STUDIES ON HISTAMINE-METABOLIZING ENZYMES IN INTACT ANIMALS. II

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In continuation of work previously reported (1, 2), we have extended in vivo studies of histamine-catabolizing enzymes. In all the species tested, rats, mice, guinea pigs, hamsters, and cats, we have observed three radioactive peaks in the urine after injection of radioactive histamine. These three peaks are readily reproducible by individual animals of any one species. Peak 1 is due to an unidentified substance; its formation can be repressed by diamine oxidase inhibitors. Peak 2 is also due to an unidentified substance; its formation can be repressed by Marsilid (1-isonicotinyl-2-isopropylhydrazine), but it is little affected by diamine oxidase inhibitors. Peak 3 is largely unchanged histamine but includes a small amount of acetylhistamine.

The term "histaminase" was proposed by Best (3) for the enzyme which attacked histamine. Later, Zeller found that the enzyme which attacked histamine also attacked other diamines, and proposed the use of the term "diamine oxidase" (4). The nomenclature is confused, as both terms are now employed by workers in this field. Our work has shown that a major histamine-metabolizing enzyme other than diamine oxidase is found in most species tested. Therefore, until the nature of this second metabolic reaction of histamine is better understood, we propose that the name "histamine-metabolizing enzyme II" be applied to the enzyme system which performs the first step in the metabolism of histamine toward the formation of the compound of Peak 2.

We also report in vivo studies paralleling those customarily performed on enzymes in vitro. Using rats, in which diamine oxidase appears to be responsible for nearly all histamine metabolism, we have studied (a) the effect of concentration of histamine on its rate of metabolism and (b) the nature, competitive or non-competitive, of the inhibition of diamine oxidase.

Using chromatographic techniques, we have made observations on the effects of hormones on histamine metabolism in mice.

Finally, histidine decarboxylase, the only enzyme known to be involved

* Supported in part by a contract with the United States Atomic Energy Commission.
in the formation of histamine, has been subjected to tests in vivo for effects of purported inhibitors and of cortisone.

**EXPERIMENTAL**

*Isotopic Compounds*—The synthesis of histamine and L-histidine labeled with C\(^{14}\) in the 2 position of the imidazole ring has been published (5). Radioactive isonicotinic acid hydrazide (6) was generously supplied by Dr. Wright H. Langham of the Los Alamos Scientific Laboratory.

*Metabolism of Histamine in Cats*—Since only rodents had been studied with radioactive histamine, a carnivore was tried. Kittens, 3 to 4 weeks of age, weighing 320 to 400 gm., were injected subcutaneously with an inhibitor, and after 20 minutes were injected subcutaneously with about 0.4 \(\gamma\) of C\(^{14}\)-histamin per gm. of body weight. Urine was collected for periods ranging from 2 to 5 hours and chromatograms were prepared. The results are shown in Fig. 1.

*Effect of Concentration of Histamine on Rate of Metabolism*—A series of solutions was prepared, all containing the same amount of C\(^{14}\)-histamine, but varying in the amount of non-isopic histamine. Rats (80 to 90 gm., fasted 19 hours) were injected ravenously with 0.60 ml. of one of the various solutions. Each rat was killed exactly 5 minutes after injection and immediately frozen. Each carcass was homogenized with 80 mg. of histamine (as the dihydrochloride) and this carrier histamine subsequently isolated and purified as the dipicrate, as previously described (7).

![Fig. 1. Radioactivity on paper chromatograms of urine of immature cats, showing effects of inhibitors on the pattern of C\(^{14}\)-histamine metabolites. Abscissa, position on paper, each division = 10 cm.; ordinate, per cent of total counts per minute on paper, each division = 10 per cent. Chromatogram 1, no inhibitor; Chromatogram 2, 10 \(\gamma\) of aminoguanidine per gm. of body weight; Chromatogram 3, 200 \(\gamma\) of Marsilid phosphate per gm. of body weight.](http://www.jbc.org/)

A chromatogram of the urine of a hamster injected subcutaneously with 0.1 \(\gamma\) of C\(^{14}\)-histamine per gm. of body weight, expressed as per cent of total radioactivity on paper, showed Peak 1 9 per cent; Peak 2, 46 per cent; Peak 3, 36 per cent.
All the samples were counted at infinite thickness and the radioactivity compared with that of the dipicrate prepared from the same amount of original solution added to the same amount of carrier. This procedure gives the per cent of originally injected histamine remaining unmetabolized after 5 minutes. If the concentration of histamine injected and the body weight of the animal are known, the rate of metabolism is readily computed. The effect of increasing concentrations of histamine on its rate of metabolism is shown in Table I.

**TABLE I**

_Effect of Concentration on Rate of Metabolism of Histamine in Rats_

<table>
<thead>
<tr>
<th>Concentration of histamine injected, γ per gm.</th>
<th>Per cent histamine metabolized in 5 min.</th>
<th>Rate of metabolism of histamine, γ per min. per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.26</td>
<td>62</td>
<td>0.03</td>
</tr>
<tr>
<td>0.53</td>
<td>59</td>
<td>0.10</td>
</tr>
<tr>
<td>2.3</td>
<td>44</td>
<td>0.20</td>
</tr>
<tr>
<td>6.8</td>
<td>33</td>
<td>0.45</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
<td>0.75</td>
</tr>
<tr>
<td>21</td>
<td>33</td>
<td>1.3</td>
</tr>
<tr>
<td>39</td>
<td>21</td>
<td>1.6</td>
</tr>
<tr>
<td>45</td>
<td>5.4</td>
<td>0.49</td>
</tr>
<tr>
<td>70</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Nature of Inhibition by Isonicotinylhydrazine—We have shown previously that isonicotinylhydrazine (Rimifon²) inhibits diamine oxidase _in vivo_ (2). The following experiment was designed to test the competitive or non-competitive nature of this inhibition by determining whether increased concentration of injected histamine could overcome the inhibition of its metabolism.

A series of solutions was prepared in which the amounts of isonicotinylhydrazine and of non-isotopic histamine varied, but the concentration of C¹⁴-histamine was the same. 0.6 ml. of each solution was injected intravenously into rats (80 to 90 gm., fasted 19 hours) which were killed after 5 minutes and immediately frozen. Per cent of injected histamine remaining in the carcass was determined as in the previous experiment. Subtraction of this value from 100 per cent gave the per cent of histamine metabolized in 5 minutes. Table II shows the effect of increasing histamine concentrations in overcoming the inhibition produced by isonicotinylhydrazine.

Nature of Inhibition by Aminoguanidine—Schuler (8) has reported that

² Rimifon and Marsilid were kindly supplied by Dr. M. J. Schiffrin of Hoffmann-La Roche, Inc.
aminoguanidine is to date the most powerful inhibitor of hog kidney diamine oxidase. We have found it to be a powerful inhibitor in vivo; it is several times as active as isonicotinylhydrazine. To test whether the inhibition in vivo of diamine oxidase by aminoguanidine was competitive or non-competitive, the following procedure was used. Female white rats, averaging 90 gm., were injected subcutaneously with varying levels of aminoguanidine (Eastman Kodak Company aminoguanidine bicarbonate, dissolved in the theoretical amount of hydrochloric acid). After about 20 minutes, the rats were injected subcutaneously with radioactive histamine, also at varying levels. Urine was collected by squeezing the animals at about 30 minute intervals, then immediately frozen and stored at subzero temperature. Urine collection was continued for 4 hours. Chromatograms were prepared as usual (1, 2). Aminoguanidine, like

other diamine oxidase inhibitors, has little effect on Peak 2. It does, however, diminish Peak 1 with a concomitant rise in Peak 3. Therefore, in order to reduce counting time, only Peaks 1 and 3 of the chromatograms were counted and the value, (counts per minute in Peak 1)/(counts per minute in Peaks 1 and 3) × 100, was determined. Injection of higher concentrations of histamine increases the per cent of histamine excreted in urine and hence increases the relative magnitude of Peak 3. Therefore, in order to establish a base from which comparisons could be readily made, the experimentally obtained values are expressed as per cent of the value obtained when no inhibitor is used (per cent of uninhibited value). The results are shown in Table III.

**Table II**

*Effect of Isonicotinylhydrazine on Per Cent Histamine Metabolized by Rats in 5 Minutes Following Intravenous Injection*

<table>
<thead>
<tr>
<th>Concentration of isonicotinylhydrazine, γ per gm.</th>
<th>Concentration of histamine</th>
<th>0.26 γ per gm. body weight</th>
<th>2.3 γ per gm. body weight</th>
<th>6.8 γ per gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>62</td>
<td>44</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>27</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>24</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>21</td>
<td>17</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

**Effect of Hormones on Histamine Metabolism**—The interaction of hormones and histamine has been the subject of many investigations, which have been reviewed by Rose (9). Definite evidence has been presented to show that histamine-destroying power rises sharply during pregnancy (10, 11) and that adrenalectomy markedly reduces the tolerance of mice
### Table III

**Effect of Various Concentrations of Histamine on Inhibition of Histamine Metabolism by Aminoguanidine**

Expressed as per cent of the uninhibited value (see the text for an explanation).

<table>
<thead>
<tr>
<th>Concentration of amino guanidine, γ per gm.</th>
<th>Concentration of histamine</th>
<th>0.05 γ per gm.</th>
<th>1.3 γ per gm.</th>
<th>11 γ per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td>90</td>
<td></td>
<td></td>
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<tr>
<td>0.5</td>
<td></td>
<td>52</td>
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<td>67</td>
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<td>41</td>
<td>57</td>
</tr>
<tr>
<td>5.0</td>
<td></td>
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<td>29</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>6</td>
<td>7</td>
<td>24</td>
</tr>
</tbody>
</table>

* Actual uninhibited values were 69, 68, and 42 per cent, respectively.

### Table IV

**Effect of Hormones on Histamine Metabolism in Female Mice**

All the mice were injected subcutaneously with 0.2 γ of C¹⁴-histamine per gm. of body weight; those pretreated with hormones received histamine 1 hour after the final injection of hormone.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Pregnant (2 wks.)</th>
<th>Adrenalectomized (3 days)</th>
<th>Estradiol*</th>
<th>Progesterone†</th>
<th>Cortisone‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak 1</td>
<td>Peak 2</td>
<td>Peak 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>44</td>
<td>32</td>
<td>20</td>
<td>46</td>
<td>23</td>
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<tr>
<td>14</td>
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<td>52</td>
<td>6</td>
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<td>36</td>
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<td>27</td>
<td>15</td>
<td>49</td>
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<td>20</td>
<td>50</td>
<td>25</td>
<td>17</td>
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<td>20</td>
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<td>27</td>
<td>28</td>
<td>47</td>
<td>14</td>
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<td>28</td>
<td>58</td>
<td>12</td>
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<tr>
<td>16</td>
<td>46</td>
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<td>38</td>
<td>44</td>
<td>14</td>
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</tr>
<tr>
<td>12</td>
<td>33</td>
<td>44</td>
<td>12</td>
<td>33</td>
<td>44</td>
</tr>
</tbody>
</table>

* The first mouse received 0.5 γ of estradiol per gm. of body weight (in olive oil) at 4 p.m., 9 a.m., and 12 noon, the second at 9 a.m. and 12 noon only.
† The first mouse received 2.5 γ of progesterone per gm. of body weight (in olive oil) at 9 a.m. and 12 noon, the second at 9 a.m. and 4 p.m. for 3 days, and at 9 a.m. of the 4th day.
‡ All three mice received 25 γ per gm. of cortisone at 9 a.m. and 4 p.m. for 3 days, and at 9 a.m. of the 4th day.
and rats to injected histamine (12). Some of the other published material on the relation of hormones to histamine requires further confirmation. In the present study, experiments were performed to test the effect of female sex hormones and adrenal hormones on histamine metabolism in female mice. Mice (Swiss albino) were pretreated as explained in Table IV. All were injected subcutaneously with 0.2 $\gamma$ of C$^{14}$-histamine per gm. of body weight, urine was collected for 4 hours, and chromatograms were prepared (2). Changes in relative magnitudes of the three radioactive peaks are used as criteria of disturbance of histamine metabolism. The results are shown in Table IV.

**Histidine Decarboxylase In Vivo**—Since histamine is formed in the guinea pig from 1-histidine but not from 1-carbon fragments (13), it seems likely that histidine decarboxylase is the only enzyme concerned with histamine formation. Martin et al. have found that d-catechin (14) and 3,4-dihydroxychalcone are inhibitors of histidine decarboxylase in vitro. This group has also shown that repeated doses of d-catechin prevent anaphylactic shock in guinea pigs (15). The effect of these two compounds on histidine decarboxylase in mice has been tested.

We had failed previously to find any effect of adrenalectomy on the rate of histamine destruction in mice (7). It was felt that a possible mechanism of the anti-inflammatory action of cortisone might be in inhibiting histidine decarboxylase, thus preventing the accumulation of bound histamine in the tissues. The effect of cortisone was also tested.

Groups of four male mice (dba strain, Rockland Farms, 22 to 26 gm.) were injected subcutaneously with the test substance at 9 a.m. and 4 p.m.

---

**Table V**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Counts/min of Histamine</th>
<th>Counts/min of Injected L-histidine</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>420</td>
<td>670</td>
<td>700</td>
</tr>
<tr>
<td>d-Catechin</td>
<td>440</td>
<td>470</td>
<td>580</td>
</tr>
<tr>
<td>3,4-Dihydroxychalcone</td>
<td>460</td>
<td>550</td>
<td>660</td>
</tr>
<tr>
<td>Cortisone</td>
<td>320</td>
<td>360</td>
<td>660</td>
</tr>
</tbody>
</table>

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8 Personal communication from Dr. G. J. Martin, who generously provided samples of both compounds.

4 d-Catechin was dissolved in warm saline; boiling destroys this compound (16). Dihydroxychalcone was dissolved in olive oil. Cortone (Merck) was kindly supplied by Dr. I. Clark.
for 2 successive days, and at 9 a.m. on the 3rd day. 1 hour later all mice
received 40 \gamma of C^{14}-L-histidine (980,000 c.p.m. per mg.) per gm. of body
weight subcutaneously. 30 hours after histidine administration, all the
mice were killed, immediately frozen, and the C^{14}-histamine content of
the entire carcass determined in the usual way (7, 13). The results are
shown in Table V.

DISCUSSION

In Fig. 1, Chromatogram 1 shows that the urine of a normal cat after
injection with C^{14}-histamine contains the three usual peaks. However,
Peak 1 is of negligible size while Peak 2 is the major metabolite. Chroma-
togram 2 shows that aminoguanidine (at a concentration which almost
completely stops diamine oxidase activity in rats) removes Peak 1 but has
little inhibitory effect on Peak 2. Chromatogram 3 shows that Marsilid
(at a concentration which produces marked reduction of Peak 2 in mice)
largely suppresses Peak 2 in cats.

Although in vitro studies show that cat tissues have diamine oxidase
activity (17), it is apparent from these experiments that diamine oxidase
has little function in the physiological metabolism of histamine in cats.
A revision of the nomenclature of histamine-metabolizing enzymes becomes
necessary to differentiate diamine oxidase, which leads to the formation
of the compound of Peak 1, from histamine-metabolizing enzyme II,
which leads to the formation of the compound of Peak 2.

Through the use of the inhibitors aminoguanidine and Marsilid, it is
possible to suppress histamine catabolism almost completely. Informa-
tion on the relative importance of diamine oxidase and histamine-metabo-
lizing enzyme II in humans may be obtained by measuring the effects
of these two compounds individually on the concentration of urinary
histamine, either under normal conditions or after injection of non-isotopic
histamine.

Table I shows that, when low levels of histamine are injected into rats,
the rate of metabolism is roughly proportional to the concentration. As
the concentration of the injected histamine is increased, a maximum is
reached, after which the rate of metabolism decreases.\(^5\) When histamine

\(^5\) The demonstration that at sufficiently high concentrations histamine inhibits
diamine oxidase may offer a clue to the discrepancy between the findings of the au-
thors (7) and of Rose and Browne (18). The latter found that adrenalectomized,
hooded rats destroyed histamine much more slowly than did normal animals.
The high concentration they injected, 24 \gamma per gm., may have been very close to
“saturation” for the histamine-metabolizing enzymes in the strain of rats employed.
A small change in histamine-metabolizing power caused by adrenalectomy might
produce an inhibitory concentration of histamine and initiate a precipitous drop in
the rate of histamine destruction. We have found no significant effect of adrena-
lectomy in mice given minute doses of histamine.
is injected at a level of 70 γ per gm. of body weight, it can all be recovered unchanged from the body of the animal after 5 minutes. Thus, at this concentration, diamine oxidase is completely inhibited by its substrate. The data of Table I produce a curve very similar to one obtained by Zeller (19) relating the concentration of histamine to its rate of metabolism by diamine oxidase in vitro. In a separate experiment, a rat injected with this totally inhibitory concentration of histamine (70 γ per gm.) showed, after 3 minutes a concentration of 72 γ per gm. in the intestine and 290 γ per gm. in the liver. Since these two organs (particularly the intestine) are the most active in histamine metabolism in the rat (20, 2) the above values are approximately the concentrations of substrate required at the site of the enzyme to produce complete inhibition.

From the maximal rate of metabolism observed, 1.6 γ per minute per gm., one can establish an approximate maximal rate of metabolism in the active organs. The above value is equal to 160 γ per minute per 100 gm. rat. As the metabolism occurs chiefly in the intestine and liver (which together weigh about 8 gm. in a 100 gm. rat), the maximal rate of destruction of histamine in these active organs is about 20 γ per minute per gm. (probably being somewhat higher in the intestine and lower in the liver). This may be compared with the value of about 7 γ per minute per gm. observed by Best and McHenry in perfused dog kidney (21).

Table II shows that, although isonicotinylhydrazine produces marked inhibition in the metabolism of low concentrations of injected histamine, if the level of injected histamine is increased, the effects of the inhibitor are almost completely overcome. Thus, the inhibition is competitive in nature. Studies have also been made to determine the concentration of isonicotinylhydrazine required in the active organs to produce inhibition. When rats are injected subcutaneously with 100 γ of C\textsuperscript{14}-isonicotinylhydrazine per gm. of body weight (which we have shown to inhibit diamine oxidase completely (2)), the concentration after 20 minutes (determined as total C\textsuperscript{14}) in the intestine is 110 γ per gm., and in the liver 130 γ per gm. When 50 γ per gm. are injected (which causes marked but not complete inhibition), the values are 56 and 60 γ per gm. respectively. These may be compared with about 70 γ per ml. required for 50 per cent inhibition of hog kidney diamine oxidase in vitro (22). Thus the concentrations required for inhibition in vivo and in vitro are of closely comparable magnitude.

Table III shows the pronounced inhibitory effect on histamine metabolism of aminoguanidine, even when given in very small quantities. Increasing the histamine concentration from 0.05 to 1.3 γ per gm. had little effect on this inhibition; however, at a level of 11 γ of histamine per gm. of body weight, the inhibitory effect of aminoguanidine is modified but by no means overcome.
The data suggest that the aminoguanidine-diamine oxidase complex is highly stable and not so readily dissociated by histamine as is the isonicotinylhydrazine-diamine oxidase complex. Chromatographic evidence previously published (2) shows that, under the same conditions as employed in this experiment, a concentration of between 50 and 100 γ per gm. of isonicotinylhydrazine is required to produce the same inhibition as given by 10 γ per gm. of aminoguanidine.

Table IV shows that pregnancy, adrenalectomy, and treatment with progesterone or estradiol do not materially change the pattern of the urinary metabolites of histamine in mice. However, cortisone produces a marked increase in the relative magnitude of Peak 3 with respect to Peaks 1 and 2. This increase in unmetabolized histamine cannot definitely be attributed to inhibition of either of the histamine-metabolizing enzymes, for the relative sizes of Peaks 1 and 2 with respect to each other are not much changed. It is likely that the cortisone influences histamine metabolism by a mechanism other than inhibition of these metabolic enzymes.

Table V affords no evidence of significant inhibition of histidine decarboxylase in mice by cortisone, d-catechin, or 3,4-dihydroxychalcone. As the number of animals used was limited by the high requirement of C14-L-histidine, the experiment is designed only to show pronounced effects. The finding of C14-histamine in the carcasses 30 hours after injection of C14-L-histidine shows that the mouse, like the guinea pig, binds some endogenous histamine in stable condition.

**SUMMARY**

1. Use of inhibitors in vivo shows that, in all the species tested, there are two histamine-metabolizing enzymes. One is diamine oxidase; the other is unknown. It is proposed that the name "histamine-metabolizing enzyme II" be applied to the latter until its nature is better understood.

2. In vivo studies, paralleling those customarily performed on enzymes in vitro, are reported. They deal with the effect of concentration of histamine on its rate of metabolism, and with the nature of the inhibition produced by diamine oxidase inhibitors.

3. Effects of certain hormones on the metabolism of histamine in mice were tested; cortisone produces an alteration of histamine metabolism which is described.

4. No inhibition of histidine decarboxylase in intact mice was observed after treatment with either cortisone or with two in vitro inhibitors.

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STUDIES ON
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ENZYMES IN INTACT ANIMALS. II
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