MECHANISM STUDIES OF GLYCOGEN AND GLYCERIDE GLYCEROL BIOSYNTHESIS*

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It is well known that isotopic tracer studies of a complex system of interrelated reactions can determine the existence and magnitude of reaction sequences for transferring atoms from a given reactant to a particular product and also in many cases, usually through studies involving asymmetrically labeled reactants and including degradation of the products, establish parameters with which postulated mechanisms for the sequences must concur. Such studies have been reported for the system of interrelated reactions yielding liver glycogen; various non-uniformly labeled acetates, propionates, butyrates, lactates, and pyruvates (1–4) and valerate-1-C¹³-4-C¹⁴ have been used as reactants (5). We have observed the appearance in liver glycogen and carcass glyceride glycerol of C¹⁴ administered intraperitoneally to normal, intact, male albino rats as glycerol-1-C¹⁴ (6) and are now reporting degradation studies establishing the C¹⁴ distribution within those molecules.

EXPERIMENTAL

The experimental conditions for the synthesis, administration, and metabolism of glycerol-1-C¹⁴ and the procedures for isolating glycogen from the liver and crystalline glycerol tribenzoate† from the carcass glycerides were as reported previously (6, 7). The glucose from the liver glycogen

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1 A small quantity of non-radioactive glycerol was added to an aliquot of melted carcass lipides (after removal of acetone-insoluble lipides) and mixed thoroughly; the mixture was added to a water-petroleum ether system and partitioned between the two solvents. The aqueous phase was filtered and extracted three times with 4 volumes of petroleum ether; the glycerol remaining in the aqueous phase was benzoylated and the glycerol tribenzoate recrystallized as described before. Glycerol tribenzoate samples were mounted on sample pans and counted as described below. No radioactivity was detectable within the limits of precision of the measurements, indicating that the glycerol-C¹⁴ found in the carcass glyceride hydrolysates was present in the carcass glycerides in esterified form and not as free glycerol mechanically carried through the separation process into the glyceride fraction.
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was degraded by the method of Topper and Hastings (2), which involves radioactivity measurements of (a) the glucosazone, (b) the 1,2-phenyl-hydrzone of mesoxalaldehyde arising from periodate oxidation of glucosazone and representing carbons 1, 2, and 3, (c) the formic acid arising from periodate oxidation of glucosazone and representing carbons 4 and 5, (d) the formaldehyde arising from periodate oxidation of glucose and representing carbon 6, (e) the hydrogen cyanide arising from a Wohl degradation and representing carbon 1, (f) the hydrogen cyanide arising from a double Wohl degradation and representing carbon 2. From these data the radioactivity of each glucose carbon atom was calculated in a manner direct enough to avoid large cumulative uncertainties except in the case of carbons 4 and 5. The sum of the radioactivities of carbons 4 and 5 is known directly (c), and the resolution involves an assumption of symmetry equating the radioactivities of carbons 3 and 4 (2). The facts that the specific molecular radioactivity of (b) was half that of (a), that (c) equaled (b) minus (e), and that (d) equaled (e) support this assumption of isotope distribution symmetry about the center of the glucose carbon chain in this case. Varying quantities of non-radioactive carriers were added during the degradations, but in every measurement the observed uncorrected counting rate was at least 4 times the background and at least 2700 counts were recorded on the instrument. The glycerol tribenzoate was hydrolyzed as described before (6) and degraded by periodate oxidation as described previously, a procedure we have demonstrated to be unaccompanied by significant rearrangements or exchange (8). All radioactivities were determined with a windowless Q gas flow tube operated in the Geiger region, with sample mountings as described previously.

RESULTS AND DISCUSSION

The radioactivity distributions in the liver glycogen relative to carbon 1 set at 1.0 and in the carcass glyceride glycerol relative to either primary alcohol carbon set at 1.0 are given in Table I. Several routes for glycogen formation from smaller intermediates are known (1-5). Apparently the possible route involving initial oxidation to acetate-2-C\textsuperscript{14}, leading through the tricarboxylic acid cycle (9, 10) and producing glucose-1,2,5,6-C\textsuperscript{14} from oxalacetate-3-C\textsuperscript{14} and oxalacetate-2-C\textsuperscript{14}, a route of interest because it results in the appearance of C\textsuperscript{14} in positions 2 and 5 of glucose without CO\textsubscript{2} or C\textsuperscript{14}O\textsubscript{2} as an intermediate, did not play a major part in glycogen formation in these animals, as it would lead to the appearance of more C\textsuperscript{14} in positions 1 and 6 than in positions 3 and 4, contrary to the observed results. The results are consistent with initial oxidation and phosphorylation to dihydroxyacetone phosphate or D-3-phosphoglyceraldehyde or both followed by the known reactions of glycolysis, the appearance of C\textsuperscript{14} in
positions 2 and 5 of glucose, and the increase of C\textsuperscript{14} concentration in positions 3 and 4 over that in positions 1 and 6 ascribable largely to CO\textsubscript{2} fixation by \(\beta\) carboxylation of pyruvate.

The relatively high quantity of C\textsuperscript{14} found in the secondary alcohol carbon of the glyceride glycerol indicates that randomizing processes played an important part in its formation. This suggests that one of the routes for its biosynthesis includes a reduction to glycerol or to a substituted glycerol of an intermediate in a higher state of oxidation, since all the randomizing processes at present accepted involve as intermediates materials in a higher state of oxidation than glycerol or its substitution products.

**Table I**

*Distribution of C\textsuperscript{14} in Liver Glycogen Relative to C-1 Set at 1.0 and in Carcass Glyceride Glycerol Relative to C-1 or C-3 Set at 1.0*

<table>
<thead>
<tr>
<th></th>
<th>Liver glycogen</th>
<th>Carcass glyceride glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>C-2</td>
<td>0.19</td>
<td>0.44</td>
</tr>
<tr>
<td>C-3</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>C-4</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>C-5</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>C-6</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

**Summary**

The distribution of the C\textsuperscript{14} in the liver glycogen and carcass glyceride glycerol of normal, intact, male albino rats (approximately 350 gm.) 24 hours after the intraperitoneal administration of approximately 30 mg. doses of glycero\textsuperscript{1}-C\textsuperscript{14} has been established through measurements of the radioactivities of suitable degradation products.

The distribution of liver glycogen C\textsuperscript{14} is consistent with initial oxidation and phosphorylation to dihydroxyacetone phosphate or d-3-phosphoglyceraldehyde or both, followed by the known reactions of glycolysis, the randomization of C\textsuperscript{14} occurring primarily through known processes involving CO\textsubscript{2} fixation by \(\beta\) carboxylation of pyruvate. The high incorporation of radioactivity observed into the secondary alcohol position of the carcass glyceride glycerol, approximately 18 per cent of the total in the molecule, indicates that randomizing processes played a part in its formation in these experiments and suggests that reduction of an intermediate in a higher state of oxidation to glycerol or to a substituted glycerol oc-
curred as part of at least one of the pathways leading from free glycerol to carcass glyceride glycerol.

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BIBLIOGRAPHY

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