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THE ROLE OF THE ANTRUM AND THE VAGUS NERVE IN THE FORMATION OF GASTRIC MUCOSAL HISTAMINE

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SUMMARY

1. The effects of the vagus nerve and of antral gastrin on the rate of histamine formation (histamine forming capacity, HFC, i.e. histidine decarboxylase activity) in the parietal cell region of the gastric mucosa has been investigated in the following stomach preparations: gastric fistula, denervated Heidenhain pouch, antral resection with gastrojejunostomy, gastrojejunostomy with exclusion of the duodenum and in the intact stomach. The determinations of mucosal HFC were made on fasting rats and on re-fed animals when the effect of feeding was studied.

2. In fasting rats with a gastrojejunostomy and the antrum intact the mucosal HFC of the innervated stomach was about 4 times higher than in the corresponding preparation with the antrum resected. In the innervated main stomach the mucosal HFC was about twice as high as in the denervated pouch, indicating that the vagus and endogenous antral gastrin each contribute to maintaining mucosal HFC in the fasting state.

3. Acidifying the stomach caused a substantial lowering of the mucosal HFC presumably by inhibiting antral gastrin release, whereas acid in the stomach did not interfere with the elevation of mucosal HFC evoked by injection of gastrin.

4. Injection of gastrin elevated mucosal HFC in the innervated main stomach and in the denervated pouch to approximately equal levels. With the dose of gastrin employed there was about a fourfold increase in the HFC of the pouch mucosa.

5. In antrectomized rats enhanced vagal influence, evoked by insulin injection or by feeding, raised the mucosal HFC. In rats with the antrum intact and the stomach acidified, insulin injection produced an increased HFC. Thus, a vagal effect on mucosal HFC exists independent of participation of antral gastrin.

6. The stable choline esters carbachol and methacholine act directly on the parietal cell without involving mucosal HFC. The vagus nerve and

gastrin, however, are assumed to provide secretory stimulation by means of accelerated histamine formation.

7. The interrelation between increased histamine formation and hydrochloric acid secretion is discussed.

INTRODUCTION

Among natural body constituents histamine has been extensively studied for its stimulatory action on the parietal cells. In all animal species studied this amine is abundantly present in the region of the mucosa containing parietal cells for which histamine is alleged to serve as a physiological excitant.

In the gastric mucosa, as in other tissues, histamine is formed by the agency of histidine decarboxylase. The essential characteristics of this enzyme, obtained from the gastric mucosa of rats, have been described by Schayer (1957) and by Kahlson, Rosengren & Thunberg (1963). In reports from this laboratory histidine decarboxylase activity in tissues is referred to as histamine forming capacity (HFC). Employing a highly sensitive and specific method HFC has been demonstrated in the gastric mucosa of all species studied: man, dog, cat, rat, guinea-pig, hamster, mouse and frog. A conspicuous feature is the coincidence of very high mucosal HFC and very low sensitivity of the acid-secreting cells to injected histamine, and vice versa (Kahlson, Rosengren, Svahn & Thunberg, 1964).

Pertinent to the present study is the finding that injection of gastrin evokes a steep and sustained elevation of HFC in the parietal cell region of the mucosa preceded by a fall in mucosal histamine content, events which were taken as evidence of a feed-back relation between histamine content and histidine decarboxylase activity (Kahlson *et al.* 1964; Kahlson, Rosengren & Thunberg, 1967). A similar, high elevation of mucosal HFC was also observed by these workers after feeding, vagus excitation and distension of the stomach. The participation of gastrin release in these various kinds of stimulation was, however, not investigated at that time. The present study was largely undertaken to determine if vagus excitation by itself, independent of concomitant gastrin release, is capable of accelerating mucosal histamine formation.

METHODS

Animals. Female rats of Sprague–Dawley strain weighing about 200 g were used, except in the experiments with methacholine, in which male rats bred at the Institute of Physiology, Lund, were also used. The rats were fed a standard pellet diet (type 142, Teknosan, Malmö, Sweden) and given water *ad libitum*. Food was with-

held for 18 hr before the actual experiment. In one set of experiments in which fasting rats were fed the rats were given food for the 3 hr during which they were trained to eat.

Drugs. Insulin (Vitrum), 2·5 i.u./kg body weight, hog gastrin II, about 5 μg/kg, methacholine chloride, 200 μg/kg, and carbacholine chloride, 20 μg/kg, were given subcutaneously. Controls received 0·9 % (w/v) NaCl solution.

Tissue sample. The rat was killed by cervical fracture and exsanguinated by opening the carotid arteries. Gastric mucosa was obtained from normal rats, from rats provided with a gastric fistula, with a Heidenhain pouch, or from rats subjected to antrectomy and gastrojejunostomy, or gastrojejunostomy only. The parietal cell region was removed by scraping with a scalpel after the stomach had been washed with 0·9 % NaCl solution and pinned flat.

Determination of HFC in vitro. The rate of histamine formation, that is histidine decarboxylase activity, was determined, by a procedure originally devised by Schayer, adapted for use in our laboratory, and fully described by Kahlson *et al.* (1963). Briefly, the method involves the following steps. The minced tissues are incubated for 3 hr at 37° C under nitrogen, in beakers containing 100–200 mg tissue, 40 μg 2-ring-[^{14}C]L-histidine (base), 10^{-4} M aminoguanidine sulphate, 10^{-1} M sodium phosphate buffer, pH 7·4 and 0·2 % (w/v) glucose, all made up to a final volume of 3·2 ml. At the end of the incubation, carrier histamine and perchloric acid are added. After filtration radioactive histidine is separated from radioactive histamine on an ion exchange resin (Dowex 50 W-X4, 100–200 mesh) and after conversion of the histamine to pipsyl histamine the radioactivity of formed histamine is determined at infinite thickness in a flow counter. With the [^{14}C]histidine and measuring equipment used 1 μg [^{14}C]histamine formed corresponded to about 5000 counts/min. Activity is expressed in μg [^{14}C]histamine formed per g tissue in 3 hr.

Stomach preparations

The operations were done under ether anaesthesia. Food was withheld 15 hr before the operation, except for the gastric fistula rat which had normal access to food before and after the operation. In rats subjected to the more elaborate kinds of operation, feeding was reinstituted on the second day after the operation in the form of a nutritionally adequate paste, the composition of which is fully described in a report by Kahlson, Rosengren & Westling (1958), followed by pellets given freely 5 to 7 days after operation. To aid post-operative recovery 6 ml. 5·5 % (w/v) glucose was injected on completion of the operation, and the following day glucose (5 ml.) and 0·9 % NaCl solution (5 ml.) were injected. Afterwards the rats drank water freely. No experiments were performed until 2 to 3 weeks after the operations.

Gastric fistula. This fistula was prepared by a technique, the principle of which has been described by Lane, Ivy & Ivy (1957). In the present experiments this preparation was employed in order to acidify the antrum with 0·1 M hydrochloric acid rinsed through the fistula at a rate of 3·5 ml./hr. In this type of experiment the unanaesthetized rat was kept in a Bollman restraining cage.

Heidenhain pouch. The pouch was prepared as devised by Alphin & Lin (1959), and its mucosal HFC was determined about 2 weeks after the operation.

Antrum resection. The operation of antrectomy was carried out in two stages. At the first stage an anastomosis was established between the upper segment of the jejunum and the glandular part of the stomach involving a 5 mm long section of the greater curvature. Care was taken to omit from the anastomosis any part of the antrum. At the second stage 10 days later, resection was carried out along the line A to B as illustrated in Fig. 1. The remaining stomach was closed by ligatures along this line. The resection of the antrum extended to the duodenal bulb which was

closed by sutures. The pertinent surgical procedures are described in the monograph by Lambert (1965). In the groups serving as controls a gastrojejunostomy was first established, and at a second stage of the operation the stomach was sectioned at the pyloric region and the openings of the stomach and duodenal bulb closed by sutures.

Acid secretion. Since this investigation is concerned specifically with gastric mucosal histamine formation, acid secretion was not collected.

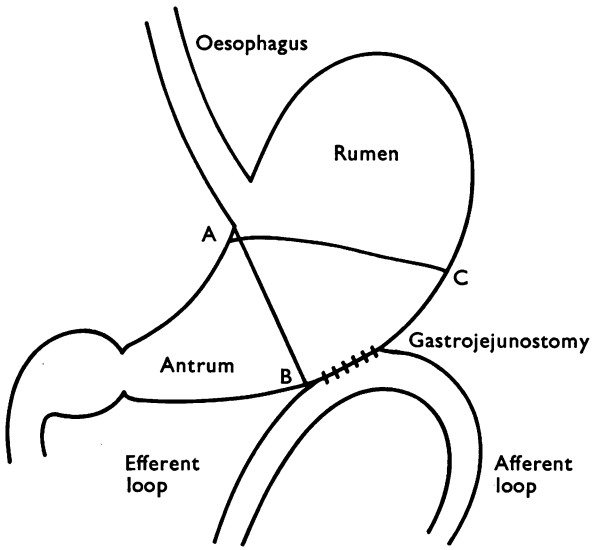

Fig. 1. Stomach of the rat showing the resection line A to B. Line A to C indicates the ridge between rumen and glandular stomach.

RESULTS

Effect of stable choline esters. The influence of these parasympathomimetic compounds was studied since it is known that vagus excitation evokes a substantial acceleration of histamine formation in the gastric mucosa (Kahlson *et al.* 1967). Methacholine and carbachol were injected in twenty-four rats, and groups of six were killed at 1 hr and at 3 hr after the injection. The results are given in Table 1. Neither compound altered mucosal HFC significantly, although both had been shown to evoke profuse hydrochloric acid secretion in the doses employed (S. E. Svensson, unpublished). This result would imply that these compounds activate the secretory device without a concomitant acceleration of histamine formation which ensues on vagal stimulation. Apparently, these compounds act directly on the secreting cells, like histamine, the injection of which does not accelerate histamine formation. In order to confirm the observations on Sprague–Dawley rats methacholine was injected in ten male rats of the Lund Physiology Institute strain, another ten rats serving as controls.

The rats were killed 2 hr after the injection. Here also injection of methacholine did not alter the mucosal HFC (Table 2).

Observations on the Heidenhain pouch. In twelve fasting rats, divided in two groups, the mucosal HFC of the pouch was compared to that of the main stomach (Table 3). The mucosa of the main stomach is under the

TABLE 1. HFC (μg/g) of gastric mucosa in fasting Sprague–Dawley rats after injection of choline esters. The means ± S.D. are also given. The figures obtained after injection of carbachol and methacholine do not differ significantly from those of the controls

	Carbachol		Methacholine	
Control	1 hr	3 hr	1 hr	3 hr
2·8	1·8	3·6	4·3	2·5
2·2	3·3	2·7	4·2	3·6
4·8	3·7	9·8	2·5	3·5
2·0	3·2	1·4	5·9	2·4
3·4	2·6	3·1	2·1	6·5
4·8	2·4	2·5	5·8	3·9
3·3 ± 1·2	2·8 ± 0·7	3·9 ± 3·1	4·1 ± 1·6	3·7 ± 1·5

TABLE 2. HFC (μg/g) of gastric mucosa in fasting male rats of the Institute strain 2 hr after injection of methacholine. The means ± S.D. are also given

Control	Methacholine
4·5	2·5
2·9	5·4
2·0	4·2
3·9	1·1
2·2	4·2
1·5	2·3
2·1	2·0
3·7	2·1
1·5	2·5
1·6	1·9
2·6 ± 1·1	2·8 ± 1·3

influence of the vagi as well as of endogenous gastrin, whereas in the Heidenhain pouch the mucosa is under the influence of endogenous gastrin alone. This difference in stimulatory influence is reflected in differences in HFC of the mucosa of the two stomach preparations. From the mean figures obtained, 5·5 μg/g for the pouch as against 10·5 μg/g for the main stomach, it would appear that in the interdigestive state endogenous gastrin and the vagus nerve both contribute in maintaining mucosal histidine decarboxylase activity. It should be noted that the Heidenhain pouch, even in the interdigestive state, has been shown to secrete hydrochloric acid, although at a low rate (Kahlson *et al.* 1964; Lilja & Svensson,

1967). These workers have further shown that high rates of secretion can be initiated by injecting suitable amounts of gastrin. In the present study we found that after injection of gastrin mucosal HFC increased in both stomach preparations, to 22·1 μg/g in the pouch, and to 26·8 μg/g in the main stomach (Table 3).

Effect of acidification of the antrum. Vagal stimulation, evoked by insulin hypoglycaemia or injection of 2-deoxyglucose, is known to elevate HFC of the gastric mucosa (Kahlson *et al.* 1967). This phenomenon could be due to a direct vagal effect on the histamine-forming cells or to release of

TABLE 3. HFC (μg/g) in the mucosa of the pouch and the main stomach of twelve fasting Heidenhain pouch rats, of which six were injected with saline and six with gastrin. The means ± S.D. are also given. The significance of the differences between the means of different groups is indicated at the foot of each column

Control		Gastrin	
1	2	3	4
Pouch	Main stomach	Pouch	Main stomach
7·8	17·0	17·0	24·0
9·1	12·0	36·0	20·0
3·7	4·9	8·8	34·0
5·8	15·0	49·0	55·0
2·2	7·8	12·0	11·0
4·5	6·5	10·0	27·0
5·5 ± 2·6	10·5 ± 4·9	22·1 ± 16·5	26·8 ± 15·8
	1 − 2: $P < 0·02$	1 − 3: $P < 0·02$	2 − 4: $P < 0·02$
			3 − 4: N.S.
			$P > 0·05$

gastrin by the vagus, or to the combined effects of both. In an attempt to resolve this problem the following experiments were performed. Thirty-six rats provided with a gastric fistula were divided in six groups. Group I and II were injected subcutaneously with 0·9 % NaCl, group III and IV were injected with insulin, 2·5 i.u./kg, and group V and VI with gastrin II, 5 μg/kg. In the groups I, III and V, the stomach was perfused with 0·9 % NaCl and in groups II, IV and VI with 0·1 M hydrochloric acid. The perfusion commenced 1 hr before the injections and was discontinued 2 hr after injection of gastrin in groups V and VI and 3 hr after injections in the remaining groups. The timing was so set that the animals were sacrificed at the hour when the mucosal HFC, as known from earlier reports, was expected to be at peak level. The results are summarized in Table 4. In the first place it will be noted that gastrin, as well as insulin-induced hypoglycaemia, evoked elevations of mucosal HFC as described in the previously quoted reports. Next, it is apparent from Table 4 that acid in

TABLE 4. HFC (μg/g) of gastric mucosa in fistula rats injected with saline, insulin or gastrin. In groups I, III and V the stomach was perfused with 0·9 % NaCl, and in groups II, IV and VI with 0·1 M hydrochloric acid. The means ± S.D. are also given. The significance of the differences in the means of different groups is indicated at the foot of each column

I NaCl + NaCl	II NaCl + HCl	III Insulin + NaCl	IV Insulin + HCl	V Gastrin + NaCl	VI Gastrin + HCl
5·7	3·5	16·0	14·0	11·0	9·7
3·4	2·7	24·0	9·4	18·0	13·0
8·0	4·3	20·0	11·0	23·0	16·0
14·0	2·1	27·0	13·0	14·0	24·0
13·0	4·5	28·0	15·0	14·0	26·0
9·2	3·3	24·0	19·0	17·0	27·0
8·9 ± 4·1	3·4 ± 0·9 I–II: $P < 0·01$	23·2 ± 4·5 I–III: $P < 0·01$	13·6 ± 3·4 II–IV: $P < 0·01$ III–IV: $P < 0·01$	16·2 ± 4·2 I–V: $P < 0·02$	19·3 ± 7·3 V–VI: N.S. $P > 0·05$

the stomach exerts a restraining influence on insulin induced elevation of mucosal HFC. This is presumably due to inhibition of gastrin release by the vagus when the stomach is acidified and is strongly supported by the fact that acid in the stomach did not reduce the elevation of HFC evoked by injection of gastrin. It will subsequently be shown that both endogenous gastrin and vagal influence contribute in maintaining histidine decarboxylase activity even in the fasting state. Acid in the stomach, assumed to inhibit release of endogenous gastrin, would therefore be expected to lower the mucosal HFC. This assumption has been tested by the experiments in group II. With acid in the stomach the HFC fell from 8·9 to 3·4 μg/g within 4 hr, indicating that the influence of endogenous gastrin in maintaining interdigestive enzyme activity is of considerable magnitude.

Observations on antrectomized rats. In rats in which the antrum had been resected two problems were investigated: first, the result of eliminating endogenous gastrin and secondly, to determine the effect of the vagus nerve *per se*. This series comprised twenty-four rats divided in four groups. Seven additional gastrojejunostomized rats with the antrum intact served as controls. These controls displayed a substantially higher mucosal HFC than that found in rats not subjected to any kind of surgery or handling. In this group the increase in HFC was about the same as in the rats provided with a gastric fistula (Table 4, group I) and for the main stomach mucosa in Heidenhain pouch rats (Table 3, col. 2). The reason for the raised HFC in these three groups has not yet been investigated since this information is not essential for the conclusions presented in this study. The rather wide range of mucosal HFC values found in the various groups of control will be commented on in the Discussion.

Removal of the antrum was followed by a substantial fall in histidine decarboxylase activity from 8·0 μg/g in the controls (Table 5) to 2·3 μg/g. This result again brings into prominence the major role of endogenous gastrin in sustaining mucosal histidine decarboxylase activity.

In the antrectomized rat the effect of vagus excitation can be investigated without interference by vagal gastrin release. An injection of insulin, 2·5 i.u./kg, evoked an increase of mucosal HFC to 4·4 μg/g (Group 2, Table 5). Substantial as this elevation appears, the rise in enzyme activity ensuing on this kind of vagal excitation was only about half the value seen in the controls with gastrojejunostomy and the antrum intact. The possibility that larger doses of insulin would increase the enzymic activity still further was not investigated, since it is known that higher doses of insulin than those mentioned above inhibit hydrochloric acid secretion (Kim, Ridley & Tuegel, 1968).

It is possible that vagal discharge by insulin injection does not reproduce the vagal drive which occurs during feeding, and that insulin may

influence mucosal HFC by mechanisms other than vagal excitation. In order to test the effect of vagal stimulation on feeding the fasting antrectomized rat was given food. Feeding for 3 hr elevated mucosal HFC from 2·3 μg/g to 5·7 (Table 5). It should be noted that the stomach becomes distended on feeding and that this by itself may possibly elevate the HFC by a mechanism involving enhanced vagal stimulation of enzyme activity. The magnitude of the effect evoked by distension would be difficult to assess experimentally, unless an arrangement for sham-feeding could be

TABLE 5. HFC (μg/g) of gastric mucosa in antrectomized fasted rats after injection of saline, insulin, gastrin and feeding, and in fasted rats with a gastrojejunostomy only. The means ± S.D. are also given. The significance of the differences in the means of different groups is indicated at the foot of each column

1 NaCl	2 Insulin	3 Feeding	4 Gastrin	5 Gastroje-junostomy only
2·0	2·3	6·0	6·7	9·4
0·8	7·1	12·0	5·4	7·7
1·9	3·3	3·3	5·3	10·0
3·0	5·4	3·9	3·9	9·0
2·5	3·3	4·1	6·2	9·0
3·8	5·1	5·0	8·4	5·6
				5·6
2·3 ± 1·0	4·4 ± 1·8	5·7 ± 3·2	5·9 ± 1·5	8·0 ± 1·8
	1 – 2: $P < 0·05$	1 – 3: $P < 0·05$	1 – 4: $P < 0·01$	1 – 5: $P < 0·01$

established. Even with the antrum removed it was to be expected that injection of gastrin would elevate mucosal HFC, and in six antrectomized rats gastrin, 5 μg/kg, was injected and the animals killed 2 hr afterwards. As a result mucosal HFC rose from 2·3 to a mean of 5·9 μg/g.

DISCUSSION

Histamine formed in the region of the gastric mucosa containing the parietal cells might serve various purposes, the most obvious being the stimulation of hydrochloric acid secretion and its accompanying local vasodilatation. In previous works on this topic the experimental design was such as not to permit assessment of the individual contributions of the vagus and the antrum in sustaining or effecting alterations in gastric mucosal histidine decarboxylase activity (Kahlson *et al.* 1964, 1967). In experiments in which the vagi were stimulated the concomitant release of endogenous gastrin was not excluded since the antrum was left intact. On feeding the individual contributions of the vagus, gastrin and distension of the stomach therefore could not be determined. Only the gross changes

of mucosal HFC caused by the combined influence of these three stimuli could be measured.

The present experiments were so designed as to enable assessment of the separate influence of the antrum and the vagus on gastric mucosal histidine decarboxylase activity in non-fed rats. This objective was achieved by applying the various surgical techniques which over the years have been developed by workers interested in studies on the influence of the antrum and the vagus nerves on the pattern of gastric secretion. In the present study the rats referred to as controls and the actual experimental rats were subjected to the same surgical procedures. In this sense all control groups were different, or were subjected to different kinds of handling. This should be emphasized when reflecting over the considerable range in HFC values of the various groups of control, differences in values which, however, do not detract from the arguments as presented.

The HFC values in the various controls (Table 3, col. 2, Table 4, group I and Table 5, group 5) were found to be higher than in controls not subjected to surgery (Tables 1 and 2). It was not investigated whether this elevation was the result of surgery. It should, however, be realized that in the controls subjected to surgery the antral region is likely to be less acid than in the intact controls. Establishing a Heidenhain pouch, obviously, reduces the parietal-cell mass remaining in the main stomach. In the gastric fistula rats perfusing the stomach with 0·9 % NaCl will dilute gastric juice. The situation of gastrojejunostomy enables alkaline duodenal contents to regurgitate into the stomach and to neutralize gastric juice. Consequently, the possibility cannot be excluded that gastrin release in the various fasted controls may differ in rate.

Removal of the antrum caused a fall in the mucosal HFC of the parietal cell region of about 70 %. The denervated Heidenhain pouch of a rat with the antrum intact, and thus likely to be under the influence of endogenous gastrin, displayed an enzymic activity of only half of the value found in the innervated main stomach. These observations indicate that endogenous gastrin and vagal influence both contribute in sustaining mucosal histidine decarboxylase activity in the main stomach of the fasting rat.

The Heidenhain pouch responds readily to gastrin since injection of 5 μg/kg elevated the HFC to four times the value found in the fasting control group. In the main stomach, which is under vagal influence, and in which consequently the basal HFC is higher than in the pouch, injection of the same dose of gastrin evoked a proportionally smaller elevation of HFC, although the final level was slightly higher than in the pouch. The observation on the denervated Heidenhain pouch shows that the vagal influence is not essential for gastrin injection to produce a substantial elevation of mucosal HFC.

On the other hand vagus excitation is effective in the absence of antral gastrin. This has been shown in two types of experiment: in antrectomized rats, and in the acidified stomach where the release of endogenous gastrin is inhibited. In both kinds of experiment insulin injection evoked a substantial elevation of mucosal HFC. Acidifying the stomach does not render the mucosa less sensitive to gastrin since injection of gastrin elevated the mucosal HFC to about the same degree as in the controls which were perfused with saline.

Insulin hypoglycaemia used to evoke vagal excitation may induce a profound disturbance of homeostasis which could influence the mucosal HFC by several non-specific mechanisms and thus alter the magnitude and duration of the vagal effect. The same would hold for re-feeding fasting rats, although to a far lesser extent. Here vagus excitation is presumably strong initially and is likely to decline during the course of 3 hr of feeding. Notwithstanding, feeding appears a more physiological mode of vagus excitation and the results obtained by this means should be considered more reliable than those obtained with insulin. The possibility should be considered that food, or its digestion products, may excite release of an intestinal hormonal agent. In the feeding experiment, however, the duodenum was excluded and probably denervated, a circumstance under which release of intestinal gastrin appears unlikely.

Injection of stable choline esters is believed to mimic vagal influence on a variety of vagally innervated structures. The vagomimetic compounds employed are known to stimulate hydrochloric acid secretion and it thus appeared pertinent to investigate the effect of methacholine and carbachol on mucosal HFC. Neither drug, in doses which evoked profound acid secretion, raised the mucosal HFC. These compounds therefore activate the secretory device directly without dependence on any demonstrable intermediary factor. The mode of action of the vagus, as regards mucosal HFC, is different from that of the choline esters. This has been shown in antrectomized rats in which vagal excitation, evoked by injection of insulin or by feeding, brings about accelerated mucosal histamine formation. The same phenomenon occurred in rats on injection of insulin, in which gastrin release had been inhibited by acidifying the antrum. It is noteworthy that the two stable cholinesters, which are capable of reproducing so many responses evoked by vagus stimulation, do not stimulate gastrin release, as evidenced by their failure to elevate mucosal HFC, a step which is obligatory for gastrin.

The acid secretory mechanism and the mode of its stimulation, as envisaged by workers in this laboratory, is depicted in Fig. 2. Current evidence indicates that this mechanism comprises two distinct components. The new and somewhat unexpected feature is that the histamine store and

its formation apparently are located not in the parietal cell itself but in a separate structure located closeby. Employing a technique evolved by Ehinger & Thunberg (1967), in which histamine is visualized by a histochemical method involving fluorescence microscopy, Thunberg (1967) demonstrated the presence of histamine and HFC in a cell located at the base of the gastric glands. This arrangement appears functionally acceptable, and even efficient, since histamine formed at high rates in close proximity to the parietal cells would stimulate these cells, especially as the mucosa lacks mechanisms for effective inactivation of the histamine formed.

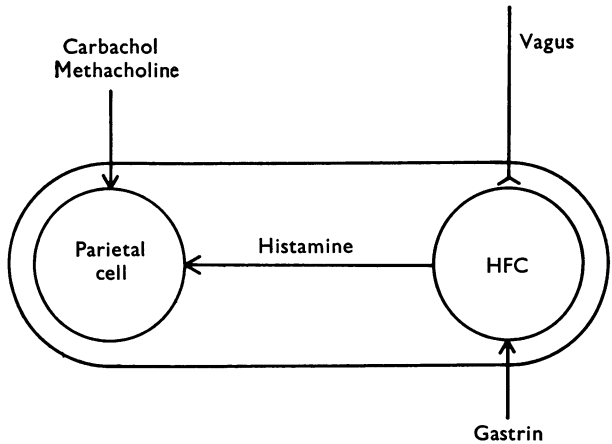

Fig. 2. Proposed arrangement of the HCl-secretory mechanism comprising the parietal cell, the cell containing and forming histamine (HFC) and the sites where gastrin, the vagus nerve, histamine and the stable choline esters (carbachol and methacholine) presumably act.

The observations, that acetylcholine released from vagal terminals promotes acid secretion through the intermediary of histamine in the cell designated HFC in Fig. 2 and that the two choline esters studied act directly on the parietal cell, may be interpreted as follows. It is assumed that acetylcholine is very rapidly destroyed and reaches the parietal cell in subthreshold amounts. Histamine, a more stable and rapidly diffusing intermediary, would secure transmission to the secretory cell and would act as an amplifying device to enable even strong secretory activity to be mediated by the vagus alone.

Every tissue in all species so far investigated by adequate techniques has been found endowed with the capacity to form histamine by the agency of histidine decarboxylase. Histamine-forming capacity has been found to be related to tissue repair and cell renewal (for references see Kahlson & Rosengren, 1968). Very recently it has been discovered that

histidine decarboxylase activity is closely related to protein synthesis in the foetal rat liver, the sole tissue as yet studied in this respect (Grahn, Hughes, Kahlson & Rosengren, 1969). In the gastric mucosa cell renewal and protein synthesis are known to proceed at continuously high rates, particularly during and after secretory activity. It appears reasonable to assume that the HFC of the non-secreting mucosa is related in large part to such processes. In forthcoming reports from this laboratory the fraction of HFC related to cell renewal and anabolic processes will be referred to as basal mucosal HFC.

The present report is concerned with the sharp elevation of mucosal HFC which ensues on feeding or on applying the individual stimuli which operate on feeding. The heightened histidine decarboxylase activity is assigned a distinct functional significance in that the steep elevation above the basal HFC stands out as a consistent, integral part of the sequence of phenomena which on feeding evoke hydrochloric acid secretion.

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REFERENCES

ALPHIN, R. S. & LIN, T. M. (1959). Preparation of chronic denervated gastric pouches in the rat. *Am. J. Physiol.* **197**, 257–259.

EHINGER, B. & THUNBERG, R. (1967). Induction of fluorescence in histamine-containing cells. *Expl Cell Res.* **47**, 116–122.

GRAHN, B., HUGHES, R., KAHLSON, G. & ROSENGREN, E. (1969). Retardation of protein synthesis in rat foetal liver on inhibiting rate of histamine formation. *J. Physiol.* **200**, 677–685.

KAHLSON, G. & ROSENGREN, E. (1968). New approaches to the physiology of histamine. *Physiol. Rev.* **48**, 155–196.

KAHLSON, G., ROSENGREN, E., SVAHN, D. & THUNBERG, R. (1964). Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol.* **174**, 400–416.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1963). Observations on the inhibition of histamine formation. *J. Physiol.* **169**, 467–486.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1967). Accelerated mobilization and formation of histamine in the gastric mucosa evoked by vagal excitation. *J. Physiol.* **190**, 455–463.

KAHLSON, G., ROSENGREN, E. & WESTLING, H. (1958). Increased formation of histamine in the pregnant rat. *J. Physiol.* **143**, 91–103.

KIM, K. S., RIDLEY, P. T. & TUEGEL, C. (1968). Effect of insulin on gastric acid secretion, histamine formation, and on the incidence of gastric lesions. *Life Sci. Oxford* **7**, 403–409.

LAMBERT, R. (1965). *Surgery of the Digestive System in the Rat.* Springfield: Charles C. Thomas.

LANE, A., IVY, A. C. & IVY, E. K. (1957). Response of the chronic gastric fistula rat to histamine. *Am. J. Physiol.* **190**, 221–228.

LILJA, B. & SVENSSON, S. E. (1967). Gastric secretion during pregnancy and lactation in the rat. *J. Physiol.* **190**, 261–272.

SCHAYER, R. W. (1957). Histidine decarboxylase of rat stomach and other mammalian tissues. *Am. J. Physiol.* **189**, 533–536.

THUNBERG, R. (1967). Localization of cells containing and forming histamine in the gastric mucosa of the rat. *Expl Cell Res.* **47**, 108–115.