THE FORMATION OF HISTAMINE IN THE RAT

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Factors affecting the determination of histidine decarboxylase activity in adult rat tissues have been studied. The optimal conditions vary from tissue to tissue, and the most potent sources of the enzyme are the pyloric part of the stomach, the liver and the duodenum, with less in the kidney. There is no relationship between the histidine decarboxylase activity of a rat tissue and the amount of histamine it contains.

The existence of a mammalian enzyme capable of decarboxylating histidine to form histamine was first demonstrated by Werle (1936) and by Holtz and Heise (1937) more than 20 years ago. These authors and their colleagues subsequently made extensive studies of the distribution of the enzyme. Waton (1956) recently confirmed and extended their observations using in the incubation mixture a specific inhibitor of histaminase, the enzyme which inactivates histamine. Using a sensitive tracer technique, Schayer (1957) has found that the optimal pH value for incubation varies from tissue to tissue.

In the present work, the optimal conditions for determining the histidine decarboxylase activity of rat tissues have been re-examined. The enzyme activity of a tissue has then been compared with its histamine content so that further light might be shed on the mode of formation and the function of histamine in this species.

Methods

Female rats of Wistar strain weighing 120–150 g. were fed on a cube diet (No. 41B, Associated London Flour Millers Ltd.), allowed drinking water *ad lib.*, and housed at 70 ± 1° F.

Formation of Histamine from Histidine by Rat Tissues

The method of Waton was first used. Values of enzyme activity in some tissues were found to be lower than those previously reported, but when the amount of tissue homogenate was doubled, consistent results were obtained. The following experiments refer to the increased amount of tissue homogenate.

Pooled tissue from freshly killed rats was cleaned and weighed, cut into small pieces, and ground in a glass mortar with a little sand and Tyrode solution (5 ml./g. tissue). The resulting homogenate was allowed to stand and the supernatant fluid extract was removed for incubation. The composition of the incubation mixture was as follows.

Tissue homogenate (800 mg.)	4·0 ml.
Phosphate buffer (M/20-K_2HPO_4)	4·9 ml.
L-Histidine (15 mg./ml., neutralised)	1·0 ml.
Aminoguanidine (10 mg./ml., neutralised)	0·1 ml.
Benzene (1 drop)	20 mg.

The substrate (histidine) was always added last. The mixture was immediately shaken and incubated for 3 hr. at 37°. The reaction was then stopped by reducing the pH of the solution to 4·0 with N HCl and by cooling to 4°. After neutralising the mixture with N NaOH, its histamine content was determined. Mixtures containing boiled homogenate or no homogenate were similarly treated and assayed for histamine. In all experiments, mixtures without the substrate were also incubated and assayed for histamine. The final volume of the mixtures was maintained at 10 ml. The histamine content of the extract incubated in the

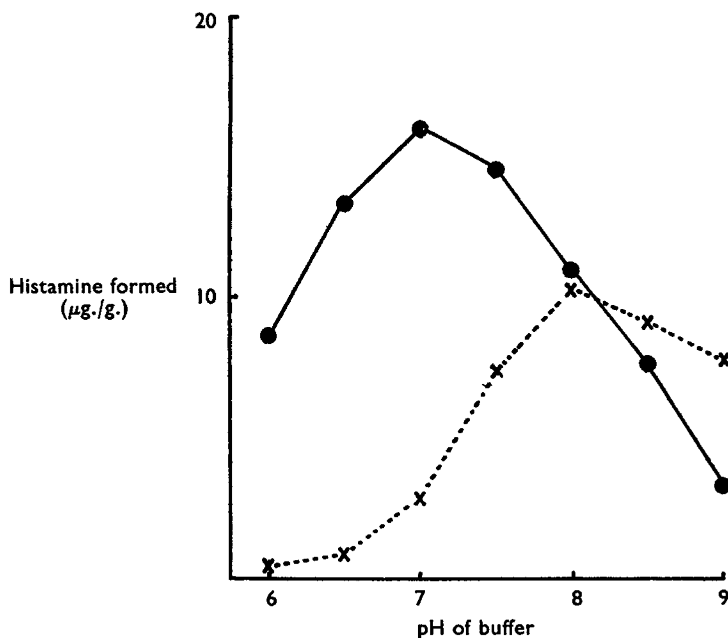

FIG. 1. The histidine decarboxylase activity of rat pyloric stomach (●——●) and liver (X - - - - X) expressed as μg. histamine formed/g. tissue/3 hr. Incubation at varying pH values.

presence of histidine less that of the extract incubated in the absence of histidine gives the amount of histamine formed from histidine during the incubation. This amount of formed histamine, when calculated per gram of tissue, was used as the index of histidine decarboxylase activity. Each result is the mean of at least three separate experiments. Each experiment uses tissue from at least 12 rats.

Extraction of Rat Tissues for Histamine

Pooled tissue from freshly killed rats was cleaned and weighed, cut into small pieces and extracted with 10 per cent (w/v) trichloracetic acid (5 ml./g. tissue) for 24 hr. Excess acid was removed by shaking the supernatant with 4 vol. of ether four times and discarding the ethereal layers. After gentle heating, the aqueous residue was assayed for its

histamine content. Each result is the mean of at least four separate experiments. Each experiment uses tissue from at least 4 rats.

Bioassay procedure. Bioassays were made on the isolated ileum of the guinea-pig. A 15 ml. bath of aerated atropinised Tyrode solution at 32° was used. On occasions, extracts were also assayed on the blood pressure of an anaesthetised cat. The specificity of the responses were checked using mepyramine maleate. All values of histamine refer to the base.

Results

Histidine decarboxylase and incubation pH. The histidine decarboxylase activity of various rat tissues was traced using phosphate buffers of various

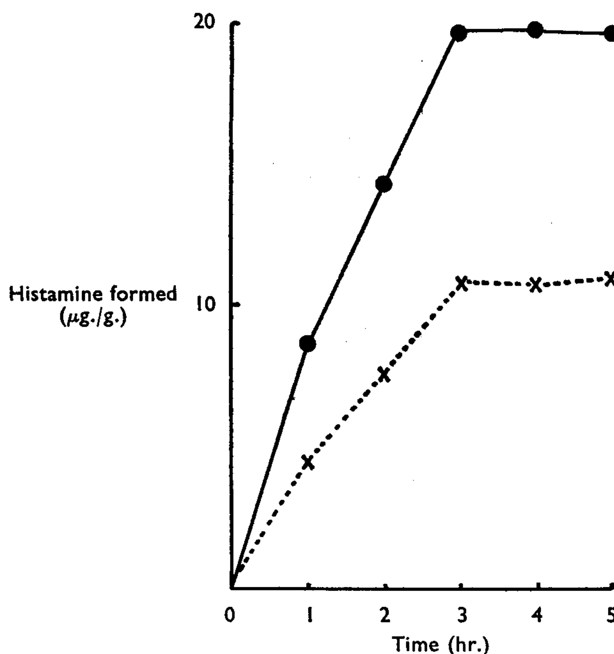

FIG. 2. The histidine decarboxylase activity of rat pyloric stomach (●——●) and liver (X - - - - X), expressed as μg. histamine/g. tissue/3 hr. Incubation for varying times at optimal pH values.

pH values. As may be seen from Fig. 1, the enzyme in the pyloric stomach showed maximal activity at pH 7·0, whereas that in the liver was highest at pH 8·0 with much less activity at pH 7·0. For kidney and duodenum, the maximal value was at pH 7·5. Only traces of enzyme activity were found over a pH range of 6·0–9·0 in jejunum, lung, abdominal skin, ileum and fundic stomach.

Histidine decarboxylase and incubation time. An incubation time of 3 hr. gave the maximal yield of histamine, no more being formed on longer incubation. The results for pyloric stomach and liver are shown in Fig. 2, and similar results were obtained for duodenum and kidney.

Histidine decarboxylase and tissue homogenate. For all tissues an optimal amount of tissue homogenate was required for enzyme activity (800 mg.). From some tissues, like liver, amounts in excess of this yielded less histamine, probably due to interfering substances. The results for pyloric stomach and liver are shown in Fig. 3.

Histidine decarboxylase and substrate. Alterations in the amount of histidine used in the incubation mixture also gave variations in the yield of histamine. Maximal enzyme activity was always obtained when

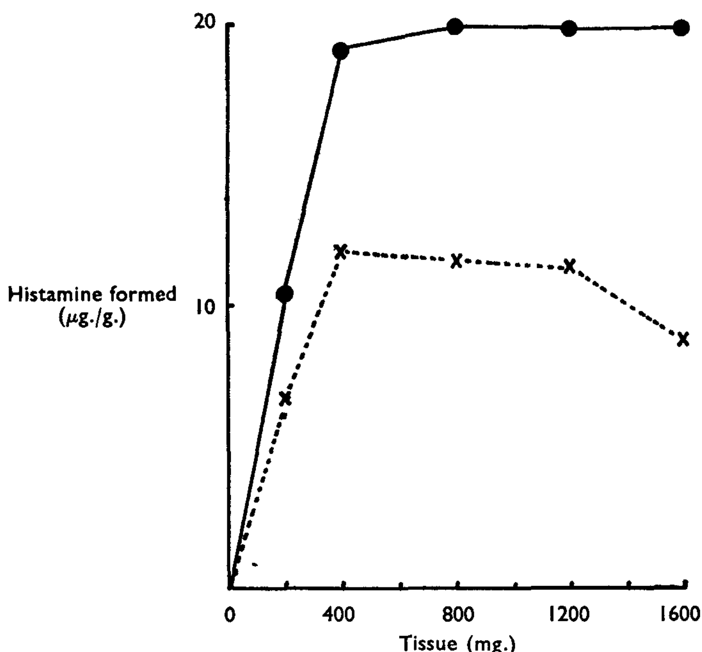

FIG. 3. The histidine decarboxylase activity of rat pyloric stomach (●——●) and liver (X - - - - X), expressed as μg. histamine formed/g. tissue/3 hr. Incubation with varying amounts of tissue.

15 mg. histidine was used, but in some experiments 5 mg. only was needed. The results for pyloric stomach and liver are shown in Fig. 4.

Histidine decarboxylase and benzene. A small quantity (20 mg.) of benzene was necessary to detect enzyme activity in rat liver, kidney and duodenum. When the amount was increased, the enzyme activity was reduced. Benzene was not necessary for, and did not potentiate, the histidine decarboxylase activity in pyloric stomach (Fig. 5).

Other Factors Affecting Histidine Decarboxylase

The presence of a small quantity (1–2 mg.) of aminoguanidine was necessary only when extracts of rat duodenum were incubated, since this tissue is one of the chief sources of histaminase. Pyridoxal, which has been reported to be a co-enzyme of histidine decarboxylase (Blaschko,

1957; Rothschild and Schayer, 1958), failed in all doses used (10–1,000 μg.) to increase the yield of histamine during the incubation of any of the four rat tissues.

Using the optimal conditions for determining the enzyme activity *in vitro*, the following values were obtained; pyloric stomach, 15·6 μg./g.; liver, 10·2 μg./g.; duodenum, 6·4 μg./g.; and kidney, 3·0 μg./g.

Histamine Content of Rat Tissues

These are shown in Table I. There is no relationship between the histamine content of a tissue and the amount of histamine-forming

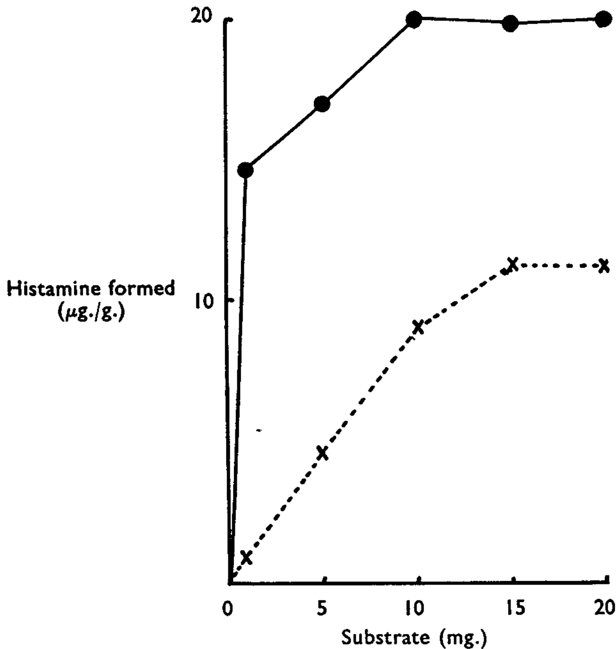

FIG. 4. The histidine decarboxylase activity of rat pyloric stomach (●——●) and liver (X - - - - X), expressed as μg. histamine formed/g. tissue/3 hr. Incubation with varying amounts of substrate.

enzyme it contains. The liver, for example, has a relatively strong histidine decarboxylase activity but very little histamine, whereas the duodenum contains much histamine and possesses less enzyme activity. It is of interest that the fundic part of the stomach, unlike the pyloric part, possesses no histidine decarboxylase activity yet it contains much histamine.

DISCUSSION

The finding that the optimal conditions for determining histidine decarboxylase activity vary from tissue to tissue agrees with the results of previous workers. Schayer, for example, found that the enzyme in rat pyloric stomach and rabbit platelets was most active at pH 7·2 whereas

for rabbit kidney it was most active at pH 8·0. The present results also confirm that, in terms of histamine formed per gram of tissue, the pyloric stomach is the most potent source of the enzyme in the rat. However, the high activity of adult rat liver has not been noted before (Kahlson,

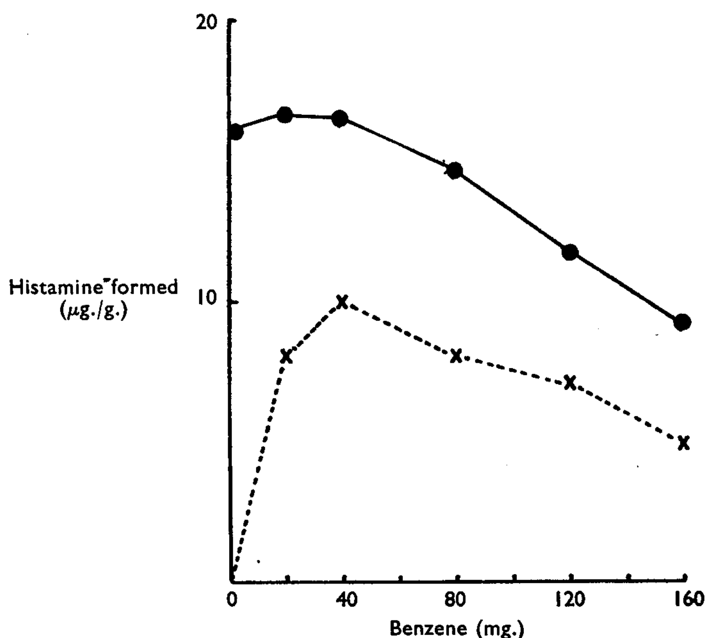

FIG. 5. The histidine decarboxylase activity of rat pyloric stomach (●——●) and liver (X - - - - X), expressed as μg. histamine formed/g. tissue/3 hr. Incubation with varying amounts of benzene.

Rosengren and White, 1960; Mackay, Marshall and Riley, 1960). This activity, unlike that of the pyloric stomach, is detectable only in the presence of a small quantity of an organic solvent such as benzene.

The weight of the liver of an adult rat is 6–10 g., and with an enzyme activity equivalent to 10·2 μg./g. this organ is capable of forming at least 60 μg. histamine in 3 hr. or 480 μg./day. Although the enzyme activity

TABLE I

COMPARISON OF THE HISTIDINE DECARBOXYLASE ACTIVITY (AS INDICATED BY THE AMOUNT OF HISTAMINE FORMED IN μg./g. TISSUE) AND THE HISTAMINE CONTENT (μg./g.) OF RAT TISSUES

Rat tissue	Histidine decarboxylase	Histamine content
Pyloric stomach	15·6	29·9
Liver	10·2	2·0
Duodenum	6·4	25·2
Kidney	3·0	1·0
Jejunum	1·2	25·0
Lung	1·2	8·0
Abdominal skin	1·0	31·3
Ileum	0·9	23·8
Fundic stomach	0·4	12·6

in the pyloric stomach is equivalent to 15·6 μg./g., this tissue weighing 0·25–0·5 g. can form only 18 μg. histamine in 3 hr., or 64 μg./day. Likewise, the duodenum and the kidney are capable of forming daily only 40 and 32 μg. histamine respectively. Thus, in terms of absolute amounts of histamine formed, the liver is by far the most potent source of histidine decarboxylase in the rat.

Kahlson (1960) has recently reported that histidine decarboxylase activity may play an important role in processes of tissue growth and repair, since a high histamine-forming capacity is found in the rat foetus, in the adult rat after partial hepatectomy, and in healing skin wounds. Waton, on the other hand, suggests that the intracellular decarboxylation of histidine is not the major pathway in the formation of histamine, and that decarboxylation occurs in the lumen of the gut and histamine is absorbed as such. Sufficient histamine may be absorbed from the alimentary tract to meet all physiological needs and local histidine decarboxylase may not be necessary. He also states that, although histidine decarboxylase is identified in a tissue by an *in vitro* method, this is not proof that such a decarboxylation occurs *in vivo*. Nevertheless, as the present work shows, the rat is capable of forming very large quantities of histamine. The pyloric stomach, liver, duodenum and kidney, for example, can form between them over 1 mg. of histamine in a day.

The presence of histidine decarboxylase in the pyloric stomach and its absence in the fundic stomach suggests that the amount of histamine available to stimulate the oxyntic and peptic cells in the fundus is controlled by the rate of formation of histamine in the pyloric portion. It is in the pyloric portion of the stomach where gastric ulcers most commonly occur. The presence of the enzyme in rat duodenum but not in jejunum or ileum is surprising, and this finding too may be linked with the fact that intestinal ulcers are found most frequently in the duodenum.

Much of the histamine in the body is held in tissue mast cells (Riley and West, 1953). In the rat, the high histamine content of the skin is reflected in its high mast cell population, and the same is true for lung. But the histamine content of the alimentary tract is not related to tissue mast cells. The fundic stomach, for example, contains a high number of mast cells per unit area yet its histamine content is relatively small and its histadine decarboxylase activity is low. The pyloric stomach contains very few mast cells but much histamine and histidine decarboxylase activity, and a similar situation is found in the duodenum. In the jejunum and ileum, there is a high histamine content, very few mast cells and very little histidine decarboxylase activity but these two tissues are the chief sources of histaminase activity in the rat. The liver and kidney possess the power to form histamine but not the power to store it. Thus, the histidine decarboxylase activity of rat tissues is not related to the amount of histamine they contain nor to the mast cell population.

Recently, whilst investigating the mechanism of action of corticosteroids in allergic diseases and asthma, we have found (Telford and West, 1960) that prolonged treatment of rats with glucocorticoids causes a marked depletion of histamine in many tissues, although the histamine

content of the pyloric stomach is increased. It is possible that these changes are brought about by an alteration in the rate of formation of histamine. The effect of glucocorticoids on the histidine decarboxylase activity of tissues is now being studied.

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