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THE EFFECT OF CIMETIDINE ON BASAL AND STIMULATED PEPSIN SECRETION IN THE ISOLATED WHOLE STOMACH OF THE RAT

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1 The isolated stomach preparation of the immature rat has been used to study the stimulation and inhibition of pepsin secretion.

2 The isolated stomach secretes a basal level of pepsin. High concentrations (10^{-3}M) of the H_2-receptor antagonist, cimetidine, and the muscarinic receptor blocking drug, atropine, did not affect this secretion in a manner which was consistently of statistical significance.

3 Concentrations of histamine of 10^{-5}M, 10^{-4}M and 10^{-3}M stimulated maximum levels of pepsin output of 126%, 155% and 299% respectively of control. There was no evidence that this secretion was secondary to the stimulation of acid secretion.

4 Cimetidine (10^{-4}M and 10^{-3}M) produced a dose-related inhibition of the pepsin output to 10^{-3}M histamine, suggesting that histamine H_2-receptors mediate this response.

5 Atropine (10^{-3}M) had no effect on the pepsin response to 10^{-3}M histamine, suggesting that muscarinic mechanisms play no part, even modulatory, in this secretion.

Introduction

In recent years isolated stomach and gastric mucosa preparations have provided much information about the control of acid secretion (Tepperman, Schofield & Tepperman, 1975; Bunce & Parsons, 1976; Wan, 1977; Main & Pearce, 1978). However, apart from some recent work on isolated gastric mucosa of the rabbit (Kapadia & Donaldson, 1978) little attention has been paid to the control of pepsin secretion. This paper describes studies on the stimulation and inhibition of pepsin secretion in the isolated stomach of the immature rat. A preliminary account of this work was given to the Physiological Society (Bunce, Grewal & Parsons, 1979).

Methods

The isolated stomach of the immature rat (35 to 45 g) was prepared by the method described by Bunce & Parsons (1976) and Bunce, Parsons & Rollings (1976). In brief, the rats were anaesthetized with pentobarbitone, the stomach exteriorized, and the oesophagus ligated. An incision was made in the rumen of the stomach and the contents washed out with warm Krebs-Henseleit solution. A second incision was made at the pyloric sphincter and polythene cannulae were inserted and tied into the stomach via these incisions. The stomach was rapidly dissected out and placed in Krebs-Henseleit solution at 37°C.

The lumen of the stomach was perfused at a rate of 1 ml/min with a modified Krebs-Henseleit solution from which the buffers ($NaHCO_3$ and KH_2PO_4) were omitted. The hydrogen ion concentration of the effluent perfusate from the stomach was continuously recorded, and the rate of acid secretion expressed as nmol/min. For the measurement of pepsin output a modification of the method of Chiang, Sanchez-Chiang, Wolf & Tang (1966) was used. The perfusate was collected at 5 min intervals and duplicate 0.625 ml aliquots mixed with an equal volume of 0.1M glycine buffer, pH 1.9. The solutions were then preincubated at 37°C for 15 min. The reaction was started by the addition of 1.75 ml of a 2% (w/v) haemoglobin solution taken to pH 1.9 by the addition of 2N HCl. The reaction mixtures were incubated for 1 h at 37°C in a shaking water bath and the reaction stopped by the addition of 4 ml of 5% (w/v) trichloroacetic acid. The solutions were filtered through Whatman no. 50 filter paper and the absorbance of the filtrate measured at 280 nm (Unicam SP800). The concentration of pepsin in each sample was measured by reference to a standard graph constructed using crystalline porcine pepsin in the concentration range 0.5 to 10.0 pepsin units per ml. Pepsin output was calculated as the product of volume (ml per 5 min) and concentration and expressed as pepsin units per 5 min.

After setting up the stomach, the preparation was left for approximately 1 h before starting the experiment. All drugs were added to the buffered solution bathing the serosal surface of the stomach.

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Drugs

The following drugs were used: pentobarbitone (Sagatal, May and Baker Ltd.), histamine acid phosphate and atropine sulphate (BDH Ltd.), bovine haemoglobin and porcine pepsin (Sigma Chemical Co. Ltd.); cimetidine was synthesized in our own laboratories.

Analysis of results

Results are expressed as mean ± s.e. of mean. Application of the Rankit test (Colquhoun, 1971) to a sample of the normalized experimental data did not provide evidence of a normal distribution, and therefore in the present work the difference between two samples was examined statistically by the Mann-Whitney U test as described by Siegel (1956). A two-tailed test was used. A P value of less than 0.05 was considered to be significant.

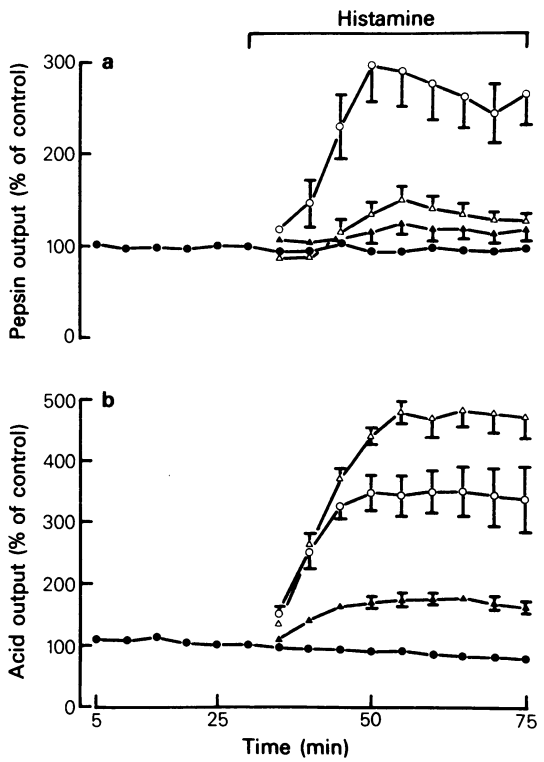

Figure 1 The gastric secretory response in the isolated stomach of the rat to histamine at concentrations of 10^{-5}M (▲, $n=6$), 10^{-4}M (△, $n=7$) and 10^{-3}M (○, $n=6$). The basal outputs of pepsin and acid are also shown (●, $n=7$). (a) Pepsin output (% of control). (b) Acid output (% of control). Vertical bars are s.e. mean.

Results

Basal secretion

The spontaneous output of pepsin was stable over the 75 min experimental period, and ranged from a mean of 18.6 ± 4.5 to 20.3 ± 5.5 ($n=7$) pepsin units per 5 min. Over the same period, acid output gradually diminished from a mean of 26.6 ± 2.5 to 18.7 ± 2.2 ($n=7$) nmol/min.

The large standard error values reveal a considerable between-preparation variability in the rate of pepsin secretion. Thus, in order to facilitate the comparison of data, subsequent results have been normalized where appropriate. This was done by expressing the acid or pepsin output at 30 min (just before the administration of an agonist or an antagonist) as 100%, and all other secretory rates observed during the course of an experiment were then expressed as a percentage of this value. Having established the level of basal secretion, the effect of histamine on pepsin secretion was next examined.

Histamine-stimulated secretion

In these experiments the basal secretion was collected for 30 min, the histamine was then added to the serosal solution and the secretion collected for a further 45 min. The effect of three concentrations of histamine was examined, 10^{-5}M, 10^{-4}M and 10^{-3}M, and the results are shown in Figure 1. Concentrations of histamine of 10^{-5}M and 10^{-4}M produced only small increases in the rate of pepsin secretion: maxima of 126±11% (n=6) and 151±15% (n=7) respectively of control. A concentration of histamine of 10^{-3}M stimulated a large increase in pepsin secretion; a maximum of 299±43% (n=6) of control. The pepsin response to 10^{-3}M histamine was therefore used in subsequent experiments for determining the effect of secretory inhibitors.

The output of acid was dose-related in the histamine concentration range 10^{-5}M to 10^{-4}M; maximum changes of 175 ± 8% ($n=6$) and 483 ± 30% ($n=7$) respectively of control. A concentration of histamine of 10^{-3}M was supramaximal for acid secretion and produced a maximum response of only 350 ± 42% ($n=6$) of control.

It has been reported by Johnson (1972) that the topical application of acid to the gastric mucosa causes pepsin secretion. To test the possibility that the stimulation of pepsin secretion observed in the present experiments was not a direct effect of histamine but a consequence of reducing the pH of the mucosal bathing fluid, the effect of bathing the surface of the stomach with exogenous acid on pepsin secretion was determined.

The effect of exogenous acid on pepsin secretion

The acid response to histamine shown in Figure 1

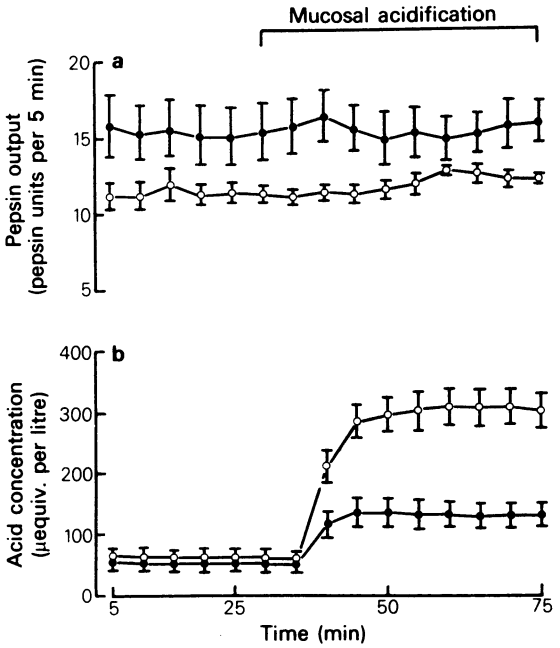

Figure 2 The effect on pepsin secretion in the rat isolated stomach of mucosal acidification to pH 4.0 (●, $n = 8$) and 3.5 (○, $n = 6$). (a) Pepsin output (pepsin units per 5 min). (b) Acid concentration (μequiv. H^+ per litre). Vertical bars are s.e. mean.

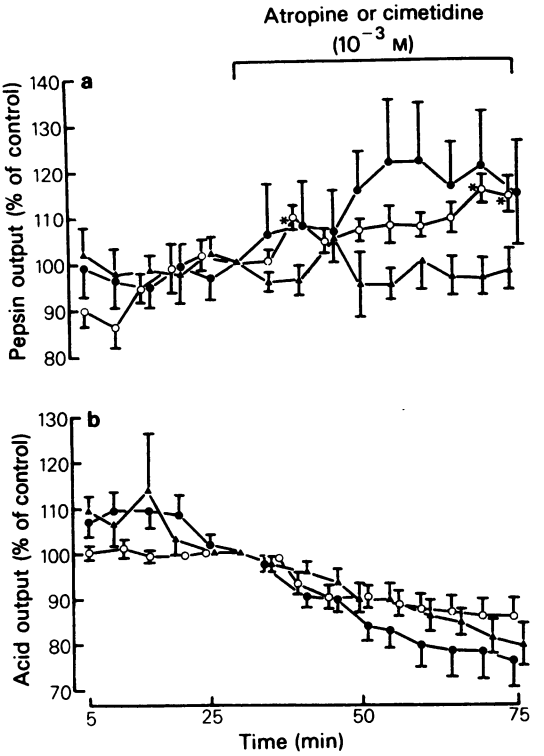

Figure 3 The basal outputs of pepsin and acid in the isolated stomach (▲, $n = 7$), and the effect of 10^{-3}M cimetidine (○, $n = 7$) and 10^{-3}M atropine (●, $n = 6$) on these responses. (a) Pepsin output (% of control). (b) Acid output (% of control). Vertical bars are s.e. mean. *Significant difference ($P < 0.05$) between the output of pepsin under basal conditions and in the presence of cimetidine.

corresponds to a pH of between 3.5 and 4.0. Therefore, after an initial 30 min control period the lumen of the stomach was perfused with unbuffered Krebs-Henseleit solution adjusted to pH 4.0 or pH 3.5 with 0.1 N HCl and then pepsin secretion was followed for a further 45 min. The results are given in Figure 2, and show that exogenous acid in this concentration range had no measurable effect on pepsin output.

Effect of secretory inhibitors on basal secretion

In these experiments the basal secretion was collected for 30 min, the secretory antagonist was then added to the serosal solution and the secretion collected for a further 45 min. Both cimetidine and atropine were used at a concentration of 10^{-3}M, and the results are shown in Figure 3. At the concentration used, both atropine and cimetidine caused a slight increase in the basal output of pepsin; the effect of atropine did not reach statistical significance at any time during the experiments and the effect of cimetidine was only significant in three of the 5 min experimental periods. Neither atropine nor cimetidine produced a significant inhibition of the basal acid output.

The failure of a high concentration of either cimetidine or atropine to produce a consistent effect on basal pepsin secretion allowed the extension of these studies to the investigation of the effect of these antagonists on histamine-stimulated secretion.

Effect of secretory antagonists on histamine-stimulated secretion

In these experiments the stomach was incubated with the antagonist alone for the first 30 min of the experiment and histamine (10^{-3}M) was added to the serosal bathing solution over the next 45 min. Cimetidine was used at concentrations of 10^{-4}M and 10^{-3}M, and the results are shown in Figure 4. In the absence of cimetidine, histamine (10^{-3}M) stimulated a maximum pepsin response of 299 ± 43% ($n = 6$) of con-

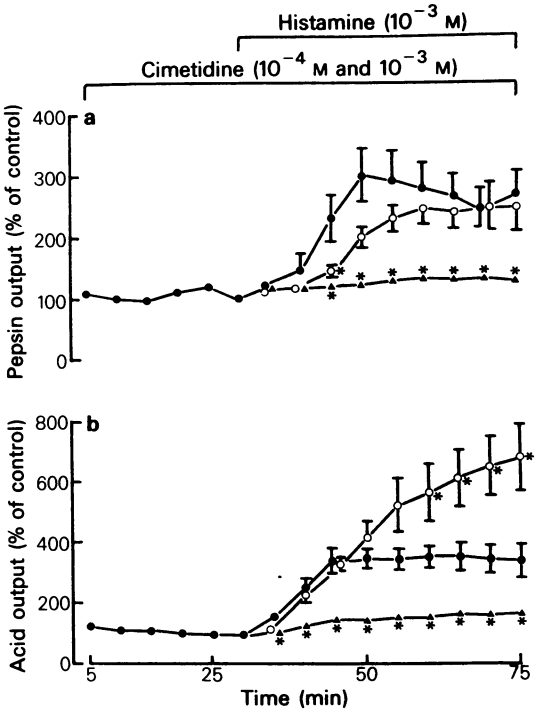

Figure 4 The gastric secretory response in the isolated stomach to 10^{-3}M histamine (●, $n = 6$), and the effect of cimetidine, 10^{-4}M (○, $n = 6$) and 10^{-3}M (▲, $n = 6$), on this response. (a) Pepsin output (% of control). (b) Acid output (% of control). *Significant difference ($P < 0.05$) between the response obtained to histamine alone and in the presence of cimetidine. Vertical bars are s.e. mean.

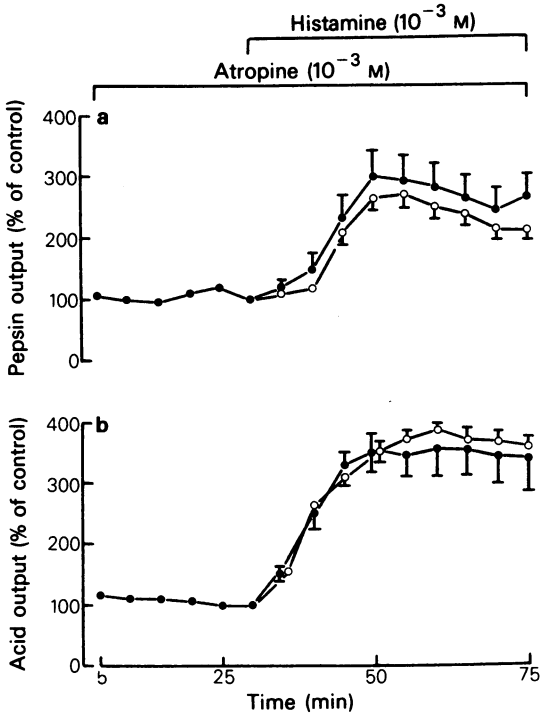

Figure 5 The gastric secretory response in the rat isolated stomach to 10^{-3}M histamine (●, $n = 6$), and the effect of 10^{-3}M atropine (○, $n = 6$) on this response. (a) Pepsin output (% of control). (b) Acid output (% of control). Vertical bars are s.e. mean.

trol. In the presence of 10^{-4}M cimetidine, histamine stimulated a maximum level of pepsin secretion of $247 \pm 39\%$ ($n = 6$) of control, and in the presence of 10^{-3}M cimetidine the maximum pepsin response was $131 \pm 10\%$ ($n = 6$) of control.

The maximum acid response to 10^{-3}M histamine was $350 \pm 42\%$ ($n = 6$) of control. At 10^{-4}M, cimetidine caused a potentiation of acid secretion to a maximum response of $671 \pm 110\%$ ($n = 6$) of control. A ten fold increase in the concentration of cimetidine produced an inhibition of histamine-stimulated secretion and the maximum acid output was $167 \pm 9\%$ ($n = 6$) of control.

The effect of atropine on the pepsin and acid responses to histamine is shown in Figure 5. At the concentration used (10^{-3}M) atropine had no significant effect on either the acid or the pepsin response to histamine.

Discussion

The isolated stomach and gastric mucosa of the rat have provided information about the control of gastric acid secretion in the absence of the vascular, hormonal and neural influences that occur in the whole animal (Bunce & Parsons, 1976; Main & Pearce, 1978). The present experiments have also established that such preparations can be used for investigation of the control of pepsin secretion.

A basal output of pepsin has previously been recorded from isolated stomach and gastric mucosa preparations of the rat (Mantovani, 1970), mouse (Villarreal, 1953) and rabbit (Kapadia & Donaldson, 1978) and a spontaneous output of pepsin was also observed from unstimulated stomachs in the present work. However, the control of this basal output is unclear since a high concentration of neither a histamine H_2-receptor antagonist (cimetidine) nor a muscarinic blocker (atropine) had an effect on the secretion which was consistently of statistical significance. Other workers have also reported that the

basal secretion of pepsin from isolated tissue preparations is resistant to inhibition by both atropine and cimetidine (Villarreal, 1953; Kapadia & Donaldson, 1978).

Histamine stimulated the secretion of pepsin in the rat isolated stomach, and such an effect of histamine has also been described in isolated tissue preparations of the mouse (Villarreal, 1953) and frog (Kasbekar & Gordon, 1979). A direct comparison of pepsin output in these latter studies with the present work is made difficult by the use of different units to define peptic activity. However, Kasbekar & Gordon (1979) found that 10^{-4}M histamine stimulated an output of pepsin from the frog isolated gastric mucosa of approximately 30 pepsin units/cm²h. The surface area of the secretory mucosa of the rat isolated stomach was approximately 2 cm², and 10^{-4}M histamine stimulated a maximum output of pepsin of about 30 pepsin units per 5 min, i.e. 180 pepsin units/cm² h (including a basal secretion of 120 pepsin units/cm² h). In contrast, Kapadia & Donaldson (1978) have reported that histamine (10^{-6}M – 10^{-2}M) has no effect on pepsin secretion from rabbit isolated gastric mucosa. The reason for these differences is unknown. For comparison with the *in vitro* studies, histamine does stimulate pepsin secretion in the rat *in vivo*, although compared with gastrin and cholinomimetics it is a relatively poor pepsigogue in this species (Johansson, Lundell, Rosengren & Svensson, 1972).

The present experiments show that higher concentrations of histamine were required for the stimulation of pepsin secretion than for the stimulation of acid secretion. Indeed, a substantial level of pepsin secretion above basal was only obtained with 10^{-3}M histamine. This result must cast some doubt on the physiological importance of histamine in the control of pepsin secretion in the rat. It is possible that although histamine itself is a poor pepsigogue, the agonist might modulate the pepsin responses to other secretory stimulants, as has been suggested for acid secretion (Soll, 1978). However, experiments in the conscious Heidenhain pouch rat have shown that histamine has little effect on the pepsin responses to gastrin and methacholine (Johansson *et al.*, 1972).

Although the simplest explanation of the present results is that histamine directly stimulates the secretion of pepsin from chief cells, the possibility exists that this output of pepsin is a consequence of a reduction in luminal pH. Evidence for such an effect of acid applied topically to the gastric mucosa has been provided by Johnson (1972) and Bynum & Johnson (1975). Two observations have been made in the present study which are inconsistent with the existence of such a mechanism in the isolated stomach. Firstly, a dose of histamine (10^{-4}M) which produced a maximal rate of acid secretion stimulated only a small increase in pepsin output. However, this latter argument should be applied with some caution since there is some evidence that pepsin output is not directly related to the concentration of acid bathing the gastric mucosa but that the output of pepsin is stimulated when the luminal acid concentration is within a certain range (Dritsas & Kowalewski, 1968). Secondly, bathing the mucosal surface with acid in the concentration range pH 4.0 to pH 3.5 (which corresponds to the acid concentration achieved by the secretion of endogenous acid) failed to stimulate the secretion of pepsin. Kapadia & Donaldson (1978) carried out similar experiments on isolated gastric mucosa of the rabbit *in vitro* and found that 50 mM HCl (pH 1.3) did stimulate pepsin secretion. Indeed, it is possible that the concentration of acid in the gastric glands in the isolated stomach is greater than that bathing the mucosal surface. For this reason the effect of perfusing the isolated stomach with solution at pH 2 was briefly examined. An increased output of pepsin was noted. However, in two out of five preparations overt damage to the mucosal tissue was observed after 30 min of acid perfusion. Thus, at the present time the precise role of acid in the stimulation of pepsin secretion in the isolated stomach is unresolved, and it is possible that perfusion of the mucosal surface with acid at pH 3.5–4.0 does not adequately mimic the milieu within the gastric glands. It is of interest to note that in experiments on the anaesthetized rat, Puurunen (1979) concluded that the gastric mucosa does not contain receptors sensitive to hydrogen ion concentration.

In the present study, cimetidine inhibited histamine-stimulated pepsin secretion in a dose-dependent manner. In contrast, at a concentration of 10^{-4}M, cimetidine potentiated the acid response to histamine and a possible explanation for this observation is that at this concentration cimetidine is reducing the effective concentration of histamine at the H_2-receptors from a supramaximal level to one which is closer to that maximal for acid secretion. However, in the presence of 10^{-3}M cimetidine the effective concentration of histamine at the receptors is further reduced to a level which stimulates a submaximal rate of acid secretion. Clearly, large concentrations of cimetidine have been used to inhibit pepsin secretion in the isolated stomach and this was a consequence of using 10^{-3}M histamine to stimulate the output of pepsin. Nevertheless, these results do suggest that H_2-receptors are involved in the pepsin response to exogenous histamine in the isolated stomach.

In studies on the isolated gastric mucosa of the frog, the H_2-receptor antagonist, burimamide, was considerably more effective as an inhibitor of the acid than the pepsin response to histamine (Kasbekar & Gordon, 1979). However, a comparison of the results in Figure 4 using 10^{-3}M cimetidine provides no clear evidence to suggest that a similar situation exists in the isolated stomach of the rat. Experiments in the

conscious rat have also revealed that H_2-receptor antagonists are relatively weak inhibitors of pepsin secretion when compared with their action on acid secretion (Lundell, 1975; Bunce, Parsons & Matheson, 1979). However, it must be emphasised that the stimulus used in these *in vivo* experiments was of cholinergic origin, and this may explain the difference between the latter results and those obtained in the present study.

A high concentration of atropine had no significant effect on the pepsin response to histamine in the isolated stomach preparation and this result indicates that muscarinic mechanisms play no part, even modulatory, in the control of this response.

References

BUNCE, K.T., GREWAL, M. & PARSONS, M.E. (1979). Pepsin secretion in the isolated rat stomach preparation. *J. Physiol.*, **269**, 55P.

BUNCE, K.T. & PARSONS, M.E. (1976). A quantitative study of metiamide, a histamine H_2-antagonist, on the isolated whole rat stomach. *J. Physiol.*, **258**, 453–465.

BUNCE, K.T., PARSONS, M.E. & MATHESON, F.R. (1979). Inhibition of pepsin secretion by metiamide and atropine in the conscious rat. *Agents & Actions*, **9**, 163–167.

BUNCE, K.T., PARSONS, M.E. & ROLLINGS, N.A. (1976). The effect of metiamide on acid secretion stimulated by gastrin, acetylcholine and dibutyryl cyclic adenosine 3′, 5′-monophosphate in the isolated whole stomach of the rat. *Br. J. Pharmac.*, **58**, 149–156.

BYNUM, T.E. & JOHNSON, L.R. (1975). Stimulation of human pepsin output by topical hydrochloric acid. *Am. J. dig. Dis.*, **20**, 607–612.

CHIANG, L., SANCHEZ-CHIANG, L., WOLF, S. & TANG, J. (1966). The separate determination of human pepsin and gastricsin. *Proc. Soc. exp. Biol. Med.*, **122**, 700–704.

COLQUHOUN, D. (1971). *Lectures in Biostatistics.* p. 80. Oxford: Clarendon Press.

DRITSAS, K.G. & KOWALEWSKI, K. (1968). The mechanism of pepsin secretion in the isolated perfused canine stomach. *Can. J. Surg.*, **11**, 85–92.

JOHANSSON, I., LUNDELL, L., ROSENGREN, E. & SVENSSON, S.E. (1972). Gastric secretion and its facilitation as related to gastric mucosal histamine. *J. Physiol.*, **226**, 431–452.

JOHNSON, L.R. (1972). Regulation of pepsin secretion by topical acid in the stomach. *Am. J. Physiol.*, **223**, 847–850.

KAPADIA, C.R. & DONALDSON, R.M. (1978). Macromolecular secretion by isolated gastric mucosa. Fundamental differences in pepsinogen and intrinsic factor secretion. *Gastroenterology*, **74**, 535–539.

KASBEKAR, D.K. & GORDON, G.S. (1979). Effects of colchicine and vinblastine on *in vitro* gastric secretion. *Am. J. Physiol.*, **236**, E550-E555.

LUNDELL, L. (1975). Elucidation by a H_2-receptor antagonist of the significance of mucosal histamine mobilization in exciting acid secretion. *J. Physiol.*, **244**, 365–383.

MAIN, I.H.M. & PEARCE, J.B. (1978). A rat isolated gastric mucosal preparation for studying the pharmacology of gastric secretion and the synthesis and release of endogenous substances. *J. Pharmac. Methods*, **1**, 27–38.

MANTOVANI, P. (1970). Effect of caerulein on peptic secretion of the rat. *Arzneim.-Forsch.*, **20**, 1588–1590.

PUURUNEN, J. (1979). Effect of intragastric acid on pepsinogen secretion in the rat. *Eur. J. Pharmac.*, **55**, 207–209.

SIEGEL, S. (1956). *Nonparametric Statistics for the Behavioural Sciences.* pp. 116–127. Tokyo: McGraw-Hill Kogakusha Ltd.

SOLL, A.H. (1978). The interaction of histamine with gastrin and carbamylcholine on oxygen uptake by isolated mammalian parietal cells. *J. clin. Invest.*, **61**, 381–389.

TEPPERMAN, B.L., SCHOFIELD, B. & TEPPERMAN, F.S. (1975). Effect of metiamide on acid secretion from isolated kitten fundic mucosa. *Can. J. Physiol. Pharmac.*, **53**, 1141–1146.

VILLARREAL, R. (1953). Pepsin secretion and synthesis *in vitro*. *Proc. Soc. exp. Biol. Med.*, **83**, 817–819.

WAN, B.Y.C. (1977). Metiamide and stimulated acid secretion from the isolated non-distended and distended mouse stomach. *J. Physiol.*, **266**, 327–346.

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