DEPENDENCE OF TISSUE HISTAMINE CONTENT ON LOCAL HISTIDINE DECARBOXYLASE ACTIVITY*

by

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Histamine is distributed in the body in a remarkable pattern: it is highly concentrated in relatively scarce cells scattered through the body, tissue mast cells and basophilic polymorphonuclear granulocytes[1-3]. Whether this peculiar pattern is determined by a similar distribution of the ability to produce histamine or the ability to store it, or by an inverse distribution of the ability to destroy it has not been investigated directly.

However, SCHAYER's experiments on the administration of radioactive histidine and histamine to guinea pigs strongly suggest that body histamine remains at or near the site where it is produced by decarboxylation[4]. SCHAYER observed accumulation of radioactive histamine in lungs, kidneys and intestines following radioactive histidine but not following radioactive histamine subcutaneously administered. In order to establish the suggested relationship between (1) histamine content and (2) histidine decarboxylase activity, and to detect the effects, if any, of (3) histamine storage capacity and of (4) histaminase activity on histamine content, a survey of mammalian tissues with respect to these four parameters has been undertaken.

Investigation of the storage capacity has so far been limited to measurements of the difference between the histamine contents of trichloroacetic acid extracts of unhydrolyzed and of acid-hydrolyzed samples of homogenate. The extra histamine in the latter is termed *combined histamine* in this report; it plus the *free histamine*, the histamine appearing in the extract of the unhydrolyzed homogenate, probably represent all or most of the histamine in the homogenate, but may not by any means reveal its full capacity to hold histamine. Included in the combined histamine are (1) any hydrolyzable derivatives of histamine—perhaps for instance acetyl histamine —that do not have the chemical properties of histamine but appear in the trichloroacetic acid extracts of the unhydrolysed homogenate, and (2) any histamine that is precipitated with the proteins of an unhydrolyzed extract but is freed from its protein attachments by hydrolysis with acid before protein precipitation.

Clear evidence for an effect on tissue histamine content of either (1) storage capacity as measured by combined histamine or of (2) histaminase activity has not been obtained so far, though these factors can hardly be without some influence on

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histamine content. The observations reported here leave little room for doubt that the factor pre-eminently responsible for the accumulation of histamine in tissues is local histidine decarbozylase activity.

METHODS

Preparation of homogenates. Homogenates of weighed samples of tissue taken from freshly killed animals were prepared in a glass homogenizer with an amount of water equal to four times the weight of the sample, and were stored at —5° C for short periods before use. The homogenates were prepared from fresh tissue except when this was known to homogenize only with difficulty or incompletely. In this case, a portion of the tissue was frozen in liquid nitrogen, pulverized by pounding[5,6] and transferred to a weighed tube in which it was thawed and weighed. Such preliminary pulverization facilitated homogenization with various tissues and was necessary with liver capsular tissue; even after this treatment beef liver capsule contained some non-homogenizable material.

Beef and sheep liver capsule were separated from the parenchyma readily by peeling; hog liver capsule was obtained by slicing off the surface of the liver and scraping the adherent parenchyma from the inner surface of the slices.

Histamine assays. The histamine content of 5% trichloroacetic acid extracts of the homogenates was measured by the microchemical method in use in this laboratory[7]. The method has been slightly modified to take care of certain non-histamine interfering substances possibly present in the homogenates. The readings given by these substances are suppressed by the use of 60 μl of dinitrofluorobenzene reagent instead of 20 μl ([7], footnote p. 119), and 60 μl was accordingly used in these measurements. Under these conditions it is desirable to wash the ketone after separation from the alkaline reaction mixture with an equal volume of carbonate buffer (pH 10)[7] to which 40 g KBr has been added per 100 ml. This washing obviates the development of an inconveniently high blank arising from excess reagent. Putrescine and cadaverine, non-histamine interfering substances that are susceptible to destruction by histaminase, are believed to be virtually eliminated from the final acid extract by this modification of the method.

To estimate the hydrolyzable histamine in the unincubated homogenates or after incubation with histidine for the measurement of histidine decarboxylase activity, sufficient 10*N* HCl was added to give a final concentration of about 1.2*N* acid. The mixture was heated in a boiling water bath for 3 hours, cooled and then treated with an amount of 10*N* NaOH exactly equivalent to the hydrochloric acid used for the hydrolysis. Trichloroacetic acid (50%) was added in the volume necessary to bring the whole mixture to 5% trichloroacetic acid concentration, and the extract put over the columns as usual. The amount of salt in these trichloroacetic acid extracts was of course greater than in extracts of unhydrolyzed homogenates but was found not to interfere with the adsorption of histamine on the Decalso column. Controls for the hydrolyzed extracts were however always run in the presence of the same amount of HCl and NaOH. In the unhydrolyzed samples the NaOH was added immediately after the hydrochloric acid and followed at once by the addition of the required amount of 50% trichloroacetic acid.

In order to correct for readings due to non-histamine substances not destroyed by histaminase — *e.g.* spermine and spermidine — Decalso column eluates were treated as follows. Following dilution with an equal volume of water, the eluates were incubated for 2 hours at 37.5° C after the addition of phosphate buffer (one-fifth volume, 0.1*M*, pH 6.8) and purified hog kidney histaminase[8]. The amount of enzyme used was found by previous tests under the same experimental conditions to be more than sufficient to destroy as much histamine as could possibly be in the sample. The slight inhibition of enzyme activity by the 17% KBr present in the incubated mixture is readily compensated for by the use of somewhat larger amounts of enzyme or by prolonging the incubation. The method was applied to eluates of incubated and/or hydrolyzed homogenates as well as of homogenates not subjected to these procedures.

Measurement of histidine decarboxylase activity. Exact estimation of the histidine decarboxylase activity of a tissue homogenate is possible only when enzymic destruction of histamine is prevented. Complete suppression of histaminase activity was not always secured by rigid exclusion of oxygen (in confirmation of WERLE[9]) nor by addition of putrescine (in disagreement with WERLE[10]), but was obtained with sodium hydrosulfite ($Na_2S_2O_4$). This reducing agent was used at a concentration (about 10 mg/ml) high enough to retain in the incubation mixture the ability to reduce safranin O energetically after an hour of incubation at 37.5° C without exclusion of air. At this concentration the hydrosulfite did not decrease the activity of decarboxylase nor interfere with the estimation of histamine. The incubation mixture filled the narrow tube used for the incubation to a height of about 12 mm. The mixture consisted of 700 μl of 0.3*M* borate buffer (pH 9.2) containing 13 μM of L-histidine, 6 mg $Na_2S_2O_4$, 2.5 μg chlortetracycline or erythromycin,

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and 8–40 mg homogenized tissue. The concentration of histidine, 20 m*M*, was somewhat higher than that used by WERLE[9]; even after hydrolysis it did not increase the blank. Erythromycin was preferred to chlortetracycline for the control of bacterial action because of its greater stability at pH 9.2, but no difference was observed in the effects of the two antibiotics. After 1 hour's incubation at 37.5° C, unless hydrolyzable histamine was to be measured, the proteins were precipitated with trichloroacetic acid and the histamine content of the extract determined. At the same time the histamine content of an extract obtained from an exactly similar mixture not incubated but acidified as soon as the homogenate was added and kept at 5° C during the incubation period, was determined. The difference between the amounts of histamine in the two extracts was taken as the measure of the decarboxylase activity of the tissue.

Measurement of histaminase activity. The measurement of histaminase activity in crude homogenates is fraught with uncertainty, partly because no method has been devised for the complete prevention of histidine decarboxylase activity, and partly because substrate concentrations that are inhibitory to histaminase in some tissues are far below the level adequate for maximal activity in others. For instance, the substrate level above which human pregnancy serum histaminase shows inhibition is about 20 μM, while the amount of histamine destroyed by the enzyme in beef plasma or by the enzyme isolated from hog kidney continues to increase with concentration up to 50 times this level, and shows no decrease with still higher substrate concentrations[11]. Of the various methods suggested by earlier workers for the control of histidine decarboxylase activity—the presence of D-histidine[10] or of suramin or trypan blue[9] in the incubation mixture, the use of acid pH[12], etc.—none of those tried was found to give complete prevention of activity under feasible conditions. Incubation of the homogenates with histidine at pH 6.8 resulted in the production of about 25% as much histamine as incubation at pH 9.2, and this degree of inhibition was assumed to be satisfactory in the absence of added histidine.

The histaminase activity of many tissues is not very great, and dilution of the homogenates to provide histamine levels certainly not inhibitory to the tissue histaminase is therefore unprofitable. In this preliminary survey of histaminase activity, the homogenate was diluted fivefold in the incubation mixture, so that the substrate concentration was 1/25 that of the histamine in the tissue unless exogenous histamine was introduced. The incubation mixture was 500 μl of 0.02 *M* phosphate buffer (pH 6.8) containing 2.5 μg chlortetracycline, 5–20 mg of homogenized tissue and 0–1.25 μg of added histamine. The mixture was shaken mechanically for 2 hours at 37.5° C, and the histamine in trichloroacetic acid extracts of these incubated samples and of identical but unincubated samples was measured by the method described above. In the unincubated samples the protein was precipitated by the addition of trichloroacetic acid prior to addition of the homogenate. The difference between the histamine content of the two samples was taken as the measure of the histaminase activity of the tissue.

RESULTS

The dependence of the free histamine content of tissues on tissue histidine decarboxylase activity is clearly evident in Fig. 1. It will be noticed that when specimens of the same tissue taken from two individuals are represented in the figure, the one higher in histamine content is as a rule proportionately higher in histidine decarboxylase activity. The two specimens of guinea pig lung exemplify this relationship most strikingly. The two specimens of beef liver parenchyma, rabbit and dog liver do not differ so much one from the other as do the specimens of guinea pig lung, but show the same relationship. Hog kidney and hog liver however seem not to follow the rule. The proportionality of the rise of histamine content to the rise of enzyme activity over the whole range of tissues is even more convincing than in the figure if the point for beef liver capsule is transposed to its actual relative position.

While the general trend toward proportionality is unmistakable, in the series of tissues there is considerable variation among the ratios of histamine content to histamine production. Without regard to the tissues containing less than 5 mg histamine per gram, for which small amounts of interfering substances present in the tissues or formed in the homogenates during incubation might well produce serious distortion of the ratio, the ratio varies from about 0.5 for rabbit intestinal mucosa to about 5 for hog liver parenchyma and hog lung. In a search for the factors producing

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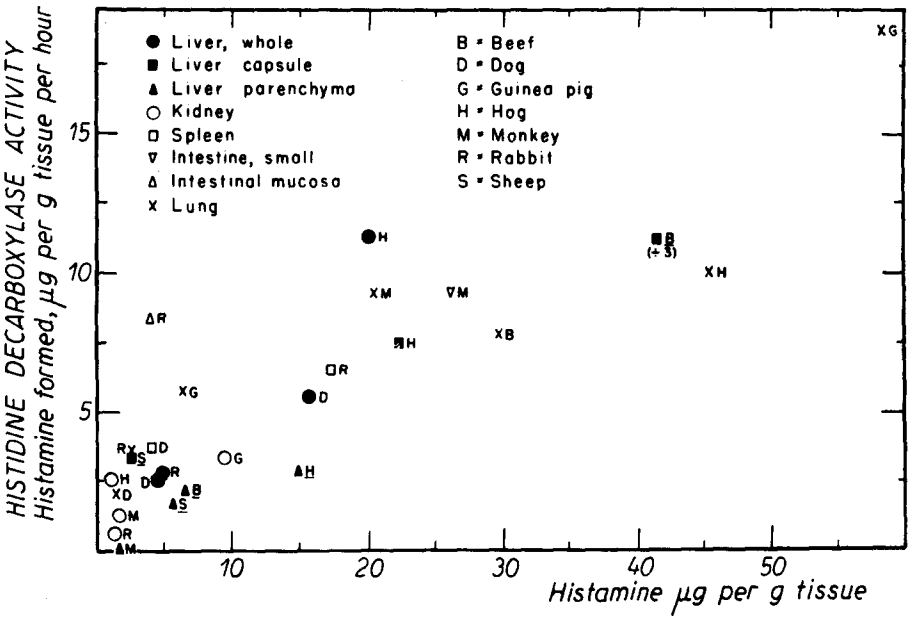

Fig. 1. Histamine content and histidine decarboxylase activity of mammalian tissues. Each point on the diagram represents one measurement or the average of all measurements (2–10 for histamine content, 2–6 for decarboxylase activity) of each parameter on a tissue from one animal. The values plotted are for free histamine, and have not been corrected for the insignificant contribution of non-histamine interfering substances in the homogenates. Underlining of the species letters indicates that the liver capsule and parenchyma homogenates came from the same liver, different from the liver used for any other liver homogenate represented. Both values for beef liver capsule have been divided by 3 in order to include this point in the figure. The values for dog lung, kidney, muscle and heart and for guinea pig liver so nearly coincide that these tissues are all represented by the point labelled dog lung. Similarly the point labelled rabbit kidney represents rabbit liver and beef liver parenchyma also. The point labelled hog kidney represents beef kidney and one hog kidney, while that labelled monkey kidney represents monkey kidney and another hog kidney.

these differences of ratio, the following three additional sets of measurements have been made on a large number of the homogenates represented in the figure.

(1). In order to discover histamine stored in such a way that trichloroacetic acid does not liberate it in active form, homogenates given no prior treatment as well as homogenates that had been incubated with histidine, were subjected to acid hydrolysis. Data from the experiments that were complete and satisfactory have been collected in Table I. As may be seen in the table, the content of combined histamine was found to be of the same order as that of free histamine in dog liver; a similar situation occurs in the lungs of dog, guinea pig and monkey (not included in the table). In all the other tissues investigated, the content of combined histamine was considerably less than the content of free histamine, and in certain tissues it was so low as to be near or below the limit of measurement. The amount of combined histamine produced during incubation with histidine may be more or less than the amount originally present; the extreme values of the ratio between these two quantities are found in the data for dog liver and for rabbit intestinal mucosa. The amount of combined histamine produced during incubation is usually less than the amount of free histamine produced; the process of combination thus seems to lag behind that of

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decarboxylation under the experimental conditions. As would be expected, decarboxylase exercises only remote control over the production of combined histamine.

Since the combined histamine measured here includes both histamine extracted in masked form by trichloroacetic acid and histamine precipitated with the proteins in the unhydrolyzed homogenate, a few hydrolysis experiments were carried out on the trichloroacetic acid extracts. It is evident from the scattered data so obtained that the acid-hydrolyzable histamine contained in different homogenates varies considerably in its make-up. As much as 90% or more of it or as little as 10% of it was recovered by acid-hydrolysis of the trichloroacetic acid extract from different tissues. This then is the fraction of combined histamine that is in the masked form referred to above.

TABLE I

FREE AND COMBINED HISTAMINE OF TISSUES

All values for each tissue obtained in the same experiment. Histamine assays of: (1) untreated homogenate, (2) hydrolyzed homogenate, (3) homogenate after incubation with histidine, (4) homogenate hydrolyzed after incubation with histidine. Figures in column *a* calculated directly from assay (1), in column *b* obtained by difference between assays (2) and (1), in column *c* by difference between assays (3) and (1), in column *d* by difference between assays (4) and (3). Conditions of incubation and hydrolysis described under METHODS.

	Original content $\mu g/g$ *tissue*		*Increase during incubation* $\mu g/g$ *tissue-hour*	
	Free *a*	*Combined* *b*	*Free* *c*	*Combined* *d*
Beef				
liver capsule	123.0	16.0	27.0	19.0
liver parenchyma	3.7	1.5	0.4	1.3
Dog liver	14.0	17.0	6.7	0.3
Guinea pig kidney	8.9	3.8	3.8	1.3
Hog				
liver capsule	21.0	3.8	3.8	0.6
liver parenchyma	15.0	2.7	1.9	0.1
Rabbit				
intestinal mucosa	3.8	0.3	8.0	2.7
kidney	1.4	0.2	0.4	0.6
lung	2.1	1.3	3.4	1.7
Sheep				
liver parenchyma	5.0	1.3	1.7	0.8

(2). The method of chemical assay for histamine is not completely specific. In the case of erythrocytes certain non-histamine substances (presumably chiefly spermine and spermidine) not susceptible to destruction by hog kidney histaminase react like histamine in the method[7]. Therefore the amount of enzyme-susceptible material was determined in the tissue homogenates (untreated, incubated with histidine *or* hydrolyzed, incubated *and* hydrolyzed). In contrast to erythrocytes, in which virtually none of the apparent histamine is susceptible to histaminase, practically all (90% or more) of the original free histamine in the tissue homogenates was destroyed

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by the enzyme. Furthermore, no convincing evidence of significant amounts of non-histamine material was found after incubation of the homogenates with histidine and/or after hydrolysis. No information at present available indicates that interference by non-histamine substances accounts for the divergent values of the ratio content to histamine produced.

(3). In spite of the difficulties of measuring histaminase activity in crude homogenates (see above under METHODS), rough estimates of this activity have been made for a number of tissues. The lowest substrate concentration covered a wide range in the series of homogenates used, since it was always 1/25 of the histamine concentration of the tissue. Many of the tissues showed little or no histaminase activity at this substrate level, and what activity they had frequently disappeared at the next higher concentration tried (usually 3–6 μM above the first concentration). This weak and easily inhibited enzyme activity was manifest in homogenates of dog liver and spleen, hog and monkey lung, both specimens of guinea pig lung, hog liver and its capsule, rabbit kidney and liver; for this group of tissues (except the histamine-rich specimen of guinea pig lung), the substrate concentrations used ranged between 2.5 and 15 μM. Little activity but no evidence of inhibition was also observed in rabbit lung. The greatest activity observed was that of beef liver capsule which destroyed 0.35 μM (39 μg) of histamine per gram per hour in 44 μM substrate. The activity was reduced 25% by increasing the substrate concentration to 56 μM. Next below beef liver capsule in activity came a group of tissues, each destroying about 0.14 micromoles per gram per hour at about 14 μM substrate concentration; this group consisted of beef liver parenchyma, hog kidney, monkey intestine and rabbit intestinal mucosa.

DISCUSSION

The conclusion drawn from this survey is that histidine decarboxylase shares the well-known and here re-emphasized vagaries of distribution of histamine. The observed degree of coincidence of the enzyme and its product is quite surprising in the complex biological systems investigated. For the cases of apparent deviation from the general relationship, various explanations are available. For instance, rather high histaminase activity in rabbit intestinal mucosa probably contributes to the unusually great deviation of the point representing this tissue from the line formed by most of the points in Fig. 1. Guinea pig lung contains a high enough content of combined histamine to account for the position of its point to the left of the line.

Earlier observations on the distribution of histidine decarboxylase activity have been few in number, qualitative rather than quantitative, and usually not related to histamine content[13]. An exception to this latter qualification is found in the work of WERLE AND ZEISBERGER on the histidine decarboxylase activity and histamine content of different portions of the gastric mucosa[14]. They came to the conclusion that there was no parallelism in the distribution of the enzyme and histamine within this tissue.

In contrast to histidine decarboxylase, histaminase seems to be distributed in the body without regard to tissue histamine content. This has been indicated by earlier observations of many workers, and is borne out by the results reported here. It is hardly surprising that this enzyme, which is believed to be identical with diamine

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oxidase and therefore to act on other substrates besides histamine, should be distributed independently of the distribution of histamine.

The order of decreasing histaminase activity of the rabbit tissues reported here is: intestinal mucosa $\gg$ lung $>$ kidney, liver, while that reported by COTZIAS AND DOLE[15] for these same tissues is: lung $\gg$ liver $>$ kidney $>$ intestinal mucosa. The differences are probably due to differences in experimental conditions: COTZIAS AND DOLE measured the ammonia produced at 10° C by dialyzed homogenates containing relatively high concentrations of added histamine. Neither set of results fits the summary statement of ZELLER[16] that in general the mammalian tissues with the highest diamine oxidase activity are kidney, intestinal mucosa, liver and lungs, in order of decreasing activity.

Dog liver, beef liver capsule and the lungs of various species, notably the guinea pig, have been found richer in combined histamine than any of the other tissues examined in this series. The concentration of combined histamine in these tissues is in general not greater than that in guinea pig skin as reported by JOHNSON[17], but their free histamine content is much greater. The significance of the presence of large amounts of combined histamine is not by any means clear at the present time, but it is interesting that most of the tissues in which it has been reported are known to be the site of marked reactions in histaminic and allergic shock.

The results reported here are entirely compatible with those of SCHAYER[4], but suggest a different interpretation of his observation of urinary excretion of radioactive histamine following administration of radioactive histidine. Because of this urinary excretion, he expressed the opinion that most of the histamine is formed in cells unable to bind it. However, it is now clear that the tissues that retain histamine most successfully are the very ones that decarboxylate histidine most actively, and the parallelism evidently holds down to the tissues with little of either function. Because of this fact it now appears that the urinary excretion of histamine observed by SCHAYER is due, not to lack of binding capacity at the site of decarboxylation, but to repletion of the binding capacity at this site, the only capacious site of binding that is as yet known in the body.

The hypothesis that heparin is a constituent of mast cells has been accepted for some time, and over the last few years the evidence that most of the body histamine is localized in the same cells has become more and more convincing. It has frequently been suggested that these two mast cell constituents enter into some sort of combination with each other. Now it appears that the enzyme producing histamine may also be located in the mast cells, since this is the simplest explanation of the conformity of the distribution of the enzyme to that of histamine in the body. Possibly decarboxylation of histidine to form histamine might prove to be the characteristic process occurring in mast cells, peculiar to these cells and indirectly responsible for the accumulation of heparin there and for other special properties of the cell. Whatever the relationship of the decarboxylation to the other processes taking place in the cell, the importance of the cell in histamine metabolism is becoming more and more evident; possessing the means of producing histamine and of storing it, it also has the fragility required for discharging its load of histamine on call. Moreover its location in the skin and along blood vessels is such as would seem desirable for delivering that load where it might be needed.

SUMMARY

1. The histidine decarboxylase activity of mammalian tissues has been estimated by measuring the increase in histamine content of tissue homogenates during incubation in a histidine-containing solution. The incubation was carried out at pH 9.2 in the presence of a strong reducing agent to prevent histaminase activity. The histidine decarboxylase activity so measured varied over a hundred-fold range in the tissues investigated.

2. The histidine decarboxylase activity of tissues varied in general in proportion to the histamine content of the tissues. Since there is strong evidence that histamine is localized almost exclusively in mast cells, these are probably also the locus of histidine decarboxylase.

3. The distribution of histaminase activity was found not to be related either directly or inversely to the histamine content of tissues.

4. The amount of combined histamine in the tissues was less than the amount of free histamine and bore little relation to the amount of combined histamine produced during incubation with histidine.

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