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HISTAMINE METABOLISM OF THE GASTRIC MUCOSA FOLLOWING ANTRECTOMY

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SUMMARY

1. Histamine metabolism of the gastric mucosa in rats subjected to antrectomy, antrectomy and substitution with pentagastrin and exclusion of the antrum has been investigated employing various *in vivo* and *in vitro* methods.

2. Mucosal histamine formation after antrectomy fell to about one third, whereas after antrum exclusion histamine formation increased many-fold.

3. After antrectomy, mucosal histamine content decreased, and increased after antrum exclusion.

4. After total gastrectomy, (*a*) whole-body histamine formation was reduced to about half, as judged by determining histamine excretion, and (*b*) pentagastrin infusion did not increase histamine excretion, showing absence of histamine mobilization from extra-gastric sources. In rats with the stomach retained, infusion of pentagastrin induced a dose-dependent increase in histamine excretion.

5. Kinetic studies in which [^{14}C]histidine was injected and the resulting urinary [^{14}C]histamine determined showed that on pentagastrin infusion after antrectomy newly formed histamine was initially mobilized to a larger extent than in the controls.

6. Antrectomized rats were subjected to substitution treatment by three injections per day of pentagastrin. After 3 weeks of substitution, histamine excretion was considerably higher than without substitution. After 6 weeks of substitution, histamine excretion was about the same in the substituted antrectomized, non-substituted antrectomized and sham-operated groups. Neither time nor substitution could, however, normalize the excretion of histamine on pentagastrin stimulation after antrectomy.

7. In non-substituted antrectomized rats, pentagastrin was less effective in elevating mucosal histamine formation than in the substituted and sham-operated groups.

8. The indispensability of the rat in this kind of study is emphasized.

INTRODUCTION

In the rat, consistent association has been disclosed between gastric mucosal histamine mobilization and gastric secretion (for references see Kahlson, Rosengren & Svensson, 1973). In the presence of the antrum, feeding, vagal excitation and gastrin injection are followed by a release of pre-formed histamine from the mucosa and a subsequent acceleration of the rate of histamine formation. After antrectomy, the interdigestive histamine formation is lowered. Yet the rate of formation can be increased upon vagal excitation or gastrin injection, although not to the same degree as with the antrum retained (Rosengren & Svensson, 1969).

The present investigation examined the time course of changes in mucosal histamine metabolism ensuing on antrectomy, and also whether the changes after antrectomy could be obviated by prolonged substitution with a gastrin analogue. A part of this work has been presented at a meeting of the Physiological Society (Lundell, 1974).

METHODS

Animals. Female rats of the Sprague-Dawley strain, weighing 250–275 g, were used. They were fed a standard pellet diet and had tap water, except for the rats subjected to total gastrectomy which post-operatively were fed a liquid semi-synthetic diet.

Drugs. Pentagastrin (Peptavlon, ICI 50.123, obtained by the courtesy of ICI-Pharma (Sweden); aminoguanidine hemisulphate (Sigma Chemical Co.); hydroxocobalamin (Hepagon novum, Astra, Sweden); Imferon (Pharmacia, Sweden) were used.

Stomach preparations. The operations were done under ether anaesthesia. Food was withheld for 16 hr before the operations.

Antrectomy was carried out by resecting the stomach 2–3 mm proximal to the clearly recognizable antrum–corpus border. The entire lesser curvature and 3–4 mm of the proximal duodenum were included in the resection. Gastrointestinal continuity was re-established by a gastro-duodenostomy end to end. Small tissue specimens from the proximal ridge of the resected antrums were fixed, sectioned and stained with Haematoxyline Congo red; histological examination revealed the presence of oxyntic gland mucosa in each section of twenty-four antrectomized rats investigated for the completeness of antrectomy.

Antrum exclusion was performed in two stages. In the first operation an isoperistaltic gastro-jejunostomy was carried out just proximal to the antrum–corpus border of the greater curvature. An entero-entero anastomosis between the efferent and afferent loop of the jejunum was done side to side, 8–10 cm distal to the gastro-jejunostomy. Ten days later the antral–corpus border was divided and the severed ends of the oxyntic gland area and the antrum were closed.

Total gastrectomy was carried out by dividing the stomach at the oesophago-gastric and gastroduodenal junctions and closing the proximal end of the duodenum.

Intestinal continuity was provided for by an oesophago-jejunal anastomosis end to side, and an entero-entero anastomosis was done as described. The post-operative

treatment has been described before (Rosengren & Svensson, 1969; Svensson, 1970*a*).

The rats subjected to total gastrectomy were injected with the vitamin B_{12} analogue hydroxocobolamin 100 μg per rat s.c. once a week and had Fe^{3+} substitution with Imferon 10 mg/rat I.M. every other week.

Sham-operation involved laparotomy with mobilization of the stomach and the distal part of the oesophagus.

Substitution with pentagastrin

Eleven antrectomized rats were substituted with pentagastrin 16 μg s.c. three times daily. The first injection was given between 07.00–07.30 hr, the second between 14.00 and 14.30 hr and the third between 21.00 and 21.30 hr, commencing immediately post-operatively. Another eleven antrectomized and twelve sham-operated rats had injections of saline only. In these experiments pentagastrin was not injected on the day of investigating histamine metabolism.

Determination of histamine formation in vivo and in vitro

The rate of whole body endogenous histamine formation and changes therein can be followed by determining the urinary excretion of free histamine in female rats fed on a histamine-free diet (Gustafsson, Kahlson & Rosengren, 1957). Whole-body histamine formation can also be determined by a procedure introduced by Schayer which involves injection of [^{14}C]histidine and determination of the subsequent excretion of [^{14}C]histamine in the urine (for references, see Schayer, 1966). Before the experiments the rats were fasted for 18 hr. During an actual experiment the rats were kept unanaesthetized in restraining cages of the Bollman type. A beaker containing a few drops of conc. HCl was placed under the rat for collection of urine in 1 or 2 hr samples. The diuresis was high and constant during the collection periods, being obtained by infusing 0·9 % NaCl solution (3·5 ml./hr) through a polyethylene tube inserted in a tail vein and connected to a motor-driven syringe. The infusion started 2–3 hr before the actual experiment. In assessing the amount of histamine mobilized, the rats were given a diamine oxidase inhibitor, aminoguanidine (10 mg/kg daily), and fed a semi-synthetic histamine-free diet for five days before the experiment. The amount of histamine excreted was determined on the isolated atropinized guinea-pig ileum. Urinary [^{14}C]histamine excreted after I.V. injection of 200 μg [^{14}C]histidine (sp. act. 9·8 mc/m-mole) was determined by the same procedure as employed in determining [^{14}C]histamine in tissue samples. Before use, the commercially obtained [^{14}C]histidine was purified to eliminate traces of [^{14}C]histamine (Kahlson, Rosengren & Thunberg, 1963) which otherwise would interfere with the determinations.

The *in vitro* determination of histamine formation was carried out by a method devised by Schayer, Davis & Smiley (1955) as adapted for use in this laboratory (Kahlson *et al.* 1963). Fasted rats receiving pentagastrin or saline infusions for varying periods of time were killed by a cervical fracture and exsanguinated. The parietal cell region was removed by scraping with a scalpel after the stomach had been opened, washed with saline and pinned flat. Mucosal tissue minced with scissors was incubated for 3 hr at 37° C under nitrogen in beakers containing 100 mg of tissue, 40 μg 2-ring-^{14}C-labelled L-histidine, 10^{-4} M aminoguanidine, 10^{-1} M sodium phosphate buffer pH 7·4 and 0·2 % glucose all made up to a final volume of 3 ml. At the end of the incubation, carrier histamine and perchloric acid were added. After filtration, histidine was separated from histamine on a column of an ion exchange resin (Dowex 50W-X4, 100–200 mesh). After conversion of the histamine to pipsyl histamine, the radioactivity was determined at infinite thickness in a flow counter.

The pipsyl samples were repeatedly recrystallized from acetone until they displayed constant radioactivity. With the [^{14}C]histidine and the measuring equipment used, 1 μg [^{14}C]histamine formed corresponded to 5000 cpm. A different method for determining histamine formation *in vitro* has been devised by Kobayashi (1963). This method involves trapping of $^{14}CO_2$ on incubating tissue samples with ^{14}C-carboxyl-labelled L-histidine. The original procedure has been adapted for use in this laboratory and has been found reliable with certain tissues, e.g. the gastric mucosa (Henningsson, Lundell & Rosengren, 1974). The $^{14}CO_2$-method as employed includes the following steps. Minced tissue was incubated in vessels containing 100 mg tissue, 40 μg ^{14}C-carboxyl-L-histidine (sp. act. 104·9 μc/m-mole), 10^{-3} M-EDTA, 5×10^{-4} M glutathione, 10^{-5} M pyridoxal-5-phosphate, 10^{-1} M sodium phosphate buffer pH 7·4 and 0·2% glucose, the total volume made up to 3 ml. In the blanks, 10^{-2} M semicarbazide was added to inhibit enzyme activities. The samples were incubated for 3 hr under nitrogen and the incubation was stopped by gentle tipping of 1 ml. 2 M citric acid from a side arm into the incubation mixture. Adequate $^{14}CO_2$ trapping into the Hyamine 10-X (0·1 m-mole) on the filter paper, placed within the incubation vessel, was achieved by continuing mechanical shaking for another 45 min. The filter paper was placed in a counting vessel containing 10 ml. Bray scintillation solution (1960) and counted in a liquid scintillation spectrometer (Packard Tri-Carb, 2002).

Determination of tissue content of histamine

A portion of each minced mucosa was used for enzyme assay and the remainder for histamine content determination which was made by a modification of the method described by Feldberg & Talesnik (1953). The tissue samples were homogenized in 2 ml. 1 M-HCl. The pestle was washed with 1 ml. 1 M-HCl and 2 ml. Tyrode solution. The homogenate was then boiled for 30 min after which it was neutralized, transferred to a flask and made up to a given volume with Tyrode solution, filtered and assayed for histamine on the atropinized guinea-pig ileum. The specificity of the assay was regularly ascertained by using mepyramine maleate.

RESULTS

Urinary histamine following total gastrectomy

Urinary excretion of histamine was studied in the fasting state and after I.V. administration of pentagastrin (8 μg/hr) in four rats subjected to a total gastrectomy. Three weeks after the operation urinary excretion of histamine in the fasting state was $9{\cdot}1 \pm 1{\cdot}10$ (S.D.) μg/2 hr. On infusing pentagastrin there was no significant increase above the fasting level now attaining $10{\cdot}3 \pm 1{\cdot}03$ μg/2 hr. Six weeks after the operation urinary histamine in the fasting state was $7{\cdot}5 \pm 1{\cdot}80$ μg/2 hr and on pentagastrin infusion $7{\cdot}6 \pm 2{\cdot}14$ μg/2 hr as against about 14 μg/2 hr in the sham-operated controls. These results are shown in Fig. 1*a* and *b* and Table 1.

Urinary histamine following infusions of different doses of pentagastrin

Mobilization of gastric mucosal histamine by various doses of pentagastrin, as reflected in the excretion of the amine, was investigated in six intact rats. Urinary histamine was collected in a 2 hr sample during saline

TABLE 1. Urinary excretion of histamine in μg, during saline or pentagastrin infusion (8 μg/hr) in rats subjected to antrectomy and substitution with pentagastrin (AES), antrectomy alone (AE) or sham-operated (SO). Experiments were performed (*a*) 3 weeks and (*b*) 6 weeks after the operations. Urine was collected in 2 hr samples, except after 4 hr when a 1 hr sample was interspersed. The number of rats in each group is indicated by n. The mean and S.D. are also given

		Saline		Pentagastrin		
Period of collection (hr)		2	2	1	2	2
(*a*) Urinary histamine (μg)	n = 6 AES	12·7 ± 4·93	14·0 ± 5·27	10·7 ± 2·49	21·2 ± 7·48	21·2 ± 4·86
	n = 6 AE	9·9 ± 3·10	7·0 ± 1·79	8·7 ± 3·73	12·6 ± 6·48	13·8 ± 4·45
	n = 6 SO	14·1 ± 2·13	12·7 ± 2·87	—	—	—
(*b*) Urinary histamine (μg)	n = 11 AES	14·9 ± 2·60	13·3 ± 3·97	13·0 ± 5·00	20·2 ± 6·33	20·6 ± 4·57
	n = 11 AE	13·5 ± 4·24	12·6 ± 4·32	9·5 ± 2·37	16·6 ± 5·43	20·0 ± 4·96
	n = 6 SO	14·6 ± 3·40	14·7 ± 2·49	15·8 ± 3·46	34·1 ± 6·20	30·9 ± 3·03

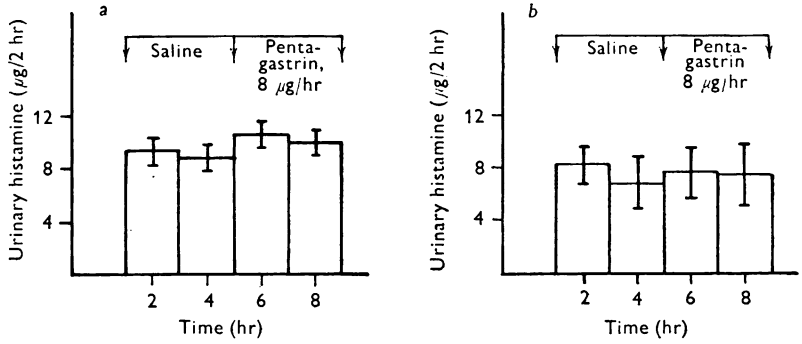

Fig. 1. Urinary excretion of histamine (μg/2 hr) during saline and pentagastrin (8 μg/hr) infusion in four rats subjected to total gastrectomy. Experiments were done (*a*) 3 and (*b*) 6 weeks after the operation. The columns represent the mean of one determination in each rat. The S.D. is also given.

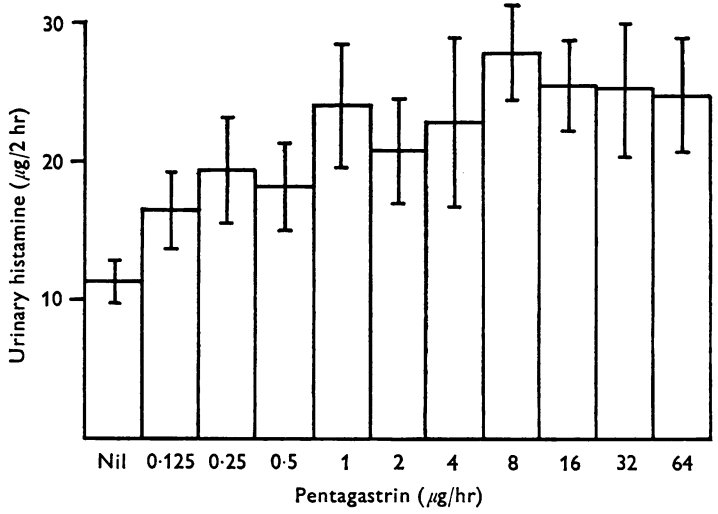

Fig. 2. Urinary excretion of histamine (μg/2 hr) in six intact rats in response to different doses of pentagastrin. Nil stands for the 2 hr period of saline infusion preceding pentagastrin infusion. The columns represent the mean of one determination in each rat. The S.D. is also given.

infusion, after which pentagastrin in the dose-range 0·125–64 μg/hr was added to the infusate for another 2 hr, followed by infusing saline for 4 hr; the dose of pentagastrin was then doubled and infused for 2 hr. This type of experiment was done twice weekly until the actual dose-range of pentagastrin had been tested in each rat. The average excretion of histamine before the administration of the first dose of pentagastrin was $11{\cdot}2 \pm 4{\cdot}14$

μg/2 hr and $11{\cdot}9 \pm 3{\cdot}12$ μg/2 hr during the 2 hr collection period preceding infusion of the second dose of pentagastrin. Infusion of pentagastrin in a dose of 0·125 μg/hr was followed by a significant rise in urinary histamine above the interdigestive level to $16{\cdot}5 \pm 2{\cdot}86$ μg/2 hr ($P < 0{\cdot}01$). On increasing the dose of pentagastrin, histamine excretion rose to a maximum of $27{\cdot}9 \pm 3{\cdot}42$ μg/2 hr, obtained on infusing pentagastrin in a dose of 8 μg/hr. Larger doses of pentagastrin were not followed by any further elevation of the urinary histamine (Fig. 2).

Urinary histamine following antrectomy and substitution with pentagastrin

Three and 6 weeks after antrectomy the excretion of urinary histamine was studied in the fasting state and after pentagastrin (8 μg/hr). Three weeks after the operation urinary histamine in the fasting state in six antrectomized substituted rats was $13{\cdot}4 \pm 4{\cdot}92$ μg/2 hr, which is very similar to that in six sham-operated rats, $13{\cdot}5 \pm 2{\cdot}48$ μg/2 hr. By contrast, in six non-substituted antrectomized rats urinary excretion of histamine was reduced by about 40 % to $8{\cdot}6 \pm 2{\cdot}88$ μg/2 hr. In the substituted group on infusing pentagastrin, urinary histamine rose to $21{\cdot}2 \pm 6{\cdot}02$ μg/2 hr and to only $13{\cdot}2 \pm 5{\cdot}33$ μg/2 hr in the non-substituted antrectomized rats ($P < 0{\cdot}005$), as seen in Table 1.

However, 6 weeks after antrectomy, the situation differed from that after 3 weeks. Histamine excretion in the fasting state was $14{\cdot}1 \pm 3{\cdot}38$ μg/2 hr in the substituted and $13{\cdot}1 \pm 4{\cdot}19$ μg/2 hr in the non-substituted antrectomized rats and $14{\cdot}6 \pm 2{\cdot}84$ μg/2 hr in the sham-operated controls. Nevertheless, on infusing pentagastrin, a difference between the three groups was revealed. In the substituted rats during the first hour of pentagastrin infusion $13{\cdot}0 \pm 5{\cdot}00$ μg of histamine/hr was excreted in comparison with $9{\cdot}5 \pm 2{\cdot}37$ μg/hr in the non-substituted antrectomized rats and $15{\cdot}8 \pm 3{\cdot}46$ μg/hr in the sham-operated ones. Further, in the subsequent two 2 hr periods the histamine excretion in the substituted antrectomized rats was $20{\cdot}4 \pm 5{\cdot}39$ μg/2 hr which is significantly less than that obtained in the sham-operated rats, $32{\cdot}5 \pm 4{\cdot}93$ μg/2 hr ($P < 0{\cdot}01$) (Table 1). Table 1 shows that in the non-substituted antrectomized rats the increase in urinary histamine in response to pentagastrin was delayed but during the last collection period reached the same level as in the substituted rats.

Determination of histamine formation and content

Seven weeks after antrectomy the rate of histamine formation of the gastric mucosa in the fasting state was $2{\cdot}3 \pm 1{\cdot}15$ μg/g.3 hr in the non-substituted and in the substituted antrectomized rats $3{\cdot}5 \pm 1{\cdot}24$ μg/g.3 hr, this difference being not significant, whereas in these two groups histamine formation was significantly less than the control group's $6{\cdot}6 \pm 1{\cdot}44$ μg/g.3 hr

($P < 0{\cdot}01$). On infusing pentagastrin (8 μg/hr) for 2 hr, the rate of histamine formation rose substantially in each group to $21{\cdot}3 \pm 6{\cdot}66$ μg/g.3 hr in the substituted antrectomized rats and $25{\cdot}7 \pm 4{\cdot}66$ μg/g.3 hr in the sham-operated group. In the non-substituted antrectomized rats the corresponding value was about 50% less than in the other two groups studied (Table 2). Determining the rate of histamine formation with the $^{14}CO_2$-method after 2 hr of pentagastrin infusion gave these results: $20{\cdot}9 \pm 4{\cdot}81$ μg/g.3 hr in the non-substituted antrectomized rats and $30{\cdot}0 \pm 4{\cdot}59$ μg/g.3 hr in sham-operated controls ($P < 0{\cdot}01$). The means and S.D. are calculated from determinations in six rats in each group. The time course of the rise in the rate of mucosal histamine formation on infusing pentagastrin (8 μg/hr) was essentially the same in the non-substituted antrectomized and sham-operated rats (Fig. 3). The increase in histamine formation tended to attain a plateau after 4 hr of pentagastrin infusion especially in the antrectomized rats. The rate of histamine formation was almost regularly 50% less in the antrectomized rats than in the controls except after 1 hr of pentagastrin infusion when the enzymic response was still larger in the latter group (Fig. 3).

The content of histamine in the gastric mucosa was $121{\cdot}5 \pm 28{\cdot}07$ μg/g in the sham-operated rats and only $31{\cdot}5 \pm 10{\cdot}94$ μg/g after antrectomy. On infusing pentagastrin (8 μg/hr) the histamine content fell to a lowest value of $6{\cdot}3 \pm 2{\cdot}81$ μg/g after 1 hr of infusion in the antrectomized rats and to $19{\cdot}6 \pm 4{\cdot}05$ μg/g after 2 hr in the control group. On prolonged pentagastrin infusion there was a partial restoration of the mucosal histamine content. After 6 hr of infusion the figures obtained in the antrectomized and sham-operated rats were $17{\cdot}9 \pm 4{\cdot}74$ μg/g and $37{\cdot}4 \pm 9{\cdot}27$ μg/g respectively (Fig. 4).

Kinetics and magnitude of histamine mobilization

The increase in histamine excretion on infusing pentagastrin may be accounted for by a release of pre-formed histamine and an acceleration of mucosal histamine formation. In order to assess the rate at which histamine leaves the mucosa on stimulation, and also the amount of histamine produced, the total amount of histamine and [^{14}C]histamine formed from injected [^{14}C]histidine were determined in the urine of six antrectomized and six sham-operated rats. Six weeks after the operation the experimental procedure was the same as described above except for injection of 200 μg [^{14}C]histidine at the commencement of pentagastrin infusion (8 μg/hr). The pattern of excretion of total urinary histamine was the same as previously described. The amount of [^{14}C]histamine excreted during the first hour of pentagastrin infusion was similar in the two groups. However, during the subsequent 2 hr periods the urinary [^{14}C]histamine was signi-

ficantly less in the antrectomized rats than in the controls ($P < 0{\cdot}01$). The ratio [^{14}C]histamine/total amount of urinary histamine, i.e. the specific activity of [^{14}C]histamine in the urine, reflects the contribution of newly formed histamine to total amount of histamine mobilized. The specific activity during the first hour of pentagastrin infusion was higher in the

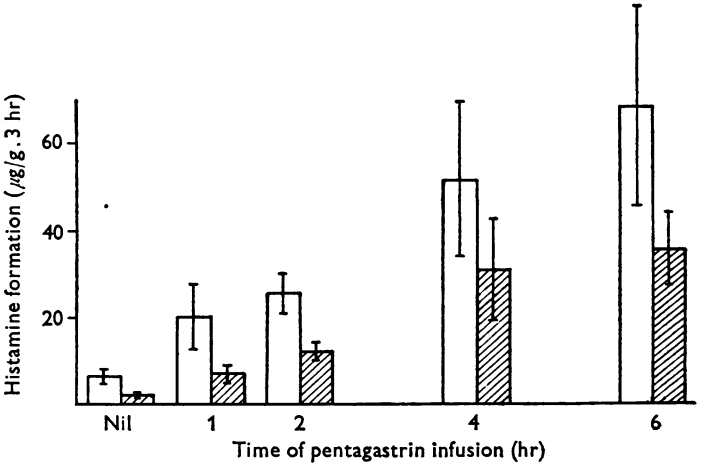

Fig. 3. Histamine formation (μg/g.3 hr) of the gastric mucosa in non-substituted antrectomized (hatched columns) and sham-operated rats (open columns) at different times after the start of pentagastrin infusion (8 μg/hr). Nil stands for saline infusion. Each column represents the mean from determinations in six rats. The S.D. is also given.

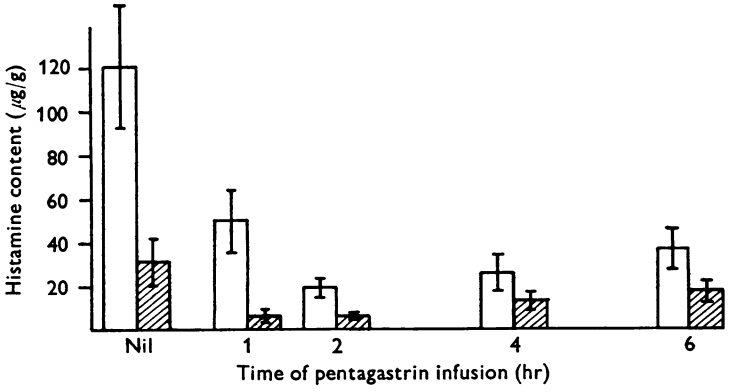

Fig. 4. Histamine content (μg/g) of the gastric mucosa in non-substituted antrectomized (hatched columns) and sham-operated rats (open columns) at different times after the start of pentagastrin infusion (8 μg/hr). Nil stands for saline infusion. Each column represents the mean from determinations in six rats. The S.D. is also given.

TABLE 2. Histamine formation (μg/g.3 hr) of the gastric mucosa after saline infusion and 2 hr of pentagastrin (8 μg/hr) infusion in rats subjected to antrectomy or sham operation. AES stands for antrectomy and substitution with pentagastrin, AE for antrectomy alone and SO for sham operation. The number of rats in each group from which the mean and S.D. are calculated is indicated by n

Saline			Pentagastrin		
AE	AES	SO	AE	AES	SO
$2{\cdot}3 \pm 1{\cdot}15$	$3{\cdot}5 \pm 1{\cdot}24$	$6{\cdot}6 \pm 1{\cdot}44$	$12{\cdot}2 \pm 2{\cdot}18$	$21{\cdot}3 \pm 6{\cdot}66$	$25{\cdot}7 \pm 4{\cdot}66$
$n = 5$	$n = 5$	$n = 6$	$n = 6$	$n = 6$	$n = 6$

TABLE 3. Urinary excretion of total histamine in μg and [^{14}C]histamine in ng after injection of 200 μg [^{14}C]histidine in six antrectomized (AE) and six sham-operated rats (SO). [^{14}C]histidine was injected at the start of pentagastrin infusion (8 μg/hr). Urine was collected in 2 hr samples, except after 4 hr when a 1 hr sample was interspersed. The means and S.D. are given and the mean ratios of [^{14}C]histamine (ng) to total histamine (μg) are included

		Saline		Pentagastrin		
	Period of collection (hr)	2	2	1	2	2
AE	Total histamine (μg)	$12{\cdot}4 \pm 3{\cdot}86$	$10{\cdot}2 \pm 2{\cdot}19$	$10{\cdot}1 \pm 2{\cdot}70$	$15{\cdot}2 \pm 4{\cdot}42$	$18{\cdot}1 \pm 4{\cdot}99$
	[^{14}C]histamine (ng)	—	—	52 $\pm 11{\cdot}3$	36 $\pm 7{\cdot}6$	20 $\pm 5{\cdot}9$
	[^{14}C]histamine/total histamine	—	—	5·2	2·4	1·1
SO	Total histamine (μg)	$14{\cdot}7 \pm 2{\cdot}55$	$13{\cdot}8 \pm 4{\cdot}09$	$19{\cdot}0 \pm 4{\cdot}43$	$33{\cdot}4 \pm 6{\cdot}94$	$32{\cdot}2 \pm 5{\cdot}09$
	[^{14}C]histamine (ng)	—	—	59 $\pm 18{\cdot}8$	61 $\pm 3{\cdot}4$	34 $\pm 3{\cdot}7$
	[^{14}C]histamine/total histamine	—	—	3·1	1·9	1·1

antrectomized group than in the controls. This difference also existed during the first 2 hr collection period, but the difference decreased in the course of prolonged pentagastrin infusion and no difference was noted during the last collection period (Table 3).

Gastric mucosal histamine metabolism following antrum exclusion

Six weeks after the exclusion of the antrum, histamine excretion in the urine was studied in six rats. The urinary excretion of histamine in the fasting state was exceedingly high ($114{\cdot}2 \pm 49{\cdot}02$ μg/2 hr) and this high excretion could be further enhanced (to $139{\cdot}2 \pm 45{\cdot}49$ μg/2 hr) by infusing pentagastrin (8 μg/hr), an increase that compared well with that recorded in sham-operated controls on pentagastrin infusion. The substantial increase in the fasting state of histamine excretion in the antrum-excluded rats as compared with the sham-operated rats is probably accounted for by an increase in the rate of histamine formation of the gastric mucosa, in the former group attaining $109{\cdot}9 \pm 19{\cdot}68$ μg/g. 3 hr. As to histamine content of the gastric mucosa in antrum-excluded rats, this value was increased to $267{\cdot}9 \pm 30{\cdot}21$ μg/g.

DISCUSSION

The importance of the antrum for the responsiveness of the parietal cells to vagal stimulation is well established (Straaten, 1933; Uvnäs, 1942; Olbe, 1964; Dinbar, Trout & Grossman, 1971; Kragelund, Amdrup, Andreassen & Jensen, 1972). Furthermore, antrectomy is followed by a suppression of the maximal secretory response to gastrin in the rat (Svensson, 1970*b*) and to pentagastrin in man (Kragelund *et al.* 1972). Related to these observations there may be other consequences of antrectomy, namely a lowered formation of mucosal histamine and a lessened gastrin-induced acceleration of histamine formation (Rosengren & Svensson, 1969; Svensson, 1970*b*).

The present study deals with changes in histamine metabolism of the gastric mucosa after extended antrectomy and also includes observations on antrum exclusion, which is known to induce a hypergastrinaemia (Accary, Dubrasquet & Bonfils, 1973). In addition, it was attempted to obviate the changes following antrectomy by substituting with pentagastrin.

This laboratory has emphasized the desirability of correlating observations on histamine metabolism made on excised tissues with corresponding changes *in vivo*. Gustafsson *et al.* (1957) and subsequent work in this laboratory have shown that in the female rat urinary excretion of histamine reflects total body histamine formation and changes therein. The usefulness of this approach has now been further substantiated. Infusion

of graded doses of pentagastrin, known to result in graded acid responses, evoked excretions of graded amounts of histamine. This histamine must have been mobilized from the stomach, since no increase in urinary histamine occurred with pentagastrin after gastrectomy. A dose-dependent effect of injected gastrin on the fall of histamine content and the rise of histidine decarboxylase activity in the gastric mucosa has previously been demonstrated (Kahlson, Rosengren, Svahn & Thunberg, 1964). The great fall in urinary histamine after gastrectomy indicates that a large fraction of urinary histamine originates in the stomach, confirming earlier observations (High, Shepherd & Woodcock, 1965; Shepherd & Woodcock, 1968).

In non-substituted antrectomized rats, 3 weeks after the operation a significant reduction in histamine excretion occurred in the fasting state as compared with the controls. Substitution with pentagastrin seemed to counteract these changes to a certain degree. Surprisingly, 6 weeks after the operation, no difference in urinary excretion of histamine was noted between non-substituted and substituted antrectomized rats, either in the fasting state or after a couple of hours of pentagastrin infusion. This would imply that a recovery had taken place in the non-substituted rats. Neither substitution nor the passing of time could, however, raise the excretion of histamine to the level obtained during pentastrin stimulation in the sham-operated controls. Determinations of the rate of mucosal histamine formation *in vitro* in the fasting state and after 2 hr of pentagastrin infusion revealed other striking differences between the three groups. Histamine formation was about 70 % less in the antrectomized rats than in the controls. On stimulation, the elevation of histamine formation was about 50 % less in the non-substituted antrectomized rats than in the controls and the substituted antrectomized rats. The increase in the interdigestive urinary histamine with the passing of time in non-substituted antrectomized rats obviously does not depend on an increased rate of gastric mucosal histamine formation, but perhaps on changes in formation in extra-gastric tissues. It should, however, be pointed out that a similar recovery did not take place in gastrectomized rats.

The present results, like those of Rosengren & Svensson (1969) and Svensson (1970*b*), emphasize the predominant role of antral gastrin in sustaining mucosal histamine formation and the responsiveness of histidine decarboxylase to stimulation by pentagastrin or gastrin. These results are at variance with reports by Johnson, Jones, Aures & Håkanson (1969) and Aures, Johnson & Way (1970), who failed to demonstrate any mucosal histidine decarboxylase activity after antrectomy, while others have reported an unaltered enzymic activity (Håkanson & Liedberg, 1970, 1972). After antrectomy the responsiveness of the enzyme to injected

pentagastrin has been reported as unaltered (Johnson *et al.* 1969; Aures *et al.* 1970) or even increased (Håkanson & Liedberg, 1972). In reports other than those derived from this laboratory, dealing with the effect of antrectomy on histidine decarboxylase activity, a modification of the method of Kobayashi (1963) has been used. In the present study an adaptation of this method gave results similar to those obtained with the method of Schayer (1966).

Antral gastrin exerts a trophic effect on the mucosa (Crean, Marshall & Rumsey, 1969; Johnson, Aures & Håkanson, 1969; Johnson, Aures & Yuen, 1969). Removal of the antrum is consequently followed by hypoplasia of the oxyntic gland mucosa (Martin, Macleod & Sircus, 1970; Capoferro & Nygaard, 1973) and a reduced DNA and RNA content (Johnson & Chandler, 1973). The importance of endogenous gastrin for the preservation of normal protein synthesis by the oxyntic gland mucosa, and the fact that the responsiveness of histidine decarboxylase to gastrin requires an intact protein synthesis system (Snyder & Epps, 1968), appear to be more reconcilable with a reduced responsiveness of the enzyme to pentagastrin after antrectomy than the unaltered or increased one reported by others.

Determinations of total urinary histamine during pentagastrin infusion will not give information on the contribution of newly formed histamine to the total amount mobilized from the gastric mucosa. Observations on the excretion of [^{14}C]histamine will, however, give information on this topic. In antrectomized rats nearly all pre-formed histamine was released within the first hour of infusion. Although the rate of histamine formation during the first hour of pentagastrin infusion in antrectomized rats was only about one third of that noted in the controls (Fig. 3), both groups excreted about the same amount of [^{14}C]histamine in that time; it would thus appear that newly formed histamine is retained in the gastric mucosa to a lesser extent after antrectomy. This situation would require mobilization of all available amounts of histamine even at the expense of maintaining normal mucosal content of the amine.

In antrum-excluded rats a strikingly high content and rate of histamine formation was noted. This again emphasizes the paramount importance of gastrin in the regulation of gastric mucosal histamine metabolism. These changes in histamine content and those appearing after antrectomy may be related to the above-mentioned reports of a growth-promoting effect of gastrin.

In the present study a combination of *in vitro* and *in vivo* methods has been employed in investigating the alterations in gastric mucosal histamine metabolism ensuing under conditions of various amounts of endogenous gastrin. The combination of these methods appears advantageous

in explaining the metabolism of histamine within the gastric mucosa, the understanding of which is essential in explaining the role of the amine in exciting the parietal cells.

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