Initial Reactions in the Metabolism of D- and L-Glyceraldehyde by Rat Liver*

BERNARD R. LANDAU† AND WILFRIED MERLEVEDE

WITH THE TECHNICAL ASSISTANCE OF HOLLIS R. WILLIAMS

From the Departments of Medicine and Biochemistry, Western Reserve University, Cleveland 6, Ohio

(Received for publication, September 27, 1962)

There appear to be several pathways in liver by which D- and L-glyceraldehyde can be converted to dihydroxyacetone phosphate and D-glyceraldehyde phosphate. Thus, the following reactions have been described in liver preparations: phosphorylation of D-glyceraldehyde to D-glyceraldehyde phosphate (Fig. 1; Pathway A) (2); formation of D-glyceraldehyde phosphate from D-glyceraldehyde via D-glycero acid (Pathway B) (3-6); and reduction of D-glyceraldehyde to glycerol with the latter’s conversion to dihydroxyacetone phosphate (Pathway C) (7-9). By either Pathway A or B, D-glyceraldehyde labeled in position 3 with C¹⁴ (indicated by an asterisk in Fig. 1) should yield via the Embden-Meyerhof pathway glycerol containing glucose units labeled in carbon atoms 1 and 6. However, via Pathway C, glycerol formed from D-glyceraldehyde-3-C¹⁴ (the label is now traced in Fig. 1 by a circle) should yield dihydroxyacetone-3-phosphate-1-C¹⁴ (10) and therefore 3,4-labeled glucose. If isotopic equilibration of trioses via triose isomerase is slow relative to hexose formation (11-13) by either Pathway A or B, more activity should be in carbon 6 than in carbon 1, whereas via Pathway C, more activity should be in carbon 3 than in carbon 4. By reaction via transaldolase, C¹⁴ from D-glyceraldehyde-3-C¹⁴ may also be introduced into carbon 6 of fructose (14).

L-Glyceraldehyde phosphate can be formed from L-glyceraldehyde (Fig. 2; Pathway A) (15), but no reaction is known for further metabolism via the Embden-Meyerhof pathway. There is evidence for the conversion of L-glyceraldehyde to D-glycer aldehyde phosphate via series of reactions (Pathway B) of which L-glyceraldehyde acid and hydroxypyruvic acid are intermediates (3, 6). L-Glyceraldehyde labeled in position 3 with C¹⁴ (indicated by an asterisk in Fig. 2) should then yield 1,6-labeled glucose with, if isotopic nonequilibration of trioses occurred, greater activity in carbon 6 than in carbon 1. L-Glyceraldehyde can be reduced to glyceral (Pathway C) (7, 16) which would yield, when labeled, dihydroxyacetone-3-phosphate-3-C¹⁴ (10), and also, therefore, 1,6-labeled glucose. However, by this latter pathway, if non-equilibration occurred, activity in carbon 1 should be greater than in carbon 6.

The relative contribution of these pathways to the metabolism of glyceraldehyde has not been assessed previously by tracings with labeled carbon. However, D-fructose-6-C¹⁴ and L-sorbose-6-C¹⁴ are probably metabolized in the liver to D- and L-glyceraldehyde-3-C¹⁴, respectively (16). Therefore, conclusions about the pathways of glyceraldehyde metabolism have been made from the labeling patterns in liver glycogen that are observed when these hexoses are administered to rats. Hers (16), noting that Muntz obtained only 1,6-labeling in glycogen with D-fructose 6-C¹⁴, has concluded that at least under Muntz’s conditions, D-glyceraldehyde is not metabolized via glycerol (Fig. 1; Pathway C). Because of the greater labeling of carbon 1 than of carbon 6 in glycogen observed by Burns et al. (17), when L-sorbose-6-C¹⁴ was administered, Hers (16) concluded that metabolism of L-glyceraldehyde was probably via reduction to glycerol (Fig. 2; Pathway C).

It is the purpose of this paper to present the labeling patterns in liver glycogen observed on administration of D- and L-glyceraldehyde-3-C¹⁴ to rats and to compare these patterns with those obtained with the use of D-fructose-6-C¹⁴ and L-sorbose-6-C¹⁴. Incubations of the labeled compounds with liver slices and D-glyceraldehyde metabolism in a perfused isolated liver have also been examined. Conditions have been selected in intact rats and in slices in which isotopic nonequilibration of trioses occurs. The results have been interpreted in terms of the pathways as depicted in Figs. 1 and 2.

EXPERIMENTAL PROCEDURE

Animals—Albino rats of the Wistar strain weighing 150 to 250 g and maintained on a stock diet ad libitum were used.

Substrates—D-Fructose-6-C¹⁴ was prepared from D-glucose-6-C¹⁴, purchased from New England Nuclear Corporation, by the method of Muntz and Carroll (18) except that before its purification by paper chromatography, it was incubated with glucose oxidase and the resulting solution was deionized so that any trace of unreacted glucose would be removed. L-Sorbose-6-C¹⁴ was purchased from the National Bureau of Standards.

D- and L-Glyceraldehyde-3-C¹⁴ were synthesized from D-fructose-6-C¹⁴ and L-sorbose-6-C¹⁴ by a small scale modification of the method of Perlin and Brice (19). The glyceraldehydes were purified by application to Whatman No. 3MM preparative chromatography paper after the addition of nonradioactive fructose or sorbose to dilute any unretracted radioactive hexose. Chromatography was performed with an n-butanol-acetic acid-water solvent, 40:10:50, of relative volume (20). Guide spots of D-glyceraldehyde, purchased from the Sigma Chemical Company, and the hexoses were identified by a phloroglucinol spray (20).

The paper area paralleling the glyceraldehyde guides was re-
moved, eluted with water, and concentrated in a vacuum. An
excellent separation of glyceraldehyde from the hexoses was
achieved, despite the spreading of the glyceraldehyde spot;
$R_f$ of sorbose $= 0.23$, fructose $= 0.25$, and glyceraldehyde $= 0.40$ to 0.63. The yields of glyceraldehydes were from 55 to
80% as determined by titrometric measurement with iodine
(19). The specific activities of aqueous solutions of the glycer-
aldehydes as determined by total combustion and assay (21)
agreed with those determined by combustion and assay of 2,4-
dinitrophenylhydrazone derivatives of the glyceraldehydes (22).
It was demonstrated by degradation (23) that essentially all
of the activity was in carbon 3 of the glyceraldehydes. Glycerol-
2-Cl4 was purchased from Orlando Chemicals, Inc., and sodium
pyruvate-2-Cl4 from Nuclear Chicago Corporation. The pyru-
vate was purified by addition to a Dowex 1 anion exchange
column in the chloride form followed by elution with dilute
hydrochloric acid and neutralization of the pyruvate-containing
fraction with dilute sodium hydroxide.

Glyceraldehydes were stored frozen in aqueous solution and
used from 2 to 12 weeks after preparation. In some experi-
ments, the solutions were concentrated in a vacuum and main-
tained at 55-60° for 2 hours immediately before use (22) or
heated at 80-85° for 2 minutes to 2½ hours (24).1

1 Wohl (25) concluded that $\alpha$-glyceraldehyde exists primarily
as a dimer in fresh aqueous solution, as evidenced by molecular
weight determinations with the use of the freezing point depression
technique. The monomeric form appeared to be produced by
aging of the solution and its formation was accelerated by heat.
Lehmann and Needham (26) attributed the differences observed
by investigators in glyceraldehyde metabolism to the use of fresh
compared to aged solutions of $\alpha$-glyceraldehyde. Baer and
Fisher (22) noted that $\beta$- and $\gamma$-glyceraldehyde freshly dissolved
in water had an optical rotation of $+14^\circ$ and $-14^\circ$, respectively,
March 1963

B. R. Landau and W. Merlevede

863

Table I

C14 distribution in liver glycogen on injection of glycerol-2-C14, pyruvate-2-C14, n-glyceraldehyde-3-C14, and D-fructose-6-C14 into rats

Rats were deprived of food for 48 hours and tubed with 0 to 3 mmoles of glucose or glycerol or both per 100 g of body weight. The labeled substrate (0.1 mmole) was injected by tail vein in a divided dose 1 and 2 hours after tubing and killing was at 3 hours.

<table>
<thead>
<tr>
<th>Labeled substrate</th>
<th>Stomach tubing</th>
<th>Glucose from glycogen</th>
<th>% Distribution of C14 in carbon</th>
<th>% C14 incorporated into glycogen</th>
<th>Experiment No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycerol</td>
<td>Glucose</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>mg/mg/100 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol-2-C14</td>
<td>3</td>
<td>3</td>
<td>1.4</td>
<td>58.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Pyruvate-2-C14</td>
<td>3</td>
<td>3</td>
<td>16.7</td>
<td>22.1</td>
<td>7.3</td>
</tr>
<tr>
<td>d-Glyceraldehyde-3-C14</td>
<td>3</td>
<td>3</td>
<td>16.8</td>
<td>6.4</td>
<td>26.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>16.3</td>
<td>4.8</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>21.4</td>
<td>8.2</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>21.6</td>
<td>11.0</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>21.9</td>
<td>12.8</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>9</td>
<td>18.8</td>
<td>3.9</td>
<td>28.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>18.4</td>
<td>7.6</td>
<td>25.6</td>
</tr>
<tr>
<td>D-Fructose-6-C14</td>
<td>3</td>
<td>3</td>
<td>33.8</td>
<td>3.3</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>38.6</td>
<td>3.6</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Experiment with Intact Rats—Rats were deprived of food for 48 hours and then given large quantities of glycerol or glucose or both in an aqueous solution by stomach tube (11, 12). The labeled substrates were injected via the tail vein, 0.05 mmole in an aqueous solution by stomach tube (11, 12). The livers were removed, and glycogen was isolated and degraded with Leuconostoc mesenteroides (29, 30).

Experiment with Liver Slices—Livers were removed from rats but that with standing for 2 days, the rotation decreased to +7° and −7°. The 14° rotation could be reobtained by evaporation in a vacuum at 55-60° for 2 hours. They consider these changes due to either a dimer-monomer, mutarotation, or hydration phenomenon. n-Glyceraldehyde inhibited glycolysis in tumor tissue to the same extent whichever preparation was used (27). Most investigators (19, 24, 28) appear to have accepted that D- as compared to L- and D-labeled substrates, the 14° rotation could be reobtained by evaporation of the solution at 80° for 1 hour before use. In Experiment 6a, the glyceraldehyde was given in a single dose 1 hour after tubing, and in Experiment 6b, 2 hours after tubing.

Radioactive Assays and Calculations—The activity of the carbons of glucose of the glycogen were assayed as CO2 in the gas phase when the activities were low, and by plating as barium carbonate and by assay in a thin end window gas flow counter when the activities were high. The % activity in each carbon has been calculated from the activity in each carbon and the sums of the activities in the six carbon atoms. The % recovery giving a measure of the adequacy of degradation is obtained by comparison of this sum with the activity in the glucose as determined by combustion (21). The percentage of the dose of C14 injected into rats which was recovered in liver glycogen is recorded. Similarly recorded is the percentage of the counts added to the medium or perfusate which was incorporated into the glycogen of the liver slices or isolated liver.
RESULTS

In Table I are presented the distributions of C\textsuperscript{14} activity found in glucose from liver glycogens after the administration to intact rats of small quantities of glycerol-2-C\textsuperscript{14}, pyruvate-2-C\textsuperscript{14}, L-glyceraldehyde-3-C\textsuperscript{14}, and D-fructose-6-C\textsuperscript{14}. Quantities of nonlabeled glucose and glyceraldehyde administered by stomach tube and methods of pretreating the glyceraldehyde were varied as indicated in the table. The glyceraldehyde and pyruvate experiments confirm the conditions employed (11, 12) as those in which asymmetry occurs. That is, more activity was in carbon 2 than in carbon 5 when glycerol-2-C\textsuperscript{14} was injected, whereas with pyruvate-2-C\textsuperscript{14}, carbon atoms 4, 5, and 6 contained more activity than the corresponding triose equivalents, carbon atoms 3, 2, and 1.

In all 7 experiments performed with L-glyceraldehyde-3-C\textsuperscript{14}, frequently as much, and occasionally more activity was present in carbon atoms 3 and 4 than in carbon atoms 1 and 6, and most of the activity in the glucose was in these carbons. In four of the experiments (2c, 3b, 6a, and 6b), there was more activity in carbon 3 than in carbon 4 and less activity in carbon 1 than in carbon 6. In the remaining three experiments, the activity in carbon atoms 1 and 6 were similar, whereas there was suggestively greater activity in carbon 4 than in carbon 3. In the glucose of glycogen from the two rats in which D-fructose-6-C\textsuperscript{14} was injected, C\textsuperscript{14} activity was predominantly in carbon atoms 1 and 6, with the greater quantity in carbon 6.

Table II records the pattern of glycogen labeling when large quantities of L-glyceraldehyde-3-C\textsuperscript{14} or D-fructose-6-C\textsuperscript{14} were injected into rats and when a small quantity of one was given in the presence of a large, nonlabeled quantity of the other. There may be a suggestion that the administration of a large

### Table II

**C\textsuperscript{14} distribution in liver glycogen on administration of L-glyceraldehyde and D-fructose**

<table>
<thead>
<tr>
<th>Substrates(^a)</th>
<th>Stomach tubing, glucose</th>
<th>Glucose from glycogen</th>
<th>% C\textsuperscript{14} incorporated into glycogen</th>
<th>% Recovery</th>
<th>Experiment No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labeled</td>
<td>Nonlabeled</td>
<td>Stomach tubing, glucose</td>
<td>Glucose from glycogen</td>
<td>% Distribution of C\textsuperscript{14} in carbon</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Fructose, 1</td>
<td>None</td>
<td>3</td>
<td>41.0</td>
<td>3.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Fructose, 0.1</td>
<td>Glyceraldehyde, 2</td>
<td>3</td>
<td>33.2</td>
<td>5.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Glyceraldehyde, 2</td>
<td>None</td>
<td>3</td>
<td>21.6</td>
<td>6.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Glyceraldehyde, 0.1</td>
<td>Fructose, 1</td>
<td>3</td>
<td>21.8</td>
<td>5.2</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22.6</td>
<td>10.9</td>
<td>16.2</td>
</tr>
</tbody>
</table>

\(^a\) Number after substrate is the quantity in micromoles injected in a divided dose.
\(^b\) L-Glyceraldehyde-3-C\textsuperscript{14} was maintained for 2 hours in a vacuum at 55-60\(^\circ\) before use. One carbon was lost in each of these degradations as well as in Experiment 7a, and the % distribution is calculated from activity in all six carbon atoms as determined by combustion.
\(^c\) Glyceraldehyde-3-C\textsuperscript{14} solution was maintained at 80\(^\circ\) for 24 hours before use.

### Table III

**C\textsuperscript{14} distribution in liver glycogen on injection of L-glyceraldehyde-3-C\textsuperscript{14} and L-sorbose-6-C\textsuperscript{14} into rats**

Experimental design as in Table I.

<table>
<thead>
<tr>
<th>Labeled substrate</th>
<th>Stomach tubing, glucose</th>
<th>Glucose from glycogen</th>
<th>% C\textsuperscript{14} incorporated into glycogen</th>
<th>% Recovery</th>
<th>Experiment No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>L-Glyceraldehyde-3-C\textsuperscript{14}</td>
<td>3</td>
<td>3</td>
<td>38.2</td>
<td>4.4</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>41.4</td>
<td>4.0</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>36.5</td>
<td>10.5</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>31.4</td>
<td>6.1</td>
<td>10.9</td>
</tr>
<tr>
<td>L-Sorbose-6-C\textsuperscript{14}</td>
<td>3</td>
<td>3</td>
<td>33.4</td>
<td>5.4</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>30.2</td>
<td>7.5</td>
<td>1.8</td>
</tr>
</tbody>
</table>

\(^a\) L-Glyceraldehyde-3-C\textsuperscript{14} solution was warmed at 80\(^\circ\) for 2 minutes before use.
\(^b\) L-Glyceraldehyde-3-C\textsuperscript{14} was maintained in a vacuum at 55-60\(^\circ\) for 2 hours immediately before use.
\(^k\) Carbon 6 lost. Per cent C\textsuperscript{14} in other carbon atoms is calculated from total activity in glucose as determined by combustion.
The distribution of C\textsuperscript{14} in glycogen isolated from liver slices after incubation with glycerol-2-C\textsuperscript{14}, pyruvate-2-C\textsuperscript{14}, and D- and L-glyceraldehyde-3-C\textsuperscript{14}

One gram of slices from livers of fed rats was incubated in 12 ml of a high potassium-bicarbonate buffer for 90 minutes at 37° (13). The buffer contained 0.1 mmole of the labeled substrate and nonlabeled substrate as indicated. At the completion of incubation, glycogen from the slices was isolated and degraded.

<table>
<thead>
<tr>
<th>Labeled substrate</th>
<th>Nonlabeled substrate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Glucose from glycogen</th>
<th>% C\textsuperscript{14} in carbon</th>
<th>% Recovery</th>
<th>% C\textsuperscript{14} injected incorporated into glycogen</th>
<th>Experiment No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol-2-C\textsuperscript{14}</td>
<td>- + + -</td>
<td>1.3</td>
<td>45.5</td>
<td>3.2</td>
<td>1.0</td>
<td>46.7</td>
</tr>
<tr>
<td>Pyruvate-2-C\textsuperscript{14}</td>
<td>- + + -</td>
<td>13.8</td>
<td>19.4</td>
<td>7.0</td>
<td>13.0</td>
<td>24.6</td>
</tr>
<tr>
<td>D-Glyceraldehyde-3-C\textsuperscript{14}</td>
<td>- + + -</td>
<td>32.8</td>
<td>1.8</td>
<td>5.4</td>
<td>5.0</td>
<td>1.1</td>
</tr>
<tr>
<td>+ - - -</td>
<td>24.1</td>
<td>2.5</td>
<td>5.9</td>
<td>8.3</td>
<td>3.0</td>
<td>56.2</td>
</tr>
<tr>
<td>- + + +</td>
<td>34.3</td>
<td>4.5</td>
<td>8.4</td>
<td>12.8</td>
<td>1.9</td>
<td>38.0</td>
</tr>
<tr>
<td>- - - -</td>
<td>39.6</td>
<td>3.1</td>
<td>7.3</td>
<td>6.6</td>
<td>2.4</td>
<td>42.5</td>
</tr>
<tr>
<td>L-Glyceraldehyde-3-C\textsuperscript{14}</td>
<td>- + + -</td>
<td>34.1</td>
<td>3.4</td>
<td>4.5</td>
<td>15.3</td>
<td>12.5</td>
</tr>
</tbody>
</table>

*Glucose, 20 mm; glycerol, 30 mm; pyruvate, 30 mm; d-glyceraldehyde, 30 mm.*

Table V records the C\textsuperscript{14} distribution in glycogen from the perfused liver when D-glyceraldehyde-3-C\textsuperscript{14} was substrate; almost as much activity was observed in carbon atoms 3 and 4 as in 1 and 6. There was more activity in carbon 6 than in carbon 1 and in carbon 4 than in carbon 3.

**DISCUSSION**

The marked activity in carbon atoms 3 and 4 of liver glycogen in the intact rat presented with D-glyceraldehyde-3-C\textsuperscript{14} indicates that a significant portion of D-glyceraldehyde entered the Embden-Meyerhof pathway via conversion to glycerol (Fig. 1; Pathway C). The occurrence of a similar distribution in the perfused liver preparation suggests the assumption that the distribution of C\textsuperscript{14} is consequent to reactions within the liver and not to metabolism of D-glyceraldehyde in other organs with subsequent entrance of labeled metabolites into the liver. Whereas activity in carbon atoms 3 and 4 was less relative to carbon atoms 1 and 6 in slices than in the intact rat or perfused system, the distributions also appear to support the occurrence of a reduction of D-glyceraldehyde to glycerol as an initial step in its metabolism. The oxidation of D-glyceraldehyde-3-C\textsuperscript{14} via glyceraldehyde phosphate to C\textsubscript{4}0\textsubscript{2} and subsequent fixation of the C\textsubscript{4}0\textsubscript{2} by the liver could also introduce activity into carbon atoms 3 and 4. However, the quantity that might be incorporated in this manner would seem to be insufficient to explain the distribution observed. Thus, to the extent that D-glyceraldehyde, D-fructose, and L-sorbose enter the Embden-Meyerhof pathway, these compounds, as D-glyceraldehyde, would be expected to be oxidized to CO\textsubscript{2} with subsequent fixation. Yet when these substrates were labeled with C\textsuperscript{14}, only a small part of the C\textsuperscript{14} found in the glucose was in carbon atoms 3 and 4.

In intact rats under the conditions selected for these experiments (11), metabolism of D-glyceraldehyde-3-C\textsuperscript{14} via glycerol might be expected to yield glucose with more C\textsuperscript{14} in carbon 3.
than in carbon 4. Although this was observed frequently, it was not a consistent observation. Since C14O2 fixation will yield more C14 in carbon 4 than in carbon 3 (33), this reaction, although relatively small, may have served partially to mask the asymmetry anticipated consequent to metabolism via glycerol. The greater activity in carbon 6 than in carbon 1 of glucose when D-glyceraldehyde-3-C14 was substrate, particularly in the liver-slice system, is in accord with either Pathway A or B of Fig. 1 or both. The contribution of transaldolase reactions (14, 35) to the asymmetry of carbon 6 compared to carbon 1 cannot be assessed from this study. The transaldolase exchange reaction (35) would also favor activity in carbon 4 relative to carbon 3.

The negligible activity in carbon 3 and carbon 4 of glucose from glycogen when D-fructose-6-C14 was administered would indicate that, if fructose is converted in large measure to D-glyceraldehyde-3-C14, the latter is metabolized via either Pathway A or B or both, and perhaps via the transaldolase reaction, but not via reduction to glycerol. Incorporation of the carbon atoms of D-fructose-6-C14 into glycerol without cleavage of the carbon skeleton may also have contributed to the greater activity in carbon 6 than in carbon 1 of glucose from glycogen.

The explanation is uncertain for the differences in the distribution of label in glycogen with fructose-6-C14 compared to D-glyceraldehyde-3-C14 as substrate. It is conceivable that the metabolism of fructose is not via glyceraldehyde as is generally supposed. Muntz and Vanko (34) observed that on administration of fructose-C14 to rats, liver glucose had a higher specific activity than its presumed precursors, glucose 6-phosphate, fructose 6-phosphate, and fructose 1, 6-diphosphate. They have suggested the possibility that fructose is not converted to glucose according to present concepts. Alternatively, metabolic compartments can exist within the liver cell (36). Thus, on entrance into the cell, D-glyceraldehyde could first experience a reducing environment and be converted to glycerol. In contrast, D-glyceraldehyde formed from fructose via fructose 1-phosphate could initially experience a phosphorylating environment and enter the Embden-Meyerhof pathway via D-glyceraldehyde phospate. That in liver slices the pathway for D-glyceraldehyde via glycerol appears relatively less significant than pathways resulting in 1, 6-labeling could then indicate that the reducing environment in the slices was less effective than in the intact liver. If such compartments exist, an overflow of glyceraldehyde from one compartment to the other might occur at high substrate concentrations. The experiments, the results of which are recorded in Table II, were intended to test these speculations. No conclusive evidence for such an occurrence was obtained.

The distribution of C14 in carbon atoms 1 and 6 of glucose from glycogen formed from L-glyceraldehyde-3-C14 and L-sorbose-6-C14, in both the intact rat and liver slices, suggests that C14 in or both these substrates are treated similarly by the liver. Presumably, L-sorbose is metabolized via L-glyceraldehyde, the L-glyceraldehyde formed within the liver following pathways similar to those followed by L-glyceraldehyde presented to the liver. Pathway A of Fig. 2 will not be considered further since there appears to be no known mechanism by which the carbon atoms of L-glyceraldehyde phosphate can be incorporated into glycogen. The introduction of L-glyceraldehyde carbon via L-glyceric acid and hydroxyproprionic acid (Fig. 2: Pathway B) and the reduction of L-glyceraldehyde via glycerol (Fig. 2, Pathway C) would yield 1, 6-labeled glucose. Whether or not both of these pathways actually participate in L-glyceraldehyde metabolism remains uncertain. A contribution from Pathway D would account for the greater labeling of carbon 6 than of carbon 1; Pathway C of itself should yield glucose containing more activity in carbon 1 than in carbon 6. However, a transaldolase exchange reaction (35) in addition to Pathway C could preferentially label carbon 6 relative to carbon 1.

Burns et al. (17) observed more label in carbon 1 than in carbon 6 of glucose from glycogen when L-sorbose-6-C14 was administered to rats under somewhat similar conditions to ours. The explanation for the difference between their results and those reported here is not certain. They did administer glucose, but not glycerol, by stomach tube. The only experiment in which we observed more activity in carbon 1 than in carbon 0 with L-glyceraldehyde-3-C14 as substrate is the experiment in which glycerol was omitted (Table III, Experiment 3c).

**Summary**

D-Glyceraldehyde-3-C14, L-glyceraldehyde-3-C14, D-fructose-6-C14, and L-sorbose-6-C14 have been injected into rats and incubated with liver slices. Liver glycogen has been isolated and degraded. Conditions have been selected where isotopic nonequilibration of trioses occurs so that asymmetry in glycogen labeling should reflect the initial entrance of the label into the Embden-Meyerhof pathway via dihydroxyacetone phosphate or glyceraldehyde-phosphate. D-Glyceraldehyde-3-C14 has also been perfused through rat liver.

Marked activity in carbon atoms 3 and 4 of glucose from glycogen was observed when D-glyceraldehyde-3-C14 was substrate. This indicates that an initial step in D-glyceraldehyde metabolism is reduction to glycerol. With D-fructose-6-C14 as substrate, activity was primarily in carbon atoms 1 and 6 of the glucose, indicating that if D-glyceraldehyde is formed from fructose within the liver, it is not reduced to glycerol but rather enters the Embden-Meyerhof pathway via glyceraldehyde-phosphate. Both L-sorbose-6-C14 and L-glyceraldehyde-3-C14 yield glucose labeled primarily in carbon atoms 1 and 6 with more activity in carbon 6 than carbon 1. This suggests that L-glyceraldehyde presented to the liver and that formed from L-sorbose within the liver traverse the same pathways. The distribution of label does not differentiate L-glyceraldehyde metabolism via D-glyceraldehyde phosphate from that via dihydroxyacetone phosphate.

**References**

29. SAKAMI, W., Handbook of isotopic tracer methods, Western Reserve University, Cleveland, 1955, p. 24.
Initial Reactions in the Metabolism of d- and l-Glyceraldehyde by Rat Liver
Bernard R. Landau, Wilfried Merlevede and With the technical assistance of Hollis R. Williams