THE METABOLISM OF HISTIDINE

II. EFFECTS OF FOLIC ACID DEFICIENCY*

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The mechanism of and possible cofactors involved in the straight deamination of histidine remain unknown. Neither pyridoxal phosphate, established as a cofactor in transamination of many amino acids, nor biotin, implicated in the straight deamination of aspartic acid, apparently plays a role in this reaction (1).

Ichihara et al. (2) have reported low levels of histidase in the livers of rats which were fed goat’s milk (presumably low in folic acid) or diets which contained Aminopterin. However, conflicting reports (3, 4) have been published as to the effect of folic acid on the activation of partially purified preparations of liver histidase. If folic acid were to function in some co-enzyme role in straight deamination reactions, additions to or revisions of present concepts (5) of the mode of action of derivatives of this vitamin would seem to be required. Thus, it was of interest to study further the effects of dietary folic acid deficiency on levels of liver enzyme systems involved in the metabolism of histidine.

EXPERIMENTAL

Methods—Male, white rats (initial weight 40 to 50 gm.) were pair-fed diets similar to those described previously (see (1) “Pyridoxine-control animals”), except that casein was fed as 20 per cent and sulfasuxidine as 1 per cent of the diets, and p-aminobenzoic acid was omitted. Appropriate changes were made in sucrose content. Folic acid was omitted from the vitamin mixtures fed to the deficient rats. In the first group of animals to be reported, no choline was fed to either control rats or rats on deficient diets (choline-folic acid deficiency). In the second group, the diets fed contained choline (simple folic acid deficiency). After 7 weeks of dietary control, animals fed diets lacking in folic acid exhibited typical symptoms of folic acid deficiency (6). Several died in severe deficiency states, and the surviving control animals were then fed 10 gm. per day of the control diet. Data were obtained from five rats on the deficient diet.

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and seven control rats in the first group and from five diet-deficient and eleven control animals in the second group. 1 week before death the mean leucocyte count for the folic acid-deficient animals was 5250 (range 3600 to 6250) and that for the control rats was 12,400 (range 8350 to 17,300). Corresponding values for hematocrit readings were 35 per cent (range 26 to 48 per cent) and 44 per cent (range 40 to 51 per cent). The mean body weights were 110 and 165 gm., respectively.

The animals were killed during the 8th week, and liver histidase, urocanase, and rhodanese levels and per cent dry weight were determined as described previously (1), except that histidase and urocanase analyses were made with a Cary recording spectrophotometer equipped to maintain the temperature of the incubation mixtures at 30°C. Since the average percentage dry weight of the livers from the folic acid-deficient animals was less (mean 28.3, range 27.3 to 29.7 per cent) than that of control rats (mean 31.2, range 30.5 to 32.0 per cent), the results are expressed as units (micromoles of appropriate substrate destroyed per minute (1)) per gm. of dry liver tissue.

Results

The results obtained with the first group of animals are shown in Table I. Choline was omitted from the diet of these animals in an attempt to hasten the depletion of folic acid in accordance with the suggestion of Stekol et al. (7). The livers from the animals fed the diet lacking in both choline and folic acid appeared to be infiltrated with fat and the supernatant material obtained after centrifugation of homogenates therefrom was milky in each case. The observed depression in mean levels of the enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Diet</th>
<th>Range</th>
<th>Mean</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>units per gm.*</td>
<td>units per gm.</td>
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<tr>
<td>Histidase</td>
<td>Control</td>
<td>0.44–1.11</td>
<td>0.80</td>
<td>&gt;0.05</td>
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<td>0.40–1.14</td>
<td>0.61</td>
<td></td>
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<tr>
<td>Urocanase</td>
<td>Control</td>
<td>0.68–1.26</td>
<td>1.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>0.41–0.76</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Rhodanese</td>
<td>Control</td>
<td>1660–2440</td>
<td>2060</td>
<td>&lt;0.05, &gt;0.01</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>1270–1910</td>
<td>1620</td>
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</tbody>
</table>

* Seven control and five diet-deficient rats.
† Probability level according to Fisher's t test (8).
* Micromoles of substrate destroyed per minute per gm. of dry liver (1): histidase, pH 9.2, 30°C; urocanase, pH 7.4, 30°C; rhodanese, pH 8.8, 20°C.
studied may have been related to an increased fat content. However, only urocanase concentrations were significantly lower ($P < 0.01$) in the livers of the diet-deficient than in those of control rats.

Data obtained from the second group of rats (simple folic acid deficiency) are shown in Table II. The levels of histidase in livers of rats severely depleted of folic acid did not differ from those of control rats. However, the levels of urocanase were significantly lower in the livers of the animals on the deficient diet. The similar concentrations of rhodanese are interpreted as an indication that liver enzyme protein synthesis in general was not affected by the simple folic acid deficiency.

**TABLE II**

*Enzyme Levels in Livers of Folic Acid-Deficient and Control Rats*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Diet</th>
<th>Range</th>
<th>Mean</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>units per gm</td>
<td>units per gm</td>
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<tr>
<td>Histidase</td>
<td>Control</td>
<td>0.66-1.10</td>
<td>0.83</td>
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<tr>
<td></td>
<td>Deficient</td>
<td>0.58-1.13</td>
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<tr>
<td>Urocanase</td>
<td>Control</td>
<td>1.10-1.53</td>
<td>1.29</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>Deficient</td>
<td>0.87-1.02</td>
<td>0.94</td>
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<tr>
<td>Rhodanese</td>
<td>Control</td>
<td>1120-2020</td>
<td>1590</td>
<td>&gt;0.05</td>
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<td></td>
<td>Deficient</td>
<td>1250-1840</td>
<td>1540</td>
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</table>

* Eleven control and five diet-deficient rats.
† Probability level according to Fisher's $t$ test (8).
‡ Micromoles of substrate destroyed per minute per gm. of dry liver (1): histidase, pH 9.2, 30°; urocanase, pH 7.4, 30°; rhodanese, pH 8.8, 20°.

**DISCUSSION**

The report by Ichihara et al. (2) of decreased levels of liver histidase in folic acid-deficient rats was based upon measurement of the rate of disappearance of histidine by a modification of the bromination procedure of Kapeller-Adler; liver preparations were analyzed after an incubation period of 2 hours at 37°. In the present work, histidase was determined by measurement of the rate of formation of urocanic acid during a 10 to 15 minute period immediately after addition of substrate to an incubation mixture by a specific spectrophotometric method. In view of the disparity of the data reported here with that of Ichihara et al. (2), it seems likely that reactions other than the straight deamination of histidine to urocanic acid were measured by the latter workers.

The present finding of decreased levels of urocanase in the livers of folic acid-deficient rats is of interest. Folic acid or some derivative thereof may be involved as a cofactor in an intermediate reaction in the conversion of urocanic acid to $\alpha$-formamidinoglutaric acid. An alternative explanation
that an accumulation of α-formamidinoglutaric acid (known to occur during folic acid deficiency (9)) might cause an inhibition of urocanase activity remains to be tested.

SUMMARY

1. Levels of urocanase, but not of histidase, are significantly lower in livers of folic acid-deficient and folic acid-choline-deficient rats than in those of control animals.

2. Liver rhodanese levels were similar in folic acid-deficient and control rats but were somewhat lower in folic acid-choline-deficient than in corresponding control animals.

3. The suggestion is made that folic acid or a derivative may serve as a cofactor in the initial steps of the degradation of urocanic acid in the rat.

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BIBLIOGRAPHY

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