STUDIES OF RIBOSE METABOLISM

II. A METHOD FOR THE STUDY OF RIBOSE SYNTHESIS IN VIVO*

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Previous studies of ribose synthesis *in vivo* have required the isolation of this sugar from nucleic acids (1-4) or from pentose-containing coenzymes (5). The demonstration by Tabor and his associates that the administration of histamine or imidazoleacetic acid (IAA) to rats results in the urinary excretion of IAA riboside (6) provides a new and considerably less cumbersome method for the investigation of ribose synthesis *in vivo*. In addition, this technique permits the isolation of ribose from the intact animal, thereby making it possible to study the effects of a variety of experimental conditions on pentose synthesis in the same animal. This communication is designed to report the details of the isolation procedure. In addition, data will be presented which suggest that the pentose components of both urinary imidazoleacetic acid riboside and visceral ribonucleic acids (RNA) are derived from a common pool.

Methods

Two 250 gm. male white Wistar rats were each injected intraperitoneally three times, at 2 hourly intervals, with 4 ml. of 0.9 per cent NaCl solution containing 3.3 µe. of glucose-2-C¹⁴ (approximately 2 mg.), 83 µmoles of imidazoleacetic acid hydrochloride, and 166 µmoles of NaHCO₃. Urine was collected during the period of the injections and for the next 16 hours. The animals were permitted Purina chow *ad libitum* until the first injection, but were offered only water during the period of urine collection. In order to compare the isotope patterns in the ribose moieties of both nucleic acid and IAA riboside, the rats were killed by decapitation at the close of the urine collection. The abdominal viscera were removed immediately and plunged into 100 ml. of ice-cold 10 per cent trichloroacetic acid. The mixture was homogenized for 1 minute in a Waring blender, and RNA ribose was isolated from duplicate aliquots of the homogenate by procedures summarized previously (4).

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IAA riboside was isolated from the pooled urine by a modification of the procedure of Tabor and Hayashi (7). The urine and washes (volume 100 ml.; 1.51 × 10⁶ c.p.m.) were acidified to pH 3.0 with 1 N acetic acid. After aeration to remove CO₂ and adjustment of the pH to 8.0 with 1.0 N NaOH, the urine was adsorbed on Dowex 1, 100 to 200 mesh, 2 per cent cross-linked, acetate form (height 12 cm., diameter 3.2 cm.). The column was washed with water and the IAA riboside eluted with 0.2 N acetic acid. The eluate was collected in 20 ml. fractions. Fractions 6 to 8 contained the riboside (37,000 c.p.m.), which was identified by the presence of radioactivity and of orcinol-reacting material (8) after, but not before, incubation with a Lactobacillus delbruckii riboside hydrolase (7). These fractions were combined and lyophilized to dryness. The residue was dissolved in 5.0 ml. of 0.05 M citrate buffer, pH 6.0, and incubated for 3 hours at 34° with the ammonium sulfate fraction of the riboside hydrolase (20 mg. of protein) used by Tabor and Hayashi (7). After hydrolysis of the riboside, the solution was deproteinized with 0.4 ml. of 70 per cent perchloric acid. The mixture was centrifuged, and the supernatant solution was brought to pH 7.0 (brom thymol blue) with 1.0 ml. of 5.0 N KOH, and then cooled to 0° for 15 minutes. The insoluble potassium perchlorate was removed by centrifugation, and the supernatant solution was deionized by passage through a mixed bed resin (MB-3, Fisher Scientific Company; height 18 cm., diameter 1.0 cm.). The effluent solution was lyophilized to dryness and the residue taken up in 0.2 ml. of water. The sugar was further purified by descending chromatography on Whatman No. 1 paper in butanol-acetic acid-water (4:1:5) (9). The strip containing ribose was located by streaking its margin with aniline phthalate reagent (10), and was eluted with water. 250 μmoles of non-isotopic d-ribose were added as carrier to duplicate aliquots of the eluate, each containing 40 μmoles of ribose and 7000 c.p.m. These samples were then degraded by techniques which were summarized in a previous paper (4), and which permit the isolation of each of the carbon atoms of the ribose molecule as BaCO₃.

The BaCO₃ samples were counted at infinite thickness and all other radioactive material at infinite thinness. A counter with a "micromil" end window and a counting efficiency of 20 per cent was used.

Materials—Imidazoleacetic acid hydrochloride was prepared by the method of Bauer and Tabor (11). Glucose-2-C¹⁴ was obtained from the National Bureau of Standards through the courtesy of Dr. H. S. Isbell. A partial purification of L. delbruckii riboside hydrolase was carried out according to the procedure of Takagi and Horecker (12). (Hydrolysis of β-ribosidic linkages by extracts of this organism was first described by Kalckar (13).)
Results

Of the total of 500 μmoles of IAA injected into two rats, 131 μmoles were recovered from the urine as the riboside. The pentose moiety of the IAA riboside contained 0.31 per cent of the radioactivity administered as glucose-2-C^14, as compared with 0.16 per cent of the injected isotope present in 400 μmoles of isolated visceral RNA ribose.

Isotope distribution was strikingly similar in ribose isolated from IAA riboside on the one hand, and from visceral RNA on the other, in each of two separate experiments (Table I). The major portion of radioactivity was in the first 2 carbon atoms of the ribose molecule, with more isotope in position 2 than in position 1. Carbon 4 was more radioactive than carbon atom 3 or 5.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Ribose source</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urinary imidazoleacetic acid riboside</td>
<td>24</td>
<td>44</td>
<td>6</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Visceral nucleic acids</td>
<td>32</td>
<td>41</td>
<td>6</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Urinary imidazoleacetic acid riboside</td>
<td>31</td>
<td>30</td>
<td>7</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Visceral nucleic acids</td>
<td>24</td>
<td>34</td>
<td>12</td>
<td>23</td>
<td>7</td>
</tr>
</tbody>
</table>

DISCUSSION

Tabor and Hayaishi's observation that rats excrete a riboside after the administration of imidazoleacetic acid or of histamine provides a new technique for the investigation of ribose metabolism. This method is simpler than previously employed measures for ribose isolation and obviates the need for killing or mutilating the animal. In addition, considerably more radioactivity had been incorporated into ribose obtained in this fashion than had entered the isolated nucleic acids. For example, in a typical experiment, 0.31 per cent of the administered C^14 was present in 131 μmoles of ribose derived from the IAA riboside, and 0.14 per cent of the administered radioactivity was found in 400 μmoles of visceral RNA ribose, a difference in specific activity of the ribose components of almost 7-fold. In our studies the urinary imidazoleacetic acid riboside accounted for 33 per cent of the administered IAA. Tabor has reported that, with

IAA riboside was also obtained from the urine of rats given glucose-2-C^14 and histamine (7, 14). The pattern of radioactivity in the pentose was similar to that observed in ribose excreted by rats given IAA and glucose-2-C^14.
sufficiently small doses of IAA, as much as 80 per cent of the administered compound may be recovered in the urine as the riboside (15).

At the present time this method is being employed to determine the effects of a variety of experimental conditions on the mechanism of ribose synthesis in the rat. Toxicity studies which are as yet incomplete suggest that it may be feasible to administer IAA to man, thereby permitting an investigation of ribose synthesis in human subjects.

The isotope distribution in the isolated ribose is consistent with synthesis via both oxidative and non-oxidative steps of the pentose phosphate pathway (16). Ribose synthesized via the oxidative loss of carbon 1 of glucose-2-$\text{C}^{14}$ would be labeled in its first position:

\[
\begin{array}{c}
C \\
\downarrow \\
C^* \\
\downarrow \\
C \\
\downarrow \\
C \\
\downarrow \\
C-P \\
\end{array}
\quad \rightarrow \quad
\begin{array}{c}
\text{CO}_2 \\
\downarrow \\
C \\
\downarrow \\
C \\
\downarrow \\
C-P \\
\end{array}
\]

Glucose 6-P \quad \text{Pentose-P}

\text{REACTION 1}

On the other hand, that produced by a non-oxidative transfer of the first 2 carbon atoms of hexose phosphate to a triose phosphate acceptor, a reaction catalyzed by the enzyme transketolase (17, 18), would be labeled in carbon atom 2:

\[
\begin{array}{c}
C \\
\downarrow \\
C^* \\
\downarrow \\
C \\
\downarrow \\
C \\
\downarrow \\
C \\
\downarrow \\
C \\
\downarrow \\
C-P \\
\end{array}
\quad \rightarrow \quad
\begin{array}{c}
\text{CO}_2 \\
\downarrow \\
C \\
\downarrow \\
C \\
\downarrow \\
C-P \\
\end{array}
\]

Fructose 6-P \quad \text{Triose P} \quad \text{Tetrose P} \quad \text{Pentose P}

\text{REACTION 2}
In the absence of more information concerning the possible effect of exchange reactions and of ribose to hexose to ribose recycling on isotope distribution, quantitative conclusions concerning the relative importance of the oxidative and non-oxidative reactions in ribose synthesis must be considered tentative. The appearance of more isotope in carbon 2 than in position 1 of ribose, however, is consistent with the predominance of the non-oxidative reactions under the conditions of our study. The substantial radioactivity in carbon 4 of ribose suggests that triose phosphate is labeled in carbon 2 as a result of aldolase cleavage of hexose diphosphate-2-C\textsuperscript{14}, followed by isomerization of the trioses. The subsequent participation of 2-labeled triose as the acceptor in the transketolase reaction (Reaction 2) would result in radioactivity in carbon atom 4 of ribose.

The similarity of isotope distribution in the ribose from IAA riboside with that in nucleic acid ribose suggests strongly that a common pool of pentose is used in the synthesis of both compounds. No ready explanation is apparent for the discrepancy between our observations and those of Marks and Feigelson (19). These workers found more than 70 per cent of the total radioactivity in carbon 2 and none in carbon 4 of nucleic acid ribose isolated from the livers of fasted rats 6 hours after the administration of glucose-2-C\textsuperscript{14}. The isotope pattern in the ribose of our animals, however, is almost identical with that observed in nucleic acid ribose isolated from a human carcinoma cell grown in tissue culture in the presence of glucose-2-C\textsuperscript{14} (4).

**SUMMARY**

A method is described for the isolation of ribose from the urine of rats given imidazoleacetic acid. The relative ease and other advantages of this procedure as compared with previously employed techniques for the investigation of ribose synthesis in vivo are emphasized. Isotope distribution in urinary ribose excreted by rats given glucose-2-C\textsuperscript{14} and imidazoleacetic acid (IAA) is similar to that found in visceral nucleic acid ribose. The pattern of radioactivity is consistent with ribose synthesis via the pentose phosphate pathway.

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