Ascorbic Acid Synthesis from D-Glucose-2-C¹⁴ in the Liver of the Intact Rat

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Evidence that glucose¹ is the ultimate 6-carbon precursor of ascorbic acid in the rat was first obtained by Jackel, Mosbach, Burns, and King (1). They administered uniformly labeled glucose-C¹⁴ to Chloretone-treated rats and recovered uniformly labeled ascorbic acid from the urine. Subsequently, Horowitz, Doerschuk, and King (2) and Burns and Mosbach (3) discovered that when glucose-1-C¹⁴ was administered to Chloretone-treated or normal rats, the urinary ascorbic acid was labeled primarily in C-6. Experiments employing glucose-6-C¹⁴ in Chloretone-treated rats resulted in urinary ascorbic acid labeled primarily in C-1 (4). These tracer studies and the nonisotopic experiments of Isherwood, Chen, and Mapson (5) led to postulation of the following scheme of ascorbic acid biosynthesis in the rat:

\[
\text{D-Glucose} \rightarrow \text{D-glucuronolactone} \rightarrow \text{L-gulonolactone} \rightarrow \text{L-ascorbic acid}
\]

Both tracer and enzymic studies have established the fact that D-glucuronolactone and L-gulonolactone can be converted to ascorbic acid by the rat (5-7). Surveys of the enzymic conversion of D-glucuronolactone to L-ascorbic acid in various mammalian organs indicate that the liver is the most likely site of synthesis (8, 9). The stereochemical similarity between glucose and D-glucuronolactone has led to the tacit assumption that glucose-C¹⁴ is the ultimate precursor of ascorbic acid, although a direct conversion has never been demonstrated in vitro.

In plant tissues, two distinct pathways of ascorbic acid formation have been demonstrated with C¹⁴-labeled substrates. One, from glucose, yields ascorbic acid with the same carbon sequence; i.e. C-1 through C-6 of glucose becomes C-1 through C-6 of ascorbic acid (10, 11). The other, from D-glucuronolactone, yields ascorbic acid with an inverted carbon sequence, i.e. C-1 through C-6 of D-glucuronolactone is converted to C-6 through C-1 of ascorbic acid (11, 12).

The results obtained with plant tissues have prompted us to reinvestigate the glucose to ascorbic acid conversion process in the intact rat, but with certain differences in experimental procedure from previously published studies. First, the experiments reported herein have been performed on nondrugged animals. Glucose-2-C¹⁴ was used to avoid possible complications arising from the loss or redistribution of C¹⁴ from terminally labeled glucose. Ascorbic acid was recovered from the liver, the presumed site of synthesis. The period of metabolic labeling was limited to 3 hours between administration of glucose-2-C¹⁴ and the time the animal was killed. Comparative data on the metabolic fate of glucose and its isotope distribution were obtained by recovery and degradation of the glycogen as well as of the ascorbic acid from the same liver. With these technical modifications, our results confirm the previously published work of others (2-5), indicating that ascorbic acid is synthesized from D-glucose in the mammalian organism by way of reactions that lead to inversion of the hexose carbon chain.

EXPERIMENTAL

Wistar male white rats were used in the three experiments outlined in Table I. Comparative data were obtained from a fasted, a fasted exercised, and a fed animal. Experiment 59-7 was designed to study certain aspects of metabolism in exercising muscle as well as ascorbic acid synthesis. The rat in this experiment was forced to swim for 30 minutes before and 3 hours after isotope administration. In Experiment 59-5 and 59-7, food was withdrawn for the 24 hours preceding and throughout the period of isotope incorporation. Glucose-2-C¹⁴ (600 μc per mmole of carbon), 72 μc per ml of distilled water, was injected interperitoneally and the animals were killed by decapitation 3 hours after injection. Each liver was homogenized in 100 ml of ice-cold 5% trichloroacetic acid in a Waring Blender for 2 minutes at high speed followed by centrifugation. The residue was re-suspended in an additional 100 ml of cold 5% trichloroacetic acid, rehomogenized, and recentrifuged. Combined trichloroacetic acid extracts were treated with an equal volume of ethanol to precipitate the glycogen and allowed to stand several hours in the cold. Glycogen was recovered by centrifugation and purified by three additional precipitations from water with ethanol. One portion of the trichloroacetic acid supernatant was treated, as described below, for recovery of ascorbic acid. The other portion was withheld for unrelated studies of nucleotide pentose.

Glycogen—A portion of each liver glycogen sample was hydrolyzed by heating for 2 hours in N H₂SO₄ over a boiling water bath. Sulfate was removed by passing the hydrolysate through a short column of IR-4D (OH⁻) exchange resin. The effluent and washes were combined, assayed for total glucose (13), diluted

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¹ Glucose refers to D-glucose and ascorbic acid to L-ascorbic acid.
Ascorbic Acid Synthesis in Rat

Recovered 32.4 mg of glycogen as glucose. Assumed a 3-fold dilution of glycogen before a 5-fold dilution with glucose and recrystallization.

The crystalline glucose was wet combusted to obtain an estimate of the total activity and degraded by fermentation with syrup under reduced pressure, and crystallized from ethanol. The products of fermentation were separated and then degraded chemically to assay the activity of each carbon.

Isotope distribution in liver ascorbic acid of rats given glucose-2-\textsuperscript{14}C

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animal status</th>
<th>C\textsubscript{4}</th>
<th>C\textsubscript{5}</th>
<th>C\textsubscript{6}</th>
<th>C\textsubscript{7}</th>
<th>C\textsubscript{8}</th>
<th>C\textsubscript{9}</th>
</tr>
</thead>
<tbody>
<tr>
<td>59-5 Fasted 24 hr</td>
<td>300</td>
<td>43</td>
<td>0.08</td>
<td>0.10</td>
<td>180</td>
<td>212</td>
<td>0.014</td>
</tr>
<tr>
<td>59-7 Fasted 24 hr, exercised</td>
<td>190</td>
<td>113</td>
<td>0.06</td>
<td>0.004</td>
<td>290</td>
<td>361</td>
<td>0.002</td>
</tr>
<tr>
<td>59-10 Fed</td>
<td>250</td>
<td>36.5</td>
<td>0.40</td>
<td>2.00</td>
<td>440</td>
<td>2</td>
<td>0.020</td>
</tr>
</tbody>
</table>

* Assumed 0.2 mg of ascorbic acid per gram of liver (wet weight)

† Twenty milligrams of carrier glycogen added during recovery.

**RESULTS**

Data summarizing that portion of \textsuperscript{14}C\textsubscript{4} incorporated into ascorbic acid and glycogen are presented in Table I. Fasting reduced the molar activity of the ascorbic acid to less than one-half the value obtained in the fed rat. Exercise reduced it still further and also markedly reduced the amount of \textsuperscript{14}C\textsubscript{4} converted to ascorbic acid. The percentage conversion of \textsuperscript{14}C to ascorbic acid in Experiments 59-5 and 59-10 was of the same order of magnitude as values obtained for the urinary ascorbic acid of normal rats administered glucose-1-\textsuperscript{14}C (3, 19).

Much more glycogen was recovered from the liver of the fed rat than from that of the fasted rats. The extremely low molar activity of the fed rat liver glycogen can be ascribed to endogynthesis.

Isotope distribution in liver glycogen-derived glucose of rats given glucose-2-\textsuperscript{14}C

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animal status</th>
<th>Relative activity in liver glycogen-derived glucose carbon atoms*</th>
</tr>
</thead>
<tbody>
<tr>
<td>59-5 Fasted</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td>59-7 Fasted, exercised</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>59-10 Fed</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

* The specific activity of C-5, which is assigned an arbitrary value of 100, is indicated by the figure in parenthesis (c.p.m. per mmole of carbon, after dilution with carrier).
† Obtained by difference, C-(1+2) less C-1.
‡ Obtained by difference, C-(4+5) less C-5.
aronous dilution. The even lower activity in the fasted liver glycogen is attributable to the experimental technique employed. Since the fasted rats were not given a load of a nonisotopic glycogen precursor along with the radioactive glucose (administered in milligram quantity), very little glycogen was synthesized during the period of isotope incorporation.

Distribution of C\(^4\) among the carbons of ascorbic acid and glycogen-derived glucose is summarized in Tables II and III. For purposes of comparison, the carbon atom containing the most C\(^4\) has been assigned an arbitrary value of 100. For glycogen this carbon was C-2, for ascorbic acid, C-5. The C\(^4\) content of the other carbon atoms has been compared to C-2 in glycogen and to C-6 in ascorbic acid. In the fed rat, the greatest amount of C\(^4\) redistributed from C-2 into other carbons of glycogen-derived glucose appeared in C-1 with lesser amounts in C-3 and C-5. In the ascorbic acid, the greatest redistribution from C-2 was into C-6 and to a lesser extent, into C-4 and C-2. In the fasted rats, the C\(^4\) pattern in the glycogen did not differ appreciably although there was detectably less C\(^4\) in C-1. The ascorbic acid patterns in the fasted rats showed an appreciable amount of redistribution of C\(^4\) into C-1 and C-2 as well as the carbons indicated for the fed animal. The present experiments provide no ready explanation for the altered patterns in the carbon atoms indicated for the fed animal. The present experiments described herein, in which ascorbic acid was recovered from normal and from Chloretone-treated rats administered glucose-1-C\(^4\) as evidence that the intact carbon chain of glucose is utilized in the conversion process, and that C-6 of glucose is oxidized to form the carboxyl carbon of ascorbic acid. The results of the experiments described herein, in which ascorbic acid was recovered from the livers of rats 3 hours after the administration of glucose-2-C\(^4\) are in accord with the earlier conclusions. When the rat is fasted before administration of the isotope, more C\(^4\) appears in C-1 and C-2 of ascorbic acid than in the corresponding carbon atoms of liver glycogen suggesting that processes which are able to utilize glucose fragments of less than 6 carbon atoms are also contributing to ascorbic acid synthesis.

**SUMMARY**

The synthesis of ascorbic acid in the livers of intact fed and fasted rats has been investigated with \(d\)-glucose-2-C\(^4\) as a tracer. The ascorbic acid and glycogen were recovered from each liver and degraded chemically to determine the isotope distribution within each carbon chain. Ascorbic acid had its greatest C\(^4\) concentration in C-5 whereas glycogen had its greatest C\(^4\) concentration in C-2 in the three experiments described. Fasting resulted in an increased amount of redistribution of the C\(^4\) in the ascorbic acid. The results are in accord with previous observations of other workers indicating that C-6 of glucose is oxidized to form the carboxyl carbon of ascorbic acid.

**REFERENCES**

Ascorbic Acid Synthesis from d-Glucose-2-C\textsuperscript{14} in the Liver of the Intact Rat
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