# CHARACTERIZATION OF HISTIDINE DECARBOXYLASE FROM RAT PERITONEAL FLUID MAST CELLS

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## SUMMARY

Following fractionation of the cells of rat peritoneal fluid it was demonstrated that histidine decarboxylase activity is almost entirely associated with the mast cells. This permits use of unfractionated peritoneal fluid cells as a source of mast cell histidine decarboxylase. Pyridoxal phosphate is the coenzyme. Results of other experiments on the characterization of this enzyme are reported.

## INTRODUCTION

The mast cell is a major repository of histamine in mammals[1,2]. Release of histamine, along with other substances from the mast cell, is believed to be of importance in certain inflammatory, allergic, and other pathological states. Consequently, characterization of histidine decarboxylase from mast cells may have a practical as well as a theoretical value.

In a previous publication, one of us[3] reported that suspensions of rat peritoneal fluid cells were able to decarboxylate L-[2-$^{14}$C]histidine and to bind the resulting [$^{14}$C]histamine in a stable manner. A soluble histidine decarboxylase could be obtained from these cells but virtually no work could be done on it owing to its low activity in the absence of the intact cell. The availability of liquid scintillation counting now makes further experiments possible. One purpose of this paper is to present evidence that the mast cells are responsible for virtually all of the histidine decarboxylase activity of peritoneal fluid**. Having demonstrated this, it was possible to use the mixture of cells for experiments characterizing this enzyme.

## METHODS

### *Preparation and assay of histidine decarboxylase from peritoneal cells*

Male, Wistar rats (200–300 g) were injected intraperitoneally with 4 ml of

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** Evidence on this point was presented in the earlier paper[3]. It was shown that while peritoneal fluid cells were active, rat buffy coat had very low activity. This reasoning was later criticized on the basis that the distribution of types of leucocytes in blood is quite different from that in peritoneal fluid. Accordingly, we have used a more direct approach to the problem of relating the histidine decarboxylase activity of peritoneal fluid to cell type.

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heparinized saline, decapitated, exsanguinated, and the abdominal wall massaged to dislodge most of the free peritoneal cells. After opening the abdominal cavity the cell suspension was removed with a dropper, centrifuged and resuspended in isotonic phosphate buffer of pH 7.4 containing 0.2% glucose. The buffered suspension was frozen and thawed 5 or 6 times and centrifuged to remove cell fragments.

The method of assay of histidine decarboxylase has been described[4,5]. It involves incubation of the enzyme preparation with L-[$^{14}$C]histidine and subsequent determination of [$^{14}$C]histamine by isotope dilution. Carrier histamine is isolated, converted to dibenzenesulfonylhistamine (BSH), and counted in a Packard Tri-Carb Liquid Scintillation Spectrometer. Enzymic activities are expressed as counts per minute per 100 mg of BSH, and are corrected for background and for blank values obtained from incubates run with heat-inactivated enzyme. Substrate concentration was 0.065 $\mu$moles of L-[$^{14}$C]histidine per ml in all experiments.

*Counting cell population of rat peritoneal fluid*

Mast-cell contents of the suspensions were estimated after dilution and staining with a solution of toluidine blue in saline. In the peritoneal fluid of Wistar rats, mast cells constitute 2 to 4% of the total cells. Total leucocyte contents were obtained following dilution with 0.1% acetic acid containing crystal violet. Dilutions were made in a white blood cell pipette; aliquots were added to both chambers of a Spencer hemocytometer and the cells counted. The values presented represent the averages of the counts obtained from duplicate samples of each suspension. The counts agreed within 3 to 7% with each other.

*Identification of the cellular fraction containing histidine decarboxylase*

Methods for the complete separation of mast cells from the remaining peritoneal fluid cells of the rat have been described[6,7]. They employ differential centrifugation in hypertonic sucrose and obtain layers of cells of different specific gravity. We found that mast cells separated in this manner had very little extractable histidine decarboxylase activity. This was due to a damaging action of the fractionation medium on the cells as is shown by the following observations: (1) when all of the cells of the peritoneal fluid were suspended for 30 min in the hypertonic sucrose medium and then completely sedimented by centrifugation in the cold, a large amount of the enzymic activity leaked out of the cells and could be recovered in the supernatant. In contrast, less than 10% of the total activity was found in the sedimented cells. (2) Sucrose in itself did not affect the activity of the enzyme once it had been released from the cells. No changes in activity were observed when sucrose, in amounts up to 75 mg/ml, was added to cell-free extracts obtained in the usual manner.

In an attempt to find a suitable method for separating active mast cells, concentrated serum albumin solutions were tried but did not show promise. Therefore, a partial separation of mast cells from leucocytes in balanced salt solution was used.

Pooled peritoneal fluid cells were suspended in HANK's balanced salt solution[8], 3 ml being used for the cells from one rat. Two ml of this suspension were placed in tapered, blood-centrifuge tubes of 6.5 ml capacity, and centrifuged for 3.5 min at approximately 500 rev./min in a horizontal International Clinical Centrifuge. The lowest 0.1 ml of the suspension were collected with a drawn-out medicine dropper. This fraction, called (A), contained from 50 to 65% of the total mast-cell content

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of the original suspension, and showed a 2.5-fold relative increase in its mast cell/leucocyte ratio when compared with the unfractionated suspension. To obtain a fraction containing a lowered mast cell/leucocyte ratio, the remaining part of the cell suspension was centrifuged at the same speed for an additional 5 min and the lowest 0.1 ml again removed. This fraction was discarded. The remaining upper layers from several tubes were pooled and centrifuged at 1800 rev./min for 5 min. This resulted in the sedimentation of all the remaining cells, and a fraction, called (B), having a mast cell/leucocyte ratio 4 to 5 times lower than that of the unfractionated fluid, was obtained. Histidine decarboxylase was extracted from the cells of each of these fractions and assayed in the manner described.

## EXPERIMENTS AND RESULTS

*Correlation between histidine decarboxylase activity and mast-cell content of rat peritoneal fluid cell fractions.* In Table I the enzymic activity extractable from a mixed cell fraction, (A), having a mast cell/leucocyte ratio of about 1:10, is compared with that obtained from a fraction, (B), having a mast cell/leucocyte ratio of less than 1:100. The number of fraction B cells were so chosen that equal amounts of leucocytes were used for extraction in both cases. The results of the three experiments shown indicate that a much higher histidine decarboxylase activity was associated with the mast cell enriched fraction. The low activities found in the (B) fractions can be attributed almost entirely to the few mast cells present.

TABLE I

HISTIDINE DECARBOXYLASE ACTIVITY OF CELL-FREE EXTRACTS OBTAINED FROM PARTIALLY FRACTIONATED RAT PERITONEAL FLUID CELLS

| Expt. | Cell fraction | Cellular content before extraction | | Ratio: mast cells/leucocytes | Enzymic activity (counts/min/100 mg BSH) |
|---|---|---|---|---|---|
| | | Mast cells | Leucocytes | | |
| I | A | $6.7 \cdot 10^5$ | $77 \cdot 10^5$ | 1:11 | 117 |
| | B | $0.7 \cdot 10^5$ | $76 \cdot 10^5$ | 1:108 | 6 |
| II | A | $7.7 \cdot 10^5$ | $84 \cdot 10^5$ | 1:11 | 84 |
| | B | $0.7 \cdot 10^5$ | $84 \cdot 10^5$ | 1:120 | 17 |
| III | A | $5.2 \cdot 10^5$ | $51 \cdot 10^5$ | 1:10 | 125 |
| | B | $0.25 \cdot 10^5$ | $53 \cdot 10^5$ | 1:210 | 12 |

*Characterization of mast cell histidine decarboxylase*; effect of pH.* Activity of the enzyme of the cell-free extract was optimal over a fairly broad zone, ranging from pH 6.5 to 7.6. Outside of these limits the activity dropped rapidly and was low at pH 6.0 and 8.0. Mast cell histidine decarboxylase did not show a sharp maximum activity as do histidine decarboxylase preparations from other sources[9,10]. All studies reported in this paper were done at pH 7.4.

* Some of the techniques commonly used in the characterization of enzymes had to be omitted owing to the extremely low substrate concentration used. This low substrate concentration is essential to the detection and quantitative estimation of mammalian histidine decarboxylase from many sources[9]. For example metal ions such as cobalt, and sulfhydryl blocking agents such as *p*-chloromercuriben.zoate might give spurious enzyme-inhibiting effects when they are in fact forming complexes with the substrate.

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*Effect of enzyme concentration.* Fig. 1 shows the effect of increasing amounts of enzyme on the decarboxylation of a constant amount of substrate. Increases in enzyme concentration over a four-fold range, produced proportional increases in activity.

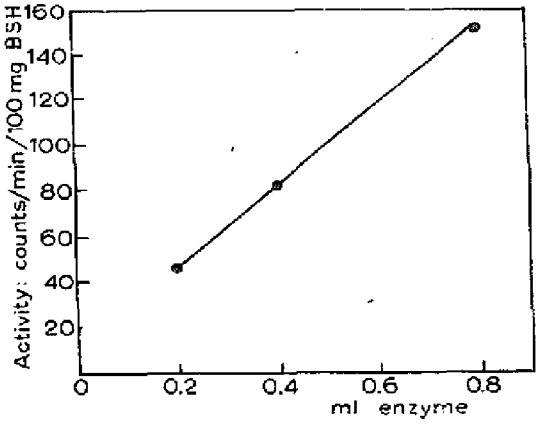


Fig. 1. Effect of concentration of histidine decarboxylase from rat peritoneal fluid mast cells on histamine formation.

*Effect of carbonyl group reagents.* As shown in Table II, mast cell histidine decarboxylase is markedly affected by semicarbazide and by hydroxylamine, both of which are potent blocking agents for carbonyl groups. This inhibition, which is observed with many amino acid decarboxylases, suggested that pyridoxal phosphate might be the coenzyme. The pyridoxal phosphate requirement of mast cell histidine decarboxylase was therefore investigated.

TABLE II

EFFECT OF HYDROXYLAMINE AND OF SEMICARBAZIDE ON THE ACTIVITY OF RAT PERITONEAL FLUID MAST CELL HISTIDINE DECARBOXYLASE

Enzyme and inhibitor were pre-incubated for 15 min at 37° prior to the addition of the substrate.

| *Inhibitor* | *Enzymic activity (counts/min/100 mg BSH)* | *% Inhibition* |
|---|---|---|
| None | 210 | — |
| Hydroxylamine, $10^{-4}$ *M* | 3 | 100 |
| Hydroxylamine, $10^{-5}$ *M* | 124 | 41 |
| None | 262 | — |
| Semicarbazide, $10^{-3}$ *M* | 0 | 100 |
| Semicarbazide, $10^{-4}$ *M* | 33 | 88 |
| Semicarbazide, $10^{-5}$ *M* | 194 | 26 |

*Effect of pyridoxal phosphate on the activity of dialyzed preparations of mast cell histidine decarboxylase.* Cell-free extracts of rat peritoneal fluid were dialyzed for 18 h against 0.1 *M* phosphate buffer in the cold. Activity was then measured and compared with that obtained following the addition of 0.120 μmoles/ml of pyridoxal-5-phosphate to the dialyzed extract.

Results (counts/min/100 mg BSH) obtained in duplicate with the dialyzed ex-

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tracts were 40 and 120 averaging 80; after adding pyridoxal phosphate activities were 1110 and 1180, averaging 1150. Another experiment confirmed these results. Thus, a marked potentiating effect of this coenzyme was evident*.

*Reversal of hydroxylamine-induced inhibition of mast cell histidine decarboxylase by pyridoxal phosphate.* The effect of pyridoxal phosphate on inhibition of mast cell histidine decarboxylase by hydroxylamine was tested. Results (counts/min/100 mg BSH) of the four groups of the experiment are:

(a) enzyme only; 126, 126 and 134: average 129 counts/min.

(b) enzyme plus hydroxylamine $10^{-4}$ $M$; all samples had zero activity.

(c) enzyme plus hydroxylamine $10^{-4}$ $M$ plus pyridoxal phosphate, approximately $10^{-4}$ $M$; all samples had negligible activity, 4 counts/min average.

(d) enzyme plus hydroxylamine $10^{-4}$ $M$ plus pyridoxal phosphate, $4 \cdot 10^{-4}$ $M$; 156, 186 and 217 counts/min, average 186**.

Another experiment confirmed these results.

TABLE III

INHIBITION OF RAT MAST CELL HISTIDINE DECARBOXYLASE BY AROMATIC AMINO ACIDS

| *Amino acid* | | *% Inhibition** |
|---|---|---|
| DL-5-hydroxy Tryptophan | $0.4 \cdot 10^{-3}$ $M$ | 25 |
| | $0.8 \cdot 10^{-3}$ $M$ | 38 |
| | $1.6 \cdot 10^{-3}$ $M$ | 50 |
| | $3.2 \cdot 10^{-3}$ $M$ | 76 |
| DL-Tryptophan | $0.8 \cdot 10^{-3}$ $M$ | 16 |
| | $3.2 \cdot 10^{-3}$ $M$ | 22 |
| DL-Tyrosine | $3.2 \cdot 10^{-3}$ $M$ | 49 |
| DL-Phenylalanine | $3.2 \cdot 10^{-3}$ $M$ | 11 |

* The averaged results of two or three separate experiments, run in duplicate, are reported. These experiments gave essentially identical results. Inhibitor and substrate were mixed prior to the addition of enzyme.

*Inhibition of mast cell histidine decarboxylase activity by aromatic amino acids.* WERLE AND KOCH[11] showed that the activity of guinea pig kidney histidine decarboxylase could be inhibited to varying extents by different amino acids. Ring hydroxylation increased the inhibitory capacity of the aromatic amino acids, tyrosine being a comparatively weak inhibitor while dihydroxyphenylalanine was a strong one. We have found a similar effect on mast cell histidine decarboxylase. The data of Table III show that the hydroxyl-substituted amino acids are much stronger inhibitors than unsubstituted ones.

* A potentiating effect of pyridoxal phosphate was also noted after its addition to a mast-cell extract which had been frozen for 18 h. The frozen enzyme gave histidine decarboxylase activities of 450 and 470 counts/min; addition of the coenzyme raised these to 970 and 1080. Thus, loss of pyridoxal phosphate probably accounts for much of the drop in activity observed in frozen mast cell histidine decarboxylase preparations[3].

** The enhancement of activity to a level higher than that of the controls has been observed repeatedly and indicates that even the fresh enzyme is not saturated with coenzyme. This is probably due to the presence in the crude extract of phosphatases which destroy pyridoxal phosphate.

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*Effect of benzene.* The histidine decarboxylase activity of extracts of rat peritoneal fluid mast cells is inhibited to the extent of 87% when one drop of benzene is added to the incubation medium. The mast cell enzyme differs, therefore, from rabbit kidney histidine decarboxylase, which, as shown by WATON[12], is enhanced in activity by the addition of benzene. The mast cell enzyme behaves in this respect like rat stomach histidine decarboxylase, which is also strongly inhibited by benzene[9].

## DISCUSSION

We have demonstrated that in rat peritoneal fluid the mast cells are a much richer source of histidine decarboxylase than the white cells. Thus it seems valid to interpret findings on histidine decarboxylase of peritoneal fluid as being due almost exclusively to the enzyme in the mast cells.

Pyridoxal phosphate is the coenzyme of rat mast cell histidine decarboxylase. It has been described as the coenzyme of many amino acid decarboxylases, and its effect in potentiating the formation of histamine by extracts from guinea pig and pig kidney incubated with L-histidine has been reported by WERLE[11] and HOLTZ[13]. However, these kidney enzymes differ considerably in properties from mast cell histidine decarboxylase and findings from studies on them are not necessarily valid for enzyme from other sources[9]. Pyridoxal phosphate seems to be much more firmly bound to the kidney enzyme than to the mast cell enzyme. WERLE failed to observe a drop in activity of dialyzed rabbit kidney histidine decarboxylase[11].

The effect of this coenzyme in reversing the blocking of mast cell histidine decarboxylase by hydroxylamine suggests that the carbonyl group of pyridoxal phosphate is involved in the enzymic decarboxylation[15].

Mast cell histidine decarboxylase interacts with other amino acids besides histidine; this can result in retardation of the rate of decarboxylation of L-histidine (Table III). METZLER[16] has reported the ability of pyridoxal phosphate to combine at neutral pH with amino acids and amines to form imino compounds (Schiff bases) which are probably intermediates in the enzymic decarboxylation of the amino acids. Such complexes could inhibit the function of the enzyme. The marked effect of ring hydroxylation on the inhibitory capacity of the amino acids tested could be explained as being due to an additional, direct binding of the amino acid to the enzyme *via* the phenolic hydroxyl group.

Finally, our finding that addition of pyridoxal phosphate increases activity to above that of the fresh enzyme suggests that the coenzyme is loosely bound in histidine decarboxylase and that in future studies excess coenzyme should be added to insure maximum activity.

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# PRODUCTION OF PENTOSE INTERMEDIATES DURING GROWTH OF *NOCARDIA OPACA* AND OTHER SAPROPHYTIC SOIL NOCARDIAS AND MYCOBACTERIA

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## SUMMARY

1. During growth in simple media *Nocardia opaca* (strain $T_{18}$) and some other saprophytic soil nocardias and mycobacteria liberate quantities of sedoheptulose and ribulose which can be detected by applying as little as 10–20 $\mu$l of medium directly to the paper chromatogram. An unknown substance which gave a spectrum similar to that of pentulose in the cysteine–carbazole reaction was also formed but this was neither ribulose xylulose, nor erythro-3-pentulose.

2. Liberation of the pentose cycle components occurs more quickly and generally in greater amounts when the medium is kept at about pH 7.0 (*e.g.* by addition of calcium carbonate, by using an increased phosphate buffer concentration, by using sodium nitrate rather than ammonium sulphate as a nitrogen source or by using sodium gluconate as a source of carbon). There seems no reason to suppose that calcium ion has any specific effect on the system.

3. Sedoheptulose and ribulose from the above cultures were identified and estimated by paper chromatography and by application of the cysteine–sulphuric acid and orcinol reactions respectively to larger quantities isolated by column chromatography. Dihydroxyacetone (characterised as the diacetate) was obtained only when the cultures were kept at about pH 7.0 (*i.e.* by addition of calcium carbonate). This and the failure to produce dihydroxyacetone from sodium gluconate suggests that the triose is mainly a product of the Emden-Meyerhof-pathway. Aldolase (with fructose diphosphate as a substrate) is present in cell-free extracts of the bacteria.

*References p. 406.*