INCORPORATION OF ACETATE CARBON INTO GLUCOSE
BY LIVER SLICES FROM NORMAL AND
ALLOXAN-DIABETIC RATS*

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In a previous report (1) we demonstrated the incorporation of palmitic
acid carbon into glucose in the intact rat and estimated the quantitative
significance of this process in the normal and alloxan-diabetic animal. Even
though the values obtained represented the lower limit of the extent of
this process, the results indicated that conversion of fatty acids to
carbohydrate is a demonstrable metabolic process.

The present investigation deals with the conversion of acetate to glu-
cose by surviving rat liver slices. In experiments with normal livers, 3
to 6 per cent of the C¹⁴ was recovered as glucose from acetate-1-C¹⁴ and
10 to 19 per cent from acetate-2-C¹⁴. Under similar conditions, diabetic
livers incorporated into glucose 5 to 8 and 14 to 24 per cent, respectively,
of the added C¹⁴. Incorporation of acetate carbon into glucose by liver
was greatly reduced when diabetic rats were injected with insulin.

An explanation of our results is offered, based on the effect of isotope
dilution upon the limits of isotope patterns attained by compounds in the
tricarboxylic acid cycle.

EXPERIMENTAL

Treatment of Animals—Rats of the Long-Evans strain were used. Dia-
betes was induced by a single intravenous injection of a 5 per cent solu-
tion of alloxan monohydrate (50 mg. per kilo of body weight). From the
time of the injection until sacrifice (about 3 weeks), daily records were kept
of food and water consumption. Weight changes and glucose excretion
in urine were measured periodically. The diabetic rats used in this study
had fasting blood sugars in excess of 200 mg. per cent approximately 1
week before they were sacrificed. A record of their diabetic histories is
given in Table I.

Both normal and diabetic rats were maintained on an adequate stock
diet until 3 days before they were sacrificed. During the last 3 days
they were fed a mixture containing 58 per cent glucose. Three of the dia-

* Aided by a grant from the American Cancer Society as recommended by the
Committee on Growth of the National Research Council.
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; 2 hours before sacrifice each was again injected subcutaneously with 10 units of unmodified insulin (Lilly).

Radioactive Substrates—Acetate was used as substrate, labeled in either the carboxyl or methyl carbon. We are indebted to Dr. B. M. Tolbert for the preparation of these compounds.

Incubation Procedure—The rats were sacrificed by cervical fracture. Liver slices approximately 0.5 mm. thick were prepared free-hand. 1 gm. of slices was gently blotted on filter paper and placed in the main compartment of the incubation flask described by Chernick et al. (2), together with 5.0 ml. of a Krebs-Henseleit bicarbonate buffer and 0.5 ml. of the substrate solution. 50 μM of sodium acetate having a C\textsuperscript{14} activity of about 100,000 c.p.m. were present in each incubation flask. The acetate was added directly to the flask in a solution made isotonic with NaCl. Incubation was carried out for 3 hours.

Analytical Procedures. C\textsuperscript{14}O\textsubscript{2}—The incubation flask was designed to permit the collection of CO\textsubscript{2} at the end of the experiment. The determination of the C\textsuperscript{14} content of this CO\textsubscript{2} has been described elsewhere (2). The contents of two incubation flasks were combined for analysis of CO\textsubscript{2} and glucose.

Glucose-C\textsuperscript{14}—The acidified medium from two incubation flasks was transferred to a 50 ml. centrifuge tube, and the flasks and liver slices were rinsed repeatedly with small portions of hot distilled water to extract soluble ma-

### Table I

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Insulin treatment</th>
<th>Sex</th>
<th>Weight when sacrificed (gm.)</th>
<th>Duration of diabetes (days)</th>
<th>Typical amount of urinary glucose excreted in 24 hrs. (gm.)</th>
<th>Fasted blood sugar (mg. per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>None</td>
<td>M.</td>
<td>190</td>
<td>30</td>
<td>9.1</td>
<td>262</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
<td>165</td>
<td>25</td>
<td>9.2</td>
<td>330</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>F.</td>
<td>155</td>
<td>14</td>
<td>7.6</td>
<td>210</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>M.</td>
<td>160</td>
<td>21</td>
<td>8.9</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>&quot;</td>
<td>120</td>
<td>21</td>
<td>5.4</td>
<td>555</td>
</tr>
<tr>
<td>11</td>
<td>Insulin†</td>
<td>F.</td>
<td>163</td>
<td>22</td>
<td>7.7</td>
<td>381</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>M.</td>
<td>165</td>
<td>21</td>
<td>6.1</td>
<td>269</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>&quot;</td>
<td>178</td>
<td>39</td>
<td>5.1</td>
<td>326</td>
</tr>
</tbody>
</table>

* Determined approximately 1 week before the rat was sacrificed.
† 50 units per kilo of body weight for 2 days.
terials from the slices. The washings were added to the tube. After centrifugation, the supernatant solution was deproteinized with Ba(OH)$_2$ and ZnSO$_4$ according to Somogyi (3). Small aliquots of the deproteinized solution were used for determination of glucose by the ceric sulfate method of Hassid (4). The remainder was concentrated to about 2 ml. by vacuum distillation, transferred to a centrifuge tube, and 3 drops of phenylhydrazine (100 mg.) and 12 drops (0.4 ml.) of glacial acetic acid were added. The mixture was heated on the steam bath for 10 to 15 minutes to form the glucosazone. After being cooled to induce crystallization, the phenylglucosazones were washed with three 12 ml. portions of distilled water and two portions of warm ether. The glucosazone crystals were suspended in 2 to 3 ml. of cold alcohol, stirred lightly, and about 10 ml. of cold water were added. The crystals were separated by centrifugation, and dried for 24 to 48 hours in vacuo at 45-50°. After subjection to microscopic examination, the glucosazones were oxidized to CO$_2$ and the latter mounted as BaCO$_3$ on filter paper plates, as described previously (5).

The specific activity of the glucose present in the incubation medium plus the hot water extracts of the slices was determined from the specific activity of the BaCO$_3$ obtained by combustion of the phenylglucosazone. The amount of glucose, measured by its reducing value, multiplied by its specific activity gives the amount of Cl$^4$ in glucose. This latter value, expressed as per cent of the acetate-C$^{14}$ added to the medium, is a measure of glucose synthesis from acetate.

Results

Slices prepared from each liver were incubated in four flasks; two contained methyl-labeled and the other two carboxyl-labeled acetate. The results of individual experiments obtained with normal livers, diabetic livers, and insulin-treated diabetic livers are recorded in Table II. The data are summarized in Table III. The C$^{14}$ content of glycogen isolated according to Good, Kramer, and Somogyi (6) was found to be negligible (less than 0.1 per cent of the added C$^{14}$).

Normal Livers—On the average, 5 and 15 per cent, respectively, of the added C$^{14}$ were recovered as glucose from carboxyl-labeled and methyl-labeled acetate. C$^{14}$O$_2$ recoveries were much higher when the substrate was CH$_3$C$^{14}$OONa than they were with C$^{14}$H$_2$COONa (34 and 15 per cent, respectively).

Diabetic Livers—The recoveries of glucose-C$^{14}$ and C$^{14}$O$_2$ observed with diabetic livers appear to be of about the same order of magnitude as those found with normal liver slices. The glucose-C$^{14}$ recoveries from both acetates were somewhat higher than those observed with normal liver slices.
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*Insulin-Treated Diabetic Livers*—The glucose-C\(^{14}\) and C\(^{14}\)O\(_2\) recoveries with this series of three livers were much lower than those found in the experiments with normal or diabetic livers. Thus, only about 1 and 3 per cent, respectively, of the added C\(^{14}\) were incorporated into glucose from carboxyl- and methyl-labeled acetate, values approximately one-fifth of those observed in corresponding experiments with diabetic livers. Average C\(^{14}\)O\(_2\) recoveries from CH\(_3\)C\(^{14}\)OONa and C\(^{14}\)H\(_3\)COONa were about 20 and 6 per cent, respectively. These values represent about one-half of the average C\(^{14}\)O\(_2\) obtained in diabetic rats.

**DISCUSSION**

When C\(^{14}\)H\(_3\)COONa was used as substrate, about 18 per cent of the added C\(^{14}\) was recovered in glucose in the experiments with the diabetic

1 The terms synthesis, glucose formation, and similar expressions throughout this paper do not necessarily imply a net gain of glucose carbon.
liver slices, and 15 per cent in the experiments with normal liver slices (Table III). Thus, normal and diabetic rat livers have the ability to incorporate acetate carbon into glucose to a considerable extent. If it is assumed that 2-carbon fragments produced by fatty acid oxidation behave similarly to acetate as regards incorporation into glucose, then the results of the present study offer further support for the view that some glucose synthesis occurs from fatty acids.

The position of the label in the added acetate had a pronounced effect on the amount of \( ^{14}C \) recovered in glucose as well as in \( CO_2 \). This was

<table>
<thead>
<tr>
<th>State of rats</th>
<th>Per cent of added acetate-( ^{14}C ) recovered per 2 gm. liver slices*</th>
<th>Ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In glucose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>From carboxyl carbon</td>
<td>From methyl carbon</td>
</tr>
<tr>
<td>Normal</td>
<td>4.8 ± 1.0</td>
<td>14.9 ± 3.2</td>
</tr>
<tr>
<td>Diabetic</td>
<td>6.8 ± 1.1</td>
<td>18.2 ± 3.2</td>
</tr>
<tr>
<td>Insulin-treated diabetic</td>
<td>0.97 ± 0.18</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>In ( CO_2 )</td>
<td>From carboxyl carbon</td>
</tr>
<tr>
<td>Normal</td>
<td>34.3 ± 3.6</td>
<td>15.1 ± 2.2</td>
</tr>
<tr>
<td>Diabetic</td>
<td>39.4 ± 7.6</td>
<td>12.2 ± 1.8</td>
</tr>
<tr>
<td>Insulin-treated diabetic</td>
<td>19.9 ± 4.7</td>
<td>6.3 ± 2.1</td>
</tr>
</tbody>
</table>

* The values are given with their mean deviation.
† The values given here are the averages of those appearing in Table II.
‡ Since this value of 2.4 was obtained values for the \( CO_2 \) ratio of twenty-two additional normal rats have been compiled and a value of 2.8 with a standard deviation of 0.16 has been obtained for the over-all \( CO_2 \) ratio. The standard deviation of individual values is 0.82.

true for livers from normal, diabetic, and insulin-treated diabetic rats. In each experiment the average value for the ratio

\[
\frac{^{14}C \text{ incorporated into glucose from } CH_4C^{14}OONa}{^{14}C \text{ incorporated into glucose from } C_2H_4COONa}
\]

referred to here as the glucose ratio, is approximately 3 (Table III), though some values ranged from 2 to 4. The ratio

\[
\frac{^{14}C \text{ recovered in } CO_2 \text{ from carboxyl-labeled acetate}}{^{14}C \text{ recovered in } CO_2 \text{ from methyl-labeled acetate}}
\]

referred to here as the \( CO_2 \) ratio, ranged from 1.6 to 3.6; the averages were 2.8, 3.2, and 3.1 for the normal, diabetic, and insulin-treated diabetic
rat, respectively (Table III). A CO₂ ratio of 2 to 4 was observed earlier by Felts et al. (7). An explanation of these ratios is offered below.

The continuous introduction of labeled acetate of constant specific activity into the tricarboxylic acid cycle results in the building up of definite isotope concentrations of C¹⁴ in the carbon atoms of each compound in the cycle. In this paper we are concerned particularly with the equilibrium isotope pattern of oxalacetate (OAA) since this determines the values for the CO₂ and glucose ratios. Below we shall show that the stable isotope pattern of compounds in the tricarboxylic acid cycle is a definite function of the degree to which the labeled molecules in the cycle are diluted.

**Table IV**

The building up of the stable isotope configuration in OAA with successive turns of the tricarboxylic acid cycle for the case of 60 per cent isotope dilution. The figures in the table represent specific activities (to two decimal places) of the carbon atoms of OAA on the basis that 100 counts enter as acetate-C¹⁴ per turn.

<table>
<thead>
<tr>
<th>Position of label in acetate</th>
<th>No. of turns of cycle completed</th>
<th>Specific activity of oxalacetate carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>COOH</td>
</tr>
<tr>
<td>Methyl carbon</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.96</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.00</td>
</tr>
<tr>
<td>Carboxyl carbon</td>
<td>1</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.00</td>
</tr>
</tbody>
</table>

by unlabeled molecules entering the cycle. Therefore, a particular value for the CO₂ or glucose ratio corresponds to a fixed amount of isotope dilution.

For example, let us assume that in each turn of the cycle a ratio of labeled to unlabeled OAA of 4:6 (designated as 60 per cent isotope dilution) is maintained; then the stable isotope pattern of OAA will be reached in the manner shown in Table IV. If 100 c.p.m. enter as C¹⁴H₃COOH, 50 c.p.m. will appear in each of the 2 center carbon atoms of OAA at the end of the first turn, assuming isotope equilibration at the succinic acid stage. 60 per cent isotope dilution will reduce this figure to 20 (see Table IV, first turn). Utilizing this OAA in the next turn with an influx of another 100 c.p.m. of C¹⁴H₃COOH, and again taking into account 60 per cent isotope dilution, gives specific activities of 4.00, 24.00, 24.00, 4.00 for carbons 1, 2, 3, and 4 of OAA (Table IV, second turn). If this process is continued, it
is quite evident that a definite limit is approached, after a few turns, for methyl-labeled acetate and after the first turn for C-1-labeled acetate. The values of the limits in Table IV are given to two decimal places.

The values of these limits for various degrees of isotope dilution can be obtained from the expressions²

\[ a \left( \frac{100 - x}{100 + x} \right) \]  
\[ a \left( \frac{(100 - x)^2}{200(100 + x)} \right) \]

where \( a \) represents the counts entering each turn as methyl-labeled acetate and \( x \) the degree of isotope dilution in per cent. Equation 1 gives the specific activities of the 2 center carbons of OAA and Equation 2 that of the carboxyl carbons. The expression for the specific activities of the carboxyl carbons of OAA obtained with C⁴ entering as carboxyl-labeled acetate is

\[ \frac{\alpha (100 - x)}{2} \]

An abbreviated tricarboxylic acid cycle, Fig. 1, shows the distribution of C⁴ entering as C¹⁴H₃COOH for the case of 60 per cent dilution after equilibrium is reached. It will be seen that the isotope patterns of all compounds remain invariant. The rate of flow through the tricarboxylic acid cycle is taken as unity, 60 per cent isotope dilution occurring only at the OAA stage. This degree of dilution corresponds to an influx of OAA at a relative rate of 1.5 (Fig. 1). While Fig. 1 shows all the dilution occurring at the OAA level, the ratios are unaffected if dilution occurs simultaneously at various points in the cycle, provided the net dilution is 60 per cent.

By the use of Equations 1 and 2, the CO₂ and glucose ratios, as defined above, become \((100 + x)/(100 - x)\) and \((500 - x)/(100 + x)\), respec-

² Equations 1 and 2 are the sum of the converging power series

\[ \alpha \left[ \frac{1}{2} \left( \frac{100 - x}{100} \right) + \left( \frac{1}{2} \right)^2 \left( \frac{100 - x}{100} \right)^2 + \ldots + \left( \frac{1}{2} \right)^n \left( \frac{100 - x}{100} \right)^n \right] \]

and

\[ \alpha \left[ \frac{1}{2} \left( \frac{100 - x}{100} \right)^2 + \frac{1}{2} \left( \frac{100 - x}{100} \right)^3 + \ldots + \left( \frac{1}{2} \right)^n \left( \frac{100 - x}{100} \right)^n \right] \]

respectively as the number of turns, \( n \), of the tricarboxylic acid cycle that have been completed approaches infinity. These series are a general mathematical statement of the process required to obtain the limiting isotope pattern of OAA for any degree of isotope dilution.
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tively. (The derivation of all ratios is indicated in Table V.) Fig. 1 shows that the isotope pattern of OAA determines not only the CO₂ and glucose ratios, but also the isotope pattern of glucose. Since carbons 1, 2, 5, and 6 of glucose have identical specific activities, as do carbons 3 and 4 (8, 9), the isotope pattern of glucose can be expressed as the ratio of the specific activity of carbon 1 to that of carbon 3. The use of Equations 1 and 2 leads to a value of 200/(100 - x) for this ratio. It can be shown that these ratios are independent of the rate of conversion of OAA to pyruvate.

Fig. 2 is a plot of these three ratios (glucose, CO₂, and glucose-labeling ratio) as a function of isotope dilution. The CO₂ curve shows that the experimentally observed CO₂ ratios, which ranged from 2.0 to 3.6 (with the exception of one low value), correspond to 35 to 56 per cent isotope dilution. The glucose ratios of 2 to 4 obtained experimentally are consistent with a wide range (20 to 90 per cent) of isotope dilution due to the relative flatness of the glucose curve. The experimental values for the glucose ratio for the insulinized diabetic animals are quite variable because of the sensitivity of this ratio to experimental error. This is due to the relatively lower level of C¹⁴ in glucose found with these rats. In one experiment glucose was degraded by a chemical method previously described (9), and the ratio (specific activity of C-1 carbon)/(specific activity of C-3 carbon)

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8 This ratio is referred to here as the glucose-labeling ratio.
TABLE V  
Derivation of Expressions Used in Text

The degree of isotope dilution is designated by $x$.

<table>
<thead>
<tr>
<th>Expression</th>
<th>Specific activities of OAA carbon</th>
<th>Expression in terms of</th>
<th>Equations 1-3</th>
<th>Isotope dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$CO_2$ ratio</strong></td>
<td>Carboxyl C of OAA from C-1 acetate</td>
<td></td>
<td>Eq. 3</td>
<td>$100 + x$</td>
</tr>
<tr>
<td></td>
<td>Carboxyl C of OAA from C-2 acetate</td>
<td></td>
<td>Eq. 2</td>
<td>$100 - x$</td>
</tr>
<tr>
<td><strong>Glucose ratio</strong></td>
<td>2 (\left(\text{center C of OAA from C-2 acetate}\right) + \left(\text{carboxyl C of OAA from C-2 acetate}\right))</td>
<td></td>
<td>Eq. 3 + (\frac{x}{100 - x}) (Eq. 3)</td>
<td>$500 - x$</td>
</tr>
<tr>
<td></td>
<td>Carboxyl C of OAA from C-1 acetate</td>
<td></td>
<td>Eq. 3</td>
<td>$100 + x$</td>
</tr>
<tr>
<td><strong>Glucose-labeling ratio</strong></td>
<td>Center C of OAA from C-2 acetate</td>
<td></td>
<td>Eq. 1</td>
<td>$200$</td>
</tr>
<tr>
<td></td>
<td>Carboxyl C of OAA from C-2 acetate</td>
<td></td>
<td>Eq. 2</td>
<td>$100 - x$</td>
</tr>
<tr>
<td><strong>$CO_2$ to glucose ratio</strong></td>
<td>2 (\left(\text{carboxyl C of OAA from C-1 acetate}\right) + \left(\text{rate of flow of OAA to pyruvate}\right)\left(\text{carboxyl C of OAA from C-1 acetate}\right))</td>
<td></td>
<td>2(Eq. 3) + (\frac{x}{100 - x}) (Eq. 3)</td>
<td>$200 - x$</td>
</tr>
<tr>
<td>for C-1 acetate</td>
<td>(\text{Rate of flow of OAA to pyruvate}) (\times) (\left(\text{carboxyl C of OAA from C-1 acetate}\right))</td>
<td></td>
<td>(\frac{x}{100 - x}) (Eq. 3)</td>
<td>$x$</td>
</tr>
<tr>
<td></td>
<td>Carboxyl C of OAA from C-2 acetate</td>
<td></td>
<td>2(Eq. 2) + (\frac{x}{100 - x}) (Eq. 2)</td>
<td>$20,000 - 300x + x^2$</td>
</tr>
<tr>
<td><strong>$CO_2$ to glucose ratio</strong></td>
<td>2 (\left(\text{carboxyl C of OAA from C-2 acetate}\right) + \left(\text{rate of flow of OAA to pyruvate}\right)\left(\text{carboxyl C of OAA from C-2 acetate}\right))</td>
<td></td>
<td>(\frac{x}{100 - x}) [2(Eq. 1) + Eq. 2]</td>
<td>$500x - x^2$</td>
</tr>
<tr>
<td>for C-2 acetate</td>
<td>(\text{Rate of flow of OAA to pyruvate}) (\left[\frac{1}{2}\left(\text{center C of OAA from C-2 acetate}\right) + \left(\text{carboxyl C of OAA from C-2 acetate}\right)\right])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fraction of OAA C derived from acetate C</strong></td>
<td>(\sum_{n=0}^{\infty} \frac{100 - x}{2} + \frac{(100 - x)^2}{(2^2 \times 100)} + \cdots + \frac{(100 - x)^n}{(2^n \times 100) \times (100 - x)^{-1}})</td>
<td></td>
<td>$\frac{100(100 - x)}{100 + x}$</td>
<td></td>
</tr>
</tbody>
</table>
was found to be 3.3. This value corresponds to 40 per cent isotope dilution (Fig. 2). The range of isotope dilution consistent with our three experimentally determined ratios is 35 to 56 per cent. Our data can thus be explained on the basis of a simple model of the tricarboxylic acid cycle and 35 to 56 per cent isotope dilution. Such a degree of isotope dilution leaves ample carbon available for synthetic purposes. For a given degree of isotope dilution, \( x \), the OAA present after equilibrium has been reached can be shown to contain a definite fraction of acetate carbon, given by the expression \( 100(100 - x)/(100 + x) \). For 35 to 56 per cent isotope dilution, 48.2 to 28.2 per cent of the total carbon present in OAA, and therefore in glucose arising from this OAA, is acetate carbon.

Insulin has no effect on the glucose, \( CO_2 \), and glucose-labeling ratios, but does affect still another ratio, \( i.e., (C^{14} in CO_2)/(C^{14} in glucose) \). Of
course this ratio can be formulated for each of the two positions of the label in acetate. The general expressions for these ratios are \( \frac{200 - x}{x} \) and \( \frac{20,000 - 300x + x^2}{500x - x^2} \) for C-1- and C-2-labeled acetate, respectively. To derive these expressions it is necessary to assume that all of the extra carbon made available by isotope dilution, due to the influx of unlabeled compounds into the tricarboxylic acid cycle, is used for glucose synthesis. This assumption assigns the largest possible value to the denominator of the "CO\(_2\) to glucose" ratio. The above expressions should therefore represent the lowest possible values for the CO\(_2\) to glucose ratio based on our model. Corresponding experimental values are indeed higher for the livers of normal, diabetic, and insulinized diabetic rats.

While all our ratios are independent of the rate of flux of compounds through the tricarboxylic acid cycle, the total amount of any compound produced is dependent on this rate of flux. The marked reduction in the incorporation of acetate carbon into glucose under the influence of insulin observed here may be due, at least in part, to a shift of acetate to fatty acid synthesis described previously (7). This would reduce the amount of labeled acetate available for entry into the tricarboxylic acid cycle, thereby reducing the total amount of C\(^{14}\) available for incorporation into CO\(_2\) as well as glucose. To explain our experimental finding that C\(^{14}\)O\(_2\) recoveries in diabetic insulinized animals were reduced by a factor of 2, while C\(^{14}\) recoveries in glucose were diminished by a factor of 6 (Table III), it is necessary to postulate a large decrease in the rate of entry of carbon into glucose. Therefore a continuous flow of carbon out of the tricarboxylic acid cycle should be available for synthetic purposes other than glucose formation. These questions are at present under further investigation.

We wish to thank Mr. Samuel Abraham for the determination of the C\(^{14}\) contents of carbons 1, 3, and 6 of the glucose sample.

**SUMMARY**

1. Liver slices prepared from normal, diabetic, and insulin-treated diabetic rats were incubated with C\(^{14}\)H\(_3\)COONa and CH\(_3\)C\(^{14}\)OONa, and the C\(^{14}\) recoveries in glucose and CO\(_2\) were measured.

2. Normal and diabetic liver slices incorporated about 5 per cent of the added C\(^{14}\) from carboxyl-labeled acetate into glucose, and 15 to 20 per cent from methyl-labeled acetate.

3. Insulin injections in diabetic rats resulted in a pronounced reduction in the glucose-C\(^{14}\) recovery from both CH\(_3\)C\(^{14}\)OONa and C\(^{14}\)H\(_3\)COONa.

4. The C\(^{14}\)O\(_2\) recoveries observed with carboxyl-labeled acetate were 2 to 3 times greater than those with methyl-labeled acetate. Large dif-
ferences were not noted between normal and diabetic livers, but $\text{C}^{14}\text{O}_2$ recovery from both substrates was reduced by one-half in livers of insulin-treated diabetic rats.

5. Values of approximately 3 were found for the ratio

$$\frac{\text{C}^{14} \text{ incorporated into glucose from C-2-labeled acetate}}{\text{C}^{14} \text{ incorporated into glucose from C-1-labeled acetate}}$$

in experiments with normal, diabetic, and insulinized diabetic rat livers, and values of 2 to 3 for the ratio

$$\frac{\text{C}^{14} \text{ in CO}_2 \text{ from C-1-labeled acetate}}{\text{C}^{14} \text{ in CO}_2 \text{ from C-2-labeled acetate}}$$

6. A scheme is presented showing how dilution of labeled molecules in the tricarboxylic acid cycle by unlabeled molecules entering the cycle makes acetate carbon available for synthetic purposes. Recovery of $\text{C}^{14}$ in glucose is explained in terms of this process. The ratios in (5) are explained in terms of stable isotope patterns occurring in the tricarboxylic acid cycle in keeping with this scheme. It is shown that these patterns are dependent on isotope dilution effects.

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INCORPORATION OF ACETATE CARBON INTO GLUCOSE BY LIVER SLICES FROM NORMAL AND ALLOXAN-DIABETIC RATS
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