Studies on the Glucuronic Acid Pathway of Glucose Metabolism

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Studies on the formation of L-ascorbic acid and L-xylulose in animals have led to the postulation (1, 2) of a cyclic pathway of glucose metabolism by way of L-glucuronic acid, L-gulonic acid, L-xylulose, D-xylulose, and the pentose phosphate sequence (Fig. 1). Preliminary experiments from this laboratory (3) designed to test this hypothesis have shown that labeled L-xylulose, L-xylulose, and the pentose phosphate sequence glucose metabolism by way of L-glucuronic acid, L-gulonic acid, gulonolactone is readily converted to liver glycogen and that the distribution of label in the glucose chain is consistent with the operation of this pathway. In the present paper, further evidence is adduced in support of this hypothesis, both from more detailed studies of the metabolism of L-gulonolactone and from the analogous behavior of L-glucuronolactone.1

EXPERIMENTAL

Wistar strain rats weighing 240 to 300 gm., and guinea pigs weighing 300 to 400 gm. were fasted for 24 hours and were then given by stomach tube 600 mg. of glucose per 100 gm. of body weight in 50 per cent aqueous solution immediately before intraperitoneal injection of labeled L-glucuronolactone or L-gulonolactone.

In experiments in which both C12 and C14 were used, the singly labeled substrates were combined, and carrier substrate was added to make a total dose of 25 mg. The average Cl3 abundance in the dose administered varied in different animals from 2.44 to 5.08 atom per cent excess. The total Cl4 given to each animal was of the order of 1.5 µc.

In the measurement of over-all catabolism of L-gulonolactone to expired CO2, fed rats and guinea pigs were used. The animals were placed in a metabolism chamber which allowed for the collection of respiratory CO2 and the urine during the 24 hours after administration of the compound. Labeled L-gulonic acid was isolated from urine by a carrier dilution procedure (5).

Labeled Compounds—Uniformly C14-labeled L-glucuronolactone (2.6 µc. per mg.) and L-gulonolactone-1-C13 (36.54 atom per cent excess C13 in C-1, 6.09 atom per cent excess C13 average for the molecule) were generously supplied by Dr. Neal Arts of the Corn Products Refining Company, Argo, Illinois. L-gulonolactone-6-C14 (1.0 µc. per mg.) was obtained from the National Bureau of Standards. Uniformly C14-labeled L-gulonolactone, L-gulonolactone-6-C14, and L-gulonolactone-1-C13 were prepared from the labeled D-glucuronic acids, respectively, by reduction with sodium borohydride (6).

Isotopic Assay: C14—The amount of C14 in expired CO2, urine,

1 A preliminary report of this work has been presented (4).

and glycogen was measured by methods used previously (6). As shown in Fig. 1, all glycogen fragments were ultimately converted to CO2 which was collected as BaCO3. A weighed amount was then acidified, and the liberated CO2 was absorbed in "Hyamine" base in methanol (7) and counted in a Packard TriCarb liquid scintillation spectrometer. Count rates ranged from about twice to 100 times background (23 c.p.m.).

Cl3—All glycogen fragments were assayed for the ratio (R) of C1302 (mass 45) to C1202 (mass 44) with a Consolidated-Nier model 21-401 mass spectrometer. Atom per cent Cl3 was calculated from the expression 100R/(1 + R). The minimal detectable enrichment of Cl3 is 0.02 atom per cent excess over the average normal abundance of 1.175 atom per cent, determined on commercial BaCO3. All C-6 and C-4-6 fragments contained the minimal detectable abundance of Cl3. All other fragments measured were enriched significantly.

Degradation of Glycogen—Glycogen was isolated (8) and hydrolyzed to glucose which was then degraded by chemical and bacterial procedures designed to yield single carbon fragments representing carbons 1, 3, and 6 of the glucose chain, as indicated in Fig. 2.

The possibility that C-1 of glycogen might contain more label than C-3 raised a question concerning the specificity of the chemical method employed in the liberation of C-3. To test this method, methyl glucoside-1-C14 and -2-C14 were oxidized with lead tetraacetate in separate experiments. CO2 derived from both labeled methyl glucosides contained not more than 1.5 per cent of the Cl4 present initially in each compound, an indication that the method is essentially specific for C-3.

A check on the isotopic purity of the methyl glucoside and potassium gluconate prepared from the glycogen hydrolysate derives from the agreement between their C13 contents, which did not vary more than 5 per cent when corrected for difference in carbon content.

RESULTS

Oxidation to CO2—Evidence for the extensive oxidation of L-gulonolactone in vivo appears in Table I. Parallel experiments with L-gulonolactone-1-C14 indicated that the major portion of the C14 in urine was present as L-gulonic acid. In previous studies it was shown that the carboxyl group of L-gulonolactone is converted to expired CO2 (6). The oxidation of L-glucuronolactone to CO2 has been demonstrated before (16).

Conversion to Liver Glycogen—Since the per cent of conversion
to glycogen is a function of the yield, it is important, when comparing the incorporation of different carbon atoms of a given substrate into glycogen, to eliminate any uncertainty arising from variations in glycogen recovery. For this reason, C\textsuperscript{3}\textsuperscript{18} and C\textsuperscript{3}\textsuperscript{14}-labeled species of each precursor were combined so that the incorporation of their terminal carbon atoms could be observed simultaneously.

The C\textsuperscript{3}\textsuperscript{13} to C\textsuperscript{3}\textsuperscript{14} ratios obtained in Experiments R358, R360, and R366 (Table II) indicate that C-6 of L-gulonolactone was from 18 to 35 times a better precursor of liver glycogen in rats than was C-1 (carboxyl carbon). In Experiments R369 and R370 the incorporation of isotope into glycogen was somewhat higher from C-6 than from the average of all 6 carbon atoms. Table IV shows that the same differential conversion of the terminal carbon atoms of L-gulonolactone to glycogen occurred in the guinea pig. When the study was carried out with D-glucuronolactone it was found, as seen in Table V, that again the incorporation of the terminal carbon atoms of this compound was unequal; C-1 was a better precursor by 25- to 40-fold than was C-6 (carboxyl carbon). From an inspection of Tables II through V it can be seen that the noncarboxyl terminal carbon atom of both precursors was incorporated to the extent of 19 to 35 per cent.

Isotope Distribution in Glycogen—Degradation of glucose derived from liver glycogen revealed that the glucose chain was unsymmetrically labeled after the administration of C-6-labeled L-gulonolactone or C-1-labeled D-glucuronolactone. From the C-1 to C-6 ratios in Table II it is seen that in rats C-1 of glucose was from 18 to 32 times more heavily labeled than C-6. In guinea pigs under the same conditions the asymmetry was 6- to 10-fold (Table IV). With D-glucuronolactone in rats the C-1 to C-6 ratio was 17 to 18 (Table V).

To gain further insight into the pathway, C-3 of glycogen was also assayed for label after the administration of L-gulonolactone-6-C\textsuperscript{13} to rats (Table III) and guinea pigs (Table IV). It is apparent that C-3 was labeled appreciably, containing from 13 to 21 per cent of the total C\textsuperscript{13} in glucose. Moreover, the C-3 to C-1 ratios were fairly constant, varying from 0.24 to 0.36.

DISCUSSION

The operation in vivo of an alternate pathway of glucose metabolism (Fig. 1) has been inferred from a study of the intermediary metabolism of D-glucuronolactone-1-C\textsuperscript{14}, -6-C\textsuperscript{14} as well as L-gulonolactone-6-C\textsuperscript{13}, -1-C\textsuperscript{13} as indicated by the extent and distribution of label in liver glycogen.

There are two possible modes of conversion of L-gulonolactone or D-glucuronolactone to liver glycogen: (a) incorporation of the intact B-carbon chain or (b) cleavage of the chain and subsequent incorporation of the fragments. The vast difference in the extent of incorporation of the terminal carbon atoms of the two precursors rules out their intact conversion.

It is then necessary to invoke a pathway involving fragmentation of the precursor molecules by which a substantial fraction (19 to 35 per cent) of the injected compound is incorporated into liver glycogen. According to the scheme in Fig. 1 it would be expected that the carboxyl carbon of L-gulonolactone and

**Liver glycogen**

\[ \text{H}^+ \]

\[ \text{Methanol} \quad \text{HCl} \quad (9) \]

\[ \text{KIO} \quad (11) \]

\[ \text{K gluconate} \]

\[ \text{Leuconostoc mesenteroides} \quad (13) \]

\[ \text{CO}_2(\text{C-1}) + \text{Ethanol} + \text{Zinc lactate} \quad (14) \]

\[ \text{CH}_3\text{COOAg} \quad \text{C}(\text{C-2,3}) \]

\[ \text{CO}_2(\text{C-4\textsuperscript{13} \textsuperscript{14}}) \]

\[ \text{Bz}_2 \quad (15) \]

\[ \text{CO}_2(\text{C-3}) \]

**Fig. 2. Degradative procedures for liver glycogen**
Excretion of C\textsuperscript{14} in expired CO\textsubscript{2} and urine during 24-hour period after administration of uniformly labeled L-gulonolactone to rats and guinea pigs*  

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animal type</th>
<th>Administered C\textsuperscript{14} in</th>
<th>CO\textsubscript{2}</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-311</td>
<td>Rat</td>
<td>%</td>
<td>46.7</td>
<td>43.3</td>
</tr>
<tr>
<td>R-312</td>
<td>Rat</td>
<td>46.7</td>
<td>40.0</td>
<td>29.4</td>
</tr>
<tr>
<td>R-323</td>
<td>Rat</td>
<td>39.4</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>G-332</td>
<td>Guinea pig</td>
<td>49.8</td>
<td>46.4</td>
<td></td>
</tr>
<tr>
<td>G-373</td>
<td>Guinea pig</td>
<td>37.0</td>
<td>43.6</td>
<td></td>
</tr>
</tbody>
</table>

* 6.0 mg. administered intraperitoneally.

Conversion of L-gulonolactone-1-C\textsuperscript{14}, 6-C\textsuperscript{14}, and of uniformly labeled L-gulonolactone-6-C\textsuperscript{14}, L-gulonolactone-6-C\textsuperscript{13} to liver glycogen in rats

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Labeled compound</th>
<th>Incorporation</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-338</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>28.5 1.55</td>
<td>18.4 48.9</td>
</tr>
<tr>
<td>R-360</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>24.1 0.81</td>
<td>29.8 61.3 34.6 18.1</td>
</tr>
<tr>
<td>R-366</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>20.1 0.10</td>
<td>54.8 69.3 2.8 21.2</td>
</tr>
<tr>
<td>R-369</td>
<td>-U-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>29.6 17.0</td>
<td>58.2 1.8 32.3</td>
</tr>
<tr>
<td>R-370</td>
<td>-U-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>32.4 20.7</td>
<td>56.3 2.2 25.6</td>
</tr>
</tbody>
</table>

* Chemical degradation.
† Uniformly labeled.

Conversion of L-gulonolactone-6-C\textsuperscript{13} to liver glycogen in rats

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Incorporation</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-372A</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>19.8 0.59</td>
</tr>
<tr>
<td>R-372B</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>26.0 0.93</td>
</tr>
<tr>
<td>R-372C</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>23.0 2.07</td>
</tr>
</tbody>
</table>

* Chemical degradation.
† Uniformly labeled.

Conversion of D-glucuronolactone-6-C\textsuperscript{14}, -1-C\textsuperscript{14} to liver glycogen in rats

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Incorporation</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-374A</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>18.7 0.46</td>
</tr>
<tr>
<td>R-374B</td>
<td>-1-C\textsuperscript{14}, -6-C\textsuperscript{13}</td>
<td>23.0 0.93</td>
</tr>
</tbody>
</table>

* Chemical degradation.

Since the appearance of our preliminary note (3) other laboratories have reported results in accord with this pathway in vivo. (a) McCormick and Touster (2) have found that xylitol-1-C\textsuperscript{14} (prepared chemically from D-xyllose-1-C\textsuperscript{14}) yields glucose, 1.3 C\textsuperscript{14} in the rat and guinea pig. (b) Posternak et al. (22, 23) have administered myo-inositol, variously labeled with deuterium, to phlorizinated rats and degraded the urinary glucose. Deuterium was found in positions 1 and 3 of glucose from inositol-6-D (22), in position 6 from inositol-2-D (23), and was absent in glucose from inositol-4-D (22). In addition, Anderson and Coots (24) have found C\textsuperscript{14}, after administration of inositol-2-C\textsuperscript{14} to rats, mainly in C-1 and C-6 of liver glycogen. These results were explained by the pathway in Fig. 1 in view of the observation that inositol is converted to D-glucuronic acid in vitro (25, 26) and D-glucuronic acid and L-galacturonic acid in vivo (27). (c) Hiatt (28) has demonstrated D-ribose biosynthesis from labeled D-glucuronolactone in a normal human subject. No conversion was observed, however, in a subject with essential pentosuria, presumably because of the inability of the pentosuric further to metabolize L-xylulose.

Additional evidence for the occurrence in vivo of the proposed pathway comes from the observation that various drugs markedly inhibit the conversion of L-xylulose, whence the low incorporation of C\textsuperscript{14} into glucose from both precursors. C\textsuperscript{14} initially in the noncarboxyl terminal would be expected to appear successively in C-5 of L-xylulose and C-1 of D-xylulose. From considerations of the pentose phosphate sequence (17, 18), the resulting C-1-labeled D-xylulose 5-phosphate (19) would yield glucose labeled in C-1 and C-3, with the major fraction of isotope in C-1. The scheme in Fig. 1 can thus account for the observations made in these experiments.²

² It is not known at present whether the lactone or acid form of each compound is the intermediate in this scheme.

² In discussing the stereochemistry of xylitol, McCormick and Touster (2) have designated its central carbon atom as a meso carbon, which is defined by Schwartz and Carter (20) as a carbon atom surrounded by two like and two unlike groups, C\textsubscript{a}b\textsubscript{b}d. Although xylitol is a meso compound its central carbon atom is not a meso atom, since it is surrounded by four unlike groups, C\textsubscript{a}(+a)(-a)b\textsubscript{b}d, the symmetry of the compound being derived from the compensation of +a by -a. The stereo differentiability of the terminal glycol moieties of xylitol resides in the configurational differences among C-2 and C-4 in contrast to the stereo inequality of like groups linked to a meso carbon atom, as observed in the acetic acid moieties bound to the central (meso) carbon of citric acid (21).
increase the rate at which glucose enters this metabolic scheme. For example, administration of barbital and Chloroform to rats leads to a marked increase in the conversion of glucose to \( \text{n-glucuronic acid} \) (29), \( \text{l-gulonic acid} \) (30), and \( \text{n-ascorbic acid} \) (31). This effect on \( \text{n-ascorbic acid} \) biosynthesis in the rat is shown by many drugs including various barbiturates, aminopyrine, and antipyrine (31). It is of considerable interest to note that in 1935 Enklewitz and Lasker (32) found that two of these drugs, aminopyrine and antipyrine, markedly increased the urinary excretion of \( \text{l-xylulose} \) in patients with essential pentosuria. It is now possible to explain their observation in terms of the scheme in Fig. 1. Administration of these drugs would be expected to increase the formation of \( \text{l-xylulose} \) from \( \text{d-glucose} \). Since the pentosuric patient is presumably not able to metabolize \( \text{l-xylulose} \), the pentose consequently would be excreted in urine.

**SUMMARY**

The metabolism of \( \text{n-gluconolactone-1-C}^{14} \), \( 6 \text{C}^{14} \), and \( \text{l-gulonic acid-6-C}^{14} \) has been investigated in the intact rat and guinea pig. The results obtained on the distribution of isotope in liver glycogen furnish further evidence for the existence of a glucuronic acid pathway of glucose metabolism. According to this scheme \( \text{d-glucose} \) is metabolized via \( \text{n-glucuronolactone}, \text{n-gulono} \). \( \text{l-xylulose}, \text{D-Xylulose}, \text{and the pentose phosphate pathway} \).

**REFERENCES**

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