METABOLISM OF L-ASCORBIC ACID IN RAT KIDNEY

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Previous studies have shown that L-ascorbic acid is extensively oxidized to respiratory CO₂ in rats (1, 2) and guinea pigs (3, 4). However, little information is available on the intermediates involved in the metabolism of the vitamin. In the present study experiments carried out with carboxyl- and uniformly labeled L-ascorbic acid point to the presence in rat kidney of an active enzyme system for the decarboxylation of L-ascorbic acid.

EXPERIMENTAL

Compounds—L-Ascorbic acid tracers, labeled with C¹⁴ specifically in carbon 1, carbon 6, and uniformly in all 6 carbon atoms, with specific activities of 0.12, 0.10, and 0.12 μc. per mg., respectively, were synthesized by published methods (7, 8). The preparation of dehydro-L-ascorbic acid-¹⁴C and 2,3-diketo-L-gulonic acid-¹⁴C has been described (2). Published methods were used for the synthesis of L-gulonolactone-¹⁴C (9) and uniformly labeled 2-keto-L-gulonic acid (10); their specific activities were 0.20 and 0.12 μc. per mg., respectively. D-Glucuronolactone-6-¹⁴C (0.40 μc. per mg.) was obtained from the National Bureau of Standards, Washington, D. C. L-Xylosone was synthesized as described previously (7).

Incubation Procedure—Homogenates of various tissues² were prepared at 5° with use of a Potter-Elvehjem glass homogenizer. Labeled L-ascorbic acid (0.35 to 0.45 mg.) was added to 5 ml. of a 20 per cent tissue homogenate in Krebs-Ringer phosphate buffer (pH 7.0) and the system was shaken for 1 hour at 37° under air. At the end of incubation, 5 ml. of 10 per cent trichloroacetic acid were added and the precipitate was removed by centrifugation. The amounts of C¹⁴ present in the supernatant fluid as L-ascorbic acid, dehydroascorbic acid, diketogulonic acid, and oxalic acid were determined by carrier dilution procedures (3, 4). Other experiments were carried out under the same conditions, except

¹ A preliminary report of this work has been presented (5, 6).
² Male rats of the Wistar strain, weighing 250 to 300 gm., were used.
that the tissue homogenates were incubated in a sealed vessel with a center well containing 1 ml. of 30 per cent KOH. At the end of incubation, 1 ml. of 30 per cent trichloroacetic acid was added to the outside compartment and the evolved C\textsuperscript{14}O\textsubscript{2} was trapped in the KOH solution while the vessel was being shaken for an additional hour. The C\textsuperscript{14}O\textsubscript{2} was collected as BaCO\textsubscript{3} and plated for counting (4). The various fractions of rat kidney were prepared in isotonic sucrose by the method of Schneider and Hogeboom (11), and were incubated with labeled L-ascorbic acid under the same conditions as employed for homogenates.

**Analytical Methods**—L-Xylose and L-xylulose\textsuperscript{8} were determined by the orcinol (12) and the cysteine-carbazole (13) methods, respectively. L-Xylosone was estimated by titration with indophenol dye after its conversion to imino-L-ascorbic acid (7).

**Results**

**Decarboxylation of L-Ascorbic Acid**—L-Ascorbic acid-1-C\textsuperscript{14} was incubated for 1 hour with homogenates of various rat tissues, and the amounts of C\textsuperscript{14} recovered as CO\textsubscript{2}, L-ascorbic acid, dehydroascorbic acid, and diketogulonic acid were measured (Table I). Marked differences exist in the metabolism of L-ascorbic acid in these tissues, kidney homogenates being the most active for oxidizing the carboxyl carbon of the vitamin to CO\textsubscript{2}. The data also show that essentially all of the labeled L-ascorbic acid, added to kidney homogenates, disappeared during incubation. Considerable formation of labeled dehydroascorbic acid and diketogulonic acid occurred in kidney, liver, and small intestine homogenates. In the case of the kidney homogenate about 2.0 per cent of the added C\textsuperscript{14} was recovered as oxalate, a compound which is formed from L-ascorbic acid in vivo (2, 3).

L-Ascorbic acid-1-C\textsuperscript{14} was incubated in kidney and liver homogenates of guinea pigs (Table II). The amounts of C\textsuperscript{14}O\textsubscript{2} formed in guinea pig kidney homogenates averaged only 6.0 per cent compared to an average of 56 per cent obtained in rat homogenates under the same conditions. Less radioactive CO\textsubscript{2} was also formed in homogenates of guinea pig liver than in those of rat liver. Two experiments with rabbit kidney homogenates, carried out under the same conditions, gave values of 5.0 and 6.1 per cent, respectively, for the amount of C\textsuperscript{14}O\textsubscript{2} recovered after incubation of the carboxyl-labeled vitamin.

In order to determine whether carbons 2 to 6 of L-ascorbic acid also contributed to the formation of CO\textsubscript{2} in the rat kidney homogenate, comparative experiments were carried out with uniformly and carboxyl-labeled L-ascorbic acid (Table III). If C\textsuperscript{14}O\textsubscript{2} originated only from the carboxyl carbon, it would be expected that the amount of radioactive

\textsuperscript{8} The authors are grateful to Dr. Gilbert Ashwell for a supply of L-xylulose.
CO₂ formed after incubation of uniformly labeled L-ascorbic acid would be about one-sixth that formed with the carboxyl-labeled vitamin. The results in Table III show that this was the case, indicating that L-ascorbic acid was decarboxylated in the rat kidney homogenate. L-Ascorbic acid-6-C¹⁴ was also incubated under the same conditions in the rat kidney homogenate system and less than 1.0 per cent of the added C¹⁴ was recovered as CO₂.

Properties of Rat Kidney Homogenate System—In Table IV are summarized some of the properties of the rat kidney homogenate system in respect to decarboxylation of L-ascorbic acid. The activity in the homogenate, with L-ascorbic acid-1-C¹⁴ as the substrate, fell from 59 per cent to 2.1 per cent when the incubation vessel was preheated for 7 minutes in a boiling water bath. The activity was not lost upon dialysis for 24 hours at 5° against Krebs-Ringer phosphate buffer (pH 7.0). No ap-

### Table I

**Metabolism of L-Ascorbic Acid-1-C¹⁴ in Rat Tissue Homogenates**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Per cent added C¹⁴ found at end of incubation as</th>
<th>CO₂</th>
<th>Dehydroascorbic acid + diketogulonic acid</th>
<th>L-Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>64</td>
<td>17</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>53</td>
<td>18</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>50</td>
<td>25</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>30</td>
<td>52</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>33</td>
<td>39</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>4.9</td>
<td>21</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>2.5</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table II

**Metabolism of L-Ascorbic Acid-1-C¹⁴ in Guinea Pig Tissue Homogenates**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Per cent added C¹⁴ found at end of incubation as</th>
<th>CO₂</th>
<th>Dehydroascorbic acid + diketogulonic acid</th>
<th>L-Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>8.4</td>
<td>38</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.5</td>
<td>18</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>6.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>7.1</td>
<td>32</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.9</td>
<td>31</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>
preciable $^{14}O_2$ was formed when the reaction was carried out anaerobi-
cally or when an acetone powder preparation was used. The decarboxyla-
tion activity was inhibited about 70 per cent by 8-hydroxyquinoline in a
concentration of $10^{-4}$ M.

The decarboxylation of various structurally related compounds was
compared with that of L-ascorbic acid in the rat kidney homogenate system
(Table V). It will be noted that these compounds were considerably
less reactive in this system.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Labeled sites</th>
<th>Per cent substrate as $^{14}O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carboxyl</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Uniform</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>Carboxyl</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Uniform</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**TABLE IV**

Decarboxylation of L-Ascorbic Acid-1-$^{14}$ by Rat Kidney Homogenate

<table>
<thead>
<tr>
<th></th>
<th>Per cent substrate as $^{14}O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>59</td>
</tr>
<tr>
<td>Buffer control</td>
<td>1.0</td>
</tr>
<tr>
<td>Boiled homogenate</td>
<td>2.1</td>
</tr>
<tr>
<td>Dialyzed homogenate</td>
<td>63</td>
</tr>
<tr>
<td>8-Hydroxyquinoline (10$^{-4}$ M)</td>
<td>18</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>4.1</td>
</tr>
<tr>
<td>Acetone powder</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Studies were carried out to localize the decarboxylation activity within
the rat kidney cell by comparing the amounts of $^{14}O_2$ formed upon incuba-
tion of L-ascorbic acid-1-$^{14}$ with the homogenate, mitochondria, micro-
somes, and the supernatant fraction (100,000 X g for 1 hour) (Table VI).
The microsomes and mitochondria had little decarboxylation activity,
whereas the supernatant fraction possessed about one-half the activity of
the homogenate. However, when the supernatant fraction was combined
with either the microsomes or the mitochondria, essentially all of the
original activity of the homogenate was restored. These results suggest
that factors present, both in the soluble fraction and in the microsomes
and the mitochondria, are required for optimal decarboxylation of L-as-
corbic acid by the rat kidney homogenate.
Studies with Dehydroascorbic Acid-$1^\text{C}^\text{14}$ and Diketogulonic Acid-$1^\text{C}^\text{14}$—The possible role of dehydroascorbic acid and diketogulonic acid as intermediates in the decarboxylation of L-ascorbic acid by the rat kidney homogenate was investigated. These compounds, labeled in their carboxyl carbon, were incubated with the rat kidney homogenate system and the amounts of radioactive CO$_2$ formed at different times were determined.

**Table V**

Decarboxylation of Various Compounds by Rat Kidney Homogenates

<table>
<thead>
<tr>
<th>Compound</th>
<th>Per cent substrate as CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Ascorbic acid-$1^\text{C}^\text{14}$</td>
<td>50</td>
</tr>
<tr>
<td>L-Gulonolactone-$1^\text{C}^\text{14}$</td>
<td>3.0</td>
</tr>
<tr>
<td>2-Keto-L-gulonic acid-$1^\text{C}^\text{14}$</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>D-Glucuronolactone-$6^\text{C}^\text{14}$</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

**Table VI**

Capacity of Various Kidney Fractions to Decarboxylate L-Ascorbic Acid-$1^\text{C}^\text{14}$

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Per cent substrate as CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>46</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>5.6</td>
</tr>
<tr>
<td>Microsomes</td>
<td>1.3</td>
</tr>
<tr>
<td>Supernatant fraction</td>
<td></td>
</tr>
<tr>
<td>&quot;    &quot; + mitochondria</td>
<td>20</td>
</tr>
<tr>
<td>&quot;    &quot; + microsomes</td>
<td>42</td>
</tr>
<tr>
<td>&quot;    &quot; + mitochondria + microsomes</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

* An amount of each fraction was used corresponding to that present in 1 gm. of wet weight of kidney.

(Fig. 1). The initial rate of decarboxylation of diketogulonic acid was greater than for dehydroascorbic acid, which was, in turn, considerably greater than for L-ascorbic acid, although the final values were about the same for each compound. For L-ascorbic acid, a 10 minute lag period was observed before any appreciable decarboxylation occurred, suggesting that L-ascorbic acid had to be converted to dehydroascorbic acid before its decarboxylation in this system.

The conversion of L-ascorbic acid to dehydroascorbic acid in rat kidney homogenates was apparently catalyzed by factors present in the microsomes and mitochondria. For instance, when L-ascorbic acid-$1^\text{C}^\text{14}$
was incubated for 30 minutes with either microsomes or mitochondria, about 35 per cent conversion of the substrate to labeled dehydroascorbic acid occurred. The ion exchange technique used in these experiments made it possible to distinguish labeled dehydroascorbic acid from labeled L-ascorbic acid (4).

![Diagram showing the percentage of substrate converted to CO₂ over time, with lines for dehydroascorbic acid, L-ascorbic acid, diketo-L-gulonic acid, and dehydro-L-ascorbic acid labeled.]

Fig. 1. Decarboxylation of L-ascorbic acid-1-[^14]C, dehydroascorbic acid-1-[^14]C, and diketogulonic acid-1-[^14]C at various times in rat kidney homogenates.

**Table VII**

<table>
<thead>
<tr>
<th>Decarboxylation of Dehydro-L-Ascorbic Acid-1-[^14]C by Rat Kidney Supernatant Fraction</th>
<th>Per cent substrate as CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant fraction*</td>
<td>63</td>
</tr>
<tr>
<td>&quot; &quot; dialyzed</td>
<td>69</td>
</tr>
<tr>
<td>&quot; &quot; + 8-hydroxyquinoline (10^-4 M)</td>
<td>33</td>
</tr>
<tr>
<td>&quot; &quot; boiled</td>
<td>8.0</td>
</tr>
<tr>
<td>Buffer control</td>
<td>8.0</td>
</tr>
</tbody>
</table>

* When L-ascorbic acid-1-[^14]C was incubated with this fraction, 20 per cent decarboxylation occurred.

Experiments were carried out which showed that carboxyl-labeled dehydroascorbic acid was decarboxylated appreciably upon incubation with the supernatant fraction (Table VII). The extent of decarboxylation was about 3-fold greater than that observed with L-ascorbic acid under the same conditions. The results showed that no loss in decarboxylation activity occurred after dialysis, but a marked drop in activity was observed when the preparation was preheated in a boiling water bath for 7 minutes. The results also showed that the decarboxylation of dehydroascorbic acid was inhibited by 8-hydroxyquinoline.

Fate of Carbon Atoms 2 to 6 of L-Ascorbic Acid—In order to gain informa-
tion on the fate of carbon atoms 2 through 6 of L-ascorbic acid, the follow-
ing $^{14}C$ balance experiment was carried out: Uniformly labeled L-as-
corbic acid was incubated in the rat kidney homogenate system and the
amount of evolved $^{14}CO_2$ was measured. The trichloroacetic acid extract
of the incubation mixture was passed through an Amberlite IR-4B column
in the acetate form (4) and the adsorbed acidic material was eluted first
with 2 N formic acid and then with 1 N HCl. The results showed that 6.2
per cent of the added $^{14}C$ was recovered in $CO_2$, 5.0 per cent in the effluent,
50 per cent in the formic acid eluate, and 30 per cent in the HCl eluate.
Evidence that the small amount of $^{14}C$ in the effluent was present as de-
hydroascorbic acid was obtained as follows: The effluent was treated
with hydrogen sulfide under conditions for the quantitative reduction
of dehydroascorbic acid to L-ascorbic acid (4). The resulting solution
was again passed through the ion exchange column, when the effluent
had no detectable $^{14}C$. These results rule out the presence in the trichloro-
acetic acid extract of labeled neutral compounds such as L-xylose or L-xylu-
lose. Although considerable $^{14}C$ was present in the formic acid eluate,
less than one-fourth of the total radioactivity in this solution was recovered
as L-ascorbic acid by the carrier dilution technique (4). The remaining
labeled material, equivalent to about 38 per cent of the $^{14}C$ added to the
incubation flask, has not yet been identified. The major portion of the
$^{14}C$ in the HCl eluate can be accounted for as diketogulonic acid since
control experiments have shown that this compound was eluted from
the ion exchange column by HCl but not by formic acid.

Additional experiments were carried out to determine whether L-ascorbic
acid was converted to pentoses in the rat kidney homogenate system. The
results showed that no detectable L-xylose or xylulose was present in the
trichloroacetic acid extract of the incubation mixture. In other exper-
iments, in which diketogulonic acid was incubated with the rat kidney
homogenate system, no detectable L-xylosone was found in the trichloro-
acetic acid extract of the tissue. The possibility that these pentoses may
be formed during incubation, and then be further metabolized, was in-
vestigated as follows: L-xylose, L-xylulose, and L-xylosone in amounts
of 0.4 to 0.5 mg. were incubated in the rat kidney homogenate system under
the identical conditions used in the experiments with labeled L-ascorbic
acid; no significant disappearance of these compounds was observed.

DISCUSSION

The results of this study point to an active system in rat kidney for
the decarboxylation of L-ascorbic acid. The most likely intermediates
involved in this reaction are dehydroascorbic acid and diketogulonic

4 Preliminary experiments in which paper chromatography was employed show the presence of several labeled compounds in this fraction.
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acid. According to such a mechanism, L-ascorbic acid is first oxidized to dehydroascorbic acid by the microsomes and mitochondria. Dehydroascorbic acid is then hydrolyzed to diketogulonic acid, which in turn undergoes decarboxylation; these reactions are catalyzed by an enzyme system in the soluble fraction of the kidney. Curtin and King (2) have obtained evidence in vivo which is in agreement with such a pathway of L-ascorbic acid metabolism in the rat, by finding that carboxyl-labeled dehydroascorbic acid and diketogulonic acid were oxidized to respiratory C\textsuperscript{14}O\textsubscript{2} at a more rapid rate than carboxyl-labeled L-ascorbic acid.

From structural considerations, the most likely products of L-ascorbic acid decarboxylation would be L-xylose and L-xylulose. Recently, evidence has been presented for the formation of L-xylose in guinea pig liver slices (16). Other studies have shown that L-gulonic acid, a precursor of the vitamin in the rat (9), is decarboxylated by enzymes in rat kidney, forming L-xylulose (17). However, no evidence was obtained in the present study for the formation of either L-xylose or L-xylulose in the decarboxylation of L-ascorbic acid by rat kidney homogenates. On the other hand, results of experiments in which uniformly labeled L-ascorbic acid was employed showed appreciable formation of labeled acidic compounds, derived from carbon atoms 2 to 6 of the vitamin. Identification of these end products will be required for an understanding of the actual mechanisms involved in this decarboxylation reaction.

SUMMARY

The results of studies with carboxyl- and uniformly labeled L-ascorbic acid show that there is in rat kidney an active system which decarboxylates L-ascorbic acid. Fractionation studies show that factors present in both the soluble and particulate fractions are required for maximal activity. Evidence was presented suggesting that dehydroascorbic acid and diketogulonic acid are intermediates in the decarboxylation of the vitamin in rat kidney homogenates. Additional results were obtained which showed no detectable formation of L-xylose, L-xylulose, or L-xylosone during the reaction.

BIBLIOGRAPHY


\* A similar mechanism has been suggested for the metabolism of the vitamin in rat liver (14). In this previous study, the disappearance of L-ascorbic acid was measured by the method of Roe and Oesterling (15) but no evidence was given for a decarboxylation reaction.
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