METABOLISM OF L-ASCORBIC ACID AND L-TYROSINE IN GUINEA PIG LIVER

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The experiments of Sealock et al. (1-3) and of Levine et al. (4) have given reasons for believing that one of the functions of ascorbic acid in the animal body is to facilitate the metabolism of L-tyrosine and L-phenylalanine.

Sealock and Silberstein (2) showed that the administration of tyrosine orally to scorbutic guinea pigs resulted in the excretion of metabolites containing the benzene ring, accounting for up to 80 per cent of the original tyrosine. Abnormal metabolites were not excreted by animals receiving L-ascorbic acid. Levine et al. (4) obtained similar results with premature infants.

Lan and Sealock (5) examined the livers of guinea pigs, both control and scorbutic, for ability to oxidize tyrosine as measured by increased QO₂ in slices. The livers from scorbutic animals showed diminished ability to oxidize the tyrosine when compared with livers from guinea pigs receiving adequate ascorbic acid. The oxidizing ability of scorbutic liver slices could be restored by the addition of ascorbic acid.

Darby et al. (6) could find no difference in rate of metabolism of L-tyrosine or conjugation of phenol (as measured by disappearance of the hydroxyphenyl groups) in liver slices from scorbutic and non-scorbutic guinea pigs. They concluded that the main defect in the metabolism of aromatic compounds by scorbutic guinea pigs is the inability of the scorbutic liver to oxidize the side chain of tyrosine rather than to oxidize the ring or conjugate the phenolic group.

However, Painter and Zilva (7) pointed out that excessive doses of tyrosine were necessary for guinea pigs to excrete metabolites containing the benzene ring, and that this abnormal excretion could only be prevented by having the guinea pig tissues saturated with L-ascorbic acid. They concluded that there was not necessarily a connection between the normal functions of L-ascorbic acid and normal metabolism of L-tyrosine.

The experiments reported below support the view that tyrosine metabolism in the liver is dependent on the presence of L-ascorbic acid.

Methods

Animals and Diets—Guinea pigs weighing between 200 and 250 gm. were fed, ad libitum, the scorbutigenic diet recommended by Dawbarn (8).
The control guinea pigs were given a daily oral supplement of 5.0 mg. of ascorbic acid. The animals receiving the scorbutigenic diet developed signs of acute scurvy in 15 to 20 days. An animal was not regarded as scorbutic until it had lost considerable weight and at autopsy showed considerable hemorrhage about the thighs.

Preparation of Liver Slices, Homogenates, and Supernatants—The animals were killed by a blow on the head and the livers removed after exsanguination. The slices were made according to Cohen's description of Deutsch's method (9).

The homogenates were prepared by macerating the whole liver in 4 to 5 times its weight of 1 per cent K$_2$HPO$_4$ (pH 7.4) for 90 seconds in a Waring blendor. After straining through muslin, the pH was readjusted to 7.4.

The homogenate, if centrifuged for 5 minutes at approximately 3000 × g, separated into a sediment with no activity towards tyrosine and a fluid with tyrosine-oxidizing properties, which is referred to below as the "supernatant." The supernatant preparation was much easier to pipette than whole liver homogenates.

Manometry—The oxygen consumption was measured in Warburg flasks of approximately 18 ml. capacity at 37°. 0.2 ml. of 20 per cent KOH plus a filter paper wick was used in the center well. After an equilibration period of 15 minutes, the taps were closed, the initial reading taken, and the substrate tipped in.

Chemical—Tyrosine was determined after the manometric incubation period by Lugg's method (10). Metaphosphoric acid (5 per cent) was used to precipitate the residual protein in the solutions from which slices were removed and in the homogenate preparations. The color developed was determined by means of a photoelectric absorptiometer. This method was found to give good reproducibility and only monohydroxyphenyl compounds were measured. Practically no blanks were found with the various tissue preparations to which no tyrosine had been added.

Nitrogen was determined by a Kjeldahl digestion (2 ml. of liver homogenate, 20 ml. of concentrated H$_2$SO$_4$, 20 gm. of K$_2$SO$_4$), the ammonia being distilled in a Markham still (11) into 1 per cent boric acid.

Results

Slices—The oxygen consumption and tyrosine metabolism in the control and scorbutic guinea pig liver slices are shown in Table I. These results show a considerable difference in the rate of disappearance of tyrosine in control and scorbutic liver slices. Also the addition of 0.3 mg. of l-ascorbic acid restores in part the ability by the scorbutic livers to metabolize tyrosine.
Homogenates—Figures for increased oxygen consumption due to added tyrosine, and for tyrosine metabolism in homogenates prepared from control and scorbutic livers, are given in Table II.

Table I

Oxygen Consumption and Tyrosine Metabolism in Control and Scorbutic Liver Slices

Approximately 100 mg. (wet weight) of liver slices in 2 ml. of Krebs-Ringer phosphate and 2.3 mg. of tyrosine suspended in 0.5 ml. of Ringer's solution. Gas phase oxygen. The figures in parentheses indicate the number of guinea pigs in the group.

<table>
<thead>
<tr>
<th>Condition of liver</th>
<th>Addition to reaction flask</th>
<th>Increased ( Q_O ) due to addition of tyrosine</th>
<th>Tyrosine metabolism disappearing per hr. per mg. of tissue, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>None</td>
<td>2.83 (3)</td>
<td>0.0382 (5)</td>
</tr>
<tr>
<td>Scorbutic</td>
<td>&quot;</td>
<td>0.49 (3)</td>
<td>0.0125 (4)</td>
</tr>
<tr>
<td></td>
<td>0.3 mg. l-ascorbic acid</td>
<td>2.40 (2)</td>
<td>0.0288 (4)</td>
</tr>
</tbody>
</table>

Table II

Effect of Addition of Tyrosine on Oxygen Consumption and Tyrosine Metabolized in 90 Minutes per Mg. of Nitrogen of Liver Homogenates

2 ml. of homogenate in each flask; 0.5 mg. of tyrosine added in 0.5 ml. of buffer. Gas phase air.

<table>
<thead>
<tr>
<th></th>
<th>Control animals</th>
<th>Scorbutic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increased oxygen consumption due to tyrosine</td>
<td>Tyrosine metabolized</td>
</tr>
<tr>
<td></td>
<td>( \mu l. )</td>
<td>( \mu M )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.71</td>
<td>0.177</td>
<td>5.35</td>
</tr>
<tr>
<td>7.95</td>
<td>0.180</td>
<td>5.20</td>
</tr>
<tr>
<td>5.29</td>
<td>0.118</td>
<td>5.15</td>
</tr>
<tr>
<td>5.15</td>
<td>0.114</td>
<td>7.30</td>
</tr>
<tr>
<td>6.80</td>
<td>0.150</td>
<td>6.50</td>
</tr>
<tr>
<td>Mean</td>
<td>6.58</td>
<td>0.148</td>
</tr>
<tr>
<td>Molecular ratio, ( O_2 ) to tyrosine</td>
<td>1.98</td>
<td></td>
</tr>
</tbody>
</table>

From these figures it is seen that there is a slight but not significant decrease in tyrosine-oxidizing capacity in scorbutic liver homogenates compared with those of the control animals. In both the control and scorbutic homogenates, 2 molecules of oxygen were consumed for each molecule of tyrosine metabolized.
**Supernatants**—Table III gives data showing the activity towards tyrosine of supernatant solutions with and without added l-ascorbic acid from the control and scorbutic animals. It is seen that l-ascorbic acid stimulated the disappearance of tyrosine from the supernatant solutions, the stimulation being significant only in the case of the scorbutic preparations. The oxygen consumption due to added tyrosine ran parallel with the tyrosine disappearance in all cases, maintaining a ratio of 2 molecules of oxygen for each molecule of tyrosine.

**Specificity of l-Ascorbic Acid in Tyrosine-Oxidizing System**—Isoascorbic acid and d-glucoascorbic acid, substances similar in structure to l-ascorbic acid, were tested for their ability to stimulate tyrosine disappearance in the supernatant preparation. Neither exhibited any effect whatever on the tyrosine-oxidizing system, even in quantities up to 2 mg. compared to 0.3 mg. of l-ascorbic acid (d-glucoascorbic acid is generally considered to have one-twentieth the antiscorbutic activity of l-ascorbic acid). The 0.3 mg. of l-ascorbic acid added to the same preparation increased the disappearance of tyrosine. It has been reported that the behavior of folic acid administered orally (12), or added to liver tissue in vitro (13), is similar to that of l-ascorbic acid. With the supernatant preparation, 0.1 mg. of folic acid stimulated tyrosine disappearance in the same manner as did l-ascorbic acid.

**DISCUSSION**

These results together with those of Lan and Sealock (5) indicate that l-ascorbic acid plays a part in the metabolism of tyrosine in the liver as judged by oxygen consumption and disappearance of hydroxyphenyl groups. However, the results do not support the contention of Darby et al. (6) that the main defect in the metabolism of aromatic compounds in scorbutic...
guinea pigs is the inability to oxidize the side chain of tyrosine rather than the inability to oxidize the ring or conjugate the phenolic group. Indeed, it is hard to reconcile this view with the experiments of Sealock et al. (1-3) in vivo, showing the excretion of metabolites containing most of the aromatic ring which has been consumed as tyrosine, but which, however, contained side chains in various but incomplete stages of oxidation.

At present it does not seem possible to give an explanation for the different behavior of liver slices and homogenates.

The ratio of 2 molecules of oxygen consumed for each molecule of tyrosine disappearing has been observed before by various investigators. In the results above, it is seen that the addition of l-ascorbic acid does not alter this ratio. This suggests that the addition of l-ascorbic acid does not alter the type of oxidation. This does not support the contention of Painter and Zilva (7) that l-ascorbic acid is functioning as an adaptation of the animal to high L-tyrosine intakes.

l-Ascorbic acid is probably the only member of the structurally related ascorbic acid family that will stimulate tyrosine disappearance. However, added folic acid will give the same effect as l-ascorbic acid, both in the whole animal (12) and in liver tissue (13). Also in folic acid deficiency, the tyrosine metabolism seems to be deranged in the liver, as in scurvy (14). These facts all indicate that both l-ascorbic acid and folic acid are essential components of the L-tyrosine-metabolizing system.

SUMMARY

1. The oxidation of tyrosine in slices of guinea pig liver was followed by measurement of oxygen consumption and determination of the disappearance of hydroxyphenyl groups. Both methods showed that oxidation of tyrosine in the liver slice depended upon the l-ascorbic acid nutrition of the animal, and in the case of liver slices from scorbutic animals the oxidation could be restored to non-scorbutic values by addition of l-ascorbic acid.

2. By use of similar techniques no difference could be seen in rate of metabolism of tyrosine in whole liver homogenates from the control and scorbutic guinea pigs. However, addition of l-ascorbic acid stimulated tyrosine oxidation in supernatant preparations from liver homogenates.

3. l-Ascorbic acid seems specific in the ascorbic acid family in this effect, as isoascorbic acid and d-glucoscorbic acid had no effect whatever on the system. However, folic acid gave results similar to those with l-ascorbic acid.

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