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Is pentagastrin-stimulated secretion mediated by histamine?

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SUMMARY Patients with duodenal ulcer disease received either a two hour pentagastrin infusion test or a similar test with the addition of a bolus of cimetidine, 200 mg, after one hour of pentagastrin. Pentagastrin induced secretion of acid and histamine, the secretion patterns of the two being similar. Total histamine output in the gastric aspirate in the first hour of pentagastrin infusion was related to total acid output ($r=0{\cdot}58$, $p<0{\cdot}01$). A similar correlation was observed during the second hour of pentagastrin infusion ($r=0{\cdot}61$, $p<0{\cdot}05$). Plasma histamine concentration rose to a peak coinciding with maximal acid secretion. After cimetidine blockade, gastric acid fell rapidly but gastric histamine output did not change. Plasma histamine concentration increased further. These results suggest that pentagastrin induced gastric histamine release is not affected by an acid inhibiting dose of cimetidine. Cimetidine caused histamine release into the circulation in both healthy volunteers and patients after total gastrectomy. The rise in plasma histamine concentration, however, was transient. In contrast, during pentagastrin infusion, the plasma histamine concentration remained high. These data support the hypothesis that histamine release induced by pentagastrin is a major stimulant of gastric acid secretion.

The mechanism by which the gastric parietal cells are stimulated to produce acid is uncertain. A variety of models have been proposed, with one or more receptor sites on the parietal cell sensitive to different chemical transmitters.[1 2] In many of these models gastrin acts directly on the parietal cell to cause acid release. Gastrin is a potent stimulant of acid secretion, and it has been reported to cause stimulation of metabolic activity in isolated parietal cell preparations.[3] It is well recognised, however, that there are difficulties in interpretation of isolated cell studies, and evidence of significant parietal cell response to gastrin has not been confirmed.

An alternative view is that gastrin acts indirectly, by releasing a transmitter in the gastric mucosa. One candidate for this role is histamine. We have observed in previous studies that pentagastrin, a synthetic analogue of gastrin, causes release of histamine into the gastric juice and into plasma, with depletion of gastric mucosal histamine stores.[4] Histamine release is related in time and degree to that of acid, the amount of histamine released correlating with gastric acid secretion. This parallel release is compatible with the hypothesis that pentagastrin acts indirectly, by causing mucosal histamine release. Histamine may then act on the parietal cell to cause acid secretion.

In order to test this hypothesis we have examined the effect of cimetidine, an H_2-blocking agent, on pentagastrin induced acid secretion and histamine production. If pentagastrin acts directly on the parietal cell, and histamine release is a passive phenomenon associated with gastric secretion, then H_2 blockade should reduce both acid and histamine secretion. Alternatively, if histamine secretion is actually stimulated by pentagastrin then H_2 blockade of the parietal cell should not affect the secretion of histamine induced by pentagastrin infusion, but only gastric acid secretion. The effect of cimetidine on pentagastrin induced gastric acid secretion and histamine production should therefore enable a distinction to be made between these two mechanisms.

Methods

SUBJECTS

Patients with duodenal ulcer diseases were allocated randomly into two groups. Group A was eight patients who received a two hour pentagastrin infusion test (6 μg/kg/h) after fasting overnight.

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Group B was 16 patients who, in addition to the two hour pentagastrin infusion, also received cimetidine 200 mg intravenously after one hour of pentagastrin. Gastric aspirate was collected in 10 minute fractions for measurement of acid and histamine. Venous blood samples were taken from a brachial vein at 10 minute intervals for histamine assay.

Eight healthy volunteers and two patients after total gastrectomy were given cimetidine 200 mg intravenously after overnight fasting. Blood samples were taken at 10 minute intervals for histamine assay.

Acid and histamine were assayed by procedures published in detail previously.[4] Briefly, gastric acid was measured by titration to pH 7·0 using an automatic titrimeter (Copenhagen). Gastric histamine was assayed fluorometrically after extraction and purification procedures as outlined previously.[4] Plasma histamine was assayed by an enzymatic-isotopic method.[4]

The gastric acid and histamine output were corrected for gastroduodenal loss by use of an inert non-absorbable marker (phenol red) to perfuse the stomach of the patient, and duodenogastric reflux by measurement of sodium ions in the gastric aspirate.[5] The mean difference between the corrected and uncorrected values was 5·9±6·6%.

Statistical comparisons were made using the Wilcoxon's matched-pairs signed-rank test. P values <0·05 are recorded as statistically significant. The effects of pentagastrin on acid and histamine production were compared with basal values. After cimetidine blockade, further statistical comparisons were made between values obtained after cimetidine and the values of the sample fraction immediately before cimetidine injection.

Results

1 EFFECT OF PENTAGASTRIN ON ACID AND HISTAMINE OUTPUT

Measurements of acid and histamine, in gastric aspirate and blood plasma, are listed in Table 1 and Table 2 for Group A and Group B patients respectively.

In Group A patients, gastric acid concentration increased from basal levels, median 31 mmol/l, to peak levels, median 121 mmol/l ($p<0·001$), and output rose from basal median 0·5 mmol to peak median 6·3 mmol ($p<0·001$) in the 10 minute fractions. Gastric histamine concentration fell from basal median 27·7 nmol/l to 15·6 nmol/l ($p<0·01$) before returning to basal values after 90 minutes pentagastrin. Histamine output, however, rose from basal median 0·52 nmol to 1·32 nmol ($p<0·01$) because of the rising volume of gastric secretion. Plasma histamine also rose from basal median 3·43 nmol/l to a peak median 5·19 nmol/l ($p<0·05$) before falling back to basal levels after one hour of pentagastrin infusion.

For Group B patients, during the first hour of pentagastrin infusion, comparable changes in acid and histamine were measured. Acid concentration increased from basal 42 to 123 mmol/l ($p<0·001$); output from 0·8 to 8·5 mmol ($p<0·001$). Gastric histamine concentration fell from basal 29·8 to 14·0 nmol/l ($p<0·01$), but output rose from 0·79 to 1·37 nmol ($p<0·01$). Plasma histamine also rose from

Table 1 *Acid and histamine during pentagastrin infusion test; Group A patients (n=8)*

Gastric fraction (no)	Concentration						Output			
	Gastric acid (mmol/l)		Gastric histamine (nmol/l)		Plasma histamine (nmol/l)		Gastric acid (mmol)		Gastric histamine (nmol)	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
2	26	0–86	58·1	13·7–89·9	2·02	0·31–2·82	0·4	0·0–0·7	0·52	0·16–0·94
3	31	0–75	27·7	14·4–89·9	3·43	1·42–6·30	0·5	0·0–2·8	0·49	0·16–1·89
4	38	16–78	51·9	12·9–120·7	5·06	2·66–5·84	0·7	0·2–3·0	1·13	0·23–2·17
5	81*	19–106	23·9	13·4–103·8	5·09*	3·63–6·35	3·2	0·2–8·0	1·18*	0·20–2·80
6	98*	41–124	16·3	11·7–82·0	5·11*	2·82–14·07	4·2	1·0–6·9	0·70	0·31–3·28
7	114*	40–141	15·7	8·1–44·0	4·47*	1·87–10·00	5·2	1·2–7·8	0·84*	0·23–2·13
8	107*	57–133	20·1	7·3–99·0	5·19*	3·19–9·23	6·3	1·6–9·6	1·19*	0·17–5·74
9	118*	57–129	15·6	7·6–42·3	4·36	3·62–9·40	5·8	1·7–13·1	0·86*	0·60–1·90
10	116*	59–137	15·7	7·6–31·7	4·64	3·07–6·29	5·8	1·7–7·9	0·86	0·17–1·10
11	114*	64–141	15·7	6·5–49·8	4·50	1·49–7·98	5·6	1·6–8·8	0·79	0·15–2·15
12	121*	45–141	17·4	2·2–41·1	3·43	0·22–4·55	6·1	1·4–8·7	0·85	0·33–2·10
13	102*	52–136	25·1	16·7–47·9	3·41	1·44–5·23	4·3	1·7–10·6	0·94*	0·64–2·67
14	85*	43–139	27·0	16·7–37·7	5·35	1·22–7·48	5·4	1·5–6·3	1·12*	0·66–1·54
15	92*	17–135	37·7	20·0–76·9	3·07	0·66–5·96	4·1	0·7–6·9	1·32*	0·66–3·31

* Higher than fraction (2) and (3); $p<0·05$; Wilcoxon's signed-rank test.

Table 2 *Acid and histamine during pentagastrin and cimetidine; Group B patients (n=16)*

Gastric fraction (no)	Concentration						Output			
	Gastric acid (mmol/l)		Gastric histamine (nmol/l)		Plasma histamine (nmol/l)		Gastric acid (mmol)		Gastric histamine (nmol)	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
2	35	0–80	28·6	11·4–98·1	3·52	1·65–8·20	1·0	0·0–2·6	0·75	0·24–1·91
3	42	0–97	29·8	9·1–110·6	3·56	0·79–7·96	0·8	0·0–2·6	0·79	0·11–1·88
4	45	0–125	28·3	11·4–100·6	6·47*	3·14–15·17	1·1	0·0–9·2	0·86*	0·26–5·44
5	90*	35–129	21·0	7·5–63·2	5·17*	3·05–14·76	4·1	0·8–9·9	1·20*	0·34–3·96
6	112*	47–139	14·0	7·5–33·3	5·97	2·14–12·70	6·6	2·1–13·9	1·13*	0·46–5·75
7	123*	70–131	16·3	5·0–45·1	4·06	1·52–16·10	8·4	3·2–15·9	1·37*	0·28–4·65
8	123*	77–145	18·7	4·1–45·1	5·69*	1·03–16·08	6·7	2·5–13·9	1·17*	0·23–4·11
9	112*	66–134	15·4	3·2–32·1	5·23	1·66–10·59	6·6	2·9–13·7	1·31*	0·14–2·59
10	100*	25–140	22·0	4·6–176·8	6·51*	2·05–17·85	4·4	0·6–10·8	1·27*	0·39–3·59
11	61	0–109	53·2*	5·9–176·1	12·43*	4·67–29·86	1·0	0·0–4·7	1·17*	0·39–4·31
12	61	2–118	39·0	23·9–144·7	7·15*	3·18–21·17	1·2	0·0–5·5	1·11*	0·20–3·27
13	64	7–124	35·0	5·0–89·7	6·75*	1·75–14·47	1·8	0·5–6·1	1·01*	0·20–2·93
14	62	0–124	28·9	1·5–118·6	6·37*	1·56–13·51	1·9	0·1–4·9	0·94*	0·07–3·46
15	65	16–125	45·7*	14·0–223·6	6·87*	3·40–15·80	1·8	0·0–5·6	1·08*	0·42–7·00

* Higher than fraction (2) and (3); $p<0·05$; Wilcoxon's signed-rank test.

basal 3·56 to peak 6·47 nmol/l ($p<0·01$).

The output of histamine as assayed in the gastric aspirate closely paralleled that of acid. The total summation of histamine in the aspirate during the first hour of pentagastrin infusion, in both Group A and Group B, was related to the total acid secreted. A similar correlation was observed during the second hour of pentagastrin infusion in Group A patients (Fig. 1).

2 EFFECT OF CIMETIDINE ON PENTAGASTRIN INDUCED ACID AND HISTAMINE

Cimetidine, 200 mg intravenously, was given to Group B patients after one hour of pentagastrin infusion. Gastric acid secretion fell immediately (Table 2). Acid concentration fell from 112 mmol/l immediately before cimetidine to 61 mmol/l ($p<0·001$); output dropped from 6·6 to 1·0 mmol in the 10 minute fraction. Gastric histamine concentration, however, rose from 15·4 to 53·2 nmol/l ($p<0·01$), which is also higher than the basal value of 29·8 nmol/l ($p<0·01$) as the gastric secretion volume declined. In contrast with the concentration data, histamine output in all 10 minute fractions were not significantly different from that before cimetidine, though they were higher than the basal median 0·79 nmol ($p<0·01$). Plasma histamine rose from 5·23 to 12·43 nmol/l ($p<0·01$) and remained high throughout the subsequent period of pentagastrin infusion.

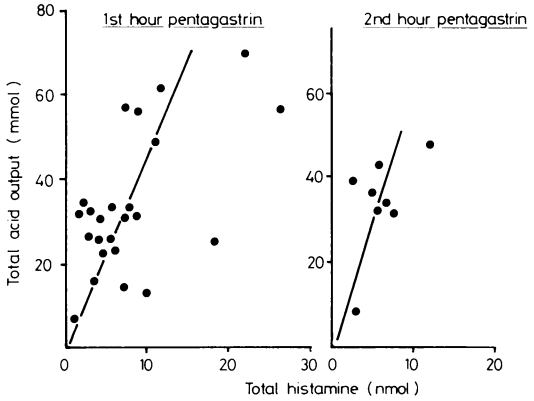

Fig. 1 *Relationship between total acid and histamine output during pentagastrin infusion*
Left: Group A and Group B: n=24; r=0·58; p<0·01
Right: Group A: n=8; r=0·61; p<0·05

Figure 2 illustrates the summation of histamine and acid output in gastric juice collected during the basal period, the first hour of pentagastrin and the second hour of pentagastrin infusion in Group A and Group B patients. The latter group also received cimetidine at the end of the first hour pentagastrin. In Group A, gastric histamine rose from basal median 3·75 nmol/h to 5·85 nmol/h ($p<0·01$) in the first hour pentagastrin and to 5·89 nmol/h ($p<0·01$) in the second hour of pentagastrin. Gastric acid rose from 3·6 to 25·9 ($p<0·001$) and then to 32·3 mmol/h ($p<0·001$). In Group B patients, histamine output rose from basal median 4·39 to 7·31 nmol/h ($p<0·001$) in the first hour of pentagastrin. With cimetidine blockade, histamine output in the second hour pentagastrin remained unchanged, media 6·87 nmol/h, which is higher than

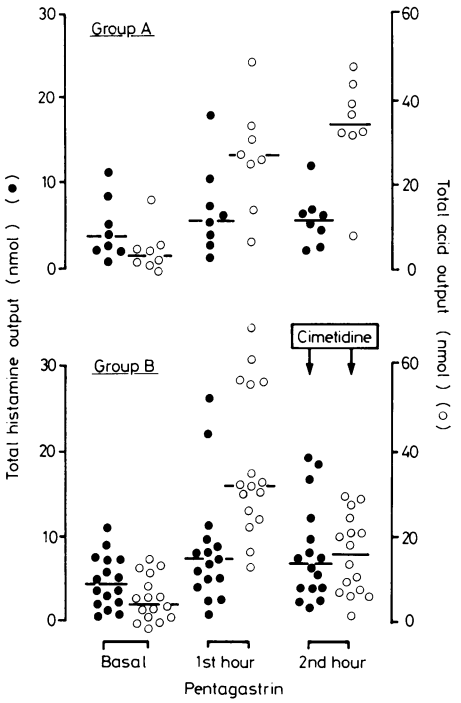

Fig. 2 *Total histamine and acid output during basal, first hour and second hour pentagastrin infusion*
Upper: Group A: n=8
Lower: Group B: n=16, cimetidine, 200 mg intravenous bolus, before second hour of pentagastrin infusion

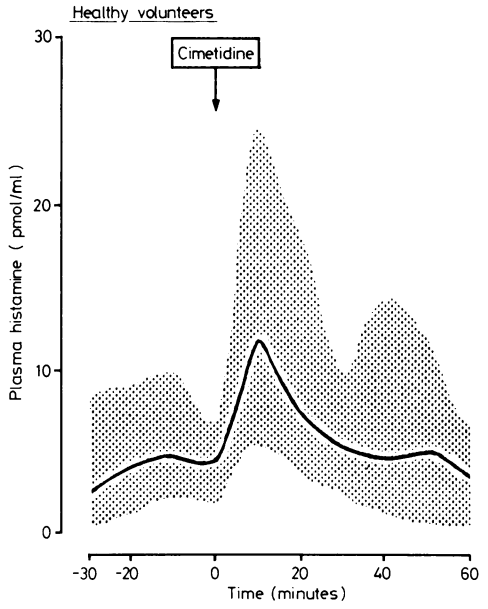

Fig. 3 *Changes in plasma histamine in eight healthy volunteers after cimetidine, 200 mg intravenous bolus*
Line: median; shaded area: range
**Significantly higher than basal (p<0·05)*

basal (p<0·001) but not significantly different from that of the first hour pentagastrin in either Group A or Group B, nor from that of the second hour pentagastrin in group A. Gastric acid rose from basal median 5 to 32·1 mmol/h in the first hour of pentagastrin infusion but fell to 16·2 mmol/h in the second hour of pentagastrin after cimetidine blockade. Thus, cimetidine reduced acid but not histamine in the gastric secretion.

3 EFFECT OF CIMETIDINE ON PLASMA HISTAMINE IN HEALTHY VOLUNTEERS AND PATIENTS WITH TOTAL GASTRECTOMY

Figure 3 shows the effect of cimetidine 200 mg intravenously, on plasma histamine concentration in healthy subjects. There was a peak between 10 and 20 minutes after cimetidine followed by a rapid fall to basal levels. Similar changes were observed in patients after total gastrectomy (Fig. 4). Thus, cimetidine induces histamine release into the circulatory systems from sources other than the stomach.

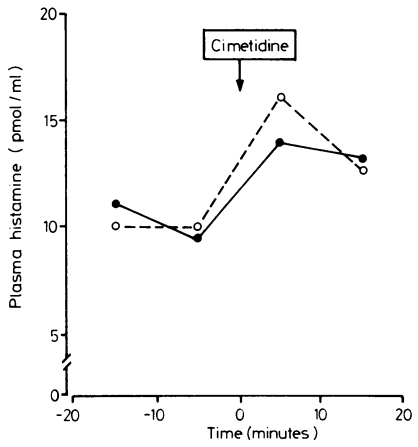

Fig. 4 *Changes in plasma histamine in two patients after total gastrectomy after cimetidine 200 mg intravenous bolus*

Discussion

Histamine is the most potent substance stimulating gastric acid secretion. It is widely distributed throughout the body and is present in abundance in the fundus of the stomach. During gastric acid secretion histamine appears in gastric juice. It is apparent that histamine is highly likely to have a role in the physiological control of gastric acid secretion. Many questions and uncertainties persist, however, as to the precise mechanism, if any, by which histamine has its action.

Many other secretagogues, including gastrin and its synthetic analogue, pentagastrin, can act on the gastric mucosa. One explanation of the phenomenon might be that histamine acts as a final common pathway for gastric acid release. In mammals H_2 receptor antagonists inhibit the secretory action of histamine, pentagastrin, 2-deoxyglucose and insulin. The secretory response of kitten fundic mucosa to acetylcholine, however, is not blocked by metiamide,[6] and the cholinergic antagonist atropine inhibits pentagastrin induced secretion in dogs.[2]

These observations are difficult to fit into a theory which proposes a single final common transmitter. An alternative to pharmacological studies is to examine the effect of secretagogues on isolated cells.[3] The responses, however, measured in such experiments are small and may not correspond to the physiological condition. These lines of evidence do not therefore allow a conclusion on the roles of gastrin and histamine in acid secretion.

It is now possible to assay histamine in biological fluids and tissues with great sensitivity and precision. Such measurements of changes in histamine during secretion may offer a better method of determining the role of histamine.

The fluorometric method developed by Lorenz[7] is suited to the assay of gastric histamine, being fast and economical. Specimens with very low concentration, such as plasma, are more conveniently assayed by the method of Schaff and Beaven.[8] The latter technique is more expensive and time consuming than fluorometric assay and the methods are therefore complementary. The precision and accuracy for both methods of histamine assay have been previously described.[4]

We have previously reported that the pattern of histamine and acid release during pentagastrin infusion is so close as to suggest a functional relationship.[4] Pentagastrin induced an increase in histamine output in gastric juice. In the present study histamine rose from basal median 3·75 to 5·85 nmol/h, and from basal 4·39 to 7·31 nmol/h in the first hour of pentagastrin for the two groups of patients respectively. The stimulated values are of similar magnitude to those reported by Parkin *et al*,[9] but 10-fold less than those reported by Peden *et al.*[10] The discrepancy may be owing to different methodologies, as Parkin's group and ours are using similar assay procedures.

After cimetidine blockade, gastric acid fell rapidly but histamine output in gastric juice did not change. The evidence of the effect of cimetidine on gastric acid secretion presented here suggests that histamine release after pentagastrin infusion is independent of gastric acid secretion, and not a passive 'washout' phenomenon. Similar findings have been reported by Peden *et al.*[10]

Cimetidine affects the enzymatic isotopic assay of histamine. It interferes with the assay as it increases the blank value in the standard curve and inhibits the enzymatic reaction at high concentrations (Fig. 5). At concentrations below 2 μg/incubation, however, the interference from cimetidine is not significant. The concentration of cimetidine in the blood of healthy human subjects after intravenous bolus injection falls rapidly at first and then at a slower rate.[5] After an intravenous bolus injection of 100 mg cimetidine, the concentrations of cimetidine in the blood at 5 minutes and 10 minutes were 4·7 and 3 μg/ml respectively.[5] As only 0·1 ml plasma was used in the assay, the estimated concentration of cimetidine in the plasma samples in the present series of experiments should be below 2 μg/incubation, and would not be expected to interfere with the histamine assay.

The release of histamine into plasma parallels gastric secretion. The pattern of histamine release

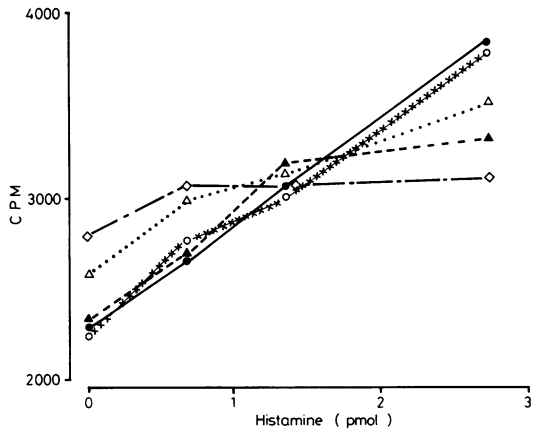

Fig. 5 *Interference from cimetidine with the standard curves obtained by single isotope assay of histamine (○) 0, (●) 0·4, (▲) 2, (△) 10, (◇) 50 μg/incubation*

into the plasma is complicated by the effect of cimetidine on other tissue histamine stores. Cimetidine when given alone induces a transient rise in histamine level in blood plasma. Other investigations reported an immediate rise and then fall to basal value in 10 minutes after cimetidine.[12] In the present study, the plasma histamine level returned to basal in 20 minutes after cimetidine (Fig. 3). Together with pentagastrin, however, a significant rise in plasma histamine level is sustained (Table 2). It is clear from our data that plasma histamine remains raised after cimetidine injection and its associated peak of histamine release, indicating sustained secretion during pentagastrin infusion despite cimetidine blockade.

These data are compatible with the hypothesis that pentagastrin acts indirectly, by releasing histamine in the gastric mucosa. The results confirm that cimetidine has no action by inhibiting histamine release. The observation is interesting in the light of the finding that mucosal histamine concentration increases during cimetidine treatment of duodenal ulceration.[13] Further experiments are in progress to study the effects of short and long term cimetidine blockade on gastric mucosal histamine and the activity of histamine methyltransferase.

These results show such a potent release of gastric histamine during pentagastrin infusion as to be compatible with a physiological pathway. Although our results cannot exclude the possibility that increase in histamine release is a non-specific effect consequent to onset of secretion,[10] the association of histamine and gastric acid release found in our previous[4] and present studies was so close that a functional relationship seems likely. Our results are compatible with the theory that pentagastrin acts indirectly on the parietal cell by causing histamine release. A further pathway, in which gastrin acts on the parietal cell by a receptor blocked by H_2-antagonists, cannot be excluded by our present data. If such a route exists its physiological significance as compared with the histamine releasing route may be small, in view of the large volume of histamine secretion induced at very low pentagastrin concentrations.

We conclude that histamine release during pentagastrin infusion is a major stimulus to gastric acid secretion.

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References

1 Code CF. Histamine and gastric secretion; a later look, 1955–1965. *Fed Proc* 1965; **24:** 1311–21.
2 Grossman MI, Konturek SJ. Inhibition of acid secretion in dog by metiamide, a histamine antagonist acting on H_2 receptors. *Gastroenterology* 1974; **66:** 517–21.
3 Soll A. The interaction of histamine with gastrin and carbamylcholine on oxygen uptake in isolated mammalian parietal cells. *J Clin Invest* 1978; **61:** 381–9.
4 Man WK, Saunders JH, Ingoldby C, Spencer J. Effect of pentagastrin on histamine output from the stomach in patients with duodenal ulcer. *Gut* 1981; **22:** 916–22.
5 Baron JH. In: *Clinical tests of gastric secretion.* London: Macmillan, 1978.
6 Tepperman BL, Schofield B, Tepperman FS. Effect of metiamide on acid secretion from isolated kitten fundic mucosa. *Can J Physiol Pharmacol* 1975; **53:** 1141–6.
7 Lorenz W, Benesch H, Barth E *et al.* Fluorometric assay of histamine in tissues and body fluids. *S Anal Chem* 1970; **252:** 94–100.
8 Shaff RE, Beavan MA. Increased sensitivity of the enzymatic isotopic assay of histamine: measurement of histamine in plasma and serum. *Anal Biochem* 1979; **94:** 425–30.
9 Parkin JV, Lorenz W, Barth H *et al.* Assay and identification of histamine in human gastric aspirate by a fluorometric-fluorenzymatic technique. Its application in patients with chronic duodenal ulcer. *Agents Actions* 1982; **12:** 17.
10 Peden N, Boyd EJS, Callachan H *et al.* The effect of Impromidine and pentagastrin on gastric output of histamine, acid and pepsin in man. *Hepato-gastroenterol* 1982; **29:** 30–34.
11 Griffiths R, Lee RM, Taylor DC. Kinetics of cimetidine in man and experimental animals. In: *Cimetidine—proceedings of the second international symposium on histamine H_2-receptor.* Amsterdam-Oxford: Excerpta Medica, 1977.
12 Lorenz W, Doenicke A, Schoning B *et al.* Histamine release. H_1+H_2. Receptor antagonists for premedication in anaesthesia and surgery. A critical view based on randomised clinical trials with Haemaccel and various antiallergic drugs. *Agents Actions* 1980; **10:** 114–24.
13 Man WK, Saunders JH, Ingoldby C, Spencer J. Effect of cimetidine on the amounts of histamine in the gastric mucosa of patients with gastric or duodenal ulcers. *Gut* 1981; **22:** 923–6.