THE TYROSINE OXIDATION SYSTEM OF LIVER

I. EXTRACTS OF RAT LIVER ACETONE POWDER*

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Many investigators of tyrosine oxidation have used systems in vitro such as slices, minces, and, more recently, homogenates of liver. During our search for a suitable preparation other than liver slices for studying the conversion of phenylalanine to tyrosine, an extract of rat liver acetone powder was tested. Although the phenylalanine to tyrosine conversion was not demonstrated, it was found that the oxidation of tyrosine was catalyzed by the powder extract. This paper will report on the conditions which were found necessary to study tyrosine oxidation by this system.

Very few papers in the literature deal with the use of acetone powder extracts as a system for studying tyrosine oxidation. Lang and Westphal (1) reported that an enzyme which attacked \( \text{L-phenylalanine and L-tyrosine} \) was present in some acetone powder preparations of dog liver. Felix et al. (2) used an acetone powder of pig kidney in their study of tyrosine oxidation. Although Felix and Schaefer reported later (3) that the supernatant of centrifuged minced rat liver had very little action on either \( \text{L-tyrosine or p-hydroxyphenylpyruvic acid} \), a fact which suggested that some of the enzymes involved are associated with the insoluble liver fraction, more recent work with homogenates (4, 5) indicates that the enzymes involved in tyrosine oxidation are soluble. Our results support this view.

For the study of tyrosine oxidation, the acetone powder extract has several advantages over homogenates. The powder is very stable for at least several weeks even at room temperature, and the extract contains the enzymes in a concentrated solution and therefore the reactions can be studied in the ordinary sized Warburg vessels with up to 10 \( \mu \text{M} \) of tyrosine. The acetone treatment and extraction steps remove or inactivate many of the other enzyme systems. As a result, the control flasks have a much lower oxygen uptake and the system is more specific for the oxidation of tyrosine. Lastly, the extract will serve as a convenient starting point for fractionation.

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procedures to separate the complex enzyme system into its component units.

**Enzymatic and Analytical Methods**

*Preparation of Acetone Powder—* The livers were removed as quickly as possible after sacrificing the animals and were placed in a beaker of ice. After about 100 gm. had been obtained, this amount was worked up as described below. The remaining steps were carried out in a cold room at 0–5°. The livers were placed in a Waring blender with 50 ml. of distilled water (previously cooled to about 5°) and homogenized for 3 minutes. The homogenate was poured, with stirring, into a beaker containing 500 ml. of acetone, previously cooled by a surrounding acetone-dry ice mixture. The resulting slurry, after standing about 10 minutes, was then homogenized in a large glass homogenizer (Potter type) of 50 ml. capacity to form a fine suspension. The suspension was diluted with an equal volume of cold acetone and filtered with suction on a large Buchner funnel, with Whatman No. 1 filter paper. The cake was not allowed to dry completely but was removed just as it began to crack. After resuspending the cake in 500 ml. of cold acetone, it was filtered again. As the cake began to crack, about 30 ml. of ether were poured over the powder and the filtration continued until the powder was light colored and dry. The rate of drying was speeded by ruffling and turning the powder with a spatula. The powder was transferred to a vacuum desiccator over sulfuric acid for several days and then stored either at room temperature or in the refrigerator.

Some early preparations were made by freezing the liver immediately in liquid nitrogen, pulverizing the frozen tissue, and dehydrating with acetone. Since this method did not yield a more active material, it was discontinued in favor of the simpler method above.

Preparations were made from the livers of Sprague-Dawley, Long-Evans, and Slonaker (Wistar) rat strains. The highest activity was found in preparations from Long-Evans male rats; the activity of powders of the livers of males usually was from 2 to 10 times higher than of the females. Preparations from Sprague-Dawley female rats had a particularly low activity. The reason for this sex difference is not clear and we plan to investigate it.

Since a comparison between preparations from young and older male rats showed very little difference in activity, rats weighing about 300 gm. were used for most of the work.

**Manometric Assay**

*Preparation of Powder Extract—* A sample of the acetone powder was weighed and cooled to -23.3° in a deep freeze unit. The calculated vol-
volume of 0.2 M phosphate buffer, pH 8.05, required for the extraction was placed in an Erlenmeyer flask and cooled to about 5° just before use.

After mixing, the extraction was carried out in the refrigerator for 10 minutes with an occasional mixing by swirling. The mixture was centrifuged at 2000 r.p.m. for 2 minutes and supernatant fluid was decanted through cheese-cloth into a large graduated centrifuge tube and cooled in a beaker of ice water until used. The extract was clear and a deep amber color.

Constituents of Warburg Flasks—The main compartment contained 1.0 ml. of 0.1 M sodium pyrophosphate buffer, pH 8.05, 0.5 ml. of boiled extract of yeast or liver adjusted to approximately pH 8.0, and 2.0 ml. of the acetone powder extract. The side arm contained 0.5 ml. of a suspension of L-tyrosine in the 0.2 M phosphate buffer or buffer alone for the control flasks. The center well held 0.2 ml. of 20 per cent potassium hydroxide to absorb carbon dioxide.

The extract was added last and the flasks were gassed with 100 per cent oxygen for 3 minutes. Equilibration in the 37° bath was for 10 minutes and the substrate was tipped in 5 minutes later. Experiments were usually run from 2 to 3 hours.

The L-tyrosine suspension was freshly prepared for each experiment.

Boiled Extract Additions—In some of the first experiments with a 9 per cent extract (weight of powder × 10 = ml. of 0.2 M phosphate buffer used for extraction), very little net oxygen uptake was found with L-tyrosine as the substrate. The addition of a concentrated boiled yeast extract increased the net oxygen uptake. Controls with boiled yeast extract and tyrosine without powder extract or with boiled powder extract showed the same oxygen uptake as the flasks without tyrosine. A concentrated liver homogenate dialysate or boiled liver extract was even more effective in stimulating tyrosine oxidation.

In later experiments, a more concentrated extract (16 or 20 per cent) was used (weight of powder × 4 or 5 = ml. of 0.2 M phosphate buffer used for the extraction). Although the more concentrated extracts showed fairly high activity without any boiled extract being added, there was still a marked stimulation by its addition. Large amounts of boiled extract were made up and frozen to keep the addition of this component a constant factor throughout a series of experiments. We believe that the extract factors were still suboptimal in some of the experiments.

Analytical Methods

Tyrosine—Tyrosine was determined by the modified Millon method of Bernhart and Schneider (6) with a Klett photoelectric colorimeter.

1 The yeast used to prepare the boiled extract was kindly supplied by the Consumer's Yeast Company, Oakland, California.
**Carbon Dioxide Production**—Carbon dioxide was determined manometrically by the direct method, as described by Umbreit et al. (7). 0.2 ml. of 4 M HCl was present in the second side arm and tipped in at the end of the experiment. Readings were continued until the liberation of bound carbon dioxide was complete.

**Acetoacetic Acid and Oxalacetic Acid**—The contents of the incubated flasks used to determine carbon dioxide production had a final concentration of 0.2 M HCl. These were centrifuged and the clear supernatants assayed manometrically at 26° by the catalytic decarboxylation methods for acetoacetic and oxalacetic acids with aniline citrate and aluminum sulfate (7). About 95 per cent of the theoretical carbon dioxide was obtained from 2.5 and 5.0 μM of known acetoacetic acid.

**Ammonia Production**—Since the flasks for determination of carbon dioxide had acid in the side arm, any ammonia liberated during the incubation was absorbed. The contents of these were centrifuged and aliquots of the supernatants used to determine ammonia. The ammonia was liberated by sodium metaborate and trapped in dilute HCl in Conway type diffusion chambers. It was then determined by nesslerization.

**EXPERIMENTAL**

**Optimal pH**—The pH of the incubation mixture was varied from 6.8 to 9.0, with phosphate buffer from 6.8 to 7.8 and with tris buffer (trimethoxy-methylamine hydrochloride) from 7.0 to 9.0. These experiments indicated that the optimal pH was between 7.8 and 8.0. A combination of phosphate and pyrophosphate buffers used over a smaller range, 7.4 to 8.0, agreed with this conclusion.

The addition of pyrophosphate increased the buffering capacity at the optimal zone. By extracting the powder with 0.2 M phosphate buffer at pH 8.05 and adding 1.0 ml. of 0.1 M pyrophosphate buffer at pH 8.05 to the incubation mixture, the initial pH was adjusted to about 7.90. During a 2 hour incubation, the pH decreased about 0.1 unit.

The pyrophosphate addition also lowered the control oxygen uptake but did not inhibit the oxidation of tyrosine in the concentration used, 0.025 M.

**Substrate Specificity**—Several compounds were tested as substrates with the same experimental conditions as previously described for L-tyrosine. All compounds were neutralized to approximately pH 8 and were made up in phosphate buffer with the exception of homogentisic acid lactone and 3,4-dihydroxyphenylalanine, since they are rapidly autoxidized at this pH.

The compounds showing a net oxygen uptake over the control flasks are listed in Table I, those showing no net increase over the controls in Table II.

The compounds active under these experimental conditions suggest that
substitutions adjacent to the phenolic hydroxy group or substitutions on the amino group prevent the oxidation. The ethyl ester was nearly as active as L-tyrosine, but at the pH used there may have been considerable

<table>
<thead>
<tr>
<th>Compounds* Showing Net Oxygen Uptake over Control Flasks</th>
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<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>L-Tyrosine</td>
</tr>
<tr>
<td>D-Tyrosine</td>
</tr>
<tr>
<td>DL-Tyrosine</td>
</tr>
<tr>
<td>L-Tyrosine ethyl ester</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compounds* Showing No Net Oxygen Uptake over Control Flasks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
</tbody>
</table>
| Carbobenzoxy-<i>L</i>-glutamyl-
  L-tyrosine | 5 | p-Hydroxy mandelic acid | 10 |
| Carbobenzoxy-<i>DL</i>-tyrosine | 10 | Phenylalanine | 10 |
| Diiodotyrosine | 10 | Phenylserine | 20 |
| Dibromotyrosine | 10 | L-Leucine | 20 |
| Dinitrotyrosine | 10 | L-Methionine | 20 |
| Mononitrotyrosine | 5 | DL-Valine | 20 |
| 3-Aminotyrosine | 10 | DL-Glutamic acid | 5 |
| Tyramine hydrochloride | 10 | DL-Alanine | 20 |
| <i>p</i>-Hydroxyphenylacetic acid | 5 | 3,4-Dihydroxyphenyl | 10 |
| <i>p</i>-Hydroxycinnamic acid | 10 | alaniné† | 5 |

* We are indebted to Dr. E. M. Gal for the tyrosine amide, homogentisic acid, <i>p</i>-hydroxy mandelic acid, and <i>p</i>-hydroxyphenylpyruvic acid, to Dr. E. F. Jensen for the ethyl and butyl esters, to Dr. H. Fraenkel-Conrat for the carboxenzoxy-<i>L</i>-glutamyl-<i>L</i>-tyrosine, to Dr. R. Y. Stanier for the <i>p</i>-hydroxyphenylacetic acid, and to Miss G. Solomon for the mononitrotyrosine.

† There was some oxygen uptake which we believe was due to non-enzymatic oxidation.

hydrolysis of the ester. The amide and the butyl ester were attacked much more slowly than L-tyrosine and further experiments need to be carried out to find out what products are formed from these compounds.

The reason for the activity of <i>D</i>-tyrosine, about one-third the rate of <i>L</i>-tyrosine, is not clear. <i>DL</i>-Alanine was practically inactive and we would have expected it to have shown more uptake of oxygen if the <i>D</i>-amino acid
ENZYMATIC OXIDATION OF TYROSINE

oxidase were functioning under these experimental conditions. The purity of the D-tyrosine is too high to account for the activity on the basis of contamination with L-tyrosine.

A comparison of the initial rates of oxygen uptake by using L-tyrosine, p-hydroxyphenylpyruvic acid, and homogentisic acid lactone showed that L-tyrosine was the slowest. This difference in rates is consistent with the possibility that the keto acid and homogentisic acid are intermediates in tyrosine oxidation. If this is true, the rate-limiting step of the series of reactions must precede the p-hydroxyphenylpyruvic acid oxidation step under these experimental conditions.

The failure to find any net oxygen uptake by using L-leucine and some of the other L-amino acids, which are better substrates for the L-amino acid oxidase than L-tyrosine, suggests that the L-amino acid oxidase is not active under these experimental conditions and argues against the first step of tyrosine oxidation being the conversion to the corresponding keto acid by the action of the L-amino acid oxidase.

Experiments with homogentisic acid lactone showed the oxygen uptake reached a plateau at 2 atoms of oxygen per molecule. With 5 μM amounts, this plateau was reached within 30 minutes. Boiled extract controls showed no appreciable autoxidation within this time.

p-Hydroxyphenylpyruvic acid required about 3.5 atoms of oxygen per molecule of substrate to reach a plateau.

Inhibitors—Only a few inhibitors have as yet been tested. The tyrosine oxidation system was strongly inhibited by 0.01 M cyanide and iodoacetate. Malonate (0.01 M) was not inhibitory and atabrine tested up to 0.001 M had no effect upon either the control or the tyrosine oxygen uptake.

Arsenite (0.01 M) lowered the control oxygen uptake rate by about 50 per cent, but did not inhibit tyrosine oxidation and behaved like pyrophosphate in this respect.

Keto Acid Stimulation—Even in the presence of boiled yeast extract, there was a marked stimulation of the rate of tyrosine oxidation by the addition of α-ketoglutarate or oxalacetate. Pyruvate was inhibitory and the stimulation by oxalacetate was shorter and less pronounced than that by α-ketoglutarate. The keto acids did not alter the control oxygen uptake rate. The rate was about tripled by the addition of 0.01 M arsenite.

2 The D-tyrosine had an optical rotation of [α]254° = +10.8° (4.8 per cent solution in 1 N HCl). The literature value (8) for D-tyrosine is [α]25° = +10.3° (4 per cent solution in 1 N HCl).

3 Homogentisic acid lactone was prepared by the method of Abbott and Smith (9). The melting point was 188–189°, uncorrected.

4 p-Hydroxyphenylpyruvic acid was prepared by using p-hydroxybenzaldehyde by the method of Herbst and Shemin (10). The product was recrystallized from water and melted at 212–214°, uncorrected.
A demonstration of the stimulation of the oxidation of tyrosine by keto acids is shown by the curves in Fig. 1. This stimulation was not found with p-hydroxyphenylpyruvic acid as the substrate.

The boiled extract obviously supplies other factors than the keto acids and we are attempting to identify these additional factors.

![Graph showing oxygen uptake over time](image)

**Fig. 1.** The stimulation of the oxidation of L-tyrosine by a mixture of α-keto glutarate and oxalacetate in the presence of arsenite. Flask contents, 2.0 ml. of 20 per cent extract of rat liver acetone powder, 1.0 ml. of pyrophosphate buffer, 0.2 ml. of sodium arsenite (to yield a final concentration of 0.01 M), 0.3 ml. of α-keto-glutarate-oxalacetate mixture (10 μM each) or 0.3 ml. of H₂O, and 5 μM of L-tyrosine in 0.5 ml. of buffer or buffer alone in the side arm.

**Oxygen Uptake, Products, and Course of Reaction**—As has been previously reported with other systems in vitro under optimal conditions, there is an uptake of 4 atoms of oxygen per molecule of L-tyrosine. We have also found this ratio repeatedly with even as much as 10 μM of L-tyrosine per flask. After the net uptake of oxygen had ceased, testing for tyrosine showed that virtually none remained.

To obtain a more complete picture of the over-all reaction, several bal-
ance experiments were performed. Oxygen uptake, carbon dioxide production, acetoacetic acid production, decrease in tyrosine, and ammonia liberation were all determined in the same experiment. Since aniline citrate decarboxylates both acetoacetic acid and oxalacetate, the latter was also determined by the aluminum sulfate decarboxylation method. Each

<table>
<thead>
<tr>
<th>Table III</th>
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<tr>
<td><strong>Balance Experiment with 5 μM L-Tyrosine</strong></td>
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</table>

The flask contents were as described under the section on methods, 20 per cent extract of rat liver powder, boiled pig liver extract supplemented with 2.0 mg. of α-ketoglutarate per flask, and HCl in the second side arm; total volume 4.2 ml. After a 2 hour incubation, acid was tipped and readings continued for 20 minutes for bound CO₂ liberation.

<table>
<thead>
<tr>
<th></th>
<th>Found</th>
<th>Theoretical*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net oxygen uptake</td>
<td>221 μl.</td>
<td>224 μl.</td>
</tr>
<tr>
<td>CO₂ liberated during incubation</td>
<td>150 μl.</td>
<td>112 μl.</td>
</tr>
<tr>
<td>CO₂ liberated by aniline citrate decarboxylation</td>
<td>81 μl.</td>
<td>112 μl.</td>
</tr>
<tr>
<td>CO₂ liberated by aluminum sulfate decarboxylation</td>
<td>0 μl.</td>
<td>0 μl.</td>
</tr>
<tr>
<td>Total CO₂</td>
<td>231 μl.</td>
<td>10.3 μM</td>
</tr>
<tr>
<td>Decrease in tyrosine</td>
<td>0.903 mg.</td>
<td>5.0 μM</td>
</tr>
<tr>
<td>Net ammonia nitrogen liberated</td>
<td>22 γ</td>
<td>1.0 μM</td>
</tr>
</tbody>
</table>

The CO₂ liberated in the control incubation flasks was 264 μl., including the initially bound CO₂. The decrease in tyrosine was calculated from the difference in the zero time and incubated tyrosine flasks, 924 γ − 21 γ = 903 γ. The control incubation flasks had 136 γ of NH₃-N, including the initial ammonia.

*The theoretical values are based upon the assumption that the products are carbon dioxide, acetoacetic acid, and fumaric acid, and that a transamination step is present.

The balance study required fourteen incubation flasks and the analyses were carried out in duplicate or triplicate. Zero time analyses were also made to determine the initial tyrosine, free ammonia, and acetoacetic acid.

The averaged results of two balance experiments are given in Table III. The oxygen uptake data are shown in detail in Table IV.

From the carbon dioxide data, it appears that some of the acetoacetic acid was decomposed during the incubation and hence more than the theoretical amount of carbon dioxide was liberated during the incubation and less by the aniline citrate. The total carbon dioxide from both sources checks with the expected value within the limit of the experimental error.
The decarboxylation by aniline citrate, although corrected for any oxalacetate, is not entirely specific for acetoacetic acid. According to Ravdin and Crandall (11), fumarylacetoacetic acid, a possible intermediate, is slowly decarboxylated by aniline citrate at 37°. The product here is more likely acetoacetic acid because of the rapid rate of decarboxylation at 26° and it parallels the rate observed when known acetoacetic acid was added to a control incubation flask. The oxygen uptake data would be the same for either product.

The failure to find free ammonia equivalent to the amount of L-tyrosine oxidized agrees with the results of Felix and Zorn (12) with minced pig liver. Since Lerner (13) could not find any significant amounts of alanine formed directly from C14-labeled tyrosine by rat liver slices, a transamination reaction would be more consistent with the present experimental data; namely, the stimulation of the reaction by keto acid.

### Table IV

**Oxygen Uptake with 5 µM of L-Tyrosine**

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Control flasks, oxygen uptake</th>
<th>Tyrosine flasks, oxygen uptake</th>
<th>Net oxygen uptake</th>
<th>Per cent oxidation completed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>34 µl.</td>
<td>83 µl.</td>
<td>49 µl.</td>
<td>21.9</td>
</tr>
<tr>
<td>20</td>
<td>60 µl.</td>
<td>150 µl.</td>
<td>00 µl.</td>
<td>40.2</td>
</tr>
<tr>
<td>30</td>
<td>84 µl.</td>
<td>208 µl.</td>
<td>124 µl.</td>
<td>55.4</td>
</tr>
<tr>
<td>40</td>
<td>100 µl.</td>
<td>250 µl.</td>
<td>150 µl.</td>
<td>67.0</td>
</tr>
<tr>
<td>50</td>
<td>116 µl.</td>
<td>286 µl.</td>
<td>170 µl.</td>
<td>75.9</td>
</tr>
<tr>
<td>60</td>
<td>134 µl.</td>
<td>320 µl.</td>
<td>186 µl.</td>
<td>83.0</td>
</tr>
<tr>
<td>75</td>
<td>156 µl.</td>
<td>356 µl.</td>
<td>200 µl.</td>
<td>89.3</td>
</tr>
<tr>
<td>90</td>
<td>174 µl.</td>
<td>383 µl.</td>
<td>209 µl.</td>
<td>93.3</td>
</tr>
<tr>
<td>105</td>
<td>191 µl.</td>
<td>407 µl.</td>
<td>216 µl.</td>
<td>96.4</td>
</tr>
<tr>
<td>120</td>
<td>208 µl.</td>
<td>429 µl.</td>
<td>221 µl.</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Plotting the log of the per cent oxidation still uncompleted versus time gave a straight line, indicating that the reaction is a first order.

\[ * (\mu l. \text{ oxygen uptake})/224 \times 100. \ (224 \text{ is theoretical complete oxidation.}) \]

DISCUSSION

As in the previous work with systems in vitro, L-tyrosine required the uptake of 4 atoms of oxygen per molecule and homogentisic acid required 2 atoms. Contrary to the results of Felix et al. (2, 3), we have found 3.5 atoms of oxygen used per molecule of p-hydroxyphenylpyruvic acid rather than between 2 and 3 atoms, as he reported. This difference may have

Subsequent to the present experimental work, evidence for the transamination of L-tyrosine by tissue extracts has been reported by others (14, 15).
been due to greater purity of our acid or the fact that we used lower sub-
strate concentrations.

Felix and coworkers used 11.1 \( \mu M \) of the keto acid, 3 hours being required
for the oxygen uptake to reach a plateau; our system showed a rapid uptake
of oxygen with 3 or 5 \( \mu M \) of substrate, with plateaus within 30 and 60 min-
utes respectively. There is some non-enzymatic oxidation of the keto
acid, but since it involves less than 3 or 4 atoms of oxygen per molecule,
any non-enzymatic oxidation would reduce the plateau level reached to
less than the theoretical value. We believe that the oxygen uptake with
the keto acid should be 4 atoms, just as with L-tyrosine. The finding that
the keto acid required 4 atoms also would allow homogentisic acid to be a
normal intermediate, while 3 atoms would not, as Felix et al. pointed out
(2).

If the final products of tyrosine oxidation are \( \text{CO}_2 \), fumaric acid, and
acetoacetic acid, as recent work suggests (11, 13), the over-all requirement
should be 5 atoms of oxygen per molecule of substrate tyrosine, and free
ammonia equivalent to the amount of tyrosine oxidized would also be ex-
pected. Since manometric experiments show general agreement in ob-
taining only 4 atoms of oxygen under optimal conditions, this difference
must be explained. If a transamination step were present, the oxygen
requirement would be lowered by 1 atom, and 4 atoms should be expected
to be required for both tyrosine and the corresponding keto acid and no
ammonia should be liberated.

Our manometric data are in agreement with the possible presence of a
transamination step. The stimulation of L-tyrosine oxidation, but not of
\( p \)-hydroxyphenylpyruvic acid oxidation, by the addition of \( \alpha \)-ketoglutarate
and the small amount of free ammonia liberated during the incubation also
support this view. Further experiments are under way to establish
whether the first step is, indeed, a transamination reaction.

Previous work and the results reported suggest that the biological oxi-
dation of L-tyrosine by liver takes place according to the following steps.

\[
\begin{align*}
\text{Tyrosine} & \xrightarrow{\text{Transamination}} \text{p-Hydroxyphenylpyruvic acid} \\
\text{Homogentisic acid} & \xrightarrow{-\text{CO}_2} \text{CH}_2\text{COOH}
\end{align*}
\]
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\[
\text{HOOC—CH═CH—CO—CH}_2—\text{CO—CH}_2—\text{COOHH}
\]
\[
\text{Fumarylacetoacetic acid}
\]
\[
\rightarrow \text{HOOC—CH—CH—COOH + CH}_3\text{CO—CH}_2—\text{COOH}
\]
\[
\text{Fumaric acid Acetoacetic acid}
\]

The proposed oxidation scheme is obviously incomplete. It remains for further work to fill in the missing steps.

SUMMARY

1. An extract of rat liver acetone powder contains an active enzyme system which, when properly fortified, rapidly oxidizes tyrosine, \( p \)-hydroxyphenylpyruvic acid, and homogentisic acid.

2. Conditions suitable for studying tyrosine oxidation by this system and some preliminary results are given.

3. The oxidation of both \( L \)-tyrosine and \( p \)-hydroxyphenylpyruvic acid requires the uptake of 4 atoms of oxygen per molecule of substrate; homogentisic acid requires the uptake of 2 atoms of oxygen.

4. Evidence is presented which supports the theory that a transamination step is present in tyrosine oxidation.

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