BIOGENESIS OF HISTAMINE*

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The origin of histamine bound in certain mammalian tissues, in which it is available for release in such conditions as tissue damage and anaphylactic shock, has not been conclusively demonstrated. It has been postulated that it arises either from dietary histidine or from exogenous histamine formed in the intestine or in tissues high in histidine decarboxylase (1). Increases, of brief duration, in the histamine content of guinea pig urine and lung have been reported to follow administration of large amounts of histidine (2, 3), but, when smaller quantities of histidine were injected, no increase of histamine could be demonstrated in urine.

In this paper it is shown that, following a small dose of radioactive L-histidine, radioactive histamine can be detected in the urine and in certain internal organs for many days. In contrast, after administration of C¹⁴-histamine, none of this substance could be detected in the same internal organs after 4 hours.

EXPERIMENTAL

Isotopic Compounds—The syntheses of L-histidine and histamine labeled with C¹⁴ in the 2 position of the imidazole ring have been published (4). The specific activity of the L-histidine was 6.7 × 10⁶ c.p.m. per mg. and that of the histamine 9.3 × 10⁶ c.p.m. per mg. by use of a flow counter.

Determination of Histamine in Urine and in Tissues—Since the amounts of histamine encountered were extremely small, it was necessary to use isotope dilution techniques to insure removal of contaminating radioactivity. Urine samples were collected in a receiving flask, kept at 0°, containing 20 mg. of non-isotopic histamine as carrier and 50 mg. of non-isotopic L-histidine as a diluent.

For examination of internal organs only those known to have a high histamine content were used (combined lungs, kidneys, and intestines). The guinea pig was killed by a blow on the head, followed by decapitation, and the organs were immediately removed and frozen with dry ice, then

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ground with 10 per cent trichloroacetic acid and sand in a mortar contain-
ing the usual amounts of carrier histamine and diluent histidine. Four
grindings and filtrations were made and trichloroacetic acid was removed
by ether extraction. Beginning at this point, the organ extracts and
urine samples were treated identically. Sodium sulfate was added to give
a 25 per cent solution and sodium hydroxide added to give pH 13 (5).
Histamine base was extracted by shaking with n-amyl alcohol four times
(two butanol extractions remove histamine completely but seem to carry
over more impurities from urine). After drying the combined alcohol
fractions thoroughly with sodium sulfate, the histamine was removed
from the alcohol by two extractions with 3 N hydrochloric acid. The acid
solution was evaporated to dryness on a hot-plate in a stream of air, the
residue dissolved in 1 ml. of water, 90 mg. of picric acid in a minimum of
boiling water added, and the hot solution treated with Norit and filtered.
To prevent crystallization of picric acid each filtrate was reheated and
seeded with histamine dipicrate. From 50 to 85 per cent of the carrier
histamine was obtained as the dipicrate which could then be recrystallized,
with Norit, to constant radioactivity. It was found that in many cases
the compound had reached constant specific activity after the first crys-
tallization; it was invariably constant after the second crystallization.
By this method minute amounts of histamine could be readily separated
from large excesses of L-histidine, urinary histamine metabolites, and uri-
nary histidine metabolites. To add further assurance that the radio-
activity being measured was due to histamine and not a contaminant,
some of the histamine dipicrate samples from the organs were converted
to free histamine. In each case, after sublimation, this compound showed
the calculated increase in specific activity.

Presence of Histamine in Tissues after Administration of C\textsuperscript{14}-Histamine—
Four guinea pigs were injected subcutaneously with 0.05 $\gamma$ of C\textsuperscript{14}-histamine
per gm. of body weight. Two were killed after 2 hours and two after
4 hours. For each time interval the tissues of one animal were examined
for total radioactivity and the tissues of the other animal used for hist-
amine determinations. The results are shown in Table I.

Presence of Histamine in Urine after Administration of C\textsuperscript{14}-L-Histidine—
Guinea pigs were injected subcutaneously with 2 $\gamma$ of C\textsuperscript{14}-L-histidine per
gm. of body weight and daily urine collections made for various intervals.
Urine was collected and analyzed for histamine and total radioactivity
as described above. The results are shown in Tables II and IV.

Presence of Histamine in Tissues after Administration of C\textsuperscript{14}-L-Histidine
—The animals used for urine analyses, and additional guinea pigs given
the same dose of C\textsuperscript{14}-L-histidine, were killed after various time intervals
and the combined lungs, kidneys, and intestines analyzed for histamine
as described above. To check for completeness of histamine extraction, 2 residues from trichloroacetic acid treatment were analyzed for histamine after hydrolysis in $6 \times$ hydrochloric acid in the presence of carrier histamine and diluent histidine. The results are shown in Table III.

Table I

| Total Radioactivity and C$^{14}$-Histamine in Tissues after Subcutaneous Administration of 0.05 $\gamma$ of C$^{14}$-Histamine Per Gm. of Body Weight to Guinea Pigs |  |
|---|---|---|---|---|---|---|---|---|---|
| | Plasma per ml. | Blood cells per ml. | Liver | Kidney | Lung | Intestine |
| Total radioactivity after 2 hrs. | 0.16 | 0.07 | 20 | 1.8 | 0.09 | 1.8 |
| Histamine content after 2 hrs. | 0.0 | 0.04 | 0.05 | 0.07 | 0.04 | 0.12 |
| Total radioactivity after 4 hrs. | 0.08 | 0.06 | 17 | 2.6 | 0.21 | 2.0 |
| Histamine content after 4 hrs. | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Table II

Counts Per Minute of Histamine in Urine Per Million C.p.m. of Injected L-Histidine (Equivalent to Per Cent $\times$ 10$^4$)

Guinea pigs administered subcutaneously 2 $\gamma$ of C$^{14}$-L-histidine per gm. of body weight.

| Guinea pig | Weight (gm.) | Days after injection |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 15 |
| HDE $\varnothing$ | 230 | 89 | 120 | 52 |  |  |  |  |  |  |  |  |
| HDF $\varnothing$ | 300 | 76 | 44 | 23 | 21 | 7 |  |  |  |  |  |  |
| HDG $\varnothing$ | 260 |  | 17 | 20 | 14 | 10 | 10 |  |  |  |  |  |
| HDH $\varnothing$ | 220 |  | 12 | 16 | 0 | 0 | 11 |  |  |  |  |  |
| HDI $\varnothing$ | 230* | 97 | 36 | 25 |  |  |  |  |  |  |  |  |

* Received 4 $\gamma$ of C$^{14}$-L-histidine per gm. of body weight. Twice the usual amount of carrier histamine added to urine and recrystallization repeated many times to demonstrate conclusively the purity of histamine dipicrate.

Counting and Calculations in Histamine Analyses—Histamine dipicrate was counted in 4.5 sq. cm. plates in flow counters of background about 20 c.p.m. Thickness corrections were made with an experimentally derived curve. Observed counts of 3 c.p.m. or more above background, after a long counting time, were considered significant and were used for quantitative treatment. Samples with less than 3 c.p.m. were reported negative. To relate the values presented in Tables II and III to actually observed counts, the following sample calculations are presented: For a
plate containing 37 mg. of histamine dipicrate the observed activity was 14 c.p.m. above background or 58 c.p.m. corrected for thickness. The total carrier histamine is equivalent to 103 mg. of histamine dipicrate; thus the total count is 58 × 103/37 = 162. The animal was injected with 3.5 × 10⁷ c.p.m. of L-histidine; therefore the reported value is 162/3.5 = 46 c.p.m. of histamine per million c.p.m. of L-histidine. The

**Table III**

C¹⁴-Histamine Content of Combined Lungs, Intestines, and Kidneys of Guinea Pigs at Intervals after Subcutaneous Injection with 2 γ of C¹⁴-L-Histidine Per Gm. of Body Weight

Expressed as counts per minute of histamine per million c.p.m. of injected histidine (equivalent to per cent × 10⁴).

<table>
<thead>
<tr>
<th>Guinea pig</th>
<th>HDQ</th>
<th>HDR</th>
<th>HDN</th>
<th>HDE</th>
<th>HDI*</th>
<th>HDF</th>
<th>HDG</th>
<th>HDH</th>
<th>HDM</th>
<th>HDK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days from injection to sacrifice</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>42</td>
<td>56</td>
</tr>
<tr>
<td>65†</td>
<td>43</td>
<td>72</td>
<td>48</td>
<td>85</td>
<td>45‡</td>
<td>46‡</td>
<td>92</td>
<td>86§</td>
<td>25†</td>
<td>48†</td>
</tr>
</tbody>
</table>

* Died.
† The picrates from these assays were converted to free histamine and sublimed without producing significant deviation from the calculated specific activity.
‡ The extraction procedure removed almost all the histamine, for, after hydrolysis of the residual material from these organs, only traces of additional histamine were found. Furthermore, this C¹⁴-histamine may be an artifact, for it has been shown that acid hydrolysis may decarboxylate traces of L-histidine (9). We have found that grinding of tissues with trichloroacetic acid at room temperature, in the presence of large quantities of C¹⁴-L-histidine, produces no C¹⁴-histamine.
§ As this animal received 4 γ of C¹⁴-L-histidine per gm. of body weight, the organs were assayed separately. Total histamine expressed in the above units was for lung 21, intestine 54, and kidney 11. Expressed as histamine per gm. of tissue: lung 8, intestine 4, and kidney 4.

The lowest observed count considered significant, 3 c.p.m., gives reported values of about 7 to 10 c.p.m. per million c.p.m. of L-histidine.

**Discussion**

The data show that exogenous histamine cannot be detected in blood, lung, kidney, intestine, and liver 4 hours after injection. The level of histamine employed, 0.05 γ per gm. of body weight, is probably many times greater than the histamine level produced by an injection of 2 γ of L-histidine per gm. of body weight. Thus it is probable that exogenous histamine is not a source of bound histamine.
After administration of a single dose of \( C^{14}\)-L-histidine, minor in magnitude compared to dietary and protein histidine, \( C^{14}\)-histamine is found for long periods of time in organs known to be rich in bound histamine.

The following sequence of events seems probable. Shortly after absorption of L-histidine into the bloodstream, the concentration of free histidine is highest, and, in those organs containing histidine decarboxylase, histamine is produced at a maximum rate. If the histamine is formed within cells possessing a binding mechanism, it may be retained in stable condition for long periods. Most of the histamine, however, is evidently formed in cells lacking a binding mechanism. It is therefore treated as exogenous histamine and undergoes further metabolism; a small percentage is excreted unchanged in the urine. Thus, bound histamine is rapidly built up in the organs and relatively large amounts of histamine appear in the urine shortly after L-histidine administration. Histamine production is probably proportional to free histidine concentration, a conclusion supported by the rough proportionality of urinary \( C^{14}\)-histamine to total urinary \( C^{14}\) (Tables II and IV).

An estimation can be made of the half life of the bound histamine in the organs. The average amount of \( C^{14}\)-histamine entering the organs daily can be obtained from the data of Table III. The average for the first 5 days after injection (a period of time of negligible duration compared to the long persistence of \( C^{14}\)-histamine in these organs) is 60 c.p.m.

\[ \text{Table IV} \]

**Daily Urinary Excretion of \( C^{14}\) after Subcutaneous Injection of \( C^{14}\)-L-Histidine**

Expressed as per cent of total administered \( C^{14}\).

<table>
<thead>
<tr>
<th>Guinea pig</th>
<th>Days after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 15</td>
</tr>
<tr>
<td>HDE</td>
<td>1.9 0.8 0.5</td>
</tr>
<tr>
<td>HDF</td>
<td>1.8 0.7 0.4 0.3 0.3</td>
</tr>
<tr>
<td>HDG</td>
<td>0.4 0.3 0.3 0.2</td>
</tr>
<tr>
<td>HDH</td>
<td>0.3 0.5 0.3 0.2</td>
</tr>
<tr>
<td>HDI</td>
<td>2.0 0.6 0.6</td>
</tr>
</tbody>
</table>

in the urine shortly after L-histidine administration. Histamine production is probably proportional to free histidine concentration, a conclusion supported by the rough proportionality of urinary \( C^{14}\)-histamine to total urinary \( C^{14}\) (Tables II and IV).

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\[ \text{1 Since carbon atom 2 of the ring of L-histidine is known to be a source of 1-carbon fragments (6), it was necessary to consider the possibility that the \( C^{14}\)-histamine was being synthesized by a route not involving L-histidine and that its radioactivity was due to incorporation of this 1-carbon fragment. A guinea pig was administered } 4.8 \times 10^4 \text{ c.p.m. of } C^{14}\text{-methylamine (} C^{14}\text{-formate being unavailable); no } C^{11}\text{-histamine could be found in the organs after 24 hours. Methylamine is largely oxidized to carbon dioxide in vivo (7) and presumably is a source of 1-carbon fragments.} \]
of histamine per $10^6$ c.p.m. of $L$-histidine. This is equivalent to 400 c.p.m. of histamine per mg. of $L$-histidine or about 0.04 $\gamma$ of histamine per mg. of $L$-histidine. Assuming the daily intake of $L$-histidine to be 80 mg., then roughly 3 $\gamma$ of new histamine are added each day to the organs (lungs, intestines, and kidneys) and presumably the same amount is lost each day to maintain equilibrium. Since assays of these organs show that the total histamine content may be about 230 $\gamma$, it is evident that turnover is slow and that the half life of bound histamine is of the order of 50 days. The histamine assays of the organs of animals killed 42 and 56 days after injection (Table III) could be in accordance with this estimate.

**SUMMARY**

An investigation has been made of the source of histamine bound in animal tissues. No $C^{14}$-histamine could be detected in the organs of guinea pigs 4 hours after injection with radioactive histamine. After injection with radioactive $L$-histidine, $C^{14}$-histamine could be detected in urine and internal organs for many days. A discussion of histamine formation and turnover is presented.

**BIBLIOGRAPHY**


* Based on observed food intake, known protein content of diet, and assumption that 2 per cent of protein is $L$-histidine. The average histidine content of several plant proteins is about 2 per cent.

* This figure was obtained by using a published value of the histamine content of guinea pig lung, 20 $\gamma$ per gm. (3), and using the observed $C^{14}$-histamine values found in the organs of Guinea Pig HDI (Table III) to estimate histamine content of intestine and kidney. It is realized that the histamine content of organs is highly variable, but it is felt that some attempt to arrive at a crude estimation of the half life of bound histamine is justified because of the inability to approach a problem of this nature with non-isotopic experiments. The relatively high requirement of the $C^{14}$-$L$-histidine imposed a limit on the number of experiments performed.

* Calculated from $\lambda t = 0.693$ where $\lambda$ (fraction turned over per day) = 0.013.
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