The Effect of Folic Acid on Tyrosine Metabolism in Guinea Pigs

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Several observations in vivo have suggested that folic acid may have a role in the oxidation of tyrosine, although no specific function of the vitamin in relation to the metabolism of this amino acid has been established. Woodruff and Darby (1) and Woodruff et al. (2) found that tyrosyuria, the excretion of p-hydroxyphenyl metabolites of tyrosine, could be corrected in scorbutic guinea pigs, not only by ascorbic acid, but also by the administration of large amounts of folic acid. However, folic acid was not effective in correcting the tyrosyuria in scorbutic monkeys (3, 4) and has given inconsistent results in scurvy in human subjects (5–8).

Experiments in vitro with folic acid have also produced variable results. Rodney, Swendeid, and Swanson (9, 10) have reported that liver homogenates prepared from rats made folic acid-deficient by being fed a sodium-lufthiaszone show a decreased ability to metabolize tyrosine, and that the activity was increased by the addition of folic acid. However, other laboratories have been unable to show any impairment of tyrosine oxidation with liver homogenates prepared from folic acid-deficient chicks (11) and rats (12). Rietsch (13) found that folic acid stimulated the metabolism of tyrosine with liver homogenates prepared from scorbutic guinea pigs. However, Knox and LeMay-Knox (14) and Williams and Sreenivasan (15) did not observe any effect of folic acid on tyrosine oxidation with dialyzed rat liver homogenates.

The present paper demonstrates that the mechanism by which folic acid maintains tyrosine metabolism in vivo in vitamin C-deficient guinea pigs consists of protecting liver p-hydroxyphenylpyruvic acid oxidase from inhibition and thereby preventing tyrosyuria. However, in contrast to ascorbic acid, folic acid and its derivatives have no effect on tyrosine oxidation in vitro, and a possible explanation for these findings will be presented.

EXPERIMENTAL PROCEDURE

Male albino guinea pigs weighing approximately 200 g were placed on a vitamin C-free diet as previously described (16, 17). Some that were maintained on the diet for 2 weeks developed typical signs of scurvy: weight loss, swollen joints, and hemorrhages in the knee joints. By this time the average ascorbic acid concentration in the liver had decreased to 2.0 mg per 100 g wet weight. Other animals were maintained on the vitamin C-free diet for only 1 week. These animals were still gaining weight and had no obvious signs of scurvy. The average ascorbic acid concentration in the liver of this moderately deficient group was 4.1 mg per 100 g wet weight.

A control group of guinea pigs was given the vitamin C-free diet supplemented with 25 mg of L-ascorbic acid per day, administered orally, and had concentrations of the vitamin from 10 to 15 mg per 100 g wet weight of liver.

Another group of male albino guinea pigs, weighing approximately 100 g, were placed on a folic acid-deficient diet for 4 weeks, according to the method of Reid, Martin, and Briggs (18). By this time the deficient animals had developed a slight leukopenia and had an average folic acid concentration in the liver of less than 3.0 μg per g wet weight, compared to a concentration of 10 μg per g wet weight in control animals on the folic acid deficient diet supplemented with 1 mg of folic acid per day.

Animals from each of these groups were fed L-tyrosine, and enzymatic and chemical studies were carried out as previously described (16, 17).

RESULTS

Effect of Folic Acid on p-Hydroxyphenylpyruvic Acid Oxidase in Moderately Vitamin C-deficient and Scorbutic Guinea Pigs in Vivo—Guinea pigs moderately deficient in vitamin C (those on the deficient diet for 7 days) were given a total of 400 mg of tyrosine, 100 mg each hour for 4 hours. Some animals also received 20 mg of folic acid, and others, various amounts of ascorbic acid (0.5, 1.0, or 2.0 mg). The latter compounds were injected intraperitoneally; half of the amount was given ½ hour before the first tyrosine feeding, and the remainder, 2 hours later. Animals were killed 1 hour after the last tyrosine feeding, and liver tyrosine transaminase and p-hydroxyphenylpyruvic acid oxidase activities were determined in freshly prepared homogenates, as previously described (17). The plasma concentration of tyrosine was determined at the time of killing, and the amount of p-hydroxyphenylpyruvic acid excreted during the 5-hour experimental period was determined in the urine (Table I). As noted previously, liver p-hydroxyphenylpyruvic acid oxidase was nearly completely inhibited in moderately vitamin C-deficient guinea pigs fed extra tyrosine (17). The intraperitoneal injection of 0.5 mg of ascorbic acid did not protect the oxidase under these conditions. However, 1 mg of the vitamin did protect the oxidase, since more than half of the expected activity was retained. Complete protection of p-hydroxyphenylpyruvic acid oxidase was found when 20 mg of ascorbic acid were injected (Table I). Large amounts of folic acid (20 mg) also completely protected liver p-hydroxyphenylpyruvic acid oxidase and prevented tyrosyuria as well as an elevation of the plasma tyrosine.
TABLE I
Protection of p-hydroxyphenylpyruvic acid oxidase by folic acid or ascorbic acid from inhibition in vitamin C-deficient guinea pigs fed extra tyrosine*

<table>
<thead>
<tr>
<th>Protective compound injected</th>
<th>No. of animals</th>
<th>Liver enzymes</th>
<th>Plasma tyrosine</th>
<th>Urine p-hydroxyphenylpyruvic acid oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid</td>
<td>mg</td>
<td>µmoles substrate metabolized/hr/g wet weight</td>
<td>mg/100 ml plasma</td>
<td>mg excreted in 3 hrs</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>27.3 ± 5.3</td>
<td>2.2 ± 0.9</td>
<td>60.0 ± 17.0</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>37.0 ± 6.8</td>
<td>1.6 ± 3.4</td>
<td>25.1 ± 0.3</td>
</tr>
<tr>
<td>20.0</td>
<td>5</td>
<td>29.0 ± 3.5</td>
<td>16.0 ± 4.3</td>
<td>3.2 ± 0.3</td>
</tr>
</tbody>
</table>

TABLE II
Protection of p-hydroxyphenylpyruvic acid oxidase by folic acid from inhibition in frankly scorbutic guinea pigs fed extra tyrosine*

<table>
<thead>
<tr>
<th>Folic acid injected intraperitoneally</th>
<th>No. of animals</th>
<th>Liver enzymes</th>
<th>Plasma tyrosine</th>
<th>Urine p-hydroxyphenylpyruvic acid oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>µmoles substrate metabolized/hr/g wet weight</td>
<td>mg/100 ml plasma</td>
<td>mg excreted in 3 hrs</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>6</td>
<td>40.0 ± 6.6</td>
<td>3.8 ± 0.8</td>
<td>46.0 ± 28.0</td>
</tr>
<tr>
<td>20.0</td>
<td>7</td>
<td>47.0 ± 8.2</td>
<td>32.9 ± 6.2</td>
<td>4.2 ± 0.1</td>
</tr>
</tbody>
</table>

* The animals received a total of 400 mg of L-tyrosine orally as previously described (17). The average ascorbic acid concentration in liver was 4.1 mg per 100 g wet weight in all of the groups except the group receiving 20.0 mg of ascorbic acid intraperitoneally, which had 15.6 mg per 100 g wet weight.

† The average p-hydroxyphenylpyruvic acid oxidase activity in six vitamin C-deficient animals not fed tyrosine was 25.8 ± 4.8 µmoles of p-hydroxyphenylpyruvic acid oxidized per hour per g wet weight of liver. The level of tyrosine in the plasma was not elevated, and no significant amount of p-hydroxyphenylpyruvic acid was excreted in the urine.

‡ Standard error of the mean is given.

The ability of folic acid to protect p-hydroxyphenylpyruvic acid oxidase from inhibition after the feeding of tyrosine was also evaluated in animals that were frankly scorbutic. As was found in animals less deficient in vitamin C (Table I), large amounts of folic acid (20 mg) completely protected p-hydroxyphenylpyruvic acid oxidase from inhibition in this group (Table II). Smaller amounts of folic acid (2 mg) showed some degree of protection of the oxidase and prevented tyrosyluria and a rise in plasma tyrosine levels. It should be noted that the concentration of liver ascorbic acid in the animals injected with folic acid was not significantly higher than in those not given folic acid. Thus, the ability of injected folic acid to protect the oxidase cannot be due to the mobilization of ascorbic acid from other tissues to the liver. In other experiments in which 1 mg of folic acid was given orally each day for 2 weeks to scorbutic guinea pigs before tyrosine feeding, there was no significant protection of p-hydroxyphenylpyruvic acid oxidase, and the animals had as high a tyrosine concentration in the plasma and degree of tyrosyluria as found in the scorbutic animals not given folic acid.

Effect of Folic Acid on p-Hydroxyphenylpyruvic Acid Oxidase in Folic Acid-deficient Guinea Pigs Fed Extra Tyrosine in Vivo—Since parenterally administered folic acid protects p-hydroxyphenylpyruvic acid oxidase in both moderately vitamin C-deficient and frankly scorbutic guinea pigs, it was of interest to determine whether the oxidase would be inhibited in folic acid-deficient guinea pigs fed extra tyrosine (Table III). It is apparent that liver p-hydroxyphenylpyruvic acid oxidase activity in folic acid-deficient animals was not inhibited by tyrosine feeding and that no tyrosyluria occurred. These results indicate that even though folic acid protects p-hydroxyphenylpyruvic acid oxidase from inhibition in folic acid-deficient animals, folic acid itself does not seem to be specifically required when there is adequate vitamin C present in the liver.

Effect of Folic Acid on Tyrosine Oxidation in Vitro—In view of the above findings in vivo, it was of interest to determine the effect of folic acid in tyrosine oxidation with liver homogenates prepared from folic acid-deficient guinea pigs. Liver, 5 g, from these animals was homogenized with 10 ml of 0.2 M sodium phosphate buffer at pH 7.5 and was centrifuged at 10,000 x g for 10 minutes. The resulting supernatant fraction was used to follow the oxidation of L-tyrosine manometrically (Fig. 1).

TABLE III
Liver p-hydroxyphenylpyruvic acid oxidase activity in folic acid-deficient guinea pigs fed extra tyrosine*

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of animals</th>
<th>Liver enzymes</th>
<th>Plasma tyrosine</th>
<th>Urine p-hydroxyphenylpyruvic acid oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles substrate metabolized/hr/g wet weight</td>
<td>mg/100 ml plasma</td>
<td>mg excreted in 3 hrs</td>
<td></td>
</tr>
<tr>
<td>Folic acid-deficient†</td>
<td>6</td>
<td>28.0 ± 6.2</td>
<td>20.6 ± 4.0</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td>Normal</td>
<td>6</td>
<td>24.0 ± 7.0</td>
<td>24.1 ± 4.8</td>
<td>12.0 ± 0.6</td>
</tr>
</tbody>
</table>

* The animals received a total of 400 mg of L-tyrosine orally as previously described (17). The average ascorbic acid concentration in the liver was 16 mg per 100 g wet weight in the folic acid-deficient group and 15 mg per 100 g wet weight in the normal group.
† The animals were kept on the folic acid-deficient diet for 4 weeks according to the method of Reid, Martin, and Briggs (38). Liver homogenates were kindly analyzed by Dr. Milton Silverman, of the National Institute of Arthritis and Metabolic Diseases, for folic acid. The average folic acid concentration was 3.0 µg per g wet weight. Control animals had an average folic acid concentration in liver of 10.3 µg per g wet weight.
‡ Plasma tyrosine levels 1 hour after the last tyrosine feeding are consistently higher in the group of animals weighing 100 g than in the older group of animals weighing 200 to 300 g.
As previously reported (19-23), ascorbic acid maintains tyrosine oxidation by preventing inhibition of p-hydroxyphenylpyruvic acid oxidase, the first oxidative step in tyrosine metabolism. In the absence of vitamin C, inhibition of the oxidase gradually occurs and becomes progressively greater until p-hydroxyphenylpyruvic acid oxidase is completely inhibited. There was no significant protective effect of added folic acid when it was tested alone or in combination with suboptimal concentrations of ascorbic acid.

The effect of folic acid, folinic acid, and prefolic acid (N4-methyltetrahydrofolic acid) on tyrosine oxidation was also determined manometrically with liver homogenates prepared from folic acid-deficient guinea pigs and normal animals (Table IV). Alone, or in combination with ascorbic acid, none of the folic acid compounds had any net stimulatory effect on tyrosine oxidation in vitro. Folic acid and derivatives of it were also assayed in the presence of TPNH and of a TPNH-generating system (glucose 6-phosphate dehydrogenase), and no protective effect on tyrosine oxidation was found under these conditions. The effect of folic acid alone, and in combination with suboptimal amounts of ascorbic acid, was also assayed under the milder spectrophotometric conditions (at room temperature and without shaking) (22). Again, in homogenates from folic acid-deficient animals, there was no protection of p-hydroxyphenylpyruvic acid oxidase from inhibition by excess p-hydroxyphenylpyruvic acid nor was there any increase in the initial rate of disappearance of substrate.

It has also been found that p-hydroxyphenylpyruvic acid oxidase purified approximately 200-fold from dog liver has no enrichment of folic acid content.

**DISCUSSION**

The data presented in this paper demonstrate that folic acid is effective in maintaining normal tyrosine metabolism in vivo in vitamin C-deficient guinea pigs fed extra tyrosine and does so by protecting p-hydroxyphenylpyruvic acid oxidase from inhibition by its substrate. Thus, folic acid can be added to the group of compounds that includes ascorbic acid, several analogues of vitamin C, 2,6-dichlorophenolindophenol, and coenzyme Q10, which have the ability to protect p-hydroxyphenylpyruvic acid oxidase from inhibition in vitro (16, 17, 23, 25, 26). However, it should be pointed out that all of the latter compounds in their reduced form also protect p-hydroxyphenylpyruvic acid oxidase from inhibition in vitro (19-22, 27), and coenzyme Q10 recently has been shown to be particularly effective in protecting p-hydroxyphenylpyruvic acid oxidase when it is assayed in the presence of small amounts of ascorbic acid (27).

Folic acid is a notable exception in this group, since it does not prevent inhibition of p-hydroxyphenylpyruvic acid oxidase when it is assayed in the presence of small amounts of ascorbic acid (27). Folic acid is a notable exception in this group, since it does not prevent inhibition of p-hydroxyphenylpyruvic acid oxidase when it is assayed in the presence of small amounts of ascorbic acid (27).

Folic acid protects p-hydroxyphenylpyruvic acid oxidase. The fact that folic acid protects in vivo, but not in vitro, may be explained if, as has been found for the other protective agents, folic acid is also required in a reduced form. Such a reduced derivative might be more effectively maintained in the intact animal than has so far been possible under the experimental conditions in vitro employed. Another explanation might be that the effectiveness of folic acid in vivo is indirect, occurring through some other agents.

**TABLE IV**

<table>
<thead>
<tr>
<th>Addition*</th>
<th>Oxygen uptake during initial 10-min incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal liver homogenate (dialyzed)</td>
</tr>
<tr>
<td></td>
<td>Net O2 uptake</td>
</tr>
<tr>
<td>None</td>
<td>µM</td>
</tr>
<tr>
<td>Ascorbic acid (5 x 10^{-4} M)</td>
<td>46</td>
</tr>
<tr>
<td>Folic acid (2 x 10^{-4} M)</td>
<td>20</td>
</tr>
<tr>
<td>Folic acid (2 x 10^{-4} M) plus ascorbic acid (5 x 10^{-4} M)</td>
<td>45</td>
</tr>
<tr>
<td>Prefolic acid (2 x 10^{-4} M)</td>
<td>23</td>
</tr>
<tr>
<td>Folic acid (2 x 10^{-4} M) plus ascorbic acid (5 x 10^{-4} M)</td>
<td>46</td>
</tr>
</tbody>
</table>

* The contents of the Warburg flasks were as described under Fig. 1. The final concentrations of folic acid or its derivatives in the presence of ascorbic acid are indicated in the table.

† Dialyzed for 2 hours, with stirring against dilute phosphate buffer, pH 7.5.
agent. Further experiments are in progress to distinguish between these possibilities.

SUMMARY

It is shown that folic acid maintains tyrosine metabolism in vivo in vitamin C-deficient guinea pigs by protecting liver p-hydroxyphenylpyruvic acid oxidase from inhibition, and thereby preventing the excretion of tyrosyl compounds in the urine.

Folic acid is similar in this respect to a number of other agents, such as ascorbic acid, analogues of vitamin C, 2,6-dichlorophenolindophenol, and coenzyme Q₁₀. Folic acid differs from these agents, however, since it fails to protect the oxidase from inhibition in vitro. This failure may be due to the difficulty in maintaining folic acid in a reduced form under experimental conditions in vitro.

No evidence has been obtained that folic acid is required as a cofactor or as an essential constituent of p-hydroxyphenylpyruvic acid oxidase in vitro, as has been previously thought, and folic acid-deficient guinea pigs fed extra tyrosine show no abnormality in metabolizing this amino acid.

Acknowledgments—We are indebted to Dr. Mary E. Reid for her advice and help in preparing the folic acid-deficient diet used in these studies, and to Dr. John C. Keresztesy for kindly supplying us with folinic and prefolic acid.

We also wish to thank Miss Rita Gardiner for analyzing the folic acid content of purified p-hydroxyphenylpyruvic acid oxidase.

REFERENCES

The Effect of Folic Acid on Tyrosine Metabolism in Guinea Pigs
Vincent G. Zannoni, G. A. Jacoby, S. E. Malawista and Bert N. La Du