Effect of Vitamin B<sub>6</sub> Deficiency on the Basal and Adapted Levels of Rat Liver Tyrosine and Tryptophan Transaminases*

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Tyrosine-α-ketoglutarate transaminase activity in the liver was increased several-fold by the injection of hydrocortisone or L-tyrosine into the rat (1, 2). Since the activity of this enzyme was measured with an excess of its coenzyme, pyridoxal-P, it was assumed that the increase in the activity represented an increase in the concentration of the protein moiety of the transaminase. Few enzymes with dissociable coenzymes have been found to respond adaptively in their levels. The purpose of the present investigation was to determine whether the protein moiety of an enzyme could be induced under conditions which did not permit all of the enzyme molecules to acquire catalytic function, i.e. during deficiency of the coenzyme. For comparison, three other transaminases in the same organ were also studied. These were tryptophan-α-ketoglutarate transaminase, phenylalanine-pyruvate transaminase, and histidine-pyruvate transaminase (3).

EXPERIMENTAL

An inbred strain of Slonaker rats was used in these experiments. A group of 12 young rats with a mean body weight of 96 gm. was placed on a vitamin B<sub>6</sub>-deficient diet obtained from the Nutritional Biochemicals Corporation. A second group of 11 rats with a mean body weight of 79 gm. was placed on a control diet which consisted of the deficient diet supplemented with 0.5 mg. of vitamin B<sub>6</sub> per gm. of diet. The rats were given approximately 8 gm. of food daily. After 13 to 14 weeks on the diets, half of the animals from each group were killed, and the levels of the several enzymes in their livers were assayed with and without the addition of pyridoxal-P. The remaining animals from each group were given intraperitoneal injections of 30 mg. of hydrocortisone per kg. 5 hours before they were killed. The hormone was given in 2 to 5 ml. of saline. During the 13 weeks the mean body weight of the control group increased by 120 per cent, whereas that of the deficient group increased by only 70 per cent.

The preparation and assay of the enzymes have been described in another communication (3). All the enzyme activities are expressed as micromoles of substrate transaminated per gm. of dry liver per hour at 25°C. The activities of tryptophan-α-

ketoglutarate transaminase have been corrected for the indolylpyruvate which disappeared during the assay. This correction amounted to approximately 15 to 30 per cent of the apparent transaminase activity (3).

RESULTS

The transaminase activities in the liver of vitamin B<sub>6</sub>-deficient animals were lower than those of the control animals when pyridoxal-P was omitted from the assay systems (Table I, "Endogenous" data). The addition of an excess of the coenzyme to the assay system resulted in greater activation of the extracts prepared from the vitamin B<sub>6</sub>-deficient animals than in those from the normal animals. It is notable that the degrees of suppression with the deficiency and of activation by pyridoxal-P were different in the four transaminase activities studied. The most marked effects were observed with tyrosine-α-ketoglutarate transaminase, both in the control and in the deficient extracts. Activation of phenylalanine-pyruvate transaminase by pyridoxal-P was not observed in extracts from control animals but was readily shown in extracts from deficient animals.

In the presence of an excess of the coenzyme and under the conditions of assay which obtained, the rate of transamination was proportional to the apoenzyme content. Under such conditions the mean activity of tyrosine-α-ketoglutarate transaminase was higher in extracts prepared from vitamin B<sub>6</sub>-deficient rats, although the difference was not statistically significant (p = 0.08). The activities of phenylalanine-pyruvate transaminase and histidine-pyruvate transaminase in the extracts from deficient animals remained slightly but significantly lower than normal (p < 0.05). There was no significant difference in tryptophan-α-ketoglutarate transaminase activities in the two groups (Table I, columns 2 and 5).

Table II shows the measured activities of the apoenzymes (i.e. activity with excess coenzyme) of the four transaminases in the livers of the control and vitamin B<sub>6</sub>-deficient animals 5 hours after hydrocortisone treatment. It will be seen that the administration of this hormone to the animals caused both the tyrosine-α-ketoglutarate apotransaminase and tryptophan-α-ketoglutarate apotransaminase activities in the liver to increase. Furthermore, the adaptive increases of these two apoenzymes were not impaired by the deficiency of the coenzyme. Hydrocortisone was without significant effect on the levels of phenylalanine-pyruvate apotransaminase and histidine-pyruvate apotransaminase in the liver.

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Transaminase Levels in Vitamin B₆ Deficiency

**TABLE I**

*Basal levels of four liver transaminases in control and vitamin B₆-deficient rats*

<table>
<thead>
<tr>
<th>Transaminase</th>
<th>5 control animals</th>
<th>6 deficient animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endogenous*</td>
<td>With coenzyme†</td>
</tr>
<tr>
<td></td>
<td>Mean ± s.e.</td>
<td>Mean ± s.e.</td>
</tr>
<tr>
<td>Tyrosine-α-ketoglutarate</td>
<td>18 ± 3.9</td>
<td>73 ± 17</td>
</tr>
<tr>
<td>Tryptophan-α-ketoglutarate</td>
<td>13 ± 1.3</td>
<td>16 ± 2.2</td>
</tr>
<tr>
<td>Phenylalanine-pyruvate</td>
<td>100 ± 9.4</td>
<td>107 ± 7.6</td>
</tr>
<tr>
<td>Histidine-pyruvate</td>
<td>33 ± 5.0</td>
<td>43 ± 4.0</td>
</tr>
</tbody>
</table>

* No pyridoxal-P added to the assay mixture.  
† With excess pyridoxal-P in assay.

**TABLE II**

*Effect of hydrocortisone on levels of four transaminases in livers of control and vitamin B₆-deficient rats*

Both control and deficient animals were given injections of hydrocortisone. The levels of the transaminase in the livers of animals 5 hours after the treatment are given below. All the activities designated in this table were measured in the presence of an excess of pyridoxal-P (determination of the apoenzyme content). The activities of the hormone-treated control animals were compared (per cent change) with those of the untreated controls (Table I, column 2), and the activities of the hormone-treated deficient animals were compared with those of the untreated deficient animals (Table I, column 5). The statistical significance of the differences was evaluated by the *t*-test.

<table>
<thead>
<tr>
<th>Transaminase</th>
<th>Control animals</th>
<th>6 deficient animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s.e.</td>
<td>Per cent change</td>
</tr>
<tr>
<td>Tyrosine-α-ketoglutarate</td>
<td>370 ± 53</td>
<td>+400, <em>p &lt; 0.001</em></td>
</tr>
<tr>
<td>Tryptophan-α-ketoglutarate</td>
<td>37 ± 2.9</td>
<td>+432, <em>p &lt; 0.001</em></td>
</tr>
<tr>
<td>Phenylalanine-pyruvate</td>
<td>120 ± 9</td>
<td>+12, not significant</td>
</tr>
<tr>
<td>Histidine-pyruvate</td>
<td>49 ± 4.1</td>
<td>+14, not significant</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Studies of the concentrations of an apoenzyme maintained by cells during deficiency of the coenzyme might reveal the presence of an important homeostatic mechanism. Such a mechanism would increase the apoenzyme concentration whenever the coenzyme concentration decreases so that the concentration of the active enzyme would be maintained. Only two pyridoxal-P-requiring enzyme systems which have been studied showed increases in the apoenzyme concentrations under conditions of vitamin B₆ deficiency. The cellular concentrations of diaminopimelic apodecarboxylase and lysine apodecarboxylase in a vitamin B₆-requiring *Escherichia coli* mutant grown in trace amounts of pyridoxine were found to be twice as high as those in cells grown in a pyridoxine-rich medium (4). Tyrosine apodecarboxylase was produced by *Streptococcus faecalis* R in cells containing little or none of the coenzyme, but the relationship of the apoenzyme content to the quantity of pyridoxal-P available was not studied (5). The levels of apokynureninase (6) and 3,4-dihydroxyphenylalanine apodecarboxylase (7) in rat liver, of glutamic apodecarboxylase in rat brain (8), and of glutamic-oxalacetate apotransaminase and glutamic-pyruvate apotransaminase in rat liver (9) and duck ventricle (10) were not significantly affected by vitamin B₆ deficiency. The levels of cysteine sulfinic acid apodecarboxylase in rat liver (11, 12), of glutamic-oxalacete apotransaminase in rat kidney and heart (13), and of 5-hydroxytryptophan apodecarboxylase in rat kidney (14) decreased in vitamin B₆ deficiency. The results from the present studies indicated that there was a possibly significant compensatory increase in the apoenzyme concentration of tyrosine-α-ketoglutarate transaminase during deficiency of the coenzyme. Under the same conditions no increases were observed in the concentrations of three other apotransaminases: tryptophan-α-ketoglutarate, phenylalanine-pyruvate, and histidine-pyruvate. It may be significant that the levels of the two apotransaminases which could not be adaptively increased fell during the deficiency of the coenzyme, whereas the levels of the two apotransaminases which could be adaptively increased remained the same or rose with the deficiency.

During the course of the present investigation the protein moiety of a new enzyme, tryptophan-α-ketoglutarate transaminase, was found also to be increased in the liver by the administration of hydrocortisone. The increases of this apotransaminase and of the tyrosine-α-ketoglutarate apotransaminase induced by hydrocortisone were not affected by vitamin B₆ deficiency in the animal. This suggested that the protein moieties of these enzymes were produced by the liver cells at unimpaired rates even when the molecules could not be completely functional because of the lack of the coenzyme. It was recently reported that the protein moiety of another liver enzyme, glutamic-pyruvate apotransaminase, could also be increased by the administration of hydrocortisone and that the increase was not affected by vitamin B₆ deficiency in the rat (9).

The different adaptive behavior of the apotransaminases and the different degrees of activation in vitro by pyridoxal-P confirmed other evidence, not presented, that four separate enzymes...
were involved in the transaminations studied. The 4- to 5-fold increase in the tyrosine-transaminating activity caused by the administration of hydrocortisone distinguished this enzyme from the transaminase acting on tryptophan, which increased only 2- to 3-fold in the same livers. In all preparations the addition of pyridoxal-P produced greater activation of the tyrosine transaminase than of the tryptophan transaminase. The phenylalanine-pyruvate and histidine-pyruvate transaminase were set apart from the former two enzymes by their lack of response to hydrocortisone. The failure of pyridoxal-P to activate the phenylalanine-pyruvate transaminase activity unless the extracts were prepared from vitamin B₆-deficient rats indicated that this enzyme was different from the one that acted on histidine. The latter was activated by pyridoxal-P even in extracts prepared from normal rats (Table I).

SUMMARY

1. The activities of four different transaminases, tyrosine-α-ketoglutarate, tryptophan-α-ketoglutarate, phenylalanine-pyruvate, and histidine-pyruvate were studied in the livers of control and vitamin B₆-deficient rats. The lowered activities of these enzymes under conditions of vitamin B₆ deficiency were primarily attributable to a depletion of the coenzyme, pyridoxal phosphate, and not to significant alteration of the concentrations of the protein moieties of these enzymes.

2. The concentration of tryptophan-α-ketoglutarate apotransaminase in the liver, like that of the tyrosine-α-ketoglutarate apotransaminase, was increased by hydrocortisone treatment of the animals. The concentrations of phenylalanine-pyruvate and histidine-pyruvate apotransaminases were not changed by hydrocortisone treatment.

3. The increases of the protein moieties of tyrosine-α-ketoglutarate and tryptophan-α-ketoglutarate transaminases induced by hydrocortisone were not affected by deficiency of the coenzyme in the animal.

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REFERENCES

Effect of Vitamin B₆ Deficiency on the Basal and Adapted Levels of Rat Liver Tyrosine and Tryptophan Transaminases
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