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STUDIES ON MAMMALIAN HISTIDINE DECARBOXYLASE

BY

N. G. WATON*

From the Department of Pharmacology, University of Edinburgh

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Histamine is present in most mammalian tissues, but its mode of formation is still not clear. According to Blaschko (1945) there are two main theories: (1) Histamine is a vitamin, formed outside the body by bacterial decarboxylation of dietary histidine in the alimentary tract. (2) Histamine is a metabolite, formed from circulating histidine by the histidine decarboxylase present in some tissues of the body.

That bacteria form histamine by decarboxylation of histidine is well known (Ackermann, 1910, 1911; Berthelot and Bertrand, 1912; Mellanby and Twort, 1912; Kendall and Gebauer, 1930; Matsuda, 1933; Gale, 1940; Epps, 1945). Gale (1953) showed that the bacterial enzyme had several important differences from the other amino acid decarboxylases which had been studied.

The occurrence of a mammalian histidine decarboxylase was demonstrated by Werle (1936) and by Holtz and Heise (1937a). They showed that, after rabbit kidney had been incubated with histidine in buffered Tyrode solution, histamine was present in the supernatant fluid obtained. Werle and co-workers (Werle, 1936, 1940, 1942; Werle and Herrmann, 1937; Werle and Krautzun, 1938; Werle and Heitzer, 1938; Werle and Daumer, 1940; Werle and Koch, 1949), together with Holtz and co-workers (Holtz and Heise, 1937a, 1937b; Holtz, Heise and Spreyer, 1938; Holtz, Credner and Reinhold, 1939; Holtz and Credner, 1941, 1944) carried out extensive investigations upon the distribution of this enzyme in various tissues of different animal species and investigated many of its physical and chemical properties. They were unable to isolate the enzyme, though Werle and Heitzer (1938) claim to have achieved a partial purification. More recently, evidence of a mammalian histidine decarboxylase has been obtained by Schayer (1952), who injected guinea-pigs with ^{14}C-labelled histidine and histamine. But these studies do not conclusively demonstrate the general occurrence of an enzyme capable of decarboxylating histidine in all mammals, as the experiments were confined to a limited range of mammalian species.

The properties and the distribution in laboratory animals of mammalian histidine decarboxylase, together with the distribution of histaminase and histamine, have been reinvestigated in the hope of clarifying our knowledge of the role of histamine in the organism.

METHODS

Formation of Histamine from Histidine by Mammalian Tissues

Rabbit kidneys, which are a rich source of histidine decarboxylase, were placed in 0.9% w/v NaCl, freed from all extraneous tissue, cut small and minced in a Latapie mincing machine. Where a tissue extract was required, the minced kidney was ground for 10 min. in a porcelain mortar with 1 g. silver sand and 10 ml. 0.9% w/v NaCl/g. tissue. This was centrifuged at 2,500 *g* for 10 min. and the supernatant fluid extract removed for incubation.

Generally 0.4 g. minced tissue in 6 ml. Tyrode solution was used as a source of the enzyme. Occasionally this was replaced by 4 ml. tissue extract plus 2 ml. Tyrode solution. The mixture of tissue and Tyrode solution was placed in a 25 ml. conical flask, 3 ml. M/5-Na_2HPO_4 (*p*H 8.2) and 1 ml. neutralized histidine (15 mg./ml.) were added, and the mixture incubated at 38° C. for 3 hr. with mechanical agitation.

After incubation, 1N-HCl was added from a microburette until a *p*H of 5.5–5.9 was obtained. The mixture was boiled for 1 min. to coagulate proteins and prevent further enzyme action. After cooling, the contents of the flask were filtered through Whatman No. 1 paper.

The histamine content of the filtrate was assayed on the guinea-pig's isolated ileum suspended in a 2 ml. bath of aerated Tyrode solution, with atropine, at 34° C.

Duplicate experiments were always carried out on each tissue mince or extract, and often the experiments were repeated with tissue from another animal. Blanks were carried out to estimate the histamine present in the incubation mixtures in the absence of the tissue, and again in the absence of the histidine solution.

In reporting the results, the average histamine content is given. The largest discrepancy between duplicates among 200 estimates was less than 20%.

*Present address: Department of Materia Medica and Therapeutics, University of Glasgow.

Potentiation or Inhibition of Histidine Decarboxylase

In these experiments 1 ml. Tyrode solution in the standard incubation mixture was replaced by 1 ml. aqueous solution of the test substance before incubation, and 1 ml. distilled water was added after incubation (T). To estimate histamine formation in the absence of the test substance, and to demonstrate any effect which the latter might have on the gut, a control incubation (C_1), in which 1 ml. distilled water was added before incubation and 1 ml. test solution was added after incubation, was carried out. Any difference observed between the results of the test incubation and the control (C_1) must therefore be due either to an effect of the test substance on the enzyme, or to the test substance causing a direct release of histamine from the tissue.

To determine if there was histamine release, a second control mixture (C_2) was prepared, in which 1 ml. distilled water was added in place of 1 ml. histidine solution.

The percentage inhibition or potentiation of the histidine decarboxylase was calculated from the formulae:

$$\% \text{ Inhibition} = 100 - \left(\frac{T - C_2}{C_1}\right) \times 100$$

$$\% \text{ Potentiation} = \frac{T - C_2}{C_1} \times 100$$

Inhibition of the Histaminase Activity of Cat Kidney

Cat kidney was used as a source of histaminase. An extract containing 20 mg. fresh tissue/ml. was prepared by the method described for rabbit kidney. Incubation mixtures (T') consisted of 4 ml. kidney extract+1 ml. Tyrode solution+3 ml. M/3-sodium phosphate buffer pH 7.2 (glass electrode)+1 ml. histamine solution containing 20 μg. base/ml.+1 ml. solution of test substance. The mixtures were incubated for 1 hr. at 38° C., after which 1 ml. distilled water was added, and the histamine content determined, after acidification and boiling. This gave a measure of the histamine destruction in the presence of the test substance. A control sample (C'_1) in which the test substance was omitted during the incubation period, but was added later, gave an estimate of the destruction of histamine in the absence of the test substance. A second control sample (C'_2), containing boiled kidney extract, gave the amount of histamine in the absence of enzyme activity. The percentage decrease of histaminase activity was calculated from the formula:

$$\% \text{ Decrease of activity} = \frac{T' - C'_1}{C'_2 - C'_1} \times 100$$

Inhibition of Histaminase Activity of a Hog Kidney Preparation

The experiment on the histaminase activity of cat kidney was repeated with a different histaminase preparation. This was made by shaking 7.5 mg. purified acetone dried powder of hog kidney with 20 ml. M/3-sodium phosphate buffer of pH 7.2.

Tissue Distribution of Histidine Decarboxylase, Histaminase, and Histamine

The histidine decarboxylase content of the tissue under test was determined by mincing the tissue and incubating it with histidine in buffered Tyrode solution for 3 hr. in the presence of 0.1 ml. 10^{-3} aminoguanidine solution and 1 drop (20 mg.) benzene. Otherwise the procedure was similar to that described for rabbit kidney tissue.

The histaminase content of the tissue was determined by incubating the minced tissue with 1 ml. histamine solution (20 μg./ml.) in buffered Tyrode solution. The procedure was similar to that described for cat kidney tissue, except that the mixture was incubated for 3 hr. Histaminase activity was estimated from the sum: histamine added plus histamine already present in the tissue, minus histamine found after incubation.

The histamine content of the minced tissue was obtained from samples which were added to Tyrode solution, acidified and boiled in the usual manner.

Cellular Distribution of Histidine Decarboxylase, Histaminase, and Histamine

Minced rabbit kidney was used as the source of histidine decarboxylase, minced rabbit lung as the source of histaminase and histamine. These were homogenized in a Potter–Elvjerheim homogenizer with 0.25M-sucrose solution. The homogenized mixtures were then subjected to differential centrifugation, as described by Schneider and Hogeboom (1950). Each homogenate was centrifuged at 2,500 g for 10 min.; this deposits unbroken cells, nuclei and large cell fragments. The kidney extracts were then centrifuged at 37,000 g for 20 min., to deposit the mitochondria, and finally at 90,000 g for 60 min. to deposit the microsomes. The lung extracts were centrifuged at 37,000 g for 60 min. in order to deposit all cell fragments. The final supernatant fluid

TABLE I

HISTAMINE PRODUCTION FROM HISTIDINE BY RABBIT KIDNEYS IN THE PRESENCE OF HISTIDINE AFTER INCUBATION FOR 3 HR. AT 38° C.

Each result is the mean of duplicate incubations.

Rabbit Number	Histamine Formed (μg./g.) with:		Histamine Formed μg./g. Wet Tissue
	Histidine Present	Histidine Absent	
1	17·1	0·1	17·0
2	5·3	0·1	5·2
3	17·6	0·8	16·8
4	22·8	0·8	22·0
5	3·6	0·2	3·4
6	1·4	0·2	1·2
7	11·8	0·5	11·3
8	11·8	<0·1	11·8
9	22·8	0·5	22·3
10	64·0	1·0	63·0
11	31·5	0·5	31·0
12	33·2	0·2	33·0
13	12·5	0·5	12·0
14	20·8	<0·1	20·8
15	2·3	<0·1	2·3
16	7·2	0·2	7·0
17	5·5	<0·1	5·5
18	6·0	0·2	5·8
19	3·5	0·1	3·4
20	5·2	0·2	5·0

TABLE II

INHIBITION OF THE HISTIDINE DECARBOXYLASE ACTIVITY OF RABBIT KIDNEY BY VARIOUS SUBSTANCES

Each result is the mean of duplicate incubations

Concentration:	10^{-3}M			10^{-3}M			10^{-4}M		
Substance	T–C_2	C_1	% Inhibition	T–C_2	C_1	% Inhibition	T–C_2	C_1	% Inhibition
B_1 pyrimidine	6·1	6·5	nil	7·9	8·2	nil	7·8	8·1	nil
Putrescine hydrochloride	7·5	7·0	nil	3·4	3·3	nil	3·3	3·2	nil
Cadaverine dihydrochloride	10·4§	10·7§	nil	9·6	9·4	nil	9·2	9·3	nil
Agmatine sulphate	21·4§	20·8	nil	20·0§	20·0§	nil	18·0§	18·2§	nil
Glyoxaline				5·9	6·1	nil	6·1	6·0	nil
Guanidine carbonate				6·7	6·4	nil	6·7	6·4	nil
Methylguanidine sulphate				5·9	5·9	nil	6·1	5·9	nil
Asym. dimethylguanidine sulphate				6·7	6·4	nil	6·2	6·2	nil
Aminoguanidine bicarbonate				3·7	6·1	40	6·4	6·4	nil
*Iproniazid ("Marsilid")				1·2	8·8	86	7·0	8·5	17
† Girard's reagent "T"				2·1	5·9	66	4·8	6·0	21
‡ Girard's reagent "P"				1·7	6·0	72	3·9	5·9	32
Hydrazine sulphate				nil	6·1	100	nil	6·0	100

*=1-*iso*Nicotinoyl-2-*iso*propylhydrazine. †=Trimethylammonium acetohydrazide chloride. ‡=Pyridinium acetohydrazide chloride.
§=Potentiates responses of histamine on gut.

contained only substances in solution or colloidal particles. Each supernatant fluid extract and residue was tested for histidine decarboxylase and histaminase activity and histamine content.

RESULTS

Normal Formation of Histamine from Histidine by Rabbit Kidneys

The amounts of histamine formed by the kidneys of 20 rabbits are given in Table I; further results will be found in Table V.

The results show the wide variation of histidine decarboxylase activity in the kidneys of different rabbits, the average being 15.0 μg./g. wet tissue within the limits 1.4–63.0. From these results, it will be obvious that no valid comparisons can be made unless in each experiment the test and blank incubations contain tissue from the same animal.

Substances Potentiating or Inhibiting Histidine Decarboxylase

Several substances were incubated with rabbit kidney tissue and histidine, and the results compared with those obtained in the absence of the test substance. The difference between the results gives a measure of the potentiating or inhibitory activity of the substance. In order to demonstrate the presence of histidine decarboxylase in the presence

TABLE III

HISTAMINE (μG.) IN INCUBATION MIXTURES, AFTER INCUBATION WITH CAT KIDNEY EXTRACTS AND A TEST SUBSTANCE

The % inhibition of histamine destruction is also given. Each result is the mean of duplicate incubations.

Concentration:	C'_2 μg. Hist-	C'_1 μg. Hist-	10^{-7}M		10^{-6}M		10^{-5}M		10^{-4}M		10^{-3}M	
Test Substance	amine	amine	T'	% Inhibition	T'	% Inhibition	T'	% Inhibition	T'	% Inhibition	T'	% Inhibition
Iproniazid	20·7	2·5	2·4	*0*	2·4	*0*	2·4	*0*	3·9	*8*	11·4	*49*
Glyoxaline	20·0	1·0	0·7	*0*	—	—	—	—	—	—	—	—
	20·7	2·5	—	—	7·8	*29*	—	—	—	—	—	—
	19·3	9·4	—	—	—	—	14·7	*53*	19·3	*100*	18·0	*87*
Methylguanidine sulphate	20·0	1·0	1·1	*0*	—	—	—	—	—	—	—	—
	20·7	2·5	—	—	5·8	*18*	—	—	—	—	—	—
	19·3	9·4	—	—	—	—	13·6	*42*	16·8	*75*	19·3	*100*
Asym. dimethylguanidine sulphate	20·0	1·0	1·0	*0*	—	—	—	—	—	—	—	—
	20·7	2·5	—	—	7·3	*26*	—	—	—	—	—	—
	19·3	9·4	—	—	—	—	14·7	*53*	17·9	*86*	19·3	*100*
Aminoguanidine bicarbonate	20·0	1·0	8·1	*32*	10·5	*50*	20·0	*100*	20·0	*100*	20·0	*100*
Guanidine carbonate	20·0	1·0	1·1	*0*	1·3	*2*	—	—	—	—	—	—
	19·3	9·4	—	—	—	—	10·3	*9*	12·6	*32*	16·8	*76*
Hydrazine sulphate	20·7	2·5	15·1	*69*	19·6	*94*	20·4	*99*	20·8	*100*	20·7	*100*
Girard's reagent "P"	20·7	2·5	2·4	*0*	4·9	*13*	11·5	*50*	15·8	*73*	20·6	*100*
Girard's reagent "T"	15·0*	1·4	1·4	*0*	2·6	*9*	4·7	*22*	10·3	*59*	13·9	*83*
B_1 pyrimidine	20·0	1·6	4·1	*13*	5·4	*20*	10·8	*50*	16·0	*76*	18·0	*96*
Putrescine hydrochloride	20·0	1·6	1·6	*0*	1·8	*0*	2·6	*5*	4·5	*15*	11·0	*51*
Cadaverine dihydrochloride	20·0	1·6	1·6	*0*	1·6	*0*	1·6	*0*	5·3	*20*	7·4	*31*
Agmatine sulphate	20·0	1·6	1·5	*0*	1·8	*0*	5·2	*15*	9·1	*40*	16·0	*76*

* 15 μg. histamine added.

of histaminase a search was made for an antihistaminase which would fail to inhibit, and if possible potentiate, histidine decarboxylase. Several known inhibitors of histaminase were investigated, such as putrescine (butane-1 : 4 diamine), and cadaverine (pentane 1 : 5 diamine), previously tested for antihistaminase activity by Werle (1942); B_1 pyrimidine (6 - amino - 5 - aminomethyl - 2 - methylpyrimidine), agmatine (1-amino-4-guanidobutane), guanidine, methyl guanidine, and dimethyl guanidine, all of which were tested by Arunlakshana, Mongar, and Schild (1954). Schuler (1952) tested many compounds as histaminase inhibitors amongst which the most active were amino-guanidine and hydrazine sulphate. Both these were included as well as Girard's reagent " P " (pyridinium-aceto-hydrazide chloride) and Girard's reagent " T " (trimethylamino-aceto-hydrazide chloride), since, from the early work of Werle and his co-workers, they appeared to be likely to behave as selective histaminase inhibitors.

Table II shows that, with the exception of iproniazid (1-*iso*nicotinoyl-2-*iso*propylhydrazine), Girard's reagents " P " and " T," and hydrazine sulphate (which caused marked enzyme inhibition), none of the substances tested inhibited histidine decarboxylase in the concentrations used. It also shows that none of these substances potentiated histidine decarboxylase. Those compounds which had no inhibitory action on histidine decarboxylase were then tested for inhibitory activity on histaminase, with the object of finding a substance which, at a given concentration, would inhibit histaminase completely without affecting histidine decarboxylase.

In Table III the effects of various chemicals upon histaminase activity are given. These results are summarized in a combined table (Table IV) which also includes the effects of these substances upon histidine decarboxylase (see Table II). Methyl guanidine sulphate (10^{-3}M), asym. dimethyl guanidine sulphate (10^{-3}M), aminoguanidine bicarbonate (10^{-4} and 10^{-5}M), caused 100% inhibition of the histaminase activity of cat's kidney extracts, but did not inhibit the histidine decarboxylase activity of rabbit's kidney. Aminoguanidine (10^{-4}M) was therefore chosen as an inhibitor of histaminase activity in further experiments designed to study the distribution of histidine decarboxylase in animal tissues.

The Effect of Organic Solvents on Histidine Decarboxylase

During experiments involving incubation times of 8 hr. or more, one drop (20 mg.) chloroform was added to prevent the growth of bacteria. The added chloroform considerably increased the pro-

TABLE IV

SUMMARY OF THE INHIBITORY ACTION OF VARIOUS SUBSTANCES ON THE HISTIDINE DECARBOXYLASE ACTIVITY OF RABBIT KIDNEY AND ON THE HISTAMINASE ACTIVITY OF CAT KIDNEY

Compound	Molar Concentration	% Inhibition of Histamine Destroying Activity	% Inhibition of Histidine Decarboxylase Activity
Hydrazine sulphate	10^{-3}	100	100
	10^{-4}	100	100
	10^{-5}	100	
	10^{-6}	94·0	
	10^{-7}	69·0	
Girard's reagent " P "	10^{-3}	100	75
	10^{-4}	73·4	37
	10^{-5}	50·2	
	10^{-6}	13·0	
	10^{-7}	0	
Girard's reagent " T "	10^{-3}	83·5	66
	10^{-4}	59·2	21
	10^{-5}	22 0	
	10^{-6}	8·0	
	10^{-7}	0	
B_1 pyrimidine	10^{-3}	96	0
	10^{-4}	76	0
	10^{-5}	50	0
	10^{-6}	20 6	
	10^{-7}	13·5	
Putrescine hydrochloride	10^{-3}	51	0
	10^{-4}	15·7	0
	10^{-5}	5·5	
	10^{-6}	0	
	10^{-7}	0	
Cadaverine dihydrochloride	10^{-3}	31·4	0
	10^{-4}	20	0
	10^{-5}	0	
	10^{-6}	0	
	10^{-7}	0	
Agmatine sulphate	10^{-3}	76	0
	10^{-4}	40·7	0
	10^{-5}	14·9	
	10^{-6}	0	
	10^{-7}	0	
Glyoxaline	10^{-3}	87	0
	10^{-4}	100	0
	10^{-5}	53	
	10^{-6}	29·2	
	10^{-7}	0	
Guanidine carbonate	10^{-3}	76	0
	10^{-4}	33	0
	10^{-5}	9	
	10^{-6}	2	
	10^{-7}	0	
Methylguanidine sulphate	10^{-3}	100	0
	10^{-4}	75	0
	10^{-5}	42	
	10^{-6}	18·1	
	10^{-7}	0	
Asym. dimethylguanidine sulphate	10^{-3}	100	0
	10^{-4}	86	0
	10^{-5}	53	
	10^{-6}	26·4	
	10^{-7}	0	
Aminoguanidine bicarbonate	10^{-3}	100	40
	10^{-4}	100	0
	10^{-5}	100	
	10^{-6}	50	
	10^{-7}	32	
Iproniazid	10^{-3}	49·2	86
	10^{-4}	8·2	17
	10^{-5}	0	
	10^{-6}	0	
	10^{-7}	0	
Chloroform	10^{-3}	Nil (Table VI)	Potentiation 300–400%
Benzene	10^{-3}	Nil (Table VI)	Potentiation 600–1,000%

TABLE V

EFFECT OF CHLOROFORM, BENZENE, TOLUENE AND AMYL ALCOHOL ON THE PRODUCTION OF HISTAMINE FROM HISTIDINE BY RABBIT KIDNEY TISSUE

Each result is the mean of duplicate incubations. Histamine produced expressed as μg. base/g. wet tissue.

	Organic Solvent Added Before Incubation (T')	Organic Solvent Added After Incubation (C'_1)	Organic Solvent Added Before Incubation. No Histidine (C'_2)	Ratio (T'/C'_1)
Chloroform alone				
	19·7	6·7	0·8	2·98
	34·4	10·8	0·5	3·19
	6·8	1·6	0·1	4·2
	11·1	3·2	0·2	3·5
Chloroform and benzene on the same tissue				
Chloroform ..	0·97	0·25	0·01	4·0
Benzene ..	10·0	0·25	0·01	40·0
Chloroform ..	24·9	7·6	0·4	3·5
Benzene ..	56·9	7·6	0·5	7·9
Chloroform ..	36·4	13·5	0·3	2·7
Benzene ..	99·6	13·5	0·2	7·4
Benzene alone				
	114·0	31·2	0·7	3·8
	6·7	1·25	0·3	4·7
	88·6	13·2	0·3	6·7
	15·4	1·2	0·05	12·5
Toluene, benzene and chloroform on the same tissue				
Toluene ..	19·7	2·2	0·1	9·0
Benzene ..	27·6	2·2	0·1	12·5
Chloroform ..	9·0	2·2	0·1	4·1
n-Amylalcohol, benzene and chloroform on the same tissue				
n-Amyl alcohol ..	15·0	13·6	0·2	1·1
Chloroform ..	36·4	13·5	0·2	2·7
Benzene ..	99·6	13·5	0·4	7·4

duction of histamine. A series of experiments was therefore carried out, in which one drop (approximately 20 mg.) of chloroform, of benzene, of toluene, or of amyl alcohol, replaced the 1 ml. aqueous solution of the test substance.

The results, summarized in Table V, show that chloroform, benzene and toluene potentiate histidine decarboxylase, and that the effect is on the *formation* of histamine, and is not due to *release* of histamine. Benzene increased the production of histamine some 7-fold within the limits 4 to 40; whereas chloroform increased the production 3- to 4-fold. Toluene was almost as active as benzene, whilst amyl alcohol had no significant action. It was therefore decided to use benzene to increase histamine production in all experiments involving histidine decarboxylase activity.

Some experiments were next carried out to determine the nature of the benzene effect, which was shown to a varying degree by a large number of organic solvents. The results will form the subject of another paper.

Inhibition of the Histaminase Activity of an Acetone-dried, Purified Powder of Hog's Kidney

Since there is evidence that more than one enzyme in tissues may destroy histamine (Kapeller-Adler, 1949; Zeller *et al.*, 1953; Schayer, 1953a, b), the most active substances in Table III were also tested for their action on an acetone-dried purified powder from hog's kidney. The results (Table VI) were similar to those obtained with cat's kidney (Table III) and do not therefore suggest any difference between the enzymes obtained from the kidneys of cats and of hogs.

Distribution of Histidine Decarboxylase, Histaminase, and Histamine in the Tissues of Various Species

Having obtained a substance which would potentiate histidine decarboxylase (benzene) and another which would inhibit histaminase (aminoguanidine) it was felt that a more accurate knowledge of histidine decarboxylase distribution could be obtained by using benzene and aminoguanidine in experiments designed to show the histidine decarboxylase activity of various tissues.

The results given in Table VII, and summarized in Table VIII, have been obtained from duplicate experiments on separate animals or groups of animals. The results for histidine decarboxylase activity refer to the histamine formed per g. tissue

TABLE VI

INHIBITION OF THE HISTAMINASE ACTIVITY OF HOG'S KIDNEY

Each result is the mean of duplicate incubations. The figures in parentheses are the corresponding results for the test against the histamine-destroying activity of the cat's kidney.

Test Substance	Final Molar Concentration	μg. Histamine Remaining after Incubation: With Test T'	Without Test C'_1	Histamine Blank C'_2	% Inhibition of Histaminase Activity $\frac{T'-C'_1}{C'_2-C'_1}\times 100$
Putrescine hydrochloride*	10^{-2}	10·3	5·4	10·1	100
	10^{-3}	7·8	5·1	9·9	56 (51)
Cadaverine dihydrochloride*	10^{-2}	9·9	5·4	10·1	100
	10^{-3}	7·2	5·1	9·9	44 (31·4)
Agmatine sulphate*	10^{-2}	10·5	5·4	10·1	100
	10^{-3}	8·9	5·1	9·9	80 (76)
Aminoguanidine bicarbonate†	10^{-4}	19·1	10·2	19·3	100 (100)
Guanidine carbonate†	10^{-4}	14·9	10·2	19·3	52 (32)
Asym. dimethylguanidine sulphate†	10^{-4}	16·1	10·2	19·3	65 (86)
Iproniazid†	10^{-3}	14·4	10·2	19·3	46 (49·2)
Histidine monohydrochloride*	$1·5\times 10^{-3}$	4·4	4·4	10·2	0
Benzene*	2×10^{-3}	4·9	4·6	10·2	0
Benzene†	2×10^{-3}	9·1	10·2	19·3	0
Chloroform*	2×10^{-3}	4·7	4·9	10·1	0
Chloroform†	2×10^{-3}	9·3	9·6	19·3	0

* 10 μg. histamine added to incubation mixture.
† 20 μg. histamine added to incubation mixture.

TABLE VII

TISSUE DISTRIBUTION OF HISTIDINE DECARBOXYLASE, HISTAMINASE AND HISTAMINE

Each result is the mean of duplicate incubations. For each tissue the results of experiments on 2 animals are given. Values in each of the 3 parts of the Table are from the same 2 animals of each species.

	Kidney		Liver		Whole Stomach		Duodenum		Ileum		Colon		Lung		Pancreas	
Histidine decarboxylase activity (μg. histamine formed/g. tissue)																
Man *	0·2	1·1	0	1·8	0·1	0	0	0	0	0·6						
Dog	0	0	0	0	0	0·7	0	0	0	0			0	0		
Cat	0	0	0	0	0	0	0	0	0	0			0	0	0	0
Rabbit ..	114	23	17	15	2	14	0	1·1	1·9	7	0	0	0	0	0	0
Guinea-pig ..	81	27	25	12	6	5	77	15	31	20	1·5	1·1	0·6			
Rat	4	6	0·6	6	4	5	3	3	0	0	0·9	0	0			
Mouse	287	67	5	7	5	12	1	4	0	0	0	0	0			
Histaminase activity (μg. histamine destroyed/g. tissue)																
Man *	21	20	1·5	0	1·4	0	26	14	26	35						
Dog	>170	>250	12	4	0	0	30	35					6	3		
Cat	>170	>250	0	1	4	0·5	70	36	57	37			3	4	5	2
Rabbit	0	0	0	0·4	0	0	2	11	1·0	11	1·3	1·2	3	4	1·9	1·8
Guinea-pig ..	0·9	0	6	0	0·1	0·1	5	9	6	13	0·6	0·4	1·4			
Rat	1·1	0	0·8	0	7	0	10	26	15	32	10	4	0·6			
Mouse	0	0	0	0	0·9	0	4	20	20	0	4	2	1·2			
Histamine present (μg. histamine present/g. tissue)																
Man *	1	2	0·8	3	10	48	16	65	24	57						
Dog	0	0	14	8	42	24	30	37	25	33			84	61		
Cat	0	0	0	0	16	10	40	31	36	15			29	9	1·0	0
Rabbit ..	0·5	0·1	1·3	0·5	6	3	6	14	1·5	1·6	3	4	18	7	0	0
Guinea-pig ..	1·8	0·2	3	0·3	3	10	6	17	5	13	2	3	11			
Rat	0	0	1	0	37	29	5	16	11	26	9	4	16			
Mouse	4	0	0	0	5	10	1·5	0	1·1	0	0	0	0			

* 6 hr. *post-mortem* tissue. First died of acute bronchitis, age 49 years; 2nd died cardiac failure following prostatectomy, age 82 years.

TABLE VIII

THE HISTAMINE CONTENT AND DISTRIBUTION OF HISTIDINE DECARBOXYLASE AND HISTAMINASE ACTIVITIES OF ANIMAL TISSUES

The symbols have the following meanings:

−= <2 μg./g. tissue
+= 2— 10 „ „
++= 10— 30 „ „
+++= 30—100 „ „
++++=100—250 „ „
+++++= >250 „ „

	Man	Dog	Cat	Rabbit	Guinea-pig	Rat	Mouse
Histidine decarboxylase (histamine formed)							
Kidney	−	−	−	+++	+++	+	++++
Liver	−	−	−	++	++	−	+
Stomach	−	−	−	+	+	++	++
Duodenum	−	−	−	−	+++	+	+
Ileum	−	−	−	+	++	−	−
Colon				−	−	−	−
Lung		−	+	−	−	−	−
Skin, voluntary muscle, spleen and caecum. Nil.							
Histaminase (histamine destroyed)							
Kidney	+++	+++++	+++++	−	−	−	−
Liver	−	+	−	−	−	−	−
Stomach	−		−	−	−	−	−
Duodenum	++	+++	+++	++	−	++	+
Ileum	+++	+++	+++	+	+	+++	++
Colon				+	−	+	+
Lung		−	+	+	+	−	−
Skin, voluntary muscle, spleen and caecum. Nil							
Histamine (histamine naturally present)							
Kidney	−	−	−	−	−	−	−
Liver	+	+	−	−	−	−	−
Stomach	+++	−	++	++	+	++++	+
Duodenum	+++	+++	+++	++	++	+	−
Ileum	+++	+++	+++	+	+	++	−
Colon				+	+	+	−
Lung		+++	+++	++	++	++	−
In rabbit: spleen +++; caecum ++; skin, voluntary muscle, nil.							

after 3 hours' incubation. The results for histaminase activity are in terms of the amount of histamine destroyed per g. tissue after 3 hours; histamine content is per g. tissue before incubation.

The value of aminoguanidine as a histaminase inhibitor in the histidine decarboxylase experiments is evident; in nearly all the experiments in which histaminase, but not histidine decarboxylase, was present, no histamine was destroyed even after 3 hours' incubation.

Intracellular Distribution of Histidine Decarboxylase, Histaminase, and Histamine

High speed differential centrifugation was tried as a method to separate histidine decarboxylase from histaminase. This might be successful if the enzymes were contained in different particulate cell bodies. The intracellular distribution of histamine was also determined.

TABLE IX

HISTAMINE FORMED BY RABBIT KIDNEY TISSUE EXTRACTS SUBJECTED TO VARYING GRAVITATIONAL FORCES AND BY THE RESIDUES (*italic numerals*) FROM EACH CENTRIFUGATION

The results are expressed in μg. histamine formed/g. original tissue. Each result is the mean of duplicate incubations.

Original Minced Tissue	1st Centrifugation 2,500 *g*	2nd Centrifugation 37,000 *g*	3rd Centrifugation 90,000 *g*	Presence of Benzene
7·0	6·4	6·0	6·2	−
	0·8	*0·9*	*0·2*	
31·3	27·6	37·9	31·2	+
	5·2	*2·1*	*0·4*	
3·1	4·8	3·2	3·5	−
	0	*not done*	*0·2*	
17·5	16·5	not done	16·2	+
	1·3	*0·2*	*0·3*	

Histidine decarboxylase in rabbit kidney tissue (Table IX) and histaminase in rabbit lung tissue (Table X) are either contained in the cell sap, or pass readily into it, since cell free tissue extract subjected to gravitational forces of 90,000 *g* and 37,000 *g* were as active as minced tissue. Of the histamine contained in the rabbit's lung, 66% was present in the "mitochondrial" fraction, which contains most cell granules, while the rest was present in the supernatant fluid after centrifugation at 37,000 *g* for 1 hour. The histamine may have been liberated *post mortem* or may have been originally present in the cell sap. The results are summarized in Table XI.

TABLE X

HISTAMINE DESTROYED BY RABBIT LUNG TISSUE EXTRACTS SUBJECTED TO VARYING GRAVITATIONAL FORCES, AND BY THE RESIDUES (*italic numerals*) FROM EACH CENTRIFUGATION

The results are expressed as μg. histamine destroyed/g. original tissue/1 hr. Each result is the mean of duplicate incubations.

Original Minced Tissue	1st Centrifugation 2,500 *g*	2nd Centrifugation 37,000 *g*
3·2	3·4	2·7
	0	*0*
5·8	6·0	3·5
	0	*0*

TABLE XI

THE INTRACELLULAR DISTRIBUTIONS OF HISTIDINE DECARBOXYLASE IN RABBIT KIDNEY TISSUE AND OF HISTAMINASE AND HISTAMINE IN RABBIT LUNG TISSUE

The limits of several estimations are given in parentheses.

Cell Fraction	Histidine Decarboxylase Activity	Histaminase Activity	Histamine
Cell debris	5·8% (0–16·6)	nil (< 0·2%)	6·3% (2·9–9·7)
Mitochondria	2·1% (0–12·8)	nil (< 0·2%)	76·9% (60·2–93·6)
Microsomes	0·4% (0–1·4)	72·4% (60·4–84·4)	24·3% (19·4–29·3)
Supernatant fluid	95·8% (52–150)		

DISCUSSION

The present work confirms the results obtained by Werle and Holtz and their respective co-workers, and provides additional evidence of the occurrence in certain animal tissues of an enzyme capable of decarboxylating histidine and forming histamine. The method used is believed to be more accurate than that used by earlier investigators, who either partially removed histaminase by shaking with kaolin, or failed to remove or inhibit it.

Histidine decarboxylase is present in the kidney, liver, stomach and duodenum of the rabbit, guinea-pig, mouse and rat, and in the ileum of the rabbit and guinea-pig. It is absent from the colon, caecum, pancreas, spleen, lung, skin, and skeletal muscle of these species and is also absent from the corresponding tissues of the cat, dog, and man. In rodents, histaminase and histamine appear to be present together in the duodenum, ileum, lung and caecum and sometimes in the colon. Of these tissues, only the duodenum of each animal and the ileum of rabbits and guinea-pigs contained any histidine decarboxylase. The stomach in every species tested contained large quantities of histamine and some histidine decarboxylase, but no detectable histaminase. In cat, dog, and man, histidine decarboxylase was not found, but there was high histaminase activity in the kidney, duodenum, and ileum. Small amounts of histaminase were found in cat and dog lung and pancreas. No histamine was found in the kidneys of rodents, though the kidneys appear to be the chief histamine-forming organs in these species; histidine decarboxylase was absent from many tissues which contain histamine, especially those of

man, dog, and cat. This suggests that, if tissue histidine decarboxylase is the source of histamine in the body, then histamine must be transported to and stored by these tissues.

The enzyme appears to be in the cell sap, since a tissue extract subjected to a gravitational force of 90,000 *g* had an enzyme activity comparable to that of minced tissue (Table IX). This tissue extract would contain no particulate cell components. The enzyme appears to pass readily from the cells into the surrounding vehicle and its presence in any particulate fraction could not be demonstrated. About 66% of the total histamine found in rabbit lung tissue was present in the mitochondrial fraction. The remainder was found in the supernatant fluid after centrifugation at 37,000 *g*; this may have been liberated *post mortem*. This result agrees with the work of Copenhaver, Nagler, and Goth (1953), confirmed by Hagen (1954), which showed that most of the histamine in the dog liver is in the mitochondrial fraction.

The distribution of histidine decarboxylase (Table VII) in the cat, dog, rabbit, guinea-pig, mouse, and rat differs in some respects from that shown by Werle (1943), who records the presence of histidine decarboxylase in the kidney and liver of the cat. Werle's data are based on the work of Holtz and Heise (1937b) and Holtz, Heise, and Spreyer (1938), who incubated the tissues in the presence and absence of histidine and tested for histamine content on the cat's blood pressure. Their test and control samples differed in one important respect—one did not contain histidine. The authors do not seem to have excluded the possibility that the small effect which they observed may have been due to histamine present as an impurity in the sample of histidine used.

The presence of histidine decarboxylase in the guinea-pig has been conclusively demonstrated in several tissues by Werle and his co-workers, by Holtz and his co-workers, by Schayer (1952), and by me. It is therefore quite possible that systemic histidine is converted to histamine in the guinea-pig. It does not follow that histamine is formed from histidine in the tissues of all mammals; and, even in rodents, a local decarboxylation of histidine is not necessarily the only source of histamine.

Wilson (1954) demonstrated that inhibition of the intestinal bacterial flora of rats by orally administered antibiotics or sulphonamides causes a decrease in the urinary excretion of histamine. These experiments seem to support an alternative theory—namely, that the histamine in mammalian tissues originates from histamine absorbed from the intestine, where it is formed as a result of bacterial activity. The presence of histaminase in the intestinal wall has been advanced as evidence against this theory. However, some of the histamine formed in the intestine by bacteria may be combined with protein or other molecules (Rocha e Silva, 1943; Rocha e Silva and Andrade, 1943) and may be absorbed in the form of a complex compound, in a pharmacologically inactive but easily convertible form, which is not attacked by histaminase. Histaminase may prevent the entry only of free, pharmacologically active, histamine.

The present work supports the view that the histamine in the body tissues has been absorbed from the intestine; in man, cats, and dogs, the presence of histidine decarboxylase has not been demonstrated—contrary to what would be expected if histamine were formed from histidine in the tissues of these species. Sufficient histamine may be absorbed from the gut in carnivorous animals to meet all physiological needs, so that no endogenous formation of histamine is necessary and, consequently, a histamine-forming enzyme is not required. In rodents, insufficient histamine may be absorbed from exogenous sources, and, to make good the deficit, histamine may be formed from histidine by the action of histidine decarboxylase in the kidney, liver and duodenum. It is possible that histidine decarboxylase is only present in the tissues of those animals which do not obtain sufficient histamine from their gut contents.

Summary

1. The formation of histamine in the mammalian body, from systemic histidine, by a tissue enzyme, histidine decarboxylase, has been reinvestigated. Proof of the presence of such an enzyme in the kidneys, liver and duodenal tissues of certain rodents has been presented. No histidine decarboxylase was found in tissues from man, dogs and cats.

2. Certain organic solvents powerfully potentiate histidine decarboxylase activity. The inhibition of histaminase by aminoguanidine (10^{-4}M) has been shown to be specific.

3. The distribution of histidine decarboxylase in several tissues of man, dog, cat, rabbit, guinea-pig, rat, and mouse has been studied, using benzene to potentiate histidine decarboxylase activity and aminoguanidine to inhibit histaminase activity. At the same time estimates were made of the histaminase and histamine contents of the tissues.

4. Studies of the intracellular distribution of histidine decarboxylase, histaminase, and histamine showed that histidine decarboxylase and histaminase are present in the cell sap. Two-thirds of the

histamine is in the mitochondrial fraction; the remainder is in the cell sap.

5. Intracellular decarboxylation of histidine is probably not the major pathway in the formation of histamine in carnivorous animals, and may only be a secondary route in rodents.

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