INSULIN AND THE FATE OF PYRUVATE IN THE DIABETIC LIVER*

BY M. J. OSBORN, I. L. CHAIKOFF, AND J. M. FELTS

(From the Division of Physiology of the University of California School of Medicine, Berkeley, California)

(Received for publication, June 13, 1951)

A previous report dealt with the action of insulin on the fate of the 3 carbon atoms of lactate in the liver of the alloxan-diabetic rat (1). It was shown that insulin decreased the oxidation of the α- and β-carbons of added lactate to CO₂ and stimulated, in a pronounced manner, the incorporation of these 2 carbon atoms, as an intact unit, into fatty acids. In assessing the significance of these findings, we assumed that the fate of the α- and β-carbons of lactate was identical with that of the corresponding carbons of pyruvate. The validity of our assumption with respect to the α-carbon is borne out by the findings presented here. It is shown that the fate of the α-carbon of pyruvate in the liver of the insulin-treated and untreated alloxan-diabetic rat parallels that of the corresponding carbon of lactate.

EXPERIMENTAL

Treatment of Animals—Rats of the Long-Evans strain used in this study were maintained as previously described (1). A record of their diabetic history is shown in Table I.

Five of the ten diabetic rats were injected subcutaneously, at 48 and 24 hours before sacrifice, with 50 units of protamine zinc insulin (Lilly) per kilo of body weight. Exactly 2 hours before sacrifice, each of the injected rats also received, subcutaneously, 10 units of unmodified insulin (Lilly).

Samples of heart blood for the determination of blood sugar were taken from each rat immediately before sacrifice.

Preparation of Substrates—The pyruvate-2-Cl⁴ was obtained from the Texas Research Foundation as an aqueous solution of the lithium salt. The lithium pyruvate-2-Cl⁴ was passed over a cation adsorption column, washed through with distilled water, and quantitatively recovered as the free acid. The sodium salt was obtained by titration with a standard solution of dilute sodium hydroxide to pH 7.3.

Carrier sodium pyruvate was prepared from freshly distilled pyruvic acid (2) by titration with NaOH to pH 7.3. Paper chromatograms of samples of the sodium pyruvate-2-C⁴ and the carrier sodium pyruvate

* Aided by a grant from Eli Lilly and Company.
were prepared by the method of Buchanan et al. (3), and color was developed by spraying with a solution containing equal parts of saturated picric acid and 1 N NaOH. \( R_F \) values for samples of the radioactive substrate and the carrier sodium pyruvate were in close agreement. A radioautograph showed the areas of \( ^{14} \text{C} \) activity to be identical with those that developed color.

The \( ^{14} \text{C} \) content of the sodium pyruvate-2-\( ^{14} \text{C} \) was determined as described by Felts et al. (1) for \( ^{14} \text{C} \)-lactate.

50 \( \mu \text{M} \) (5.5 mg.) of sodium pyruvate with a \( ^{14} \text{C} \) activity of \( 3.3 \times 10^5 \) c.p.m. were present in each incubation flask in Experiment 1. The con-

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Sex</th>
<th>Alloxan injection</th>
<th>Animal weight</th>
<th>Degree of diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Route</td>
<td>Dose*</td>
<td>When injected with alloxan</td>
</tr>
<tr>
<td>F.</td>
<td>Subcutaneous</td>
<td>180</td>
<td>200</td>
<td>180</td>
</tr>
<tr>
<td>1</td>
<td>Subcutaneous</td>
<td>100</td>
<td>150</td>
<td>183</td>
</tr>
<tr>
<td>2</td>
<td>Subcutaneous</td>
<td>450 (4)</td>
<td>285</td>
<td>220</td>
</tr>
<tr>
<td>3</td>
<td>Subcutaneous</td>
<td>400 (4)</td>
<td>230</td>
<td>216</td>
</tr>
<tr>
<td>4</td>
<td>Subcutaneous</td>
<td>400 (4)</td>
<td>310</td>
<td>254</td>
</tr>
<tr>
<td>5</td>
<td>Subcutaneous</td>
<td>450 (4)</td>
<td>300</td>
<td>324</td>
</tr>
<tr>
<td>6</td>
<td>Intravenous</td>
<td>44</td>
<td>135</td>
<td>199</td>
</tr>
<tr>
<td>7</td>
<td>Subcutaneous</td>
<td>175</td>
<td>170</td>
<td>300</td>
</tr>
<tr>
<td>8</td>
<td>Intravenous</td>
<td>30</td>
<td>140</td>
<td>162</td>
</tr>
<tr>
<td>9</td>
<td>Intravenous</td>
<td>30</td>
<td>140</td>
<td>220</td>
</tr>
</tbody>
</table>

* Single injection unless otherwise indicated by the figures in parentheses.
† Determined on whole blood about 1 week before sacrifice.

The \( ^{14} \text{C} \) concentration of pyruvate was varied in Experiment 2 (Figs. 1 and 2). This substrate was always added in 0.5 cc. of a solution made isotonic with NaCl. All samples were counted with a thin end window Geiger-Müller counter which had an efficiency of 5 to 7 per cent.

**Preparation of Liver Slices and Incubation Procedure**—The rats were sacrificed by cervical fracture, and their livers quickly excised. Liver slices approximately 0.5 mm. thick were prepared free-hand with a razor blade, and placed in a bicarbonate (4) or phosphate (5) buffer. The whole liver and the slices were kept at a low temperature during the slicing period. Approximately 500 mg. of slices were gently blotted on filter paper, weighed on a torsion balance, and placed in the incubation flask.

The incubation flask described by Chernick et al. (6) was used. The liver slices were placed in its main compartment, together with 4.5 cc. of
bicarbonate or phosphate buffer and 0.5 cc. of the substrate solution. The incubation procedure has been described elsewhere (6).

Methods of Analysis—The collection of C\(^{14}\)O\(_2\), the isolation and determin-

### Table II

**Experiment 1. Incorporation of Sodium Pyruvate-\(2-C^{14}\) into Fatty Acids and CO\(_2\) by Livers of Alloxan-Diabetic Rats, Untreated and Treated with Insulin**

500 mg. of liver slices were incubated for 3 hours at 37.5°. In the flasks with bicarbonate buffer the gas phase was 95 per cent O\(_2\) and 5 per cent CO\(_2\), while in those with phosphate buffer the gas phase was O\(_2\). In the latter flasks CO\(_2\)-free NaOH was introduced into the center well at zero time in order to provide continuous absorption of the expired CO\(_2\). Duplicate flasks were incubated, and each figure reported below is the average of two separate determinations.

<table>
<thead>
<tr>
<th>Pretreatment with protamine zinc insulin*</th>
<th>Blood sugar at sacrifice†</th>
<th>Liver composition</th>
<th>Bicarbonate buffer</th>
<th>Phosphate buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats: days</td>
<td>units per kg. per day</td>
<td>mg. per cent</td>
<td>Liver weight</td>
<td>Total fatty acids$</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>848</td>
<td>9.7</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>50</td>
<td>193</td>
<td>17.5</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>944</td>
<td>12.0</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>50</td>
<td>313</td>
<td>20.7</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>805</td>
<td>11.0</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>50</td>
<td>214</td>
<td>21.5</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>604</td>
<td>11.6</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>50</td>
<td>111</td>
<td>17.1</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>652</td>
<td>7.9</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>50</td>
<td>115</td>
<td>12.4</td>
</tr>
</tbody>
</table>

* In addition, Rats 2, 4, 6, 8, and 10 received 10 units of unmodified insulin exactly 2 hours before sacrifice.
† Determined on whole blood.
§ Calculated from the weight of BaCO\(_3\) after combustion of fatty acid-C\(^{14}\). In slices taken from the pool approximately 30 minutes after sacrifice.

In order to determine the total amount of CO\(_2\) expired, approximately 500 mg. of slices were incubated in phosphate buffer in an oxygen atmosphere. The expired CO\(_2\) was absorbed on filter paper saturated with CO\(_2\)-free NaOH and collected in a CO\(_2\)-free atmosphere, at the end of the incubation period, in the usual manner. An aliquot was titrated with standard HCl to the phenolphthalein and to the brom cresol green end points, and the amount of CO\(_2\) present was calculated from the titration difference.
Results

Experiment 1. Fatty Acid and Carbohydrate Contents of Livers of Untreated and Insulin-Treated, Diabetic Rats—The fatty acid content of the livers of diabetic rats was about 2 per cent of the wet weight of the liver, while that of the livers of insulin-treated animals ranged from 4.5 to 7.5 per cent (Table II).
In the injected group, the total carbohydrate content of the liver was increased, in one case rising to 10.4 per cent. The average increase was 60 per cent above the diabetic level. All samples were taken from a pool of slices approximately 30 minutes after the rats were sacrificed.

**Incubation of Liver Slices with Pyruvate-2-C^{14} in Bicarbonate Buffer**—From 32 to 40 per cent of the added C^{14} was incorporated into CO_{2} by liver slices prepared from diabetic rats. Considerable variability was observed in the conversion of the α-carbon of pyruvate into fatty acids by liver slices of these animals, the values ranging from 0.8 to 8 per cent of the added C^{14}.

The CO_{2} expired by the liver slices of insulin-treated, diabetic rats contained only 6 to 13 per cent of the added C^{14}, but from 26 to 48 per cent of the isotope was recovered in fatty acids. Moreover, since the livers of the injected rats were 80 to 90 per cent heavier than those of untreated diabetics of essentially identical body weight, the increase in synthetic capacity of the whole liver of the insulin-treated animals was even more striking than our data would indicate.

**Incubation of Liver Slices with Pyruvate-2-C^{14} in Phosphate Buffer**—The recoveries of C^{14}O_{2} and fatty acid-C^{14} were, on the whole, lower in the experiments carried out in the phosphate medium than in the bicarbonate buffer. But the stimulation in fatty acid synthesis, as well as the reduction in C^{14}O_{2} recoveries under the influence of insulin, is again evident.

In contrast to the reduced C^{14}O_{2} recoveries observed in the livers of insulin-injected rats, total CO_{2} expired by these livers increased from about 5 mg. in the uninjected to 6 to 7 mg. in the injected rats (Table II).

**Experiment 2. Concentration Study**—The following concentrations were tested: 25, 50, 75, and 100 μM of sodium pyruvate in 5 cc. of incubation medium. The results are shown in Figs. 1 and 2. The decreased C^{14}O_{2} recoveries, as well as the increased fatty acid-C^{14} recoveries in the insulin-treated rats, were observed at all of these concentrations. The curves indicate that, under the conditions of our experiment, the utilization of pyruvate is roughly proportional to its concentration in the medium.

**DISCUSSION**

The conversion of the α-carbon of added pyruvate to CO_{2} and its utilization for fatty acid synthesis by liver slices were parallel to those observed for the corresponding carbon of lactate (1). However, about twice as much C^{14}O_{2} and fatty acid-C^{14} were derived from pyruvate-2-C^{14} as from lactate-2-C^{14}. Since D,L-lactate was used in our earlier study, this difference can be explained by the relative specificity of lactic dehydrogenase for the L stereoisomer of lactate.

C^{14}O_{2} recoveries, in the experiments with the phosphate as well as the bicarbonate buffer, were severely reduced by insulin. The finding that the
livers of the insulin-treated rats contained much more carbohydrate and fat than did those of the untreated rats raised the question whether a greater dilution of the pyruvate and (or) C-2 pools could account for the difference in the C¹⁴O₂ recoveries observed in the experiments with these two types of livers. In this connection it should be noted that a dilution effect was not observed in C¹⁴O₂ recoveries when the concentrations of exogenous pyruvate were varied from 25 to 100 μM per flask (Fig. 1). The demonstration that the C¹⁴ recoveries were directly proportional to the concentration of the added substrate indicates that wide variations in the size of the pyruvate pool have little effect upon the recoveries of C¹⁴O₂ and fatty acid-C¹⁴ in either the diabetic or insulin-treated diabetic liver.

The reduction in the conversion of the pyruvate-2-C¹⁴ to CO₂ induced by insulin was not accompanied by a similar change in total CO₂ production by the liver. Indeed, the quantities of CO₂ produced by 500 mg. of liver slices obtained from insulin-treated rats were somewhat higher than those produced by the same amount of hepatic tissue of untreated diabetic rats (see the last column, Table II).¹ The effect of insulin on pyruvate oxidation is, therefore, not the result of a depression in the over-all CO₂ production by the liver.

The dramatic increase in the synthesis of fatty acids at the expense of the added pyruvate and the concomitant reduction in its oxidation observed under the influence of insulin provide confirmation of the view we expressed earlier on the rôle of insulin in shifting the fate of the C-2 fragment arising from this intermediate. Since this shift in the proportions of the C-2 fragments utilized for oxidative and synthetic reactions involved no decrease in total CO₂ production² in the liver, it would appear that the production of C-2 fragments was increased in the liver of the insulin-treated rat.

SUMMARY

1. The fate of pyruvate-2-C¹⁴ was studied in surviving liver slices prepared from (1) alloxan-diabetic rats and (2) alloxan-diabetic rats that were injected with insulin for 2 days before sacrifice.

2. From 30 to 40 per cent of the α-carbon was oxidized to CO₂ by liver slices of diabetic rats. Previous insulin injections severely reduced the oxidation of this carbon. This insulin effect was not accompanied by a decrease in total mg. of CO₂ produced (phosphate buffer).

¹ The CO₂ measurements were carried out in the phosphate buffer.

² Injections of insulin for 2 days increased the size of the liver (Table II). Thus the total CO₂ produced by the whole liver in the insulin-treated rat was far greater than that of the diabetic rat.
3. Liver slices of the diabetic rat incorporated from 1 to 8 per cent of the pyruvate-2-C\textsuperscript{14} into fatty acids. The recoveries observed with the livers of the hormone-treated rats ranged from 26 to 48 per cent, a 5-fold increase.

4. The above C\textsuperscript{14} values were observed in experiments carried out in a bicarbonate buffer, but similar insulin effects were also found in experiments with a phosphate buffer.

5. The conclusion is drawn that insulin shifts the metabolism of the C-2 intermediate derived from added pyruvate from an oxidative fate to one involving synthesis.

BIBLIOGRAPHY

INSULIN AND THE FATE OF
PYRUVATE IN THE DIABETIC LIVER
M. J. Osborn, I. L. Chaikoff and J. M. Felts


Access the most updated version of this article at http://www.jbc.org/content/193/2/549.citation

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/193/2/549.citation.full.html#ref-list-1