact stomach, and second, the thinness of the neonatal gastric tissue compared with that of the adult. In addition, histological examination revealed that the thickness of the glandular gastric mucosa was less in the 'blistered', isolated preparation than in sections from the same whole stomach (Table 1). These factors would thus contribute to a somewhat thinner, and perhaps better oxygenated, glandular tissue.

A thin preparation is not, however, the only criterion for producing a stable *in vitro* gastric mucosa. An additional difficulty mentioned earlier is in the possible damage to epithelial integrity when the muscle coats are separated from the mucosa. This problem also seems to have been satisfactorily overcome by use of the 'blistering' technique on the neonatal

mucosa. This isolation procedure was very easy and rapid to perform and seemed to cause only minimal trauma to the tissue (Pl. 1). Early attempts at peeling the piglet gastric mucosa away from the muscle layers, as is commonly done with frog gastric mucosa, were unsuccessful.

Whole stomachs from foetal and new-born rabbits have been known to secrete acid (Wright, 1962). These results together with those of the present study show that young animals may represent a good source for *in vitro* preparations of mammalian gastric mucosa due to their thinness and ease of preparation.

As the pigs grew older (more than 7 days), the 'blister' formation between the serosal muscle layer and the gastric mucosa required much more effort, perhaps due to the greater investation of the developing connective tissue in these preparations; this extra force may have produced micro-tears within the epithelium. Dissections in the case of the older animals also took more time. Such factors were undoubtedly fundamental to the questions of why the gastric mucosae from older pigs secreted at low rates (0–1 μequiv $H^+/cm^2.hr$) and could not be stimulated by histamine. It was also noted that the transepithelial p.d. of these older preparations was much more dependent on the presence of Na^+ in the mucosal bathing solution than those taken from 0–5 day old piglets (unpublished observation; also see Forte & Machen, 1975).

Although substantial secretory rates were obtained from the isolated piglet preparation, on a square cm basis they were not so high as from more intact mammalian preparations. For instance, Rehm (1956) reported values ranging from 50 to 200 μequiv $H^+/cm^2.hr$ for preparations with an intact blood supply, such as the stimulated dog stomach flap. The large difference between these high rates for the *in situ* preparations and our observed values for isolated piglet gastric mucosa would clearly be minimized by appropriately normalizing the square area basis for comparison. The number of oxyntic cells and gastric glands is greater per cm^2 in the intact preparation than in the stretched and flattened gastric mucosa that is produced by the blistering and isolation procedure. Perhaps, as suggested by Kitahara *et al.* (1969), oxyntic cell counts are the only real way to normalize the data and also to account for developmental and growth differences between neonatal and adult tissue. However, present information does not permit more quantitative comparisons.

The H^+ secretion obtained in response to various dose levels of histamine (Text-fig. 4) is similar to that observed in frog gastric mucosa. The threshold concentration required to provoke a response in piglet gastric mucosa was of the order of 10^{-8} M histamine, and secretion appears to reach a maximum at $1–6 \times 10^{-5}$ M. Very similar dose–response relationships for the bullfrog have been reported by Davidson, Lemmi & Thompson

(1966) and by Kasbekar (1967). The latter author showed that the threshold dose for histamine was between 10^{-8} and 10^{-7} M with the maximal response at approximately 10^{-4} M.

Neither Kasbekar (1967) nor Davidson *et al.* (1966) reported inhibitor effects of histamine on frog gastric mucosa such as those seen in the present study when concentrations greater than 10^{-5} M were used. There was, however, a clear levelling off in H^+ secretion at histamine concentrations greater than 10^{-5} M in both studies on frog preparations. For studies on *in vivo* mammalian preparations the inhibitory effects of large doses of histamine are well known (see Grossman, 1967). This is consistent with the inhibitory effects observed in the isolated piglet preparation, but direct correlations between *in vivo* and *in vitro* effects are not possible at this time because of obvious differences in the preparations. The inhibitory effects of large doses of histamine on this preparation may reflect non-specific effects on the acid secretion mechanism of the oxyntic cells.

In addition to its effects on H^+ secretion, maximally stimulating doses of histamine caused p.d. to increase and resistance to decrease in the piglet gastric mucosa (Text-figs. 2 and 3). As mentioned in the Results and also seen in Text-fig. 3, the p.d. was increased by 10^{-8} M histamine, the threshold concentration, but then exhibited little additional changes as the concentration was further increased to $1\text{–}6 \times 10^{-5}$ M. In contrast, the H^+ secretion (and resistance) always exhibited maximal changes after adding $1\text{–}6 \times 10^{-5}$ M histamine. This stimulation of the p.d. at sub-maximal doses may be an indication that the p.d. response and H^+ secretion of piglet gastric tissue are not so intimately related as the resistance and H^+ secretion appear to be (Text-fig. 5).

In contrast to the p.d. response observed in piglet gastric mucosa, histamine stimulation of acid secretion in *in vitro* frog or *in situ* dog preparations is accompanied by a net decrease in transepithelial p.d., i.e. the mucosal side becomes more positive (Rehm, 1962; Forte & Solberg, 1973; Rehm *et al.* 1955). It is pointed out frequently in these cited studies that histamine produced a transient rise in p.d. before H^+ secretion actually began. These authors and others (e.g. Heinz & Durbin, 1959; Rehm & Le Fevre, 1965) have interpreted the general effect of the mucosal side becoming more positive concomitant with the stimulation of H^+ secretion as the electrical manifestation of H^+ pumping from cell to mucosal fluid. The increase in p.d. associated with histamine seen here for the piglet gastric mucosa is similar to that observed in at least one other species, *Necturus maculosus*. For isolated *Necturus* gastric mucosa, Shoemaker *et al.* (1967) observed that histamine and other secretagogues which stimulated H^+ secretion produced a substantial rise in transmucosal p.d. As suggested by these authors, the fact that the mucosal side became more negative

could not be directly linked with a H^+ pump but was rather more likely related to stimulation of Cl^- transport from serosal to mucosal sides. Support for this notion is available from ion flux studies in isolated piglet gastric mucosa which show an activation of Cl^- transport in excess of H^+ secretion subsequent to stimulation by histamine (Forte & Machen, 1975).

The resistance decrease observed upon maximal histamine stimulation is well known in both amphibian (Rehm, 1962; Forte & Solberg, 1973) and mammalian (Rehm, 1953) gastric mucosae. These resistance changes can be correlated well with the increase in H^+ secretion (see Fig. 6; also Rehm, 1953). In addition to the decrease in tissue resistance, extensive ultrastructural changes occur within the oxyntic cells concomitant with H^+ secretion. Namely, there is a decrease in the number of vesicular and tubular membrane units in the cytoplasm and an increase in the apical membrane surface area in the form of a large number of cytoplasmic extensions lining the intracellular canaliculi (Sedar & Friedman, 1961; Sedar, 1965; Forte & Forte, 1970; Helander, Sanders, Rehm & Hirschowitz, 1972). Similar changes have also been observed in piglet gastric mucosa (T. M. Forte, T. E. Machen & J. G. Forte, in preparation). Such increases in membrane surface area may represent the morphological basis of the observed resistance changes associated with H^+ secretion.

The lack of any pronounced stimulatory effect of either pentagastrin or acetylcholine was unexpected since histamine elicited such a large response. At least two explanations come to mind. First, specific receptor sites for these secretagogues may not have developed at the young age of the piglet used here. A second possibility is that histamine is the final common effector for stimulation of acid secretion with the other secretagogues working through their action on histamine. In the latter case, if one were to follow the reasoning of Kasbekar *et al.* (1969), then histamine may be metabolized by or be effluxing from the piglet gastric mucosa very rapidly, much faster than the rates commonly observed in the frog. This notion has some support from the fairly rapid return toward resting levels which occurs in piglet mucosa when histamine is removed (Text-fig. 2). The absence of an available histamine pool would then explain the lack of response by this tissue to the other secretagogues.

There are several problems with the above hypothesis in its most simple form. For instance, the basis for the change in sensitivity to secretagogues between intact and isolated preparations would have to be determined. Furthermore, the isolated piglet mucosa is not completely insensitive to pentagastrin and acetylcholine. There were inhibitory effects by these agents at high concentrations. The inhibitory effects of 10^{-6}–10^{-4} M pentagastrin on histamine-stimulated H^+ secretion may be an indication of interaction between these two drugs at a common receptor site on the

serosal membrane of oxyntic cells. There is some basis for this sort of occurrence from *in vivo* experiments on dogs (Makhlouf, McManus & Knill, 1968).

Acetylcholine also elicited a response at large concentrations (4×10^{-4}–4×10^{-3} M), but only in the form of a decrease in p.d. Neither resistance nor H^+ secretion was affected. Similar effects have been reported by Ridley, Cummins & Vaughan (1965) of 10^{-2} M acetylcholine on isolated rat stomach. These effects may be on a non-oxyntic cell type.

It is recognized that more experiments are required before the consequences of the pentagastrin and/or acetylcholine responses (or lack thereof) on isolated piglet gastric mucosa can be evaluated. However, the present experiments do indicate the great potential of the *in vitro* piglet gastric mucosa for studies of the mechanism of action and interaction of gastric secretagogues.

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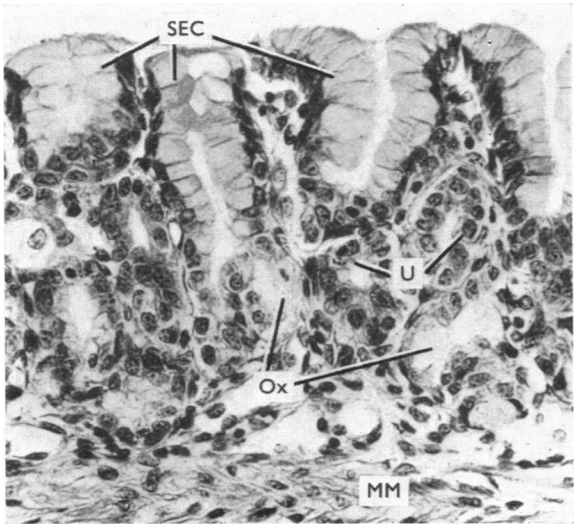
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EXPLANATION OF PLATE

Micrograph of section through piglet (four days old) gastric mucosa. Surface epithelial cells (SEC) are readily apparent at the mucosal surface. Glands contain oxyntic cells (Ox) and many unidentified cells (U). Muscularis mucosae (MM) is also visible. Section stained with H & E. Magnification × 310.