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STUDIES ON THE HISTIDINE-HISTAMINE RELATIONSHIP *IN VIVO*

BY

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In previous tracer experiments on the formation of histamine from histidine *in vivo*, animals were injected with (^{14}C)-L-histidine, killed at various intervals, and tissues assayed for (^{14}C)-histamine (Schayer, 1952 ; Schayer, 1959 ; Bjuro, Westling & Wetterqvist, 1964). For more refined studies it would be desirable to measure tissue concentrations of both (^{14}C)-L-histidine and (^{14}C)-histamine ; certain additional inferences could then be made from the observed levels of precursor and product, and from the changes in these levels with respect to time.

In the present study the tissue levels of free (^{14}C)-L-histidine and (^{14}C)-histamine were measured in the same sample. Tissues were homogenized in acidic buffer and two aliquots taken. One aliquot was incubated with a powerful specific bacterial histidine decarboxylase preparation ; the second aliquot was untreated. The (^{14}C)-histamine content of both aliquots was then determined by isotope dilution assay ; the difference provides a measure of the concentration of free (^{14}C)-L-histidine. Results of several studies on the formation and fate of newly formed histamine *in vivo* are reported.

METHODS

Female albino CF-1 mice (Carworth, Inc., New City, New York) weighing 18–21 g were used. (^{14}C)-L-histidine and (^{14}C)-histamine (specific activity 35.0 and 0.90 mc/m-mole respectively) were purchased from Nuclear Chicago. (^{14}C)-L-histidine was purified before injection to remove traces of radioactive histamine (Schayer, 1968). In all experiments with (^{14}C)-L-histidine, mice were injected intravenously with 10.9 μc (approximately 24×10^6 d.p.m.) each. (^{14}C)-histamine was injected intravenously, 0.25 μc/mouse (approximately 0.5×10^6 d.p.m.). Assays were made on pooled tissues from three to six mice.

Tissues were assayed for total (^{14}C), (^{14}C)-histamine, and (^{14}C)-L-histidine. To prepare tissue for assays, extracts were made in cold acetate buffer (0.2 M ; pH 4.7) as follows. Liver was hand homogenized in 9 ml. of buffer, lung in 4 ml., and blood was mixed with 4 ml. of buffer. Tough tissues (stomach, muscle and intestine) were minced, frozen and thawed twice, 2 ml. buffer added, and freezing and thawing repeated. Tissues and extracts were kept cold at all times before the start of assay.

Tissue-buffer preparations were transferred to graduated centrifuge tubes ; buffer was then added to give a final volume of 12 ml. for liver, muscle and intestine, and 6 ml. for small tissues. Samples were centrifuged in the cold for 5 min at 1.000 rev/min. Without removing the sediment, portions of the samples were taken for assay.

A. One quarter each supernatant was transferred to a tube and frozen ; this material was saved in case any assay was repeated.

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B. 0.2 millilitres of each supernatant was transferred to 20 ml. beakers and frozen ; this material was assayed for (^{14}C)-L-histidine.

C. Four samples (25 μl.) were transferred to counting vials ; this material was for total (^{14}C) count.

D. To the remaining supernatant plus sediment was added: (*a*) 2 ml. of histamine carrier (containing histamine dihydrochloride 66.4 mg/ml. and L-histidine monohydrochloride 50 mg/ml.) ; and (b) 3.0 N perchloric acid (1 ml.) for large samples or 3.0 N perchloric acid (0.5 ml.) for small samples. This material was for the determination of (^{14}C)-histamine.

To determine total (^{14}C), the four 25 μl. aliquots of each sample from *C* were used. To each vial was added 15 ml. of phosphor solution (2,5-*bis*-2-(5-tert-butylbenzoxazolyl) thiophene (BBOT) 4 g in toluene 600 ml. plus methylcellosolve 400 ml. and naphthalene 80 g. Then 0.10 ml. of standard (^{14}C)-toluene was added to two vials and 0.10 ml. non-isotopic toluene to the other two. Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer. Counting efficiency was calculated from the activity of the internal standards, and the total (^{14}C) calculated.

(^{14}C)-histamine was determined by isotope dilution assay of sample *D* above. After centrifuging to remove protein, the supernatants were decanted into heavy glass tubes (approximately 50–60 ml. capacity equipped with Neoprene stoppers and suitable for extractions and centrifugation). The precipitate was washed with 0.3 N perchloric acid 1 or 2 ml., and the washings combined with the supernatant. For large volume samples, 10 N sodium hydroxide 1.0 ml. and sodium chloride 4 g were added ; for small volume samples the quantities were 0.5 ml. and 2 g, respectively. The samples were shaken twice with 15 ml. portions of butanol-chloroform (3 : 1). The two combined butanol-chloroform extracts (upper layers) were shaken with 0.1 N sodium hydroxide 5 ml. saturated with sodium chloride. Thereafter carrier histamine is extracted into acid and converted into the benzenesulphonyl derivative (BSH) as described by Schayer (1966) ; however, because twice the usual amount of histamine carrier was used, reagents used in preparing BSH were increased to 1.5 times the amount described. A second recrystallization of BSH was made by dissolving the sample in hot alcohol, adding a small amount of activated carbon, filtering, and washing with small amounts of hot alcohol. The filtrate was then reheated, warm water added until slow disappearance of turbidity, and the solution allowed to cool, first at room temperature, then in the refrigerator.

Free (^{14}C)-L-histidine was determined by quantitative decarboxylation to (^{14}C)-histamine and subsequent determination of the latter as described above. Decarboxylation was effected by a modification of the method of Gale (1957). To the 0.20 ml. aliquots of tissue samples from *B* was added 1.7 ml. of acetate buffer, pH 4.7 and 0.2 N, and 0.10 ml. of a suspension of bacterial histidine decarboxylase (Mann Research Laboratories, New York, N.Y.), 10 mg/ml. of acetate buffer. Incubation at 37° C under nitrogen for 90 min resulted in quantitative conversion of free histidine to histamine (^{14}C)-histamine (endogenous plus newly formed) was determined as above ; because of the relatively high radioactivity in samples of this type, 1 ml. carrier and a single recrystallization of BSH was sufficient.

A minimum of 4,000 counts was obtained on all samples and background. Observed c.p.m. were corrected for background (about 20 c.p.m.). For BSH, c.p.m. in the sample of known weight was corrected to the c.p.m. in a theoretical yield of BSH (141 mg or 242 mg for 1 or 2 ml. carrier, respectively). Then, from the size of the aliquot assayed, and the weight of the tissue, corrections were made to c.p.m. in the entire tissue and c.p.m./g tissue. From the counting efficiency, determined with an internal (^{14}C)-toluene standard, disintegrations per minute (d.p.m.) per g of tissue were calculated. For BSH from bacterial histidine decarboxylase incubates, the concentration of endogenous (^{14}C)-histamine was subtracted ; the difference is the histamine equivalent of free (^{14}C)-L-histidine.

RESULTS

Separation of (^{14}C)-histamine from (^{14}C)-L-histidine and its metabolites

Since histamine formation is a minor pathway of histidine metabolism in mammals, extremely high activities of (^{14}C)-L-histidine must be injected in order that the (^{14}C)-

histamine formed can be measured quantitatively. In most earlier studies (^{14}C)-histamine was assayed 1 or more days after injection of (^{14}C)-L-histidine ; because at these times most radioactivity remaining in the tissues was caused by (^{14}C)-L-histidine incorporated into protein molecules, the minute quantity of (^{14}C)-histamine could be extracted relatively free of radioactive contaminants.

The present study, however, includes short-term experiments in which (^{14}C)-histamine must be assayed in the presence of overwhelming activities of free (^{14}C)-L-histidine and its metabolites. Accordingly, a preliminary experiment was performed to check the efficacy of the BSH method under these conditions.

Six mice were injected intravenously with (^{14}C)-L-histidine 10 μc each, killed 5 min later, and stomach, lung and muscle taken for assay. Tissues were ground with perchloric acid and histamine carrier as described in METHODS, extracted three times with a butanol-chloroform mixture, and the histamine converted to BSH (batch *A*).

Then to the aqueous residues from the previous extraction, which contained the bulk of the (^{14}C)-L-histidine and its metabolites, additional carrier histamine was added, the extraction repeated, and BSH again prepared (batch *B*). Radioactivity in BSH (batch *B*) should indicate whether large activities of free (^{14}C)-L-histidine and its radioactive metabolites could significantly contaminate the isolated BSH and give spuriously high values for (^{14}C)-histamine.

A 20 mg aliquot of each BSH batch was counted, the remainder recrystallized using a different solvent (see METHODS) and a second 20 mg sample counted. Results are shown in Table 1. In BSH(*A*) samples, radioactivity was essentially unchanged after recrystallization and probably almost entirely caused by (^{14}C)-histamine (Schayer & Cooper, 1956). The BSH(*B*) values suggest that high activities of (^{14}C)-L-histidine and its metabolites do not significantly contaminate BSH ; in lung and muscle the radioactivity was too low to be significant ; in BSH(*B*) from stomach, the activity was low and possibly attributable to a trace of C^{14}-histamine not extracted in (*A*). Although one crystallization of BSH seemed to ensure adequate purity, as an extra precaution against contamination, BSH from endogenous C^{14} histamine assays was recrystallized a second time.

TABLE 1

RADIOACTIVITY IN HISTAMINE CARRIER ADDED TO TISSUES OF MICE KILLED 5 MIN AFTER INTRAVENOUS INJECTION OF (^{14}C)-L-HISTIDINE

Counts per minute corrected for background. † Single estimations on pooled tissues of six mice. * See text for meaning of *A* and *B*.

Batch of benzene-sulphonylhistamine (BSH)	(^{14}C) in 20 mg BSH†		
	Stomach	Lung	Muscle
*A** First cryst.	1,457	90	24
A Second cryst.	1,446	84	21
*B** First cryst.	38	6	0
B Second cryst.	36	2	—

(^{14}C)-histamine and free (^{14}C)-L-histidine in tissues of mice at various intervals after injection of (^{14}C)-L-histidine

The next experiment was a study of the rate of disappearance of free (^{14}C)-L-histidine from blood and tissues after intravenous injection into mice, and the rate of appearance and disappearance of (^{14}C)-histamine formed from it.

Eighteen mice, fasted overnight and throughout the experiment, were injected intravenously with (^{14}C)-L-histidine. Groups of three mice were killed at 4, 10, 25, 62, 156 and 390 min, blood and tissues taken, and assayed for total (^{14}C), free (^{14}C)-L-histidine, and (^{14}C)-histamine. The intervals were selected on the basis of preliminary experiments so that each interval is 2.5 times the previous one. Results are shown in Table 2. In all tissues the concentration of free (^{14}C)-L-histidine drops rapidly while total (^{14}C) disappears more slowly. (^{14}C)-histamine is detectable at the earliest interval tested, and differs from tissue to tissue with respect to concentration and rate of change.

TABLE 2

TOTAL (^{14}C), FREE (^{14}C)-L-HISTIDINE AND (^{14}C)-HISTAMINE IN TISSUES AT VARIOUS INTERVALS AFTER INTRAVENOUS INJECTION OF MICE WITH (^{14}C)-L-HISTIDINE

Single estimations on pooled tissues of three mice.

Tissue	Time (min)	Total (^{14}C) (d.p.m./g tissue) ($\times 10^3$ omitted)	(^{14}C)-L-histidine (d.p.m./g tissue) ($\times 10^3$ omitted)	(^{14}C)-histamine d.p.m./g tissue
Blood	4	1,030	624	288
	10	600	315	130
	25	390	97	117
	62	600	49	43
	156	690	16	54
	390	530	7	41
Liver	4	6,610	5,910	845
	10	4,510	2,850	1,910
	25	2,230	604	1,090
	62	1,840	332	750
	156	1,460	192	1,030
	390	1,270	125	790
Lung	4	1,090	970	1,650
	10	660	360	618
	25	420	125	241
	62	570	61	219
	156	580	28	195
	390	400	14	152
Stomach	4	1,070	932	14,700
	10	750	499	30,800
	25	480	124	17,300
	62	480	60	29,300
	156	350	36	16,400
	390	330	22	20,800
Muscle	4	430	387	1,100
	10	—	384	1,550
	25	—	197	650
	62	150	94	1,440
	156	60	24	950
	390	70	14	1,240
Intestine	4	250	211	1,010
	10	280	176	2,890
	25	280	76	1,530
	62	320	55	740
	156	190	37	810
	390	120	12	300

(^{14}C)-L-histidine and (^{14}C)-histamine in tissues of mice 24 and 48 hours after injection of (^{14}C)-histidine

Because in the previous experiment (Table 2) (^{14}C)-histamine was found in all tissues for 390 min after injection of (^{14}C)-L-histidine, the next experiment tested the ability of tissues to retain newly formed (^{14}C)-histamine for periods up to 48 hr. Results are

shown in Table 3. Free (^{14}C)-L-histidine levels are relatively low in all tissues while the variable (^{14}C)-histamine levels sugggest that prolonged binding occurs only in some tissues.

TABLE 3

(^{14}C)-HISTAMINE AND (^{14}C)-L-HISTIDINE (FREE) IN TISSUES AT 24 AND 48 HR AFTER INTRAVENOUS INJECTION OF MICE WITH (^{14}C)-L-HISTIDINE

Single estimations on pooled tissues of three mice.

Tissue	Time (hr)	(^{14}C)-histamine (d.p.m./g tissue)	Free (^{14}C)-L-histidine (d.p.m./g tissue)
Liver	24	137	34,200
	48	83	29,000
Lung	24	689	7,100
	48	(low)	3,100
Stomach	24	5,260	4,000
	48	7,360	(low)
Muscle	24	598	3,000
	48	1,320	450
Intestine	24	372	7,680
	48	161	1,660

In vivo *evidence of the ability of mouse tissues to remove (^{14}C)-histamine from blood and to inactivate it*

(^{14}C)-histamine found in tissues of mice given (^{14}C)-L-histidine (Tables 2 and 3) might be of local origin, or it might have been formed elsewhere and carried by the blood to the tissue assayed. To attempt to resolve this problem it was necessary to obtain *in vivo* evidence on the ability of tissues to capture blood-borne histamine and to destroy it. The experimental approach used is based on two assumptions: (*a*) that within a few minutes after intravenous injection of (^{14}C)-histamine the total (^{14}C) in a tissue provides a rough measure of ability of the tissue to remove histamine from blood ; and (*b*) that the ratio of total (^{14}C) to (^{14}C)-histamine provides a crude measure of the *in vivo* histamine-destroying activity of the tissue (Schayer, 1953).

Mice were injected with (^{14}C)-histamine, killed 4 min later, and tissues assayed for total (^{14}C) and for (^{14}C)-histamine. Results are shown in Table 4. The data suggest that liver is extremely active in uptake of exogenous histamine and in its subsequent inactivation ; intestine also seems to be somewhat active in both respects.

TABLE 4

(^{14}C)-HISTAMINE AND TOTAL (^{14}C) IN TISSUES 4 MIN AFTER INTRAVENOUS INJECTION OF MICE WITH (^{14}C)-HISTAMINE

Single estimations on pooled tissues of four mice.

Tissue	(^{14}C)-histamine (d.p.m./g tissue)	Total (^{14}C) (d.p.m./g tissue)	(^{14}C)-histamine % of total (^{14}C)
Blood	6,800	32,600	20·9
Liver	1,510	193,000	0·78
Lung	5,850	32,700	17·9
Stomach	4,340	36,000	12·1
Muscle	2,620	23,400	11·2
Intestine	5,740	113,000	5·1

DISCUSSION

A number of tentative conclusions on the histidine-histamine relationship *in vivo* can be drawn from the data presented. In cases where reliable *in vitro* data exist, there is good agreement.

The data of Table 2 suggests that free histidine circulating in the blood entered the tissues where it was rapidly catabolized, or incorporated into proteins. Liver initially contains enormous activities of (^{14}C)-L-histidine. An approximation of the rate of conversion of histidine to extractable metabolites can be made from the ratio of total (^{14}C) to (^{14}C)-L-histidine. In most tissues, 25 min after injection of (^{14}C)-L-histidine, about 25–30% of the total extractable (^{14}C) was unchanged histidine; the remainder was a mixture of metabolites. The rate of incorporation of (^{14}C)-L-histidine into protein was not measured; it cannot be calculated from the data because the radioactive carbon atom, in the 2-position of the imidazole ring, is lost in the respired air to a considerable extent (Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1950). The concentration of (^{14}C)-L-histidine in blood and liver fell about 50% between the 4 and 10 min measurements (Table 2); thus it would appear that the half-life of free L-histidine in these tissues is roughly 6 min.

In contrast to the rapid disappearance of (^{14}C)-L-histidine in all tissues, the behaviour of newly formed (^{14}C)-histamine was variable. Stomach seemed to be the most active histamine-forming organ *in vivo*. In liver, stomach, muscle and intestine, the concentration of (^{14}C)-histamine remained essentially constant throughout most of the experimental period. In blood and lung there was a steady drop in concentration of (^{14}C)-histamine. In all tissues, however, the rate of loss of (^{14}C)-histamine was less than that of (^{14}C)-L-histidine; hence the ratio of (^{14}C)-histamine to (^{14}C)-L-histidine increased with time.

Since the foregoing data indicated the ability of some tissues to retain endogenously formed (^{14}C)-histamine for short intervals, longer periods were tested (Table 3). Forty-eight hours after injection of (^{14}C)-L-histidine, the (^{14}C)-histamine concentration in liver was low while the concentration of (^{14}C)-L-histidine was relatively high; because the trace of (^{14}C)-histamine present in liver could have been newly formed, the data provide no evidence of prolonged retention of histamine by liver. In lung, (^{14}C)-histamine was too low to measure at the 48 hr period. In stomach, muscle and intestine, (^{14}C)-histamine levels showed no marked change from the 24 hr to the 48 hr interval, while their (^{14}C)-L-histidine content dropped sharply. Hence it is probable that stomach, muscle and intestine are capable of retaining endogenous histamine for long periods. Thus the findings in mice are similar to those in rats; in rats injected with (^{14}C)-L-histidine, (^{14}C)-histamine was found in stomach, muscle, intestine and skin for many days, but was undetectable in lung and liver within 2 days (Schayer, 1959).

Further interpretation of the data of Tables 2 and 3 requires introduction of evidence on (*a*) uptake of blood-borne histamine by mouse tissues and (*b*) their ability to inactivate it; these data are shown in Table 4.

From the total (^{14}C) activity (Table 4), liver seemed most active per g of tissue in removing histamine from the blood; intestine was also active. On a total organ weight basis, the predominance of liver and intestine was accentuated.

In the final column of Table 4, the data show that 4 min after intravenous injection of (^{14}C)-histamine, 10–20% of the (^{14}C) in blood, lung, stomach and muscle was unmeta-

bolized histamine. In liver, however, less than 1% of the total (^{14}C) was labelled histamine. These values suggest that liver rapidly destroys the histamine which it removes from the blood. Lindahl (1958) has reported high *in vitro* histamine-methylating activity in mouse liver. The data of Table 4 are also consistent with a moderate *in vivo* histamine-inactivating ability in intestine: no *in vitro* studies of mouse intestine are known to us but rat intestine has a moderate diamine oxidase activity (Zeller, Birkhauser, Mislin & Wenk, 1939).

With the data from Table 4, it is now possible to return to Table 2 for further consideration of the data.

First, because mouse blood has no significant histamine forming ability *in vitro* (unpublished) it is assumed that its (^{14}C)-histamine originates in other tissues.

Second, because liver seemed to destroy histamine which it removed from blood (Table 4), but showed a relatively constant (^{14}C) histamine level for several hours after injection of (^{14}C)-L-histidine (Table 2), it is suggested that liver may normally convert a small amount of histidine to histamine in a locus affording some protection from hepatic histamine-destroying enzymes.

Third, (^{14}C)-histamine in lung drops continuously. Whether this histamine is formed locally, or removed from blood, cannot be decided from the present data. Mouse lung does have significant *in vitro* histidine decarboxylase activity (Schayer, 1960; 1962).

Fourth, stomach seems to be the most active histamine-forming tissue in mice as it is in rats (Bjuro *et al.*, 1964).

Fifth, since muscle showed little tendency to accumulate blood-borne histamine, the concentration being less than that of blood (Table 4), its prolonged retention of (^{14}C)-histamine (Table 2) suggests that this histamine may, in part, be formed in muscle itself. This is corroborated by the data of Table 3 which suggest long-term binding of endogenous (^{14}C)-histamine in muscle. Mouse muscle has significant *in vitro* histidine decarboxylase activity (Schayer, 1960; 1962).

Sixth, for intestine (Table 2), the ratio of (^{14}C)-histamine to (^{14}C)-L-histidine was relatively high, being second only to that of stomach. With the additional evidence that intestine can retain (^{14}C)-histamine for prolonged periods (Table 3) and that it can destroy blood-borne histamine (Table 4), it seems likely that intestine forms histamine *in vivo*.

Although an unequivocal interpretation of the present data is not always possible, extensions of this approach might clarify the significance of the findings. For example, the procedure could be extended to determine both histamine and L-histidine in the non-isotopic as well as the isotopic form. Such data would permit calculation of specific activities of precursor and product in various tissues, and their changes with respect to time. It may also be possible to assay catabolic products of histamine and to include other procedures helpful for understanding the complex life cycle of histamine in living animals.

SUMMARY

1. A method is described for determining (^{14}C)-histamine and free (^{14}C)-L-histidine in the same tissue sample.

2. Evidence is presented that the method permits a reliable assay for traces of (^{14}C)-histamine in the presence of overwhelming activities of (^{14}C)-L-histidine and its metabolites.

3. Studies are reported on the rate of disappearance of (^{14}C)-L-histidine from blood and tissues following intravenous injection into mice, and on the rates of appearance and disappearance of the (^{14}C)-histamine formed from it.

4. The data suggest that liver removed most circulating free histidine; levels of free (^{14}C)-L-histidine dropped rapidly in all tissues, the half-life in blood and liver being approximately 6 min.

5. After intravenous injection of mice with (^{14}C)-L-histidine, (^{14}C)-histamine was detectable in all tissues at the earliest time tested, 4 min. Stomach contained very large activities of newly formed (^{14}C)-histamine.

6. Four minutes after intravenous injection of (^{14}C)-histamine into mice, liver and intestine contained large activities of total (^{14}C); other tissues contained much less. Of the total (^{14}C) in liver, less than 1% was unmetabolized (^{14}C)-histamine; in most other tissues 10–20% of the total radioactivity was caused by unchanged (^{14}C)-histamine. The data suggest that liver is the most active histamine-inactivating tissue in the mouse, with intestine also somewhat active.

7. From the available data it was tentatively concluded that most or all mouse tissues tested may produce some of the histamine which they contain.

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