THE METABOLISM OF HISTIDINE

I. EFFECTS OF VITAMIN B\textsubscript{6} AND OF BIOTIN DEFICIENCY*

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(Received for publication, January 18, 1957)

Histidine is unique among the commonly occurring amino acids in that a major route (1) of its catabolism in the animal organism involves direct removal of the amino group to form an unsaturated compound, urocanic (imidazoleacrylic) acid, and ammonia. This may be considered as straight deamination in contrast to oxidative deamination and transamination reactions which have been described for most amino acids. In the latter reactions, riboflavin derivatives (2) and pyridoxal phosphate (3), respectively, have established coenzyme roles. Little is known, however, of the part that vitamin B\textsubscript{6} derivatives play in straight deamination of amino acids.

Biotin has been implicated as a cofactor of bacterial enzyme systems by which aspartic acid is deaminated to form fumaric acid (4). The action of aspartase in bacteria is analogous to that of histidase in mammalian and other species:

\[
R-\text{CH}_2-\text{CH}-\text{COOH} \rightarrow R-\text{CH}=\text{CH}-\text{COOH} + \text{NH}_2
\]

where

\[
\begin{align*}
R &= \text{HOOC--} \text{ in aspartic acid (aspartase)} \\
R &= \text{HC==C--} \text{ in histidine (histidase)}
\end{align*}
\]

Thayer and Horowitz (5) reported a several fold increase in the amount

* Supported in part by a grant from the National Institutes of Health, United States Public Health Service (grant No. A650, M and N).

\dagger Aided by a fellowship from The National Foundation for Infantile Paralysis, Inc.
of L-amino acid oxidase produced by *Neurospora* grown in the presence of limited, as compared with higher, concentrations of biotin. The oxidase was more active toward histidine than toward other amino acids tested.

In view of these relationships of biotin and of the multiple role of vitamin B₆ derivatives in amino acid metabolism, it was of interest to investigate the effects of deficiencies of each vitamin on liver enzyme systems concerned with the straight deamination of histidine. In addition to histidase and urocanase, levels of aspartic-glutamic transaminase and rhodanese were measured. Rhodanese, which is not known to be linked to any major metabolic process, was assayed as an index of possible effects of the vitamin deficiencies on enzyme protein synthesis. The levels of transaminase provided an estimate of the degree of vitamin B₆ deficiency in the tissues of the animals fed diets lacking in this vitamin.

From these studies, evidence has been obtained that an adaptive increase in liver histidase accompanies depletion of vitamin B₆ or biotin in the tissues of the rat. It was also found that histidase, urocanase, and rhodanese activities per gm. of liver increase during normal growth.

**EXPERIMENTAL**

*Pyridoxine Deficiency*—Male, white rats of the Wistar strain (initial weight, 40 to 50 gm.) were fed a high protein (40 per cent casein) diet, devoid of pyridoxine, which contained deoxypyridoxine and isoniazide. The diet contained, in addition to the casein, in gm. per 100 gm., sucrose 45.8, powdered cellulose 5, salts (6) 2, corn oil 4, cod liver oil 2, choline 0.2, and vitamin mixture 1. In the vitamin mixture for the control animals were 50 mg. each of pyridoxine hydrochloride, thiamine hydrochloride, and riboflavin, 20 mg. each of biotin and folic acid, 200 mg. each of niacin, calcium pantothenate, and p-aminobenzoic acid, 20 gm. of inositol, and powdered sucrose to make 100 gm. The vitamin mixture used in the diet for the deficient animals was the same, except that pyridoxine was omitted and 100 mg. each of deoxypyridoxine and isoniazide were added per 100 gm. The intake per 10 gm. of food was thus 50 γ of pyridoxine hydrochloride or 100 γ of both of the antagonists. After 18 days of feeding of the deficiency diets, the usual symptoms (7) of pyridoxine deficiency were observed. The animals were killed at intervals during the following 10 days.

One group of animals was fed *ad libitum* up to a maximum of 10 gm. of food per day per rat. A second group was pair-fed. Several animals died in severe deficiency states. Data were obtained from ten control and five deficient animals fed *ad libitum* and from eight pairs of the pair-fed group.

Since the mean body weight of the deficient animals, at the time of
death, was less than half that of the control rats (54 gm. versus 115 gm. in the pair-feeding experiment), groups of normal rats of similar weight ratios were also studied. Twelve rats each were fed 10 gm. per day of the control diet. Four of these animals were killed during 1 week to provide a group of average weight of 75 gm.; the remainder were killed 2 or 3 weeks later, at which time the mean body weight was 164 gm.

Biotin Deficiency—Other male rats (initial weight 40 to 48 gm.) were fed diets similar to that described for the pyridoxine-control animals, except that cascin was replaced by commercial dried raw egg white. Biotin was omitted from the vitamin mixture used in preparation of the deficient diet for half of the rats. The remainder served as controls and were given 40 γ of biotin by intraperitoneal injection three times a week during the experimental period. The animals were pair-fed. After 7 weeks of dietary control, those which received no biotin exhibited various signs of biotin deficiency (8). Data were obtained from nine pairs. The average of the weights of the deficient animals at time of death during the 8th week was 122 gm.; that of the control animals, 146 gm.

Assay Procedures—The rats were killed by decapitation. A portion of liver was taken for determination of total nitrogen (Kjeldahl method) and of dry weight (overnight at 105°). The remainder was ground with 5 volumes of cold 1 per cent KCl solution per gm. of tissue in a Potter-Elvehjem homogenizer immersed in an ice bath. After removal of an aliquot for rhodanese determination, the homogenate was centrifuged at 6000 × g for 10 minutes at 0°. Histidase, urocanase, and aspartic-glutamic transaminase were measured in the supernatant fluid.

The maximal rates of formation (at pH 9.2) and of disappearance (at pH 7.4) of urocanic acid, as measured at 277 mμ, the absorption maximum of the latter, were used to determine histidase and urocanase levels, respectively. Portions of supernatant material (0.05 or 0.10 ml.) were incubated with histidine or urocanic acid and buffer in 1 cm. Beckman cells as described by Mehler and Tabor (1).

For determination of transaminase activity, the coupled reaction scheme was utilized in which the rate of oxalacetate formation is followed by conversion of the latter to malate in the presence of excess malic dehydrogenase with the concomitant oxidation of reduced diphosphopyridine nucleotide (DPNH). The rate of disappearance of DPNH was measured at 340 mμ. The procedure and reagents were essentially those described by Karmen (9) for determination of serum transaminase, except that 2.0 ml. of 0.1 M phosphate buffer, pH 7.4, were used in a total volume of 3.0 ml. 0.1 ml. of a solution made by diluting the supernatant fluid 25-fold was used in place of serum.

For assay of histidase, urocanase, and aspartic-glutamic transaminase,
the temperature of incubation was 28°. Increase in temperature due to heat output from the hydrogen lamp of the Beckman DU spectrophotometer was minimized by insertion of the standard test tube attachment between the light source and cuvette holder. Preparations from deficient and corresponding control animals were usually analyzed simultaneously or within 1 hour of each other.

Rhodanese activities were measured by the colorimetric method of Cosby and Sumner adapted by Rosenthal et al. (10), modified as suggested by Rosenthal and Vars (11) by the use of 0.1 M 2-amino-2-methyl-1,3-propanediol sulfate buffer, pH 8.8. 0.1 ml. portions of one to five dilutions of the whole homogenates were used for analysis. Temperature of incubation for rhodanese was 20°.

Urocanic acid was made by enzymatic deamination of histidine with preparations from histidine-adapted strains of Pseudomonas fluorescens (1, 12). The molar extinction coefficients (at 277 μm) of the products obtained from several runs were between 18,500 and 18,600. In subsequent calculations, the latter figure was utilized for histidase and urocanase, and the extinction coefficient for reduced diphosphopyridine nucleotide reported by Horecker and Kornberg (13) was used for transaminase activities.

Enzyme activities were calculated in terms of micromoles of substrate destroyed per minute per gm. of liver, a unit being the amount of enzyme which catalyzes the destruction of appropriate substrate at the rate of 1 μmole per minute under the conditions used. Differences between the groups of animals were similar, whether activities were expressed as units per gm. of wet or dry weight or of total nitrogen of liver, or were based upon the protein content of the supernatant fluid from the homogenates. The averages of the dry weights of liver tissues from the pyridoxine-deficient rats and their pair-fed controls and from the biotin-deficient rats and the pair-fed controls were respectively 30.9, 29.3, 31.3, and 31.9 per cent; corresponding values for per cent total nitrogen were 3.67, 3.76, 3.85, and 3.90; averages for protein content of supernatant fluids, determined according to Lowry et al. (14), were 18.2, 18.1, 19.3, and 18.4 mg. per ml., respectively.

**Results**

In Table I is a summary of the data obtained for histidase, urocanase, aspartic-glutamic transaminase, and rhodanese in the livers of vitamin B₆-deficient and control rats. Only histidase and urocanase were determined in the livers of the animals fed ad libitum. The results were so similar to those obtained with the pair-fed rats that they are presented together in Table I. Due to manipulative losses, values for aspartic-
glutamic transaminase and rhodanese were available from only seven of the eight pairs of rats of the pair-feeding experiment.

The levels of histidase in the livers of the deficient animals are significantly greater than in livers of control rats. The depressed levels of transaminase are regarded as evidence of decreased effective amounts of tissue pyridoxal phosphate. The similarity of rhodanese and urocanase concentrations in both groups is interpreted to mean that liver enzyme protein synthesis was not generally affected by lack of pyridoxal phosphate.

Although the deficient animals were of the same age as the control rats, they weighed considerably less. The elevated levels of liver histidase, however, cannot be ascribed to differences in body weight. As shown in Table II, concentrations of this enzyme, as well as of urocanase and rhodanese, are normally lower in smaller than in larger (older) rats. Aspartic-glutamic transaminase activities per gm. of tissue did not change significantly with age during the period studied.

In Table III are comparable data for biotin-deficient and pair-fed control rats. Histidase levels were also somewhat elevated in the livers of animals deficient in biotin. The differences are significant at a probability level of between 1 and 5 per cent, according to Fisher's "t" test (15). Urocanase, aspartic-glutamic transaminase, and rhodanese activities were not significantly different from control values.

### Table I

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Diet</th>
<th>No. of animals*</th>
<th>Range</th>
<th>Mean</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidase</td>
<td>Control</td>
<td>18</td>
<td>0.232-0.426</td>
<td>0.315</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>13</td>
<td>0.244-0.702</td>
<td>0.415</td>
<td></td>
</tr>
<tr>
<td>Urocanase</td>
<td>Control</td>
<td>18</td>
<td>0.298-0.517</td>
<td>0.377</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>13</td>
<td>0.236-0.458</td>
<td>0.362</td>
<td></td>
</tr>
<tr>
<td>Aspartic-glutamic transaminase</td>
<td>Control</td>
<td>7</td>
<td>30.1-77.0</td>
<td>51.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>7</td>
<td>10.6-21.4</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
<td>Rhodanese</td>
<td>Control</td>
<td>7</td>
<td>496-731</td>
<td>605</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>7</td>
<td>480-623</td>
<td>547</td>
<td></td>
</tr>
</tbody>
</table>

* These numbers include both rats which were fed ad libitum and those which were pair-fed for histidase and urocanase, and pair-fed rats for transaminase and rhodanese.
† Probability level according to Fisher's "t" test (15).
‡ Micromoles of substrate destroyed per minute per gm. of liver (see the text): histidase, pH 9.2, 38°; urocanase, pH 7.4, 38°; transaminase, pH 7.4, 28°; rhodanase, pH 8.8, 20°.
### TABLE II

**Enzyme Content of Rat Liver**

<table>
<thead>
<tr>
<th>Weight of rat (gm.)</th>
<th>Histidase unit per gm.*</th>
<th>Urocanase unit per gm.</th>
<th>Aspartic-glutamic transaminase units per gm.</th>
<th>Rhodanese units per gm.</th>
<th>Total nitrogen per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>0.188</td>
<td>0.184</td>
<td>53.4</td>
<td>363</td>
<td>2.71</td>
</tr>
<tr>
<td>71</td>
<td>0.230</td>
<td>0.294</td>
<td>46.8</td>
<td>483</td>
<td>3.49</td>
</tr>
<tr>
<td>80</td>
<td>0.221</td>
<td>0.267</td>
<td>61.2</td>
<td>517</td>
<td>3.53</td>
</tr>
<tr>
<td>87</td>
<td>0.184</td>
<td>0.267</td>
<td>58.4</td>
<td>361</td>
<td>3.13</td>
</tr>
</tbody>
</table>

Mean ....... 0.206 0.253 55.0 431 3.22

<table>
<thead>
<tr>
<th>Weight of rat (gm.)</th>
<th>Histidase unit per gm.*</th>
<th>Urocanase unit per gm.</th>
<th>Aspartic-glutamic transaminase units per gm.</th>
<th>Rhodanese units per gm.</th>
<th>Total nitrogen per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>0.291</td>
<td>0.348</td>
<td>66.7</td>
<td>575</td>
<td>3.66</td>
</tr>
<tr>
<td>148</td>
<td>0.392</td>
<td>0.405</td>
<td>47.9</td>
<td>799</td>
<td>3.84</td>
</tr>
<tr>
<td>148</td>
<td>0.396</td>
<td>0.524</td>
<td>58.0</td>
<td>834</td>
<td>3.98</td>
</tr>
<tr>
<td>164</td>
<td>0.315</td>
<td>0.353</td>
<td>42.8</td>
<td>651</td>
<td>3.48</td>
</tr>
<tr>
<td>174</td>
<td>0.304</td>
<td>0.396</td>
<td>51.6</td>
<td>549</td>
<td>3.44</td>
</tr>
<tr>
<td>176</td>
<td>0.322</td>
<td>0.396</td>
<td>44.9</td>
<td>578</td>
<td>3.40</td>
</tr>
<tr>
<td>180</td>
<td>0.386</td>
<td>0.469</td>
<td>55.7</td>
<td>750</td>
<td>3.95</td>
</tr>
<tr>
<td>183</td>
<td>0.386</td>
<td>0.405</td>
<td>49.0</td>
<td>674</td>
<td>3.67</td>
</tr>
</tbody>
</table>

Mean ....... 0.349 0.412 52.2 676 3.68

* Micromoles of substrate destroyed per minute per gm. of liver (see the text): histidase, pH 9.2, 28°C; urocanase, pH 7.4, 28°C; transaminase, pH 7.4, 28°C; rhodanese, pH 8.2, 20°C.

### TABLE III

**Enzyme Levels in Livers of Biotin-Deficient and Pair-Fed Control Rats***

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Diet</th>
<th>Range units per gm.†</th>
<th>Mean units per gm.</th>
<th>F†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histidase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.171-0.325</td>
<td>0.241</td>
<td>&lt;0.05, &gt;0.01</td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>0.208-0.462</td>
<td>0.303</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Urocanase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.213-0.342</td>
<td>0.284</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>0.207-0.421</td>
<td>0.314</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aspartic-glutamic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transaminase</td>
<td>Control</td>
<td>26.5-80.9</td>
<td>50.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>40.5-74.2</td>
<td>57.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Rhodanese</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>557-750</td>
<td>669</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>468-744</td>
<td>645</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Nine pairs.

† Probability level according to Fisher's "t" test (15).

‡ Micromoles of substrate destroyed per minute per gm. of liver (see the text): histidase, pH 9.2, 28°C; urocanase, pH 7.4, 28°C; transaminase, pH 7.4, 28°C; rhodanese, pH 8.2, 20°C.
The disparity between mean enzyme concentrations in the livers of the two sets of control animals of the vitamin B₆ and biotin deficiency studies is believed to be related to the dietary protein sources used. This question will be considered in a subsequent paper.

**DISCUSSION**

Dietary vitamin B₆ is required for maintenance of the liver enzyme system by which histidine undergoes transamination in the rat (16). The observed elevation in histidase levels in the livers of the pyridoxine-deficient animals is interpreted as an adaptive increase in this enzyme which catalyzes straight deamination in response to denial of a route of degradation of histidine which is pyridoxal phosphate-dependent; i.e., transamination.

The increase in histidase levels in the livers of rats made deficient in biotin is analogous to the adaptive increase in L-amino acid oxidase production by *Neurospora* grown in media which contained limited amounts of this vitamin. These findings would seem to implicate biotin in some, as yet undiscovered, role in amino acid metabolism.

In early work, Edlbacher and Becker (17) observed increased levels of histidase in livers of thiamine-deficient animals. Conflicting reports (18, 19) have appeared from Japanese workers as to the effect of folic acid in activation of partially purified preparations of liver histidase. Ichihara *et al.* (20) have reported a summary of experiments in which liver histidase was decreased in rats fed diets containing Aminopterin or which were low in folic acid. Addition of folic acid and glutathione to crude enzyme preparations from these animals restored activity to levels found in control rats. The data were based upon disappearance of histidine in various incubation mixtures rather than on the rate of formation of urocanic acid.

It is of interest that histidase, urocanase, and rhodanese are increased to a greater extent than the total liver nitrogen during rapid growth, whereas aspartic-glutamic transaminase levels remain unchanged. The observed increase in histidase is in agreement with the findings of Ross and Ely (21). Cohen and Hekhuis (22) found higher levels of transaminase in the livers of older, as compared with younger, cats. Beaton *et al.* (23) reported that liver aspartic-glutamic transaminase levels increased with age; however, the rats in their study were older (i.e., weighed more initially) than the ones used in the present work.

**SUMMARY**

1. Significantly elevated levels of histidase accompanied decreased levels of aspartic-glutamic (and presumably of histidine) transaminase in the livers of vitamin B₆-deficient rats. Urocanase and rhodanese concentrations were little different from control values.
2. Liver histidase levels were also higher in biotin-deficient than in corresponding control rats (with a difference significant at a probability level of between 5 and 1 per cent). No significant difference was noted in aspartic-glutamic acid transaminase, in urocanase, or in rhodanese concentrations.

3. Concentrations of liver histidase, urocanase, and rhodanese, but not of aspartic-glutamic transaminase, increase during rapid growth of the rat.

4. Changes in enzyme activity are discussed in terms of metabolic adaptation to depletion of tissue cofactors.

The expert technical assistance of Mrs. Donna Bullock and of Mr. Leo R. Black is gratefully acknowledged. We should like to thank Dr. Harry Vars and Dr. Otto Rosenthal for suggestions regarding the rhodanese procedures, and Dr. Alan H. Mehler for cultures of histidine-adapted Pseudomonas used in this work.

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