THE METABOLISM OF L-AND D-HISTIDINE BY SLICES OF LIVER AND KIDNEY*

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Whether oxidative deamination is the favored initial step in the catabolism of histidine, as may be the case with many of the amino acids, is open to question. The supporting evidence of Krebs (1) is less striking with L-histidine than with most other natural amino acids. Kidney slices yielded somewhat more ammonia in the presence of histidine and oxygen than in the absence of either, and liver slices produced small amounts of urea and ammonia, apparently at the expense of amino nitrogen and with a measurable oxygen uptake.

According to Edlbacher and his associates (2) L-histidine is also attacked in the liver by histidase, an enzyme which leaves the α-amino group intact, but hydrolyzes the imidazole ring in such a way as to permit ultimate conversion of the histidine to glutamic acid. Like glutamic acid, L-histidine is glycogenic (3, 4).

Production of urocanic acid from histidine by reductive α-deamination has long been considered a possibility. Recently Edlbacher and Heitz (5) have isolated a hepatic enzyme (urocanase) able to convert urocanic acid quantitatively to L-glutamic acid, presumably via isoglutamine, the α-carbon in this instance having its origin in the imidazole ring.

That some oxidative or hydrolytic α-deamination of histidine may occur seems likely. In the rat, imidazolelactic acid (6, 7) and imidazolepyruvic acid (6) can serve as dietary substitutes for histidine, and D-histidine can be inverted to form the L isomer (8). Schoenheimer, Rittenberg, and Keston (9) noted that histidine isolated from the tissues of rats fed N15 as ammonia contained an excess of this isotope in its α-amino group.

Without doubt D-histidine is less readily utilized than the natural isomer (4, 8). It is said not to be attacked by histidase (2). According to Krebs (1) it is deaminated in the liver and kidney by D-amino acid oxidase. This has not been substantiated by tests with the reconstituted enzyme system. In such tests, Klein and Handler (10) observed only slow oxidation, Karrer and Frank (11) none.

* The experimental data presented in this communication are taken from a dissertation submitted by Robert M. Featherstone in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry in the Graduate College of the State University of Iowa.
Most of the studies on histidase have been made with liver extracts, which probably lack L-amino acid deaminase activity (1). The present communication records tests which were undertaken in an effort to determine whether oxidative deamination or histidase activity predominates in liver tissue and whether appreciable deamination of histidine occurs in the kidney. Slices of rat liver and rat kidney were employed. Oxygen uptake and changes in amino nitrogen and in ammonia (or ammonia and urea) were determined simultaneously. Both L- and D-histidine were used in media buffered with phosphate or with bicarbonate at the physiological pH of 7.4, as well as at pH 8.4 which Krebs found more nearly optimum for the activity of D-amino acid oxidase and Edlbacher more suitable for the activity of histidase.

EXPERIMENTAL

The L-histidine used in these studies was isolated as the L-histidine monohydrochloride monohydrate from spray-dried blood by the method of Cox, King, and Berg (12). It was racemized as directed by Duschinsky (13) and the D-histidine was prepared from the racemate as the monohydrochloride monohydrate, essentially as outlined by Conrad and Berg (8). Solutions of 2 gm. of the monohydrochloride monohydrate and 1 equivalent of hydrochloric acid in 100 cc. of water gave \([\alpha]_D^{25} = +8.32^\circ\) for the L and \(-8.20^\circ\) for the D form (cf. (8, 14)). Both isomers melted at 251–253°. None of the preparations responded to Sullivan's naphthoquinone test for cystine, which Darby and Lewis (15) have reported may contaminate preparations of histidine monohydrochloride, particularly if the histidine is not first separated as the dihydrochloride, as was done in our procedure. The ninhydrin “carboxyl nitrogen” method of assaying free amino acids (16) yielded 99 per cent or more of the calculated “carboxyl nitrogen” on aliquots containing as little as 0.04 mg. of histidine. Since the amino acid was to be used in a system sensitive to mercury and its preparation had involved its isolation initially as the mercuric sulfate complex, it was tested for residual mercury. No evidence that even minute traces were present could be obtained by the Reinsch, SnCl₂, or dithizone tests, or by spectrographic analysis. For use in the tissue slice studies the histidine monohydrochloride monohydrate was dissolved in ammonia-free water together with an equivalent weight of sodium hydroxide. The requisite amount of histidine was employed to provide a 0.02 M solution after its admixture with the buffered medium in which the tissue was suspended.

The tissue slices were prepared from the livers or kidneys of rats from Sprague-Dawley stock which had been maintained on a diet of Purina dog chow. The animals were killed by a blow on the head. The slices were suspended in phosphate or bicarbonate media, prepared as directed by
Krebs and Henseleit (17). Addition of the histidine solution caused no appreciable change in pH.

Standard manometric procedures were used to determine oxygen utilization. Data for experiments in which bicarbonate buffers were used were obtained by employing the apparatus described by Dixon and Keilin (18). The gassing in these instances was with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide, rather than with the usual 100 per cent oxygen. Oxygen data were recorded for a period of 1½ or 3 hours before the slices were treated with acid from the side sac. The slices were then removed for weighing, and the solutions for analysis.

When urea and ammonia were determined together, as was usual in the studies with liver slices, the aliquots were incubated for 30 minutes with urease in the form of a finely ground Arloco tablet. The total ammonia was measured by distillation and titration, essentially as described by Keys (19). The same procedure was used for estimating ammonia in the tests with kidney slices. The standard acid and alkali solutions were checked against each other daily and the normality of the standard alkali was redetermined each month by titration against potassium acid phthalate. Blank determinations were made routinely on all other solutions and reagents used in the study and corrections were applied as found necessary.

Amino nitrogen was measured by the ninhydrin “carboxyl nitrogen” method (16). To verify the calculated quantity of amino nitrogen added to the respirometers, the same volumes of amino acid, buffer solution, and acid as were used in the studies were mixed together and analyzed.

RESULTS AND DISCUSSION

The data, summarized in Tables I and II, were calculated conventionally as Q values, Q representing the apparent change per mg. of dry tissue per hour in c.mm., of oxygen, or non-gaseous substance expressed in c.mm., 1 millimole being considered equivalent to 22,400 c.mm. The average Q (experimental minus control) values tabulated represent differences between averages of triplicate or quadruplicate tests in which the amino acid was added to the flasks, and averages of simultaneous tests in which slices were incubated with the buffered medium alone. A plus sign indicates that the average oxygen uptake by the flasks containing the amino acid was the greater. To avoid confusion, readings of the Dixon-Keilin differential manometer were expressed in the same way, even though this is contrary to the usual practice.

Q_{\text{urea + NH}_3} includes both urea and ammonia expressed as ammonia, Q_{\text{NH}_3} only ammonia. A plus difference indicates a greater average content, after incubation, in the experimental than in the control flasks, a negative difference the converse.

Some free amino acid nitrogen (carboxyl nitrogen) was always found in
the media of the control flasks after incubation. In determining changes in the media of the experimental flasks, obviously the added amino acid nitrogen was considered. If it is assumed that the liberation of amino acid nitrogen from tissue is not appreciably altered by the presence of added amino acid, then a minus difference in $Q_{\text{amino N}}$ values may be said to indicate utilization of amino acid nitrogen; a plus difference would indicate either lack of utilization or its masking by an excessive simultaneous liberation of amino acid nitrogen from the tissue.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utilization of Oxygen and Amino Nitrogen and Production of Ammonia and Urea by Slices of Rat Liver Incubated with Media Containing $L$- or $D$-Histidine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Average $Q$ (experimental minus control)</th>
<th>pH</th>
<th>Buffer</th>
<th>Special addition to media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O$_2$</td>
<td>Urea + NH$_3$</td>
<td>Amino N</td>
<td></td>
</tr>
<tr>
<td>$L$-Histidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>-0.38</td>
<td>+11.10</td>
<td>-6.26</td>
<td>7.4</td>
</tr>
<tr>
<td>III</td>
<td>-6.51</td>
<td>+3.20</td>
<td>-10.28</td>
<td>8.4</td>
</tr>
<tr>
<td>V</td>
<td>0.00</td>
<td>+5.97</td>
<td>-6.05</td>
<td>7.4</td>
</tr>
<tr>
<td>VII</td>
<td>+6.24</td>
<td>-6.72</td>
<td>8.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>VIII</td>
<td>+17.07</td>
<td>-5.57</td>
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<td>&quot;</td>
</tr>
<tr>
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<td>+0.60</td>
<td>+13.85</td>
<td>-6.91</td>
<td>8.4</td>
</tr>
<tr>
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<td>+8.90</td>
<td>-10.30</td>
<td>8.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>XI</td>
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<td>-9.54</td>
<td>8.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>$D$-Histidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
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<td>-2.28</td>
<td>-2.35</td>
<td>7.4</td>
</tr>
<tr>
<td>IV</td>
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<td>+0.37</td>
<td>-2.12</td>
<td>8.4</td>
</tr>
<tr>
<td>VI</td>
<td>-1.73</td>
<td>-3.32</td>
<td>-2.81</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Table I records the results of several series of liver slice tests with $L$- and $D$-histidine. In every trial with $L$-histidine an increase in urea plus ammonia occurred. In only one of the four series in which oxygen uptake was measured was there any indication that oxygen was consumed. The exception (Series IX) was one in which the medium was saturated with octyl alcohol which, according to Krebs (1), inhibits the activity of $L$-amino acid deaminase. In none of the tests in which the $L$-histidine media were saturated with octyl alcohol was there evidence of suppressed ammonia and urea production. Comparisons with control tests indicated quite the contrary. Disappearance of amino nitrogen was also greater. The data suggest that the changes were chiefly non-oxidative.

The decrease in amino nitrogen indicates that the change may not be
confined to the production of glutamic acid by histidase, which liberates 2 equivalents of ammonia but does not affect the amino nitrogen. Non-oxidative metabolism of the glutamic acid produced, formation of urocanic acid, conversion to histamine, and production of peptides are all possibilities, but the data afford no indication as to which or how many of these may be involved.

Trial tests on DL-alanine without octyl alcohol strongly suggested that oxidative deamination occurred. In the presence of octyl alcohol considerable inhibition of ammonia and urea formation and of amino nitrogen utilization was observed.

The data on D-histidine show little or no production of urea plus ammonia and a loss of amino nitrogen much smaller than with L-histidine. The evidence fails to support either oxidation by D-amino acid oxidase or cleavage by histidase. The former observation agrees with the evidence which Klein and Handler (10) and Karrer and Frank (11) obtained in tests with reconstituted D-amino acid oxidase, the latter observation with the claim of Edlbacher (2) that histidase does not attack D-histidine.

Table II records the data obtained in experiments with kidney slices and D- or L-histidine. When D-histidine was tested in the phosphate-buffered media, no oxygen uptake occurred and little or no amino nitrogen disappeared; in the bicarbonate media, the oxygen uptake, ammonia production, and diminution of amino nitrogen all favored the possibility that some oxidative deamination may have taken place. When arsenious
oxide was added to the system, the disappearance of amino nitrogen became
marked, but oxygen uptake and ammonia formation diminished sharply. 
According to Krebs arsenious oxide does not interfere with deamination by 
N-amino acid oxidase, but prevents oxidation of the deaminized residue 
beyond the ketonic acid stage.

Comparative tests indicated that DL-alanine (in phosphate-buffered 
media) was much more vigorously attacked. A greater loss of amino 
nitrogen was observed when arsenious oxide was added to the system, but 
the oxygen uptake and the production of ammonia were not as markedly 
affected as in comparable tests with D-histidine.

Incubation of L-histidine with kidney slices induced no oxygen uptake 
and no appreciable production of ammonia. These results are contrary to 
those noted in a single test by Krebs in which he obtained differences in 
Q values of +2.10 for oxygen, +0.91 for ammonia, and −2.40 for amino 
nitrogen. Krebs’ contention that D-histidine is more readily deaminated 
in the kidney than is the L isomer is apparently based solely on differences 
inQNHa of +8.42 for D-histidine and +1.85 for the L-amino acid (1).

SUMMARY

Data obtained on oxygen uptake, amino nitrogen diminution, and urea 
and ammonia production, upon incubating media containing histidine with 
liver slices, seem to indicate that histidase plays a more important rôle than 
L-amino acid deaminase in the metabolism of the natural isomer of this 
amino acid by this tissue. Comparable tests indicate that the D isomer of 
histidine is not attacked by histidase and is not readily oxidized by N-amino 
acid oxidase.

Analogous tests with kidney slices afford some indication that D-histidine 
may undergo slight oxidative deamination in the kidney, but no evidence 
that L-histidine is either oxidatively deaminized or split hydrolytically in 
that organ.

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