A Comparison of Glucose Utilization by Mouse Liver and by the Transplantable Hepatocarcinoma C954

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SUMMARY

The following aspects of carbohydrate metabolism were compared in liver and in the transplantable hepatocarcinoma C954 carried by the C57-leaden mouse strain: (a) glucose production from endogenous sources; (b) glucose-6-phosphatase and isomerase activities; (c) glycogen synthesis; (d) extent of glucose utilization for CO2, lactate, and fatty acid formation; (e) pathways of glucose catabolism; and (f) operation of the tricarboxylic acid cycle.

Liver slices produced a large amount of glucose from endogenous sources. No such formation was detected in the experiments with the hepatocarcinoma slices. This inability of the tumor to form glucose from endogenous sources was associated with the absence of glucose-6-phosphatase in homogenates prepared from it. The presence of hexosephosphate isomerase was demonstrated in both tumor and liver. No glycogen synthesis from glucose was observed in slices of the hepatocarcinoma.

The extent of utilization of glucose for lactate and fatty acid formation by liver and tumor slices was studied with various concentrations of labeled glucose in the medium. At all concentrations studied the recoveries of C14 as lactate and fatty acids in the experiments with tumor slices surpassed those observed with liver.

In host liver slices the yields of C14O2, C14-labeled fatty acids, and lactate-C14 from glucose-1-C14 and -6-C14 were approximately equal. In the hepatocarcinoma C954 slices the C14O2 yields from glucose-1-C14 were 2-3 times those from glucose-6-C14. The recoveries of C14-labeled fatty acids and lactate-C14 from glucose-6-C14 exceeded those from glucose-1-C14 in the experiments with the hepatocarcinoma.

The participation of the hexosemonophosphate oxidative pathway in glucose metabolism of this tumor was estimated by two methods. A considerable portion of glucose catabolism in the tumor proceeded via this direct oxidative pathway. It would appear that, in the liver of the tumor-bearing mouse, the Embden-Meyerhof scheme is by far the major pathway for conversion of glucose carbon to fatty acids.

Chromatographic-radioautographic analysis of water-soluble compounds derived from glucose breakdown by the slices of the hepatocarcinoma offers evidence for the operation of the tricarboxylic acid cycle in this neoplasm.

Evidence suggestive of a defect in the conversion of glutamate to glutamine by the hepatocarcinoma is presented.

A previous report from this laboratory dealt with the oxidative metabolic patterns in the transplantable hepatocarcinoma C954 carried by mice of the C57-leaden strain. The incorporation of C14 of acetate-1-C14, acetate-2-C14, propionate-1-C14, octanoate-1-C14, pyruvate-2-C14, and glucose evenly labeled with C14 into respiratory CO2 and various intermediate products by the liver and tumor slices was studied by a chromatographic-radioautographic method that made possible the identification and measurement of twelve or more labeled intermediates. The pattern of labeling of various intermediates observed in the experiments with this hepatocarcinoma differed quantitatively from that observed with either host or normal liver. Evidence for the operation of the

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tricarboxylic acid cycle in the hepatocarcinoma was provided by the following findings: (a) oxidation of labeled 2-carbon fragments generated from acetate and octanoate to C\textsuperscript{14}O\textsubscript{2}; (b) incorporation of isotope from 2-carbon fragments into compounds of the cycle as well as into glutamate, aspartate, and alanine, all of which arise from cycle compounds. It was also shown that the oxidation of acetate to CO\textsubscript{2} by a suspension of the hepatocarcinoma cells was inhibited by malonate, a competitive inhibitor of succinate oxidation.

One other phase of the metabolism of the hepatocarcinoma C\textsubscript{954} was studied. When slices of this tumor were incubated with glucose-1-C\textsuperscript{14} and -6-C\textsuperscript{14}, the C\textsuperscript{14}O\textsubscript{2} yields from the former were several times greater than those from glucose-6-C\textsuperscript{14} (5). In similar experiments with normal mouse (leaden strain) liver and host liver, the yields of C\textsuperscript{14}O\textsubscript{2} from these two singly labeled hexoses were about the same. It was therefore inferred that a pathway of glycolysis other than the classical Embden-Meyerhof operated in this neoplastic tissue. The limitations in the use of C\textsuperscript{14}O\textsubscript{2} data obtained from experiments with individually C\textsuperscript{14}-labeled hexoses for evaluating glycolytic pathways have been pointed out by a number of investigators (6, 36, 64).

Following the appearance of our first reports (5, 15), we observed that the hepatocarcinoma C\textsubscript{954} possessed a high capacity for lipogenesis from glucose, and it was this finding that made possible the more precise study of glycolytic pathways recorded here. In addition, we have investigated three other aspects of carbohydrate metabolism in the hepatoma: (a) the conversion of glucose to lactate and other water-soluble intermediates; (b) the interrelation between the two enzymatic reactions, hexosephosphate isomerase and glucose-6-phosphatase; and (c) synthesis of glycogen from glucose. A preliminary report of some of the findings has appeared (2).

MATERIALS AND METHODS

ANIMALS AND THEIR TREATMENT

Male mice of the C57-leaden strain carrying the hepatocarcinoma C\textsubscript{954} were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. (This tumor was discovered in 1940 as a spontaneous parenchymal-cell tumor of the liver in an old male mouse of the leaden strain [16].) The tumor was maintained in this laboratory by serial subcutaneous transplantations on the upper back region of male mice. The hepatocarcinoma grew rapidly and was free of necrotic and cirrhotic changes. We are indebted to Dr. Stuart Lindsay for the following histological description of the tumor: Most of the neoplasm was composed of sheets of cells without glandular or tubular formation (Fig. 1). In a few areas, however, the cells appeared in broad, irregular cords (Fig. 2). Little stroma was present. The neoplastic cells resembled hepatic parenchymal cells but were smaller and more pleomorphic. The cytoplasm was relatively sparse, and mitoses were numerous.

The tumors were allowed to grow for 12–14 days after implantation, at which times they were excised for study. The mice rarely survived longer than 3–4 weeks after tumor inoculation. The tumor-bearing as well as the normal mice were 5–6 weeks old when they were killed by cervical fracture. They were fed an adequate commercial stock diet throughout their stay in the laboratory.

Tissue Slice Experiments

Substrates.—Glucose-1-C\textsuperscript{14} and -6-C\textsuperscript{14} were obtained from the National Bureau of Standards, Washington, D.C. Glucose evenly labeled with C\textsuperscript{14} (glucose-E-C\textsuperscript{14}) was prepared by the photosynthetic procedure of Putman and Hassid (47) and was isolated in chromatographically pure form. The equality of labeling of each carbon was confirmed by chemical degradation (10).

Buffer.—Krebs-Henseleit bicarbonate buffer was prepared according to Umbreit et al. (57). The pH of each batch of the aerated (95 per cent O\textsubscript{2}, 5 per cent CO\textsubscript{2}) buffer was between 7.3 and 7.4.

Preparation of slices and incubation procedure.—Slices of liver and hepatoma, prepared free-hand with a razor blade, were first placed in the ice-cold buffer and then carefully washed with this buffer. Only firm tumor tissue was used. Two hundred and fifty mg. of the washed slices, approximately 0.5 mm. thick, were blotted on filter paper and transferred to the main compartment of an incubation flask (13) that contained the labeled substrate in 2.5 ml. of buffer. The incubation flasks were provided with a center well for the collection of respiratory CO\textsubscript{2}. The flasks were gassed for 30 seconds with a mixture of 95 per cent O\textsubscript{2} and 5 per cent CO\textsubscript{2}, then capped with rubber serum stoppers, and incubated for 3 hours, with mechanical shaking, in a water bath maintained at 37°C. At the end of the incubation period 0.4 ml. of 30 per cent KOH was injected through the rubber serum cap into the center well, and immediately thereafter 0.4 ml. of 5 N H\textsubscript{2}SO\textsubscript{4} was injected into the main compartment of the flask. The flasks were shaken for an additional 30 minutes to insure complete absorption of the respiratory CO\textsubscript{2} by the alkali in the center well. Other experimental details are given in Tables 4 and 5.
GLUCOSE-6-PHOSPHATASE AND HEXOSEPHOSPHATE ISOMERASE OF MOUSE LIVER AND HEPATOCARCINOMA

Homogenates were prepared as described in text. Aliquots were taken from the supernatant solution for enzyme assay. The assay system consisted of homogenate, 74.0 \( \mu \)moles of glucose-6-phosphate (dipotassium salt) dissolved in 1.0 ml. of water, and enough citrate buffer to make the final volume 3.0 ml. Incubated at 37 °C for 1 hour in air.

TABLE 1

<table>
<thead>
<tr>
<th>Tissue*</th>
<th>Incubation time (min.)</th>
<th>Inorganic PO₄ released (( \mu )moles)</th>
<th>Fructose-6-PO₄ produced (( \mu )moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>10</td>
<td>15.3</td>
<td>19.6</td>
</tr>
<tr>
<td>Liver</td>
<td>20</td>
<td>38.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Liver</td>
<td>30</td>
<td>42.6</td>
<td>7.0</td>
</tr>
<tr>
<td>Liver</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>10</td>
<td>1.6</td>
<td>24.4</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>20</td>
<td>1.0</td>
<td>24.2</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>30</td>
<td>1.0</td>
<td>24.4</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>60</td>
<td>2.2</td>
<td>24.4</td>
</tr>
</tbody>
</table>

* 1.0 ml. of liver homogenate was used. It yielded 50.4 mg. of dry material. One ml. of hepatoma homogenate was also used, and it yielded 50.5 mg. of dry material.

† A measure of the glucose-6-phosphatase reaction (see text).

‡ A measure of the hexosephosphate isomerase reaction (see text).

washed with copious quantities of water to remove all water-soluble compounds. The slices, along with the glass wool, were then transferred to centrifuge tubes, and the tissue was saponified with sodium ethylate. The fatty acid fractions were isolated in chloroform and assayed for \( ^{14} \)C activity by the method reported earlier (13).

Glycogen-\( ^{14} \)C determinations.—In other experiments washed slices were added to a hot 30 per cent KOH solution. The glycogen was isolated from the mixture, purified by reprecipitation and dialysis, and assayed for radioactivity by the method previously presented (28).

Paper chromatographic analysis of water-soluble compounds.—When chromatographic analysis of the water-soluble compounds was required, enzymatic reactions in the slices were stopped by placing the flask in boiling water for about 1 minute. After the stopped flask was cooled in an ice bath, alkali (30 per cent KOH) was injected into its center well and 0.1 ml. of 1 N HCl into its main compartment. The pH of the medium was thereby reduced below 2, which served to release all its CO₂. The aqueous extract, containing all the water-soluble compounds of the slices and of the incubation medium, was electrolytically desalted and examined by the chromatographic-radioautographic technic described elsewhere (35). The developing solvents used were phenol saturated with water, in the first dimension, and a mixture of n-butanol, acetic acid, and water (200 ml.:44 ml.:100 ml.) in the other. Identification of the \( ^{14} \)C-labeled compounds in this fraction (aqueous extract) and assay of their individual \( ^{14} \)C activity have been described in a previous report (15, 35).

**Determination of the specific activity of recovered glucose at the end of the incubation period.**—The glucose-\( ^{14} \)C areas on the chromatograms were located (35), cut out, and quantitatively eluted with water. Known aliquots of this eluate were assayed for their glucose content by the method of Mendel and Hoogland (44) and for their \( ^{14} \)C activity.

**Homogenate Experiments**

**Preparation of homogenates.**—3.0 gm. of each tissue was homogenized with 20 ml. of 0.1 M citrate buffer (pH 6.5) in a Potter-Elvehjem, all-glass homogenizer, with a tolerance of 0.5 mm. The

TABLE 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Volume of homogenate (ml.)</th>
<th>Inorganic phosphorus released (( \mu )moles)</th>
<th>Fructose-6-phosphate produced (( \mu )moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.1</td>
<td>9.6</td>
<td>16.9</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>0.1</td>
<td>0.5</td>
<td>15.4</td>
</tr>
<tr>
<td>Liver</td>
<td>0.5</td>
<td>33.6</td>
<td>9.8</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>0.5</td>
<td>1.0</td>
<td>18.5</td>
</tr>
<tr>
<td>Liver</td>
<td>1.0</td>
<td>45.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>1.0</td>
<td>1.7</td>
<td>19.0</td>
</tr>
</tbody>
</table>

homogenization was carried out at 0°C. The resulting homogenate was diluted to 40.0 ml. with citrate buffer, and the mixture was centrifuged at 1000 \( \times g \), in a refrigerated centrifuge, for 5 minutes at 0°C. Aliquots of the supernatant solution were taken for enzymatic assay.

**Incubation procedure.**—Varying amounts of the homogenates, as indicated in Tables 1–3, were in-
incubated with 74 µmoles of either dipotassium glucose-6-phosphate or dipotassium fructose-6-phosphate in a total volume of 3.0 ml. The incubation was carried out in air at 37°C. The enzymatic reactions were stopped with 1 ml. of a 10 per cent trichloroacetic acid solution, and the precipitated protein was removed by centrifugation. The resulting supernatant fraction and washings were adjusted to 10 ml. with water.

Glucose-6-phosphatase.—This enzyme was studied by measuring the appearance of inorganic phosphate from added glucose-6-phosphate (40). Two 1-ml. aliquots of the deproteinized solution were taken for determination of inorganic phosphate as described by Fiske and Subbarow (24). The values agreed within 2 per cent.

Products of the glucose-6-phosphatase reaction.—It was shown that the moles of free glucose were approximately equal to the moles of inorganic phosphate released. Thus, the products of the glucose-6-phosphatase reaction in our liver homogenate experiments are glucose and inorganic phosphate.

Hexosephosphate isomerase.—This enzyme was measured in two ways—by the appearance of fructose-6-phosphate when glucose-6-phosphate was added as substrate, and by the disappearance of fructose-6-phosphate when this phosphorylated ketose served as substrate. Two 1-ml. aliquots of the deproteinized solution were taken for determination of fructose as described by Roe (50). A factor of 0.61 was used to convert the Roe values to fructose-6-phosphate (57). The values agreed within 2 per cent.

### Table 3

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Tissue</th>
<th>Homogenate volume (ml.)</th>
<th>Substrate</th>
<th>Phosphorus released (µmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liver</td>
<td>0.5</td>
<td>Glucose-6-phosphate</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td>Liver + hepatocarcinoma</td>
<td>0.5 + 0.5</td>
<td>Glucose-6-phosphate</td>
<td>39.0</td>
</tr>
<tr>
<td>2</td>
<td>Liver</td>
<td>1.0</td>
<td>Glucose-6-phosphate</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>Liver + hepatocarcinoma</td>
<td>1.0 + 1.0</td>
<td>Glucose-6-phosphate</td>
<td>47.0</td>
</tr>
<tr>
<td>3</td>
<td>Liver</td>
<td>1.0</td>
<td>Fructose-6-phosphate</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>Liver + hepatocarcinoma</td>
<td>1.0 + 1.0</td>
<td>Fructose-6-phosphate</td>
<td>25.3</td>
</tr>
</tbody>
</table>

In the experiments with liver homogenates, aliquots of the protein-free filtrate (trichloroacetic acid solution) were deionized with Duolite A-3 and C-4 ion-exchange resins, and the water eluates were evaporated to dryness in vacuo at 40°C. The deionized residues were dissolved in water, and aliquots of the resulting solution were taken for colorimetric analysis of glucose by the method of Mendel and Hoogland (44) and for two-dimensional paper chromatographic analysis as described above. Spraying such chromatograms with benzidine:trichloroacetic acid:95 per cent ethanol (500 mg.:20 ml. of a 20 per cent aqueous solution: 80 ml.) revealed only one carbohydrate spot which was identical in position with that of authentic glucose.

It is conceivable that the production of inorganic phosphate in the experiments with the liver homogenates could be the result of glycogen synthesis from the added glucose-6-phosphate. However, it was shown that the moles of free glucose were approximately equal to the moles of inorganic phosphate released. Thus, the products of the glucose-6-phosphatase reaction in our liver homogenate experiments are glucose and inorganic phosphate.

### RESULTS AND DISCUSSION

#### A. GLUCOSE PRODUCTION FROM ENDogenous SOURCES

Washed slices of host liver and of the hepatocarcinoma were incubated with glucose-E-C<sup>14</sup>. Pure glucose was isolated, by chromatographic procedures, from aqueous extracts prepared from the incubation mixtures. In the case of the liver, as was to be expected, a fall (from 1140 to 220 counts/min/µg) was observed in the specific activity of the glucose isolated at the end of the incubation period, thus demonstrating glucose formation by the liver slices. It is difficult to determine the exact amounts of glucose formed from endogenous sources in these experiments with host liver slices, but the fivefold dilution observed in the specific activities of the glucose isolated at the end of the incubation period can leave no doubt that an extensive formation of this hexose from endogenous sources had occurred.
The specific activity of the glucose was unaltered in the experiments with the hepatoma slices. This demonstrates that the tumor slices did not produce glucose from endogenous sources.

B. GLUCOSE-6-PHOSPHATE AND HEXOSE-PHOSPHATE ISOMERASE

To investigate further the tumor's inability to produce free glucose, glucose-6-phosphatase and hexosephosphate isomerase were compared in homogenates prepared from normal and neoplastic tissues. Table 1 shows that the production of inorganic phosphate from glucose-6-phosphate (through the action of glucose-6-phosphatase) by the hepatocarcinoma C94 was extremely low, whereas the extent of activity of this enzyme in liver homogenates was considerable.

An absence of glucose-6-phosphatase has also been reported in the Novikoff hepatoma by Weber and Cantero (59). It has, however, been shown to be present in the transplanted hepatoma originally induced by chrysochlorin (11).

Hexosephosphate isomerase activity in both liver and tumor homogenates was so high that the equilibrium of the isomerase reaction. The lack of glucose-6-phosphatase to fructose-1,6-diphosphate occurred under these conditions, because ATP was not added. Glucose-6-phosphate is not oxidized when no TPN is added. Thus, the data in Table 1 show that, with increasing incubation times, during the hepatoma experiments, confirms the virtual absence of glucose-6-phosphatase in homogenates of this tissue.

Table 2 shows that, when the amounts of liver homogenate were increased from 0.1 to 1.0 ml., the production of inorganic phosphate from glucose-6-phosphate rose at the expense of glucose-6-phosphate. In similar experiments with hepatoma homogenates, where phosphatase activity was very low, the change in fructose-6-phosphate concentration, in the hepatoma experiments, confirms the virtual absence of glucose-6-phosphatase in homogenates of this tissue.

Table 2 shows that, when the amounts of liver homogenate were increased from 0.1 to 1.0 ml., the production of inorganic phosphate from glucose-6-phosphate rose at the expense of glucose-6-phosphate when no TPN is added. Thus, the data in Table 1 show that, with increasing incubation times, during the hepatoma experiments, confirms the virtual absence of glucose-6-phosphatase in homogenates of this tissue.

The recoveries of C\textsuperscript{14}O\textsubscript{2} from glucose-E-C\textsuperscript{14} were much higher in the experiments with the hepatocarcinoma than in those with liver when the concentration of added glucose was low, but this difference was not observed when the amounts of glucose added to the medium were 50 and 100 \(\mu\)moles.

Whether or not the total carbohydrate utilized by the hepatoma slices was greater than that utilized by the liver slices cannot be decided by the experiments carried out here. The liver slices contained about 2 per cent glycogen, and the possibility of a preferential utilization of this glycogen over the added glucose cannot be ruled out. This would not apply to the tumor slices which, as pointed out below, contained practically no glycogen.

Glycogen formation.—Earlier work has shown that the glycogen content of hepatomas is extremely low (63), and we have confirmed this finding with the hepatocarcinoma C\textsubscript{94}. There was no measurable glycogen formation from glucose-C\textsuperscript{14} by the hepatoma slices under our incubation conditions. On the other hand, liver slices converted about 2 per cent of the added glucose-1-C\textsuperscript{14}, -6-C\textsuperscript{14}, and -E-C\textsuperscript{14} to glycogen.

Weber and Cantero (60) have reported that Novikoff hepatoma does not contain the enzyme phosphoglucomutase, and an absence of this enzyme in the hepatocarcinoma C\textsubscript{94} could explain our failure to demonstrate incorporation of C\textsuperscript{14} into glycogen when slices of that tumor were incubated with glucose-C\textsuperscript{14}.

Hers (29) has shown that, when glucose-1-C\textsuperscript{14} is converted to glycogen by the rat, almost all the C\textsuperscript{14} recovered from the glucose isolated from liver.

Abbreviations: ATP for adenosine triphosphate; TPN for oxidized triphosphopyridine nucleotide; and TPNII for reduced triphosphopyridine nucleotide.
Conversion of glucose to fatty acids.—Only a few reports dealing with lipogenesis in hepatocarcinomas have appeared (3, 12, 65). The incorporation of glucose carbon into fatty acids of the Novikoff hepatocarcinoma has been shown by Abraham et al. (6), that a reliable measure of the operation of these two pathways cannot be obtained from the yields of C14O2 from glucose-1-C14 than from glucose-6-C14 in experiments with a variety of tumor tissues (5, 21, 37, 48, 58, 63), and on the basis of such observations have inferred that a pathway other than the Embden-Meyerhof, presumably the hexosemonophosphate oxidative pathway, operates in these tissues. It was pointed out, however, by Abraham et al. (6), that a reliable measure of the operation of these two pathways cannot be obtained from the yields of C14O2 from glucose-1-C14 and -6-C14. Katz and Wood (36), who have discussed in detail the use of the ratio C14O2 derived from glucose-1-C14 to C14O2 derived from glucose-6-C14 to estimate the two pathways of glucose metabolism, have concluded that there is no simple relation between this C14O2 ratio and the relative proportions of glucose catabolized via the pentose cycle and the Embden-Meyerhof pathways.

Table 4

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glucose added to medium (μmoles)</th>
<th>Added glucose recovered as:</th>
<th>Added glucose recovered in A+B+C (Per cent) (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CO2 (A)</td>
<td>Fatty acids (B)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Per cent) (μmoles)</td>
<td>(Per cent) (μmoles)</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>1</td>
<td>19.0</td>
<td>0.19</td>
</tr>
<tr>
<td>Liver</td>
<td>1</td>
<td>7.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>10</td>
<td>12.2</td>
<td>1.22</td>
</tr>
<tr>
<td>Liver</td>
<td>10</td>
<td>5.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>50</td>
<td>4.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Liver</td>
<td>50</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>100</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>
It has been reported for a variety of tumors that carbon 6 of glucose is a better precursor of lactate, fatty acids, and other triosephosphate-derived compounds than is carbon 1 (2, 12, 37). This was again observed in our experiments with the hepatocarcinoma C954. Thus, it is shown in Table 5 that the fraction of C14 of glucose-1-C14 converted to fatty acids was 50-80 per cent of that observed with glucose-6-C14. In two experiments (mice 3 and 4, Tables 5 and 6), in which both lactate-C14 and C14-labeled fatty acids were isolated from incubation mixtures containing either glucose-1-C14 or -6-C14, the values for the ratio

\[
\frac{\text{C14-fatty acids derived from glucose-1-C14}}{\text{C14-fatty acids derived from glucose-6-C14}}
\]

were about equal to those for the similar ratio for C14-lactate recoveries.

The ratio

\[
\frac{\text{triose derived from glucose-1-C14}}{\text{triose derived from glucose-6-C14}}
\]

has been taken as a direct measure of the participation of the hexosemonophosphate oxidative pathway in glucose metabolism, and a method of calculating the relative extent of operation of the two pathways, based on production by acetyl-CoA units (as measured by fatty acid production) from glucose-1-C14 and -6-C14, has been presented (6). The assumptions underlying this calculation have been stated (4). In this calculation it was assumed that glucose-6-phosphate and fructose-6-phosphate are not in isotopic equilibrium. In view of the high hexosephosphate isomerase activity in the hepatocarcinoma C954, this assumption now seems questionable. Katz and Wood (36) have presented

### Table 5

**Conversion of Glucose Carbons 1 and 6 to CO2 and Fatty Acids by Mouse Liver and Hepatocarcinoma C954 Slices**

250 mg of tissue slices were incubated for 3 hours at 37°C. The medium consisted of 3.5 ml of Krebs-Henseleit bicarbonate buffer (pH 7.3) containing 2 μmoles of the labeled glucose. Gas phase was 95 per cent O2 and 5 per cent CO2.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Tissue</th>
<th>Substrate</th>
<th>Per cent of added C14 recovered as:</th>
<th>Per cent of glucose-derived fatty acids formed via the hexosemonophosphate oxidative pathway as calculated by method of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CO₂</td>
<td>Fatty acids</td>
</tr>
<tr>
<td>1</td>
<td>Liver</td>
<td>Glucose-1-C¹⁴</td>
<td>3.9</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-6-C¹⁴</td>
<td>4.2</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Hepatocarcinoma</td>
<td>Glucose-1-C¹⁴</td>
<td>13.7</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-6-C¹⁴</td>
<td>7.0</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>Liver</td>
<td>Glucose-1-C¹⁴</td>
<td>4.1</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-6-C¹⁴</td>
<td>4.0</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Hepatocarcinoma</td>
<td>Glucose-1-C¹⁴</td>
<td>17.4</td>
<td>1.2</td>
</tr>
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an alternative method of calculation in which they
assume complete isotopic randomization at the
hexosephosphate level. In the last two columns
of Table 5 the participation of the hexosemono-
phosphate oxidative pathway in glucose metabo-
lism, as calculated by the two methods, is pre-
sented. In column 6 the method of Abraham et al.
(4, 6) was used, and in column 7 the results were
obtained by the use of Figure 3 in the paper of
Katz and Wood (36).

These calculations based on recoveries of C\textsuperscript{14}-
labeled fatty acids from glucose-1-C\textsuperscript{14} and -6-C\textsuperscript{14}
confirm our earlier supposition on the routes of
glucose metabolism in mouse liver and hepato-
carcinoma C\textsubscript{954} based on C\textsuperscript{14}O\textsubscript{2} ratios (5).

D. CHROMATOGRAPHIC ANALYSIS OF WATER-SOL-
UBLE COMPOUNDS DERIVED FROM CARBONS 1
AND 6 OF Glucose: Evidence for a Func-
TIONING TRICARBOXYLIC ACID CYCLE IN THE
HEPATOCARCINOMA C\textsubscript{954}

The water-soluble, C\textsuperscript{14}-labeled compounds
formed during the metabolism of glucose-1-C\textsuperscript{14} and
and glucose-6-C\textsuperscript{14} were analyzed by the radio-
chromatographic procedure (15, 35). Typical
radioautographs are shown in Figure 3, and the
percentage of the C\textsuperscript{14} recovered in the various
products is recorded in Table 6.

In the experiments with liver slices, about 70
per cent of the added C\textsuperscript{14} was recovered as glucose
at the end of the incubation period. The corre-
sponding figure for the experiments with the hepato-
carcinoma slices was about 1 per cent. The major
portion of the C\textsuperscript{14} in the hepatocarcinoma experi-
ments was found as lactate.

Weinhouse and his associates have shown that
the tricarboxylic acid cycle operates in a variety
of tumors (61, 62). The results of the chromato-
graphic analysis of the water-soluble C\textsuperscript{14}-labeled
products formed from glucose-1-C\textsuperscript{14} and -6-C\textsuperscript{14} by
the hepatocarcinoma C\textsubscript{954} suggest that this cycle
also operated in this tumor. By this sensitive
method of analysis, compounds which represent
several peripheral reactions of the tricarboxylic
acid cycle were detected without resorting to
trapping methods which may distort the metabo-
lism of a tissue by creating an artificially large
pool. In the present study with the variously
labeled glucoses, we were able to demonstrate sig-
nificant incorporation of C\textsuperscript{14} into dicarboxylic and
tricarboxylic acids of the cycle (Fig. 3). It should
be recalled here that Brown et al. (15), while
studying the oxidative metabolic patterns of this
tumor with C\textsuperscript{14}-labeled acetate, octanoate, and
propionate, also demonstrated the presence of C\textsuperscript{14}
in these polyfunctional acids.

A difference in the metabolic patterns of liver
and hepatocarcinoma C\textsubscript{954} was brought out in con-
nection with the incorporation of glucose carbon
into glutamine. The value for the ratio\textsuperscript{2} of C\textsuperscript{14} re-

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Glucose carbon labeled & Compound on chromatogram & \multicolumn{2}{c|}{Per cent added C\textsuperscript{14} recovered in experiments with:} & \multicolumn{2}{c|}{Hepatocarcinoma slices} \\
\hline
\hline
 & & Liver slices & & & \\
\hline
1 & Glucose & 71.4 & 72.4 & 1.4 & 1.1 \\
6 & Glucose & 70.4 & 73.3 & 0.8 & 0.7 \\
1 & Lactate & 1.7 & 1.1 & 24.0 & 26.0 \\
6 & Lactate & 1.7 & 1.1 & 29.1 & 31.9 \\
1 & Alanine & 0.4 & 0.0 & 1.2 & 2.0 \\
6 & Alanine & 0.7 & 1.7 & 1.7 & 2.3 \\
1 & Aspartate & 0.5 & 0.4 & 0.4 & 0.7 \\
6 & Aspartate & 0.0 & 0.0 & 0.3 & 0.6 \\
1 & Glutamate & 0.7 & 0.5 & 4.0 & 4.7 \\
6 & Glutamate & 0.5 & 1.5 & 5.2 & 5.6 \\
1 & Glutamine & 0.9 & 0.9 & 0.4 & 0.9 \\
6 & Glutamine & 1.2 & 1.6 & 0.4 & 1.7 \\
\hline
\end{tabular}
\caption{Chromatographic Analysis of Water-soluble Compounds Arising from Incubation of Glucose-C\textsuperscript{14} with Mouse Liver and Hepatocarcinoma C\textsubscript{954} Slices}
For experimental details see Table 5 and text
\end{table}
covered in glutamate to that in glutamine in the experiments with liver slices metabolizing glucose-1-C\textsuperscript{14} was 0.7; in the corresponding experiment with tumor slices it was 7.6. In the experiments with glucose-6-C\textsuperscript{14} the values were 0.7 and 8.2 for liver and hepatocarcinoma, respectively. This shift in the ratio reflects the tumor's lowered capacity to convert glutamate to glutamine, an observation made previously by Brown et al. (15) when glucose-E-C\textsuperscript{14} was used as substrate. These observations are consistent with the findings of Roberts et al. (49) that the glutamine levels of tumor tissue are far below those in normal tissue.

**COMMENT**

An interesting property of the hepatocarcinoma C\textsubscript{954} is its high capacity for synthesizing fatty acids from glucose. Since it is difficult to determine whether hepatoma cells are derived from parenchymal liver cells, bile duct epithelium, or other cells of the liver, Pitot and Potter (46) have questioned the validity of comparing the metabolism of a hepatoma with that of liver. For example, on the basis of enzymic studies, they suggest the possibility that the Novikoff hepatoma cell is derived from a cell that resembles bile duct epithelium more than parenchymal liver cells. Since the liver is one of the more active sites of fatty acid synthesis, the high capacity for lipogenesis from glucose observed in the hepatocarcinoma C\textsubscript{954} favors the parenchymal liver cell as the origin of this tumor.

The fatty acid-synthesizing capacity of the hepatocarcinoma C\textsubscript{954} may reflect increased amounts of acetyl-CoA made available by the high glucose utilization. However, in view of the demonstration of an extensive operation of the hexosemonophosphate oxidative pathway in the tumor, it might be argued that the augmented fatty acid synthesis resulted from TPNH generated in the oxidation of glucose-6-phosphate and gluconic acid-6-phosphate. Even though it is well established that TPNH is an absolute requirement for the oxidation of glucose-6-phosphate and gluconic acid-6-phosphate, but, according to Glock and McLean (37), this coenzyme (over 90 per cent of it) exists in tissues in the reduced form (TPNH). Thus, the extent of operation of the hexosemonophosphate oxidative pathway in a tissue might depend upon the presence in that tissue of a mechanism for oxidation of TPNH. This is borne out by the experiments of Hers (30) demonstrating that glucose utilization by rat liver slices via the hexosemonophosphate oxidative pathway can be increased by addition of substrates (glucosone and glucuronolactone) that oxidize TPNH, thereby making TPN available. Fatty acid synthesis can also be considered a mechanism for regenerating TPN from TPNH.

**ACKNOWLEDGMENTS**

We are indebted to Dr. J. Katz for valuable discussions in the course of this work.

**REFERENCES**


Abraham et al.—Glucose Utilization by Mouse Liver and Hepatocarcinoma C947


46. Pito, H. C., and Potter, V. R. An Enzymic Study of the Cellular Origin of the Dunning and the Novikoff Hepa-


54. ———. Studies on the Relationship Between Glucose Oxidation and Intermediary Metabolism. II. The Role of Glucose Oxidation in Lipogenesis in Diabetic Liver. Ibid., pp. 1196–1201.


60. ———. Studies on the Relationship Between Glucose Oxidation and Intermediary Metabolism. II. The Role of Glucose Oxidation in Lipogenesis in Diabetic Liver. Ibid., pp. 1186–1201.


Fig. 3.—Chromatographic analysis of water-soluble compounds derived from carbons 1 and 6 of glucose. A, liver slices incubated with glucose-l-C\textsuperscript{14}; B, hepatocarcinoma slices incubated with glucose-l-C\textsuperscript{14}; C, liver slices incubated with glucose-6-C\textsuperscript{14}; D, hepatocarcinoma slices incubated with glucose-6-C\textsuperscript{14}.
A Comparison of Glucose Utilization by Mouse Liver and by the Transplantable Hepatocarcinoma C954

S. Abraham, I. L. Chaikoff and P. Cady


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