THE HYDROXYLATION OF PHENYLALANINE AND ANTIPYRINE IN PHENYLPYRUVIC Oligophrenia

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In normal individuals the major portion of ingested phenylalanine is converted into tyrosine. In the disease phenylpyruvic oligophrenia, however, ingested phenylalanine is converted mainly into phenylpyruvic and phenyllactic acids (1), which are excreted in the urine. This phenomenon has been ascribed to the inability of the body to convert phenylalanine to tyrosine (2) and a consequent shunting of phenylalanine through metabolic pathways which are normally of minor significance. The experiments presented here demonstrate that the failure of phenylpyruvic oligophrenic individuals (phenylketonurics) to hydroxylate phenylalanine is not absolute; they can form tyrosine to a small but definite degree. In these individuals the hydroxylation of certain other aromatic compounds is apparently unaffected.

EXPERIMENTAL

Reagents and Materials—We are indebted to Eli Lilly and Company for supplying us with antipyrine prepared for intravenous use. The 3-C14-dL-phenylalanine (0.5 mc. per mm) was obtained from Tracerlab, Inc. An acetone-dried preparation of Streptococcus faecalis, prepared according to Epps (3), was used for the decarboxylation of phenylalanine and tyrosine to their corresponding amines.

Methods—Radioactivity was measured in a gas flow windowless counter having a background of 1.8 c.p.m. and an efficiency for C14 of about 45 percent (4). Each compound was suspended in an appropriate solvent, transferred onto a tared planchet, and evaporated to dryness. The values were corrected for self-absorption and expressed as counts per minute per micro-mole of compound.

Tyrosine and phenylalanine were isolated from plasma proteins after hydrolysis of the proteins and concentration of the hydrolysates as described in the preceding paper (5). Tyrosine was precipitated by adjustment of the hydrolysate to pH 6. The isolated tyrosine was purified to isotopic homogeneity by repeated recrystallizations carried out by dissolv-
ing the material in dilute acid, treating the solution with activated charcoal, and then adjusting to pH 6. Possible contamination by small amounts of labeled phenylalanine was avoided by an additional recrystallization of the isolated tyrosine from a solution containing large amounts of non-radioactive DL-phenylalanine. Several of the tyrosine samples were converted to tyramine with the S. faecalis decarboxylase. The pH was then adjusted to 9.5 and the tyramine was extracted into isoamyl aclohol and returned to an aqueous phase by shaking the alcohol extract with a small volume of 0.1 N HCl. The acid extract was evaporated to dryness, the residue taken up in 0.5 ml. of absolute alcohol, and the tyramine hydrochloride precipitated by the addition of 20 ml. of ether. Suspensions of tyramine hydrochloride in ether were transferred to planchets for counting.

Phenylalanine in the hydrolysates was converted to phenylethylamine hydrochloride by the procedure described in the previous paper (5). Suspensions of phenylethylamine hydrochloride in ether were transferred to planchets for counting.

Phenylpyruvic acid was extracted from acidified urine into ether. The ether was evaporated to dryness and the residue was treated with 3,5-dinitrophenylhydrazone (6). The resulting hydrazone derivative was purified to constant specific activity. Suspensions of the hydrazone in water were transferred to planchets for counting.

Antipyrine in plasma was assayed by the method of Brodie and Axelrod (7).

Plasma L-phenylalanine was determined by a specific decarboxylase method described in the preceding paper (5).

Experiments with Isotopically Labeled Phenylalanine—About 20 μc. of 3-C\textsuperscript{14}-DL-phenylalanine (6.5 mg.) were administered orally to two phenylketonuric children, aged 3 and 4 years, and to two human subjects who had terminal cancer but who had no signs of liver damage. Samples of blood were withdrawn at various time intervals, and the specific activity of phenylalanine and tyrosine isolated from the plasma proteins was measured. The data in Table I indicate that labeled phenylalanine administered to normal subjects was rapidly incorporated into proteins not only as phenylalanine but also as tyrosine. The ratio of the specific activity of tyrosine to phenylalanine (T:P) in plasma protein, at any time, may be used as a rough index of the extent of the conversion of phenylalanine to tyrosine. In the phenylketonurics values for T:P were much lower than in the controls. The incorporation of phenylalanine into protein was about the same for the phenylketonurics as for the controls, but that for tyrosine was low. It can be concluded, therefore, that little conversion of phenylalanine to tyrosine is occurring. The block is, however, not complete, since small amounts of tyrosine are formed.
A similar experiment was performed on a normal dog. Values for T:P were the same as those obtained in the control subjects.

The urine of one of the phenylketonurics was collected over a period of 54 hours, and the total amount and specific activity of the excreted phenylpyruvic acid were determined. This experiment showed that about two-thirds of the ingested isotopic DL-phenylalanine was excreted as phenylpyruvic acid.

**Table I**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time after administration</th>
<th>Phenylalanine(^*) c.p.m. per μM</th>
<th>Tyrosine(^*) c.p.m. per μM</th>
<th>Ratio, tyrosine to phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>3</td>
<td>21.9</td>
<td>5.67</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>28.5</td>
<td>6.83</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>24.4</td>
<td>6.07</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>20.6</td>
<td>5.98</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>16.8</td>
<td>5.75</td>
<td>0.34</td>
</tr>
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<td>Control, Patient A</td>
<td>6</td>
<td>11.2</td>
<td>2.46</td>
<td>0.22</td>
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<tr>
<td></td>
<td>24</td>
<td>10.4</td>
<td>2.79</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>7.84</td>
<td>2.24</td>
<td>0.29</td>
</tr>
<tr>
<td>&quot; &quot; B</td>
<td>6</td>
<td>28.1</td>
<td>5.30</td>
<td>0.19</td>
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<tr>
<td></td>
<td>24</td>
<td>24.6</td>
<td>4.90</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>21.0</td>
<td>4.70</td>
<td>0.22</td>
</tr>
<tr>
<td>Phenylketonuric, Patient J</td>
<td>24</td>
<td>55.0</td>
<td>0.91</td>
<td>0.016</td>
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<tr>
<td>&quot; &quot; &quot; C</td>
<td>48</td>
<td>39.0</td>
<td>0.89</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>46.0</td>
<td>0.70</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>33.0</td>
<td>0.69</td>
<td>0.021</td>
</tr>
</tbody>
</table>

\(^*\) 20 to 50 μM of compound were deposited on the planchets for counting.

\(\dagger\) The values in parentheses were determined on tyramine hydrochloride formed from the tyrosine by treatment with bacterial tyrosine decarboxylase.

"Metabolism of Antipyrine in Phenylketonurics"—Numerous compounds including many drugs are metabolized in the body through the hydroxylation of an aromatic ring. Experiments were undertaken to determine whether other aromatic hydroxylations were affected in subjects with phenylpyruvic oligophrenia. A typical example of a compound which undergoes hydroxylation *in vivo* is antipyrine, which has been shown to yield 4-hydroxyantipyrine (8) by a rather non-specific hydroxylation mechanism (9). The rate of disappearance of antipyrine from plasma is a good
measure of its rate of hydroxylation, since only a negligible fraction of ingested antipyrine is excreted unchanged by the kidney. 1 gm. of antipyrine was administered intravenously to each of the two phenylketonurics. Samples of blood were withdrawn for the measurement of plasma antipyrine at 2, 4, and 7 hours following the administration of the drug. The values for antipyrine when plotted against time were found to decline exponentially at the rate of about 20 per cent per hour. Values for the metabolism of antipyrine in a group of normal adults have been reported to range from 3 to 12 per cent per hour (10). The values for the phenylketonurics were, if anything, higher than the reported normal values. The conclusion may be drawn that there was no impairment of the antipyrine-hydroxylating mechanism in these subjects, indicating that the chemical lesion in this disorder does not apply to aromatic hydroxylations in general; it may well be confined to the conversion of L-phenylalanine to tyrosine.

TABLE II
Plasma Phenylalanine after Oral Administration of L-Phenylalanine to Phenylpyruvic Patients

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Dose (gm.)</th>
<th>Patient J (mg. per cent)</th>
<th>Patient C (mg. per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>37.0</td>
<td>34.2</td>
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<td>40</td>
<td>1</td>
<td>80.6</td>
<td>60.5</td>
</tr>
<tr>
<td>160</td>
<td>1</td>
<td>92.9</td>
<td>99.5</td>
</tr>
<tr>
<td>280</td>
<td>1</td>
<td>95.2</td>
<td>102.8</td>
</tr>
<tr>
<td>400</td>
<td></td>
<td>95.2</td>
<td>89.8</td>
</tr>
</tbody>
</table>

It is possible that the small amount of phenylalanine which is converted to tyrosine in the phenylketonurics does so through the non-specific mechanism which oxidizes antipyrine and other aromatic drugs. To investigate this several gm. of L-phenylalanine were administered to the phenylketonurics, as described below, and the experiment with antipyrine was repeated. Although the plasma levels of L-phenylalanine were maintained throughout the experiment at about 100 mg. per cent, the rate of antipyrine oxidation was not inhibited. It may be concluded that antipyrine and phenylalanine do not compete for a common tissue catalyst.

Plasma Levels of L-Phenylalanine in Phenylketonurics—8 gm. of L-phenylalanine were administered orally to each of the two phenylketonurics in divided doses, 6 gm. at the start, followed by 1 gm. at 2 hour intervals. Samples of blood were withdrawn for the measurement of plasma L-phenylalanine at the intervals shown in Table II. Plasma L-phenylalanine reached a plateau at levels of about 40 to 50 times those for fasting normal subjects.
**DISCUSSION**

It is generally accepted that phenylpyruvic oligophrenia is a genetic disorder and that the gene which normally controls the enzyme system responsible for the conversion of L-phenylalanine to tyrosine is missing. The present finding that in this disorder some tyrosine can be formed from L-phenylalanine requires modification of this theory. Several explanations of these findings are possible, based upon the recent studies on the enzyme, L-phenylalanine oxidase, which specifically catalyzes the oxidation of L-phenylalanine to tyrosine (11). (1) The amount of L-phenylalanine oxidase present in the liver may be smaller than usual, but not completely absent; (2) L-phenylalanine oxidase may be completely lacking, but a small amount of tyrosine may be formed from phenylalanine through an alternative pathway or by intestinal bacteria; (3) the amount of L-phenylalanine oxidase may be normal, but certain cofactors might be missing to activate the enzyme; (4) the amount of L-phenylalanine oxidase may be normal, but an inhibitor of this reaction might be present. Each of these theories will be investigated by studies on L-phenylalanine oxidase in biopsy material from livers of phenylketonurics. There is no obvious connection between the mental deficiency of phenylketonurics and their inability to convert phenylalanine to tyrosine. The absence of L-phenylalanine oxidase from brain (11) rules out the possibility of a direct relationship. However, a cofactor or inhibitor of phenylalanine oxidase might also influence another metabolic system which could be essential for normal brain metabolism.

The maintenance of such an extremely high plasma level of L-phenylalanine after its oral administration to the phenylketonurics indicates that the alternative pathway for L-phenylalanine metabolism, conversion to phenylpyruvic acid, is a relatively slow process. In individuals whose tyrosine-forming ability is normal, large doses of L-phenylalanine are so rapidly metabolized that the plasma level rises only slightly and then returns rapidly to normal values (12).

**SUMMARY**

3-C\(^{14}\)-DL-Phenylalanine was administered orally to a dog, two normal adults, and two phenylketonuric children. Tyrosine and phenylalanine were subsequently isolated from the plasma proteins and their specific activities determined. The ratio of the activity of tyrosine to phenylalanine (T:P) was used as an index of the extent of conversion of phenylalanine to tyrosine. Significant values for T:P were found in the phenylketonurics. However, in the controls and in the dog, T:P values were 10 to 15 times greater. It is apparent that the hydroxylation of L-phenylalanine to tyrosine does take place in the condition of phenylpyruvic oligophrenia, but to a diminished degree. The ability of phenylketonurics
to hydroxylate other aromatic compounds, such as antipyrine, is not affected.

BIBLIOGRAPHY

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