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Histidine decarboxylase in the stomach of the rat

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Two enzymes capable of decarboxylating L-histidine *in vitro* have been identified in rat and mouse stomach; one, located in the fundic portion, shows maximal activity at a pH value of 5·6, whilst the other, in the pyloric portion, requires a pH of 7·6 for optimal activity. The enzyme in rat fundus is stable when stored at low temperatures and is inhibited only slightly by benzene, α-methyldopa and α-methylhistidine; the pyloric enzyme, on the other hand, is rapidly destroyed on storage at low temperatures, and is slightly stimulated by benzene but much inhibited by α-methyldopa and α-methylhistidine. Dopa and 5-hydroxytryptophan compete with the substrate for the pyloric histidine decarboxylase but have no effect on the fundic enzyme. Starvation inhibits the activity of the fundic enzyme but has only a slight effect on the pyloric enzyme. It is concluded that the two enzymes in the stomach capable of forming histamine differ from the specific and non-specific histidine decarboxylases found in other tissues.

At least two enzymes capable of forming histamine *in vitro* have been described in recent years; one is specific for L-histidine whereas the other is non-specific and decarboxylates other aromatic amino-acids as well as L-histidine (Lovenberg, Weissbach & Udenfriend, 1962). It is probable that the specific enzyme is the more important for the *in vivo* formation of histamine (Dawson, Maudsley & West, 1965). The properties of the histidine decarboxylase in rat stomach have not been fully established although it has generally been considered to be a specific enzyme for L-histidine (Waton, 1956; Schayer, 1957; Telford & West, 1961a). Recently, Håkanson & Owman (1966) showed that the enzyme in rat stomach is also capable of decarboxylating dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP).

The present work was designed to determine the nature of histidine decarboxylase in rat stomach responsible for the formation of histamine *in vitro*. Code (1965) concluded that histamine is the final common local chemostimulator of the parietal cells of the gastric mucosa, and so an attempt has been made to study the relationship between histidine decarboxylase activity and gastric function.

Experimental

Male Sprague-Dawley rats weighing 150–170 g were used in all experiments. They were fed on 41B cube diet, allowed drinking water *ad libitum*, and housed at 70° ± 1° F (21° C). At different times, groups of at least seven animals were killed by a blow on the head and rapidly decapitated.

Preparation of stomach extracts. The stomachs were removed, opened along the line of the lesser curvature, washed with isotonic saline (0·9% w/v sodium chloride solution), blotted dry on filter paper, weighed, cut into small pieces with scissors, and homogenized with isotonic saline

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(5 ml/g) in a glass homogenizer. After centrifugation at 5,000 × *g* in a refrigerated centrifuge for 15 min, aliquots of the supernatant (equivalent to 50 or 100 mg tissue) were removed for the incubation experiments. On other occasions, the stomachs were divided into two portions; the thin fundic part was separated from the thick pyloric part, an intermediate area about 2 mm wide on either side of the demarcation line being discarded.

Histidine decarboxylase activity of the extracts. This was estimated using the method developed by Kobayashi (1963), in which the tissue extract is incubated with carboxyl-labelled histidine under standard conditions and the $^{14}CO_2$ formed is estimated in a counter. The incubation mixture consisted of [*carboxyl*-^{14}C]DL-histidine (0·1 μc in 0·1 ml distilled water containing 10 μg L-histidine), stomach extract equivalent to 50 or 100 mg tissue, streptomycin (25 mg in 0·1 ml water), and phosphate buffer (M/15) sufficient to produce a final volume of 3 ml. Streptomycin was used to inhibit bacterial decarboxylation of histidine occurring during the incubation (Callingham, Kobayashi, Maudsley & West, 1965). When no tissue was present, the results obtained were similar to those found when using boiled tissue; they represent the blank values. Incubation was allowed to proceed for 2 hr at 37° in a shaking incubator, during which time the evolved $^{14}CO_2$ was absorbed on to filter paper impregnated with hyamine hydroxide. The reaction was then stopped by the addition of 0·3 ml M citric acid, but shaking continued for a further hour to allow the hyamine to absorb completely the $^{14}CO_2$. The radioactivity of the filter paper was determined in a Packard Tricarb liquid scintillation counter (at an efficiency of counting of 70%); a coefficient of variation of reproducibility of about 1% was obtained by allowing time for 10,000 counts to accumulate. Each value in the figures is the mean of at least two separate estimations and has been corrected for the blank value.

Histamine content of the tissues. The method used was that described by Parratt & West (1957). Briefly, the tissues were extracted with 10% w/v trichloroacetic acid (5 ml/g), the excess acid was removed by ether, and the solutions were assayed on the isolated atropinized guinea-pig ileum. The specificity of the responses was confirmed by mepyramine maleate. The values of histamine refer to the base.

Starvation. Food was withheld from a group of rats for 24 hr and the histidine decarboxylase activities of their stomachs were then estimated and compared with those of freely-fed animals.

Results

Effect of pH on the enzyme activity of rat stomach. The histidine decarboxylase activity shows two peaks (Fig. 1); the higher peak has an optimal pH value of about 5·6 whilst the lower peak occurs at about pH 7·6.

Only one peak of activity is present in each of the extracts made after dividing the stomach into two portions (Fig. 2). The optimal pH value of the fundic portion is about 5·6 whilst that of the pyloric portion is

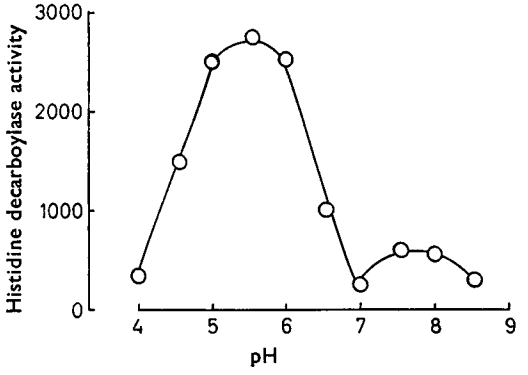

FIG. 1. The effect of pH on the histidine decarboxylase activity of rat stomach, expressed as counts/min/100 mg tissue. Note the two peaks of activity at pH values of 5·6 and 7·6.

about 7·6. These optimal pH values have been used in all subsequent incubation experiments. The results in Fig. 2 also show that the maximal enzyme activity in the fundic portion is about 10 times that in the pyloric part.

FACTORS AFFECTING THE REACTION RATE OF THE TWO ENZYMES

Substrate concentration. When the radioactive histidine is diluted with known amounts of non-radioactive L-histidine (10–1,000 μg) before incubation, and the $^{14}CO_2$ evolved is measured after incubation, estimates can be made of the amounts of histamine formed. For the pyloric enzyme, there is a linear relationship at pH 7·6 between the amount of substrate and the enzyme activity but the graph of activity against substrate concentration for the fundic enzyme at pH 5·6 is not linear but hyperbolic

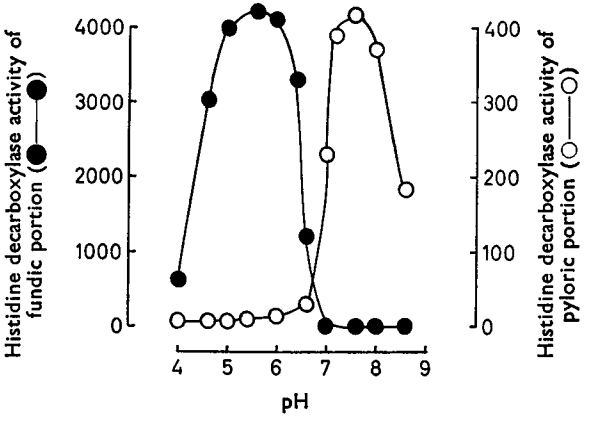

FIG. 2. The effect of pH on the histidine decarboxylase activities of the fundic (●——●) and pyloric (○——○) portions of rat stomach, expressed as counts/min/50 mg tissue. Note the different scales.

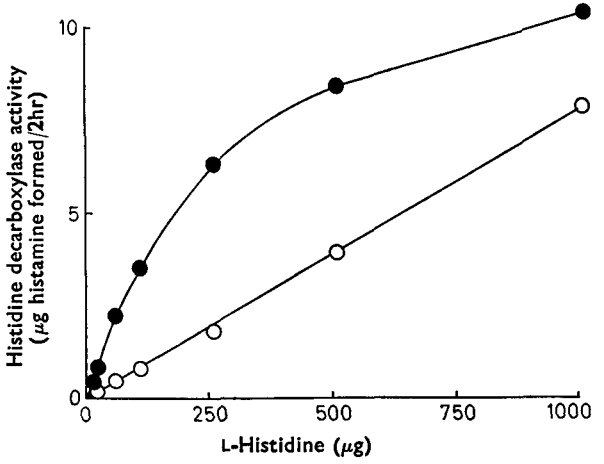

FIG. 3. The effect of substrate concentration on the histidine decarboxylase activities of the fundic (●——●) and pyloric (○——○) portions of rat stomach, expressed as μg histamine formed by 50 mg tissue in 2 hr.

(see Fig. 3). The substrate affinity of the enzyme in the fundic portion ($K_m = 1{\cdot}07 \times 10^{-4}$ g/ml, about 7×10^{-4} M) is nearly three times that of the pyloric portion ($K_m = 2{\cdot}67 \times 10^{-4}$ g/ml, about $1{\cdot}7 \times 10^{-3}$ M).

Enzyme concentration. When the enzyme concentration in the incubation mixture is increased by using more tissue extract, there are similar increases in the rates of the reaction of both preparations when measured at their optimal pH values (see Fig. 4).

Incubation time. There are linear relationships between times of incubation and enzyme activity of the two portions of stomach when estimates are made at the optimal pH values (Fig. 5).

Storage. The pyloric enzyme activity rapidly disappears when extracts are kept at −15° and only 10% of the activity remains after

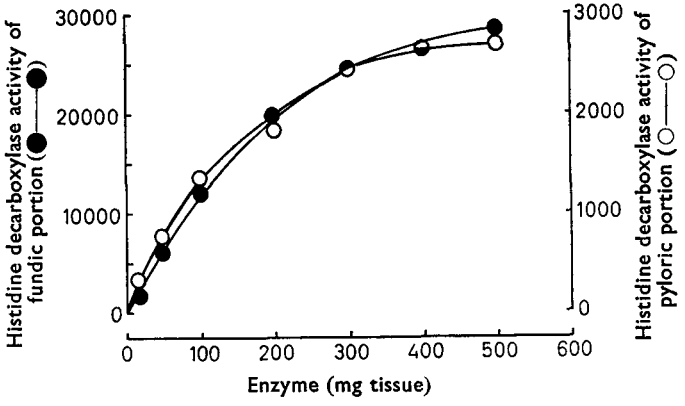

FIG. 4. The effect of enzyme concentration on the histidine decarboxylase activities of the fundic (●——●) and pyloric (○——○) portions of rat stomach, expressed as counts/min. Note the different scales.

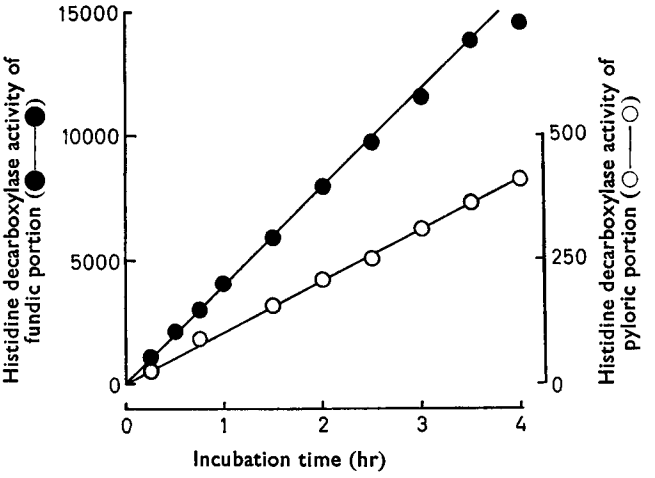

FIG. 5. The effect of incubation time on the histidine decarboxylase activities of the fundic (●——●) and pyloric (○——○) portions of rat stomach, expressed as counts/min/50 mg tissue. Note the different scales.

14 days. On the other hand, the fundic enzyme is little affected by this treatment (Fig. 6).

Effect of benzene. Benzene (20 mg) potentiates the histidine decarboxylase activity of the pyloric portion by about 20% but has little or no effect on the enzyme in the fundic portion.

STUDIES ON ENZYME SPECIFICITY

Effect of α-methylhistidine and L-*α-methyldopa.* Both substances inhibit the histidine decarboxylase activity of the pyloric portion but have

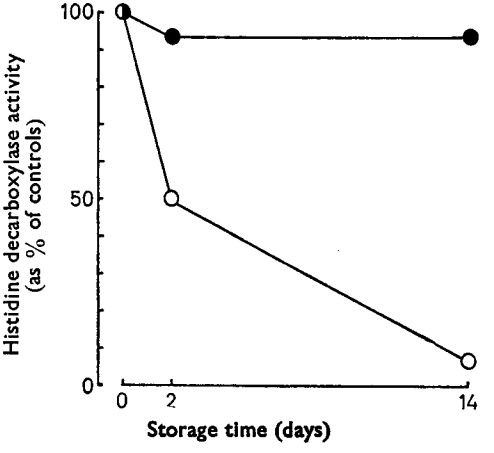

FIG. 6. The effect of storage at −15° C on the histidine decarboxylase activities of the fundic (●——●) and pyloric (○——○) portions of rat stomach, expressed as percentages of control values.

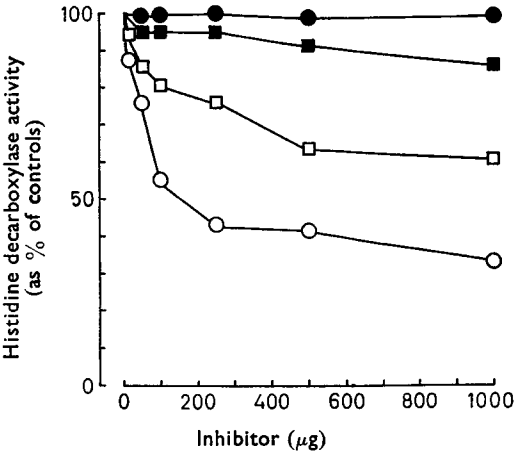

FIG. 7. The effect of L-α-methyldopa (circles) and α-methylhistidine (squares) on the histidine decarboxylase activities of the fundic (solid symbols) and pyloric (open symbols) portions of rat stomach, expressed as percentages of control values.

little effect on that in the fundic portion, even when used in concentrations up to 1,000 μg in the incubation mixture (see Fig. 7).

Effect of dopa and 5-hydroxytryptophan. Both substances have no effect on the fundic histidine decarboxylase but compete with the substrate, L-histidine, for the pyloric enzyme, markedly reducing the output of $^{14}CO_2$ (Fig. 8).

Stereospecificity. Non-radioactive L-histidine added to the incubation mixture of each enzyme reduces the output of $^{14}CO_2$ due to reduction of the specific activity of the original mixture. On the other hand, D-

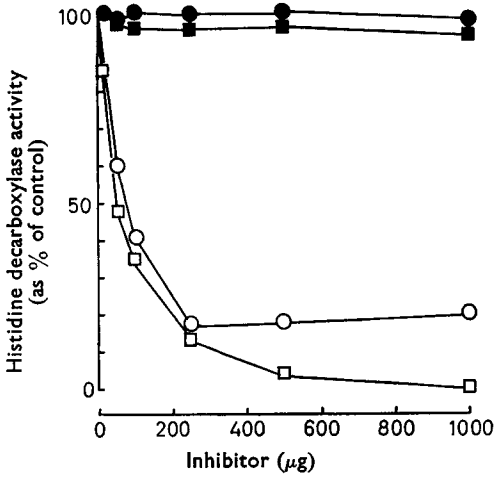

FIG. 8. The effect of DL-dopa (squares) and DL-5-hydroxytryptophan (circles) on the histidine decarboxylase activities of the fundic (solid symbols) and pyloric (open symbols) portions of rat stomach, expressed as percentages of control values.

histidine in concentrations up to 1,000 μg in the incubation mixture of 3 ml has no effect on either enzyme.

EFFECT OF STARVATION

The enzyme activity of the fundic portion is markedly inhibited by starvation, only 3% of the total activity remaining; in the pyloric portion, however, activity is only reduced to 79% of the control value. Whereas the histamine content of the fundic portion is unaffected by starvation (control value, 4 μg/g), that in the pyloric portion is reduced by 33% (control value, 36 μg/g). It is thus possible that the histamine in the fundic portion is normally kept at a low basal level and the histamine formed by the high enzyme activity in this portion of the stomach is catabolized at a fast rate or transported to other parts of the body.

Histidine decarboxylase in mouse stomach. As in rat stomach, two enzymes capable of forming histamine have been identified in mouse stomach. One resides in the fundic portion and has an optimal pH value for activity of 5·6; the other in the pyloric part has an optimal pH value between 7·6 and 8·0 (see Fig. 9).

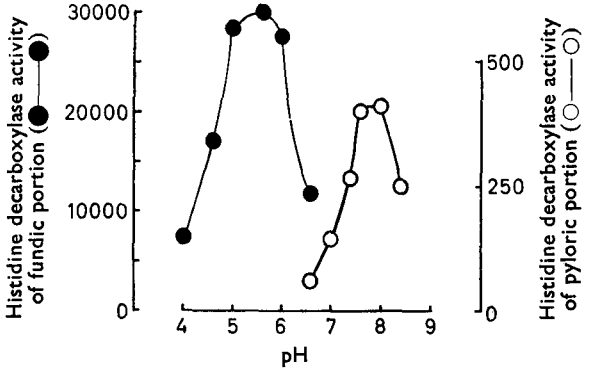

FIG. 9. The effect of pH on the histidine decarboxylase activities of the fundic (●——●) and pyloric (○——○) portions of mouse stomach, expressed as counts/min/50 mg tissue. Note the different scales.

Discussion

The results of the present work show that an enzyme capable of forming histamine *in vitro* resides in the thin fundic portion of rat stomach and that it differs in reactivity from the enzyme located in the thick muscular pyloric portion. The failure to find enzyme activity in the fundic portion (Håkanson & Owman, 1966) may be the result of making incubation experiments either at pH values other than the optimal (pH 5·6) or after fractionation with ammonium sulphate which markedly reduces activity.

The fundic enzyme has many of the characteristics of the specific histidine decarboxylase found in rat foetal liver (Telford & West, 1961b) and in rat hepatoma (Mackay, Riley & Shepherd, 1961). It has a high

affinity for the substrate (K_m of 7×10^{-4} M), and requires an acid pH value (pH of 5·6) for optimal activity; it is only slightly inhibited by α-methylhistidine which is considered to be one of the more potent and specific inhibitors of the specific enzyme (Robinson & Shepherd, 1962).

The pyloric enzyme has many of the characteristics of the non-specific histidine decarboxylase; it requires an alkaline pH value (pH of 7·6) for optimal activity, it is inhibited by α-methyldopa, it is able to use dopa and 5-HTP as substrates, and it is potentiated by benzene (Telford & West, 1961a; Schayer, 1963). However, it is inhibited by α-methylhistidine and has a higher affinity for the substrate (K_m of $1{\cdot}7 \times 10^{-3}$ M) than does the non-specific enzyme (Mackay & others, 1961).

The fundic enzyme is greatly reduced in activity by a physiological stress such as starvation whereas this procedure has little effect on the pyloric enzyme. As the optimal pH value for activity of the fundic enzyme is near to that of rat stomach secretion (pH 4·5; Lane, Ivy & Ivy, 1957) and it is more than 10 times as active as the pyloric enzyme, the fundic enzyme has to be considered as a major source of gastric histamine. Further work is, however, needed to show that these results obtained with *in vitro* tests apply in the intact animal.

Levine (1965) found that oesophageal ligation (thereby preventing food from entering the stomach) lowers gastric acid secretion in the rat by 97%. Starvation in the present work reduced the histidine decarboxylase activity of the fundic portion of the stomach by about the same amount. This indicates a relationship between the enzyme activity in the fundic portion and the rate of acid secretion, and supports the view of Code (1965).

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