THE ENZYMATIC CONVERSION OF PHENYLALANINE TO TYROSINE*

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The enzymatic oxidation of phenylalanine to tyrosine has been studied as part of an investigation of the intermediates and enzyme systems involved in the biosynthesis of epinephrine. Womack and Rose (1) indicated that phenylalanine was converted to tyrosine in vivo by showing that tyrosine is not essential when sufficient phenylalanine is available. Definite proof was provided by Moss and Schoenheimer (2), who demonstrated that isotopically labeled phenylalanine given to rats appeared both as labeled phenylalanine and labeled tyrosine in the body proteins.

Information concerning the tissue catalysts responsible for the oxidation of phenylalanine to tyrosine is meager. Embden and Baldes (3) perfused livers with phenylalanine and showed an increase in tyrosine. Bernheim and Bernheim (4) demonstrated that surviving liver slices, on incubation with phenylalanine, produced an increased amount of phenolic material which showed many of the properties of tyrosine.

The experiments reported in the present paper demonstrate that liver slices and homogenates can convert phenylalanine to tyrosine. The properties and requirements of a soluble system that is capable of catalyzing the reaction are described.

Materials—Phenylalanine and tyrosine were obtained from the Nutritional Biochemicals Corporation. There was less than 0.1 per cent of the d isomer in the L-phenylalanine and about 0.2 per cent of the L isomer in the D-phenylalanine.1 These determinations were made by the method of Meister, Levintow, Kingsley, and Greenstein (5). 3-C14-DL-Phenylalanine was obtained from Tracerlab, Inc. Samples of DL-cyclohexylglycine, cyclohexyl-DL-alanine, and cyclohexyl-DL-aminobutyric acid were obtained from Dr. Alton Meister of the National Cancer Institute, the β-2-thienylalanine from the Nutritional Biochemicals Corporation (Lot 3045), and p-hydroxyxynamic acid and p-hydroxyxymandelic acid from Dr. Emery M. Gall of the University of California, Department of Bio-

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1 The authors are indebted to Dr. Alton Meister for these analyses.
chemistry. Diphosphopyridine nucleotide (DPN), purity 0.73, was prepared according to the method of Kornberg and Pricer (6). Triphosphopyridine nucleotide (TPN), purity 0.85, was prepared according to the method of Kornberg and Horecker (7). Reduced diphosphopyridine nucleotide (DPNH\(_2\)), purity about 0.65, prepared from DPN by the method of Ohlmeyer (8), was obtained from Dr. Arthur Kornberg. The practical grade of 1-nitroso-2-naphthol (Eastman Kodak Company) was found to be satisfactory for tyrosine assay. Alcoholic solutions were stable indefinitely. Samples obtained from several other sources were found to be grossly impure and unsuitable for the analysis. The tyrosine decarboxylase used was contained in a crude acetone powder of *Streptococcus faecalis*. The preparation also possessed phenylalanine decarboxylase activity which was about one-seventh of that for tyrosine.

**Analytical Method**—Tyrosine was determined by a colorimetric method based on the reaction of 1-nitroso-2-naphthol with o- and p-substituted phenols.\(^2\) Applied to deproteinized liver extracts, the method yields lower blank values than do other procedures for tyrosine assay. Tyrosine added to extracts is recoverable quantitatively.

Cysteine, glutathione, and other reducing substances interfere with the nitrosonaphthol reaction. When such substances were present, tyrosine was assayed by the nitration procedure reported by Snell and Snell (9). Satisfactory results may be obtained with this method by very careful control of conditions.

**Occurrence of Enzyme Activity**—Table I summarizes the experimental data on the occurrence of phenylalanine-oxidizing activity in animal tissues and in microorganisms. Livers of the rat, guinea pig, rabbit, dog, chicken, and human convert phenylalanine to tyrosine. The enzyme system is found only in the liver, at least in the case of the rat, no measurable differences in rat liver enzyme concentration being correlated to age, sex, or strain. The three microorganisms tested were not able to carry out the conversion.

**Identification of Enzymatically Formed Tyrosine**—The phenolic material formed by the action of rat liver slices and homogenates on l-phenylalanine was identified as tyrosine by the following procedures.

1. Treatment with 1-nitroso-2-naphthol yielded a product which had light absorption characteristics and solubility properties identical with those of the product formed by treating tyrosine with the reagent.

2. Tyrosine decarboxylase converted the material to a product which, like tyramine, could be extracted into isomyl alcohol at pH 9.6 and could then be extracted from the alcohol with dilute acid. The decarboxylated material also reacted with 1-nitroso-2-naphthol to yield a product with

\[^{3}\) Cooper, J. R., and Udenfriend, S., unpublished.\]
light absorption characteristics identical with those of the known tyramine derivative.

3. On paper chromatograms the product moved with an \( R_f \) identical with that of tyrosine.

When \( ^{34}\text{C} \)-DL-phenylalanine was used as substrate, the isolated tyrosine also contained \( ^{34}\text{C} \), indicating that tyrosine was formed by the direct oxidation of phenylalanine. This rules out the possibility that phenylalanine merely stimulates tyrosine production through some other route.

Experiments on Tissue Slices—The conversion of L-phenylalanine to tyrosine in liver slices required oxygen and proceeded unabated for many hours when incubated at 37\(^{\circ}\). Since the slices could also destroy L-tyrosine at a fairly rapid rate, balance studies were not attempted. In Table II are presented data from an experiment in which rat liver slices were incubated with L-phenylalanine and L-tyrosine.

Preparation of Soluble System from Liver—All operations were carried out in a cold room (0-3\(^{\circ}\)). Solutions and equipment were allowed to cool to the temperature of the cold room before use.

Rats were stunned and then decapitated to permit the blood to drain. The livers were immediately removed and homogenized in a Waring blender with 2 to 3 times their weight of isotonic KCl. The homogenate was centrifuged at 13,000 r.p.m. for 1 hour in a Servall centrifuge. After centrifugation, a small amount of fatty material was removed from the

### Table I

<table>
<thead>
<tr>
<th>Organism</th>
<th>Tissue</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Liver*</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Lung†</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>Kidney†</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>Brain†</td>
<td>0</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Liver*</td>
<td>0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&quot; * +</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>&quot; + +</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>&quot; * +</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>&quot; + +</td>
<td></td>
</tr>
<tr>
<td>S. faecalis</td>
<td>Cell suspension</td>
<td>0</td>
</tr>
<tr>
<td>E. coli</td>
<td>&quot; &quot;</td>
<td>0</td>
</tr>
<tr>
<td>Yeast</td>
<td>&quot; &quot;</td>
<td>0</td>
</tr>
</tbody>
</table>

\( + = \) present; \( 0 = \) absent.

* Tested in both slices and homogenates.
† Tested in homogenates only.
‡ Tested in slices only.
surface and the supernatant fluid was then dialyzed against isotonic KCl containing 0.01 M phosphate buffer, pH 6.7. Since the activity deteriorated even at 0°, dialysis was carried out for no longer than 3 hours with an efficient rocking device, permitting 4 to 6 liters of solution to pass through the apparatus per 100 ml. of supernatant solution.

Dialyzed preparations could be stored in the deep freeze for at least 2 months. It was found convenient to divide a large preparation into several small fractions for storage in the deep freeze. The preparation could also be lyophilized with little loss in activity. The dried material also required storage in the deep freeze.

All the activity of homogenates was recoverable in the supernatant solutions. Substitution of NaCl for KCl did not influence the activity

| Table II | Incubation of L-Phenylalanine and L-Tyrosine with Rat Liver Slices |
|---|---|---|---|---|
| | Sample No. | Incubation time | Substrate | Tyrosine formed | Tyrosine destroyed |
| L-Phenylalanine | 1 | 2 | 0 | 0.06 | |
| | 2 | 1 | 23.9 | 0.39 | |
| | 3 | 2 | 23.9 | 1.00 | |
| | 4 | 3 | 23.9 | 1.42 | |
| L-Tyrosine | 5 | 2 | 4.00 | | 1.30 |
| | 6 | 2 | 4.00 | | 1.08 |

150 mg. slices were incubated in 4 ml. of Ringer's bicarbonate buffer, pH 7.4, at 37° in an atmosphere of 95 per cent oxygen-5 per cent CO2.

of the resulting preparations. Homogenization in hypertonic sucrose, to preserve mitochondria, did not yield homogenates with increased activities. Further centrifugation of the dialyzed preparation in the ultracentrifuge at 125,000 × g for 1 hour yielded a small amount of inactive sediment and a supernatant containing all the original activity.

Properties of Soluble System from Liver—The crude liver extract had an appreciable oxygen uptake in the absence of added L-phenylalanine. This made it impossible to determine the oxygen utilized for tyrosine formation. The ability of the preparation to deacetylate acetylamino acids and to hydrolyze peptides made it difficult to study the effect of the liver extract on derivatives of L-phenylalanine. Sufficient purification was achieved so that L-phenylalanine could be completely recovered as tyrosine and phenylalanine in the presence of DPN, oxygen, and the liver extract (see below). This indicated the absence of other enzymes which could metabolize phenylalanine and tyrosine. In Fig. 1 is shown a chromatogram from an experiment in which C14-DL-phenylalanine was incu-
bated with the enzyme preparation. All the C\textsuperscript{14} originally added was found in the phenylalanine and tyrosine spots. No other radioactive spots were found. The conversion of only half of the phenylalanine to tyrosine is due to the inactivity of the unnatural enantiomorph (see below).

**Test System**—The test system utilized to study the requirements of the enzyme consisted of 1.25 ml. containing 0.15 ml. of 1 M phosphate buffer, pH 6.7, 0.5 ml. of enzyme preparation, 2 \( \mu \text{M} \) of L-phenylalanine, 0.55 \( \mu \text{M} \) of DPN, and 5.0 \( \mu \text{M} \) of nicotinamide. The mixture in a 20 ml. beaker was shaken in air, at 25\( ^\circ \), on a Dubnoff metabolic shaking incubator. At the end of the incubation period 0.6 ml. of 15 per cent trichloroacetic acid was added and the sample was transferred to a conical tube for centrifuging. After centrifuging, a 1 ml. portion was taken for tyrosine assay.

**Requirements**—The preparation required both oxygen and pyridine nucleotide for activity. DPN and TPN were almost equally effective in fresh undialyzed preparations. Preincubation of such preparations for 30 minutes in oxygen at 37\( ^\circ \) reduced the effectiveness of DPN to negligible values, whereas the TPN effectiveness was reduced by approximately one-half. Aging of the preparations for several hours, either in the ice box
or at room temperature, reduced the effectiveness of TPN much more than that of DPN. In the dialyzed preparations, DPN and DPNH₂ were many times more effective than TPN (Fig. 2).

No activity was observed under strict anaerobic conditions. Optimal conditions were obtained in air when a sufficiently large gas-liquid interface was maintained and when the samples were shaken at a rate sufficient to produce maximal equilibration. A decreased activity was observed in pure oxygen as compared to air. This was presumably due to irreversible inactivation of the enzyme system by oxygen. Methylene blue could not substitute for oxygen in the reaction. Hydrogen peroxide, produced enzymatically by xanthine oxidase action, had no effect on the conversion.

Substrate—The relationship between the amount of L-phenylalanine added and tyrosine formed is shown in Fig. 3. No tyrosine was formed from D-phenylalanine. However, with undialyzed preparations some tyrosine appeared, probably as a result of racemization of the phenylalanine. N-Acetyl- and N-chloroacetyl-L-phenylalanine did not yield the corresponding tyrosine derivatives. No tyrosine ethyl ester appeared when L-phenylalanine ethyl ester was used as substrate. DL-Phenylglycine, phenylserine, phenylpyruvic acid, phenylethylamine, benzoic acid, hippuric acid, cinnamic acid, and mandelic acid were tested with the liver extract. In no case was the corresponding p-phenol formed.

Optimal Temperature—The maximal initial rate of tyrosine production took place at about 35°. However, in the presence of oxygen the enzyme system was rapidly inactivated at this temperature. Also, at temper-
Fig. 3. Relationship between L-phenylalanine concentration and tyrosine formation. Incubation time 75 minutes; 25°; pH 6.7; 0.55 μM of DPN; dialyzed preparation.

**TABLE III**

Inhibitors of Tyrosine Formation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Per cent inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azide</td>
<td>8.0 × 10⁻⁵</td>
<td>100</td>
</tr>
<tr>
<td>Cyanide</td>
<td>8.0 × 10⁻⁵</td>
<td>78</td>
</tr>
<tr>
<td>Fluoride</td>
<td>8.0 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>Arsenite</td>
<td>8.0 × 10⁻⁵</td>
<td>22</td>
</tr>
<tr>
<td>Arsenate</td>
<td>8.0 × 10⁻⁵</td>
<td>0</td>
</tr>
<tr>
<td>β-2-Thienylalanine</td>
<td>1.2 × 10⁻³</td>
<td>66</td>
</tr>
<tr>
<td>Cyclohexyl-DL-glycine</td>
<td>2.4 × 10⁻³</td>
<td>0</td>
</tr>
<tr>
<td>Cyclohexyl-DL-alanine</td>
<td>2.4 × 10⁻³</td>
<td>26</td>
</tr>
<tr>
<td>Cyclohexyl-DL-α-aminobutyric acid</td>
<td>1.6 × 10⁻²</td>
<td>43</td>
</tr>
<tr>
<td>DL-Phenylalanine</td>
<td>2.4 × 10⁻³</td>
<td>0</td>
</tr>
<tr>
<td>DL-α-Alanine</td>
<td>5.0 × 10⁻³</td>
<td>0</td>
</tr>
<tr>
<td>DL-Phenylglycine</td>
<td>2.4 × 10⁻³</td>
<td>9</td>
</tr>
<tr>
<td>Phenylserine</td>
<td>2.4 × 10⁻³</td>
<td>0</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2.4 × 10⁻³</td>
<td>85</td>
</tr>
<tr>
<td>Acetanilide</td>
<td>2.4 × 10⁻³</td>
<td>0</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>4.2 × 10⁻²</td>
<td>0</td>
</tr>
</tbody>
</table>

The concentration of L-phenylalanine used in all these experiments was 2.4 × 10⁻² M.

* The values presented are the average of at least two experiments. Values less than 10 per cent cannot be considered significant.

atures of 35° and higher tyrosine was slowly destroyed. At 25° the reaction proceeded more slowly but continued at a constant rate for about an hour. At this lower temperature there was no measurable destruction of
tyrosine. The calculated initial rate of tyrosine formation, in micromoles per minute per gm. of liver, is 0.1 at 25° and 0.3 at 35°.

Optimal pH—In phosphate buffers, the maximal rate of tyrosine formation occurred at pH 7. Citrate buffers inhibited the reaction.

Inhibitors—The degree to which various substances were able to inhibit the conversion of phenylalanine to tyrosine is shown in Table III. The marked inhibition by cyanide and azide plus the requirement of oxygen suggests a heavy metal oxidase. The marked inhibition by 3-thienylalanine and cyclohexylalanine is very interesting, especially since compounds such as phenylglycine and phenylserine had little or no effect. L-Tyrosine, the end-product of the reaction, did not inhibit the reaction. However, L-phenylalanine itself was definitely inhibitory (Fig. 3).

DISCUSSION

The conversion of L-phenylalanine to tyrosine is catalyzed by an enzyme system found in mammalian livers. The presence of such an enzyme in animals may explain why tyrosine, in contrast to phenylalanine, is not an essential amino acid (1). In yeast (10) and Escherichia coli (11), where independent pathways for phenylalanine and tyrosine synthesis have been demonstrated, enzymatic conversion of phenylalanine to tyrosine does not seem to occur.

The amount of activity found in liver suggests that conversion to tyrosine is an important pathway of L-phenylalanine metabolism. Dakin (12) and Embden and Baldes (3) were among the first to suggest this. Jervis and coworkers (13, 14) have presented evidence that one of the defects in the inherited disease, phenylpyruvic oligophrenia, is the inability to convert phenylalanine to tyrosine, resulting in an accumulation of phenylalanine, phenylpyruvic acid, and phenyllactic acid. This suggests that L-phenylalanine metabolism normally proceeds mainly via tyrosine.

The high degree of specificity of this enzyme system makes it improbable that it catalyzes the numerous other aromatic hydroxylations carried out in animal tissues. Structural considerations suggest that 3-thienylalanine and cyclohexylalanine are probably inhibitors of the competitive type. The failure of cyclohexylglycine and cyclohexylaminobutyric acid to inhibit indicates that there is some specificity with regard to inhibitors, perhaps determined by the alanine portion of the molecule.

The enzyme system which catalyzes the conversion of phenylalanine to tyrosine is apparently not a simple one. The requirements of the system for both oxygen and pyridine nucleotide suggest that at least two enzymes are involved. The variation in the ratio of DPN activation to TPN activation under different conditions indicates a further degree of complexity.

Raper (15) has shown that H2O2, in the presence of Fe++, can convert
phenylalanine to tyrosine. However, the failure of catalase to inhibit and 
H₂O₂ to stimulate this preparation would seem to rule out a peroxidative 
type of system.

Further studies on the properties of the catalytic system and on the 
reaction mechanism will have to await the purification and fractionation 
of the enzymes involved in the reaction.

We wish to thank Mr. Carroll T. Clark for his valuable technical 
assistance.

SUMMARY

1. Surviving liver slices and extracts catalyze the conversion of L-phenyl-
alanine to tyrosine.
2. The enzymatically formed tyrosine has been identified by isotopic 
and chromatographic procedures.
3. A soluble system has been obtained from rat liver which, in the pres-
ence of DPN and oxygen, can catalyze the conversion of L-phenylalanine 
to tyrosine.
4. The enzyme system is highly specific. D-Phenylalanine and many 
derivatives and homologues of L-phenylalanine are unable to substitute 
for L-phenylalanine as substrate in this oxidation.

BIBLIOGRAPHY

192, 535 (1951).
New York, in press.
New York, in press.
12. Dakin, H. D., Oxidations and reductions in the animal body, Monographs on 
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