THE ROLE OF PTEROYLGLUTAMIC ACID IN TYROSINE OXIDATION BY RAT LIVER TISSUE

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In so far as their function in nutrition is defined, the vitamins have been found to be essential components of several enzyme systems (1). In this connection there is evidence that the vitamin, pteroylglutamic acid (PGA), is necessary in an enzyme system concerned with oxidation of the amino acid tyrosine. Experiments have shown that livers from rats with a PGA deficiency produced by incorporating succinylsulfathiazole in the diet have a lowered capacity to oxidize tyrosine (2), and that tyrosine metabolites disappear from the urine of vitamin C-deficient guinea pigs when PGA is administered (3). More indirect is the evidence that untreated pernicious anemia patients, who presumably are deficient in PGA (4), cannot properly oxidize tyrosine (5).

It is the purpose of this paper to present in detail our experiments on the oxidation of tyrosine by liver tissue from rats with a PGA deficiency induced by succinylsulfathiazole (2). It will be shown that the rate of tyrosine oxidation for these livers is lower than for normal liver and that the rate can be increased by the addition of PGA, but not by pteroylheptaglutamic acid, a naturally occurring conjugate of PGA, or by liver extract containing the antipernicious anemia (APA) principle.

These results will be compared and contrasted with those obtained in a new series of experiments in which the PGA inhibitor 4-aminopteroylglutamic acid is employed to produce a PGA deficiency state.

EXPERIMENTAL

Liver tissue was obtained from rats which had been maintained on the succinylsulfathiazole-containing diet of Daft and Sebrell (6) until leucopenia developed. This leucopenic state was taken as evidence that the animal was deficient in PGA. Further proof of the existence of a PGA deficiency was afforded by actual determination by a microbiological assay (7) of the PGA content of liver tissue from these animals. As is shown in Table I, the concentration of PGA in liver from rats receiving succinylsulfathiazole was much lower (less than 10 per cent) than that of liver from rats without this dietary supplement, when assayed either directly or after treatment under conditions simulating those in a Warburg experiment.
To determine the rate of tyrosine oxidation, livers from three to five animals were pooled and homogenized in a Waring blender for 15 seconds, with m/15 phosphate buffer of pH 7.2 in an amount to give a concentration of 0.25 gm., wet weight, per ml. The oxidation of 0.5 gm. of L-tyrosine by 2 ml. of the suspensions was determined over a 2 hour period in the Warburg apparatus at 37°. In computing oxygen uptake, averages of three flasks were used. Nitrogen determinations on the different liver suspensions showed a relatively constant protein content per ml. To determine the fate of the tyrosine after incubation analysis for residual tyrosine was carried out on the pooled contents of the flasks used with each substance. The values so obtained agreed well with those calculated from the oxygen consumption. The oxygen uptake due to the utilization of the tyrosine was observed for normal and PGA-deficient liver tissue, and for PGA-deficient liver tissue to which PGA or PGA conjugate was added.

### TABLE I

<table>
<thead>
<tr>
<th>Rat group No.</th>
<th>Diet supplement</th>
<th>Preincubation treatment of liver</th>
<th>PGA content of liver</th>
<th>γ per gm.</th>
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<tr>
<td>1</td>
<td>None</td>
<td></td>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>1</td>
<td>&quot;</td>
<td>2 hrs., 37°, pH 7.2</td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>Succinylsulfathiazole</td>
<td>&quot;</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>2 hrs., 37°, pH 7.2</td>
<td></td>
<td>0.22</td>
</tr>
</tbody>
</table>

A summary of the studies under these conditions (Table II) shows that in all of the experiments the oxidation of tyrosine by liver suspensions from the succinylsulfathiazole-induced PGA-deficient animals was less than that from normal rat livers. The addition of 10 γ of crystalline PGA per flask at least partially restored the oxidation. However, tyrosine oxidation was not affected by the conjugate, pteroylheptaglutamic acid, containing an equivalent amount of PGA.

Data in Table II also show the effect of liver extract containing the APA principle on the rate of tyrosine oxidation by PGA-deficient liver tissue. To the PGA-deficient homogenate 0.1 ml. per flask of phenol-free, 15 unit liver extract was added. No stimulation of tyrosine oxidation was noted, and in fact a decrease was observed with normal and PGA-deficient liver suspensions. Dilution of the liver extract to the point at which no inhibition occurred did not show any effect on tyrosine oxidation.

Recent experiments have reported the effects of a powerful antagonist of PGA, 4-amino-PGA, in producing a PGA deficiency syndrome in vari-
ous animals including the rat (8, 9). It was of interest, therefore, to de-
termine whether this inhibitor of PGA could affect the rate of tyrosine
oxidation by liver tissue. Accordingly, 4-amino-PGA was added to nor-
mal liver suspensions and the tyrosine oxidation values were obtained as
in the previous experiments. No effect on tyrosine oxidation was observed
(Table III) when the antagonist was added in amounts up to 160 times
(200 γ per ml.) the determined PGA content of liver (5 γ per gm.).

**Table II**

*Tyrosine Oxidation by Liver Tissue from Rats with Succinylsulfathiazole-Induced PGA Deficiency*

The results are expressed in c.mm. of O₂.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Normal liver</th>
<th>PGA-deficient liver</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PGA added</td>
</tr>
<tr>
<td>1</td>
<td>66.5</td>
<td>17.5</td>
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<tr>
<td>2</td>
<td>77.6</td>
<td>19.6</td>
</tr>
<tr>
<td>3</td>
<td>48.6</td>
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<td>4</td>
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<td>5</td>
<td>67.0</td>
<td>32.3</td>
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<td>6</td>
<td>56.2</td>
<td>28.3</td>
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<tr>
<td>7</td>
<td>48.5</td>
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<td>56.2</td>
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<td>9</td>
<td>64.7</td>
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<tr>
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<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>63.0</td>
<td>47.4</td>
</tr>
</tbody>
</table>

Mean...... 61.3 26.8 37.4 23.0

* 0.1 ml. of liver extract per flask.
† 0.0001 ml. of liver extract per flask.

It was found, however, that liver tissue from rats fed a diet containing
4-amino-PGA until a PGA deficiency syndrome developed was unable to
oxidize tyrosine at a rate comparable to that of liver tissue from control
rats (Table III). In contrast to results obtained with liver tissue from
rats with a succinylsulfathiazole-induced PGA deficiency, the addition of
PGA to liver tissue from inhibitor-fed rats in amounts up to 1 mg. per ml.
of liver had no effect in restoring tyrosine oxidation. The effect of liver
extract was also studied, and again no stimulation of tyrosine oxidation
was noted with amounts of liver extract ranging from 0.0001 to 0.1 ml. per
flask. Three experiments were carried out in which 1.04 γ of a vitamin
B₁₂ concentrate were added to flasks containing PGA-deficient liver
homogenate, but no stimulation of tyrosine oxidation was observed under these conditions. However, liver tissue from rats receiving the same amount of inhibitor with a supplement of either PGA or liver extract in sufficient quantities to maintain a normal blood picture (8) had a tyrosine oxidation rate equivalent to normal liver controls. Since ascorbic acid has an effect on tyrosine oxidation in scorbutic guinea pigs (10), ascorbic acid was added to liver homogenates from both succinylsulfathiazole- and

**TABLE III**

Tyrosine Oxidation by Liver Tissue from Rats with Inhibitor-Induced PGA Deficiency

- Experiment No. 1 to 8 for Normal liver and PGA-deficient liver
- Control, Inhibitor added, PGA added, Liver extract added
- Vitamin B₆ concentration added, Treated in rats

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Normal liver</th>
<th>PGA-deficient liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>60.1</td>
<td>24.7</td>
</tr>
<tr>
<td>2</td>
<td>42.4</td>
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<td>4</td>
<td>63.0</td>
<td>48.5</td>
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<tr>
<td>5</td>
<td>56.4</td>
<td>36.5</td>
</tr>
<tr>
<td>6</td>
<td>64.7 61.3 (200 γ)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>46.3 45.9 (250 γ)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>61.2 66.8 (500 γ)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>57.7 58.1</td>
<td>33.8</td>
</tr>
</tbody>
</table>

* Amount of PGA per flask.
† PGA administered.
‡ Amount of liver extract per flask.
§ Liver extract administered.
‖ Amount of 4-amino-PGA per flask.

inhibitor-induced PGA-deficient animals. The addition of 0.25 mg. of ascorbic acid per flask had no effect on the tyrosine oxidation rate.

**DISCUSSION**

It has been shown that liver tissue homogenates from rats with a PGA deficiency syndrome induced by dietary supplements of either succinylsulfathiazole or a PGA inhibitor are unable to oxidize tyrosine at a normal rate. Further, the addition of PGA itself, under experimental conditions already described, increases the tyrosine oxidation rate of these liver homogenates. This is taken as evidence that PGA is an essential component of the tyrosine oxidative process.
In most of the experiments with succinylsulfathiazole-induced PGA-deficient liver tissue the addition of PGA in vitro effected only a partial restoration of the tyrosine oxidation rate, although a greater amount of PGA was added than is present in normal rat liver. It has been established that PGA is present in animal tissue predominantly as a conjugate (11), and the question therefore arises as to whether this conjugate is the metabolically active form of the vitamin. Since PGA conjugate (pteroylheptaglutamic acid), in contrast to PGA itself, had no effect on increasing the tyrosine oxidation rate of PGA-deficient liver tissue, it must be concluded that for its role in the tyrosine oxidative process, at least, PGA must function not in the conjugated but in the free form.

It has recently been shown (12) that a biotin antagonist could not displace the vitamin in vitro, but only affected the action of biotin added to biotin-deficient liver preparations. The finding that 4-amino-PGA will not affect the oxidation of tyrosine by rat liver tissue in vitro but that liver homogenates from 4-amino-PGA-fed rats are unable to oxidize tyrosine may be of similar significance in attempting to explain the mechanism of action of the antagonist. The results obtained with tyrosine oxidation of liver tissue from rats fed diets containing both 4-amino-PGA and PGA itself are proof that as far as tyrosine oxidation is concerned in vivo the action of 4-amino-PGA is completely reversed by PGA.

Reversal in vivo of 4-amino-PGA action was also obtained when liver extract was substituted for PGA. Hence, apparently there is a substance in liver extract that acts either directly or indirectly on the tyrosine oxidation process. Additional evidence for this is to be found in the recent report of Sealock and Lepow (13), wherein they show that APA liver extracts will return urinary tyrosyl values of scorbutic guinea pigs to normal levels. They were unable to demonstrate any effect on oxidation of tyrosine in vitro by liver slices from scorbutic guinea pigs. The experiments reported here are similar in that no effect on tyrosine metabolism was shown by either liver extract or vitamin B12 concentrate in vitro. Whether this substance in liver extract affecting tyrosine oxidation is actually the APA liver principle or vitamin B12 remains to be determined. Some evidence is afforded by experiments showing that patients with pernicious anemia excrete large amounts of tyrosine metabolites, a condition that is corrected by treatment with liver extract (5).

With scorbutic guinea pigs it has been found (13) that tyrosine oxidation is impaired, and that the addition of ascorbic acid will restore it to normal. Woodruff and Darby (3) reported that PGA would return urinary tyrosyl values to normal in scorbutic animals, and Johnson and Dana (14) showed that ascorbic acid produced improvement in vivo of a succinylsulfathiazole-induced PGA deficiency in rats. This improve-
ment was not complete, and PGA was necessary for marked reticulocyte response. However, it has been shown here that the addition of ascorbic acid to the PGA-deficient liver suspensions had no effect on tyrosine oxidation under the conditions of these experiments.

SUMMARY

Liver tissue from rats with a succinylsulfathiazole-induced PGA deficiency shows a decreased oxidation of tyrosine compared with normal liver tissue. The oxidation of tyrosine can be partially restored by the addition in vitro of PGA, but not by PGA conjugate or by liver extract.

Liver tissue from rats with PGA deficiency induced by feeding 4-amino-PGA also shows a decreased tyrosine oxidation. This effect cannot be reversed by the addition in vitro of PGA, liver extract, or vitamin B₁₂ concentrate but can be reversed by the administration in vivo of either PGA or liver extract. The addition of 4-amino-PGA in vitro to normal rat liver tissue does not affect the rate of tyrosine oxidation.

BIBLIOGRAPHY

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