Metabolism of [2-14C]Acetate and Its Use in Assessing Hepatic Krebs Cycle Activity and Gluconeogenesis*

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To examine the fate of the carbons of acetate and to evaluate the usefulness of labeled acetate in assessing intrahepatic metabolic processes during gluconeogenesis, [2-14C]acetate, [2-14C]ethanol, and [1-14C]ethanol were infused into normal subjects fasted 60 h and given phenyl acetate. Distributions of 14C in the carbons of blood glucose and glutamate from urinary phenylacetylglutamine were determined. With [2-14C]acetate and [2-14C]ethanol, carbon 1 of glucose had about twice as much 14C as carbon 3. Carbon 2 of glutamate had about twice as much 14C as carbon 1 and one-half to one-third as much as carbon 4. There was only a small amount in carbon 5. These distributions are incompatible with the metabolism of [2-14C]acetate being primarily in liver. Therefore, [2-14C]acetate cannot be used to study Krebs cycle metabolism in liver and in relationship to gluconeogenesis, as has been done. The distributions can be explained by: (a) fixation of 14CO2 from [2-14C]acetate in the formation of the tricarboxylic acid cycle and glutamate in liver and (b) the incorporation of 14C into glutamate in a second site, proposed to be muscle. [1,3-14C]Acetone formation from the [2-14C]acetate does not contribute to the distributions, as evidenced by the absence of 14C in carbons 2-4 of glutamate after [1-14C]ethanol administration.

A model of Krebs cycle metabolism and gluconeogenesis has been described and tested (1). Relative flow rates and carbon exchange in the Krebs cycle were calculated from the distribution of 14C in the carbon of glutamate from urinary phenylacetylglutamine after administering [3-14C]lactate and phenyl acetate. Comparing the distributions of 14C in blood glucose and the glutamate and their specific activities provided evidence for the distribution in glutamate reflecting that in hepatic a-ketoglutarate.

[2-14C]Acetate has been proposed as a substrate for determining the correction factor for carbon exchange in the Krebs cycle in liver during gluconeogenesis (2-7) and has been used in studies in vivo in rats (8) and dogs (9) and humans (10-13). The purpose of this study was to investigate the fate of acetate by determining the distribution of 14C from [2-14C]-acetate in the carbons of glucose from blood and of glutamate from urinary phenylacetylglutamine under the same conditions as for [3-14C]lactate. This allows a direct comparison of the fate of the carbons of acetate and lactate and tests the validity of using [2-14C]acetate, as has been done (8-11, 13).

EXPERIMENTAL PROCEDURES

Subjects—Six healthy women (ages 25-41 yr and weighing 54-75 kg) were studied. They consumed a diet containing at least 200 g of carbohydrate for at least 3 days before fasting. None were taking any medication. The experimental protocol was approved by the Human Investigation Committees at Huddinge University Hospital and University Hospitals of Cleveland. Informed consent was obtained from each subject.

Procedure—The subjects were studied after 60 h of fasting as previously described for [3-14C]lactate administrations (1). Each subject ingested 1.6 or 1.9 g of sodium phenyl acetate at 10-15-min intervals for a total of 4.8 or 5.7 g (30 or 36 mmol). Each portion was dissolved in Coca-Cola Light® (Fripps Brewery, Brümma, Sweden) to mask its taste. At the time the second portion of phenyl acetate was ingested, an infusion of a 14C-labeled compound through a peripheral vein was begun at a constant rate and was continued for 6 h. Four subjects were given [2-14C]acetate, one [2-14C]ethanol, and one [1-14C]ethanol. Sixty ml of blood was drawn from another peripheral vein every 1.5 h, and urine was collected in 1.5-h intervals throughout the period of infusion.

The 14C-labeled substrates were given in trace amounts; 40 μCi was infused into each subject. Each subject, throughout the 6-h of infusion, was encouraged to drink 240 ml of water every hour.

Materials—Sodium phenyl acetate was prepared as previously described (1). Sodium [2-14C]acetate, [2-14C]ethanol, and [1-14C]ethanol were purchased from Amersham International (Buckinghamshire, England). [2-14C]Acetate gave a single peak of 14C with the mobility of acetate on HPLC using an Aminex HPX-87H column (Bio-Rad) at room temperature with 0.01 N H2SO4 as solvent. [1-14C]Ethanol and [2-14C]ethanol were, beyond the evidence of purity from the manufacturer, subjected to HPLC using a fermentation monitoring column (Bio-Rad) with 0.002 N H2SO4 as solvent. [1-14C]Ethanol had 5% 14C-labeled impurity that was detected by the absorption of 14C from urine (1). The specific activities of β-hydroxybutyrate and urea were isolated from urine (1). The specific activities of β-hydroxybutyrate and urea were determined (1). Glutamic acid from phenylacetylglutamine was isolated and degraded to obtain the 14C distribution of 14C in each of its carbons. Each of the glutamic acid degrated had between 2000 and 5000 dpm of 14C.

Analyses—Plasma glucose concentration was determined enzymatically (14). From the remainder of the blood samples, glucose was isolated and degraded (15, 16) to obtain the percent distribution of 14C in each of its carbons. Each of the glucose degradated had between 2000 and 5000 dpm of 14C. From phenylacetylglutamine, β-hydroxybutyrate, and urea were isolated from urine (1). The specific activities of β-hydroxybutyrate and urea were determined (1). Glutamic acid from phenylacetylglutamine was isolated and degraded to obtain the percent distribution of 14C in each of its carbons (1). Between 1.8 and 3.2 mmol of glutamic acid was isolated from each sample, and between 9000 and 40,000 dpm was used in each degradation.

Calculations—To correct the distributions in glutamate and glucose...
for incorporation via fixation of $^{14}$CO$_2$, formed from the [2-$^{14}$C]acetate, recourse was made to the method previously reported (1). The expressions used were:

$$[^{14}C]Glutamate_a = \frac{[^{14}C]urea_a}{[^{14}C]urea_b} \times [^{14}C]glutamate_b \tag{1}$$

$$[^{14}C]Glucose_a = \frac{[^{14}C]urea_a}{[^{14}C]urea_b} \times [^{14}C]glucose_b \tag{2}$$

where $[^{14}C]glutamate_a$ and $[^{14}C]glutamate_b$ are the $^{14}$C specific activities of glutamate due to $^{14}$CO$_2$ fixation on administering [2-$^{14}$C]acetate and [2-$^{14}$C]bicarbonate, respectively; $[^{14}C]glucose_a$ and $[^{14}C]glucose_b$ are the corresponding specific activities of glucose; and $[^{14}C]urea_a$ and $[^{14}C]urea_b$ are the corresponding specific activities of urea.

RESULTS

The percent incorporations of $^{14}$C into the carbons of glutamate (designated by K with subscripts) from urinary phenylacetylglutamine excreted by each subject are recorded in Table I. With [2-$^{14}$C]acetate and [2-$^{14}$C]ethanol administration, carbon 4 had the most $^{14}$C (41.0-52.3%), Carbons 2 and 3 had similar amounts (15.8-24.0%), and carbon 1 had about half the $^{14}$C activity of carbons 2 and 3 (9.4-15.8%). Carbon 5 had 0.2-5.9%. [1-$^{14}$C]Ethanol administration resulted in $^{14}$C incorporation in carbons 1 and 5, with ~3 times as much in carbon 5 as in carbon 1.

Distributions of $^{14}$C in the carbons of blood glucose (designated by G with subscripts) from the subjects given [2-$^{14}$C]acetate and [2-$^{14}$C]ethanol are shown in Table 1. Carbons 1, 2, 3, and 6 have similar percentages (17.4-22.2%). Carbons 3 and 4 have somewhat more than half the $^{14}$C incorporations of the other carbons (10.1-12.5%).

Specific activities of urinary urea, glutamate from urinary phenylacetylglutamine, and expired $^{14}$CO$_2$ from the subjects given [2-$^{14}$C]acetate and [2-$^{14}$C]ethanol are shown in Table II. Carbons 3 and 6 have similar percentages (14.9-21.7%). Carbons 1, 2, and 3 have about half the $^{14}$C activity of carbons 4 and 5 (19.8-29.6%). Carbons 4 and 5 have somewhat more than half the $^{14}$C incorporations of the other carbons (20.0-27.9%).

Specific activities of urinary glucose, and expired $^{14}$CO$_2$ from the subjects given [2-$^{14}$C]acetate and [2-$^{14}$C]ethanol are recorded in Table III. Recorded in the last column of Table III are data from Table I for subjects given [2-$^{14}$C]bicarbonate in the fasted state.

Table IV gives the mean $^{14}$C distributions in glutamate from urine of the subjects given [2-$^{14}$C]acetate and the distributions corrected for the incorporation of $^{14}$C from $^{14}$CO$_2$ from the [2-$^{14}$C]acetate. The average $^{14}$C distributions in blood glucose from the subjects given [2-$^{14}$C]acetate and the distributions corrected for $^{14}$CO$_2$ incorporation are given in Table V. Corrections for $^{14}$CO$_2$ incorporation in glutamate and glucose were obtained using Equations 1 and 2 under "Experimental Procedures" and the data from Table III as shown in Ref. 1. Corrections were 38, 29, and 21% in carbon 1 of glutamate in the 1.5-3, 3-4.5, and 4.5-6 h urine samples and 60 and 61% in carbons 3 and 4 of glucose at 4.5 and 6 h, respectively.

Plasma glucose concentration (mean ± S.E.), after 60 h of fasting, was 3.8 ± 0.4 mmol/liter and, after 6 h of infusion, 3.2 ± 0.2 mmol/liter.

DISCUSSION

Based on the distribution of $^{14}$C in glutamate from the glutamine conjugate of phenyl acetate, [3-14C]lactate has proven to be a satisfactory substrate for estimating the rates of the different reactions involved in the Krebs cycle and in gluconeogenesis (Table VIII of Ref. 1). These estimations were possible because apparently the major portion of the [3-$^{14}$C]lactate yielding those distributions was metabolized directly in liver. Therefore, the distribution in the glutamate was representative of events occurring in liver per se. The situation is very different with [2-$^{14}$C]acetate.

We propose, as illustrated in Fig. 1, that in contrast to the metabolism of [3-$^{14}$C]lactate, there is considerable oxidation of [2-$^{14}$C]acetate to $^{14}$CO$_2$ in muscle and that a substantial portion of labeled glutamate is formed from [2-$^{14}$C]acetate in muscle, converted to glutamine, conjugated with phenyl acetate, and excreted in urine. Thus, the distribution of $^{14}$C in the glutamate is not representative of the events occurring in liver per se, but a combination of events occurring in peripheral tissue and liver. It is for this reason that the $^{14}$C pattern of the glutamate (α-ketoglutarate) is not a reflection of the $^{14}$C pattern of the oxalacetate of liver from which glucose is formed via P-enolpyruvate. Furthermore, estimates that have been made of carbon exchange in the Krebs cycle in liver in vivo using [2-$^{14}$C]acetate are not valid. The reasons for these conclusions are discussed below.

Fate of Carbons of Acetate—An abbreviated scheme of the Krebs cycle and of gluconeogenesis in liver is shown in Fig. 1 (for a more complete scheme, see Fig. 1 of Ref. 1). The carbons of oxalacetate are designated by O, α-ketoglutarate by K, fumarate by F, P-enolpyruvate by E, pyruvate by P, lactate by L, and glucose by G. Certain relationships should prevail if [2-$^{14}$C]acetate is metabolized in liver per se in a closed system, i.e., with no carbons other than those of the [2-$^{14}$C] acetyl-CoA and unlabeled CO$_2$ entering the cycle. If the spe-

<table>
<thead>
<tr>
<th>Subject</th>
<th>14C-Compound infused</th>
<th>Urine collection</th>
<th>14C in carbons K</th>
<th>Recovery</th>
<th>K/Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.E.</td>
<td>[2-$^{14}$C]Acetate</td>
<td>1.5-3</td>
<td>9.7</td>
<td>17.3</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5-6</td>
<td>9.4</td>
<td>21.5</td>
<td>24.0</td>
</tr>
<tr>
<td>A.J.</td>
<td>[2-$^{14}$C]Acetate</td>
<td>1.5-3</td>
<td>10.1</td>
<td>16.7</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5-6</td>
<td>10.0</td>
<td>22.8</td>
<td>21.7</td>
</tr>
<tr>
<td>M.T.</td>
<td>[2-$^{14}$C]Acetate</td>
<td>3-4.5</td>
<td>11.8</td>
<td>19.7</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5-6</td>
<td>13.1</td>
<td>21.1</td>
<td>22.0</td>
</tr>
<tr>
<td>K.H.</td>
<td>[2-$^{14}$C]Acetate</td>
<td>3-4.5</td>
<td>13.8</td>
<td>18.9</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5-6</td>
<td>15.8</td>
<td>19.5</td>
<td>23.0</td>
</tr>
<tr>
<td>M.L.H.</td>
<td>[2-$^{14}$C]Ethanol</td>
<td>4.5-6</td>
<td>12.5</td>
<td>22.6</td>
<td>20.5</td>
</tr>
<tr>
<td>U.H.</td>
<td>[1-$^{14}$C]Ethanol</td>
<td>0-3</td>
<td>22.7</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-6</td>
<td>28.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Value is sum of K$_2$ + K$_3$ + K$_5$.  

| Table I | Distribution of 14C in the carbons of glutamate from urinary phenylacetylglutamine | | | | |
Metabolism of Acetate

**TABLE II**

<table>
<thead>
<tr>
<th>Subject</th>
<th>14C-Compound infused</th>
<th>Time sampled</th>
<th>%</th>
<th>%</th>
<th>Recovery</th>
<th>G1/G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.E.</td>
<td>[2,14C]Acetate</td>
<td>4.5</td>
<td>18.7</td>
<td>19.3</td>
<td>11.6</td>
<td>19.8</td>
</tr>
<tr>
<td>A. J.</td>
<td>[2,14C]Acetate</td>
<td>4.5</td>
<td>19.5</td>
<td>18.6</td>
<td>10.2</td>
<td>12.0</td>
</tr>
<tr>
<td>M. T.</td>
<td>[2,14C]Acetate</td>
<td>4.5</td>
<td>18.7</td>
<td>18.7</td>
<td>12.1</td>
<td>11.6</td>
</tr>
<tr>
<td>K. H.</td>
<td>[2,14C]Acetate</td>
<td>6</td>
<td>18.6</td>
<td>18.8</td>
<td>11.1</td>
<td>11.9</td>
</tr>
<tr>
<td>M. L. H.</td>
<td>[2,14C]Ethanol</td>
<td>4.5</td>
<td>19.4</td>
<td>18.0</td>
<td>10.1</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* Corrected for loss of G6 in the degradation procedure.

**TABLE III**

Specific activities of urinary urea, glutamate from urinary phenylacetylglutamine, urinary β-hydroxybutyrate, blood glucose, and expired CO2 from fasted subjects given [2-14C]acetate and 14C bicarbonate.

<table>
<thead>
<tr>
<th>Compound measured</th>
<th>Time sampled</th>
<th>Labeled compounda</th>
<th>dpm/μmol</th>
<th>%</th>
<th>%</th>
<th>Recovery</th>
<th>K2/K1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>0-1.5</td>
<td>0.04 ± 0.01 (4)</td>
<td>0.59 ± 0.19 (4)</td>
<td>1.5-3</td>
<td>0.49 ± 0.13 (3)</td>
<td>3.51 ± 1.20 (4)</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>3-4.5</td>
<td>1.56 ± 0.26 (4)</td>
<td>7.34 ± 0.88 (4)</td>
<td>1.5-3</td>
<td>4.56 ± 0.36 (4)</td>
<td>10.5 ± 0.86 (4)</td>
<td>2.9</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0-1.5</td>
<td>3 ± 1 (3)</td>
<td>3.2 ± 0.5 (4)</td>
<td>1.5-3</td>
<td>9 ± 3 (3)</td>
<td>4.5 ± 0.6 (4)</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>3-4.5</td>
<td>24 ± 5 (3)</td>
<td>4.5 ± 0.6 (4)</td>
<td>3-4.5</td>
<td>48 ± 3 (4)</td>
<td>5.1 ± 0.6 (4)</td>
<td>2.2</td>
</tr>
<tr>
<td>β-Hydroxybutyrate</td>
<td>3-4.5</td>
<td>41 (1)</td>
<td>12.8 ± 1.5 (4)</td>
<td>4.5-6</td>
<td>43 ± 9 (3)</td>
<td>12.1 ± 2.5 (4)</td>
<td>1.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.5</td>
<td>44 ± 4 (4)</td>
<td>29 ± 3 (4)</td>
<td>4.5-6</td>
<td>54 ± 4 (4)</td>
<td>30 ± 3 (4)</td>
<td>2.3</td>
</tr>
<tr>
<td>CO2</td>
<td>1</td>
<td>3 (3)</td>
<td>43 ± 12 (4)</td>
<td>2</td>
<td>7 (1)</td>
<td>52 ± 9 (4)</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10 (1)</td>
<td>49 ± 3 (4)</td>
<td>3</td>
<td>10 (1)</td>
<td>47 ± 5 (4)</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>16 (1)</td>
<td>47 ± 3 (3)</td>
<td>5</td>
<td>16 (1)</td>
<td>47 ± 3 (3)</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>18 (1)</td>
<td>50 ± 3 (3)</td>
<td>6</td>
<td>18 (1)</td>
<td>50 ± 3 (3)</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**TABLE IV**

Mean distribution of 14C in the carbons of glutamate from urinary phenylacetylglutamine from subjects given [2-14C]acetate and those distributions corrected for incorporation of 14CO2.

<table>
<thead>
<tr>
<th>Time sampled</th>
<th>14C in carbons</th>
<th>K2/K1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5-3</td>
<td>9.9</td>
<td>17.0</td>
</tr>
<tr>
<td>1.5-3 corrected</td>
<td>6.1</td>
<td>17.7</td>
</tr>
<tr>
<td>3-4.5</td>
<td>12.8</td>
<td>19.3</td>
</tr>
<tr>
<td>3-4.5 corrected</td>
<td>9.1</td>
<td>20.1</td>
</tr>
<tr>
<td>4.5-6</td>
<td>12.1</td>
<td>21.3</td>
</tr>
<tr>
<td>4.5-6 corrected</td>
<td>9.5</td>
<td>21.9</td>
</tr>
</tbody>
</table>

**TABLE V**

Mean distribution of 14C in the carbons of blood glucose from subjects given [2-14C]acetate and those distributions corrected for incorporation of 14CO2.

<table>
<thead>
<tr>
<th>Time sampled</th>
<th>14C in carbons</th>
<th>G1/G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>18.8</td>
<td>18.4</td>
</tr>
<tr>
<td>4.5 corrected</td>
<td>21.9</td>
<td>21.5</td>
</tr>
<tr>
<td>6</td>
<td>18.5</td>
<td>18.8</td>
</tr>
<tr>
<td>6 corrected</td>
<td>21.8</td>
<td>22.2</td>
</tr>
</tbody>
</table>

* All values are given as mean ± S.E. with the numbers in parentheses representing number of subjects.

† Data are from Ref. 1.

The specific activity of carbon 2 of acetyl-CoA is set to 100 on repeated cycling, the relative distribution of 14C in oxalacetate will be O1 = 50, O2 = 100, O3 = 100, and O4 = 50 and in α-ketoglutarate K1 = 50, K2 = 100, K3 = 100, K4 = 100, and K5 = 0, thus the ratio K2/K1 and K4/K3.

When gluconeogenesis occurs (the system is open), pyruvate is carboxylated to oxalacetic acid, which is converted to P-enolpyruvate, the precursor of glucose. The ratios of incorporations of 14C from [2-14C]acetate to carbon 1 of α-ketoglutarate (K2/K1) and carbon 1 to carbon 3 of glucose (G1/G6) are a function of the rate of pyruvate carboxylation relative to Krebs cycle flux. These ratios can be <2 in only two circumstances, because there is 14CO2 fixation by pyruvate and because of [1-14C]acetyl-CoA formation. The only way [1-14C]acetyl-CoA can be formed is if the [2,3-14C]oxalacetate is converted P-enolpyruvate to [2,3-14C]pyruvate, which is then decarboxylated to [1,2-14C]acetyl-CoA. 14C from [1-14C]acetyl-CoA would then be incorporated via the cycle into carbon 1 of α-ketoglutarate and into carbon 3 of glucose. Under all circumstances, K2 = K3 and G2 = G3. If there is complete isotopic equilibration of oxalacetate with fumarate, K2/K1 will equal G1/G6. The greater the rate of pyruvate carboxylation relative to Krebs cycle flux, the greater the K2/K1 and K4/K3 ratios.

Distributions of 14C from [2-14C]Acetate in Glucose and Glutamate—In the 60-h fasted human, gluconeogenesis occurs (10), and there is minimal decarboxylation of pyruvate (1). The low rate of pyruvate decarboxylation is further evidenced by the small percentage of 14C in carbon 5 compared to carbon 4 of glutamate (Table I). Carbons 1 and 2 of acetyl-CoA are the precursors of carbons 5 and 4 of α-ketoglutarate. Therefore, there was minimal formation of [1-14C]acetyl-CoA from the [2-14C]acetate. Nevertheless, G1/G6 ratios were <2 (1.5–

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* Data are from Ref. 1. 
† All values are given as mean ± S.E. with the numbers in parentheses representing number of subjects. 
§ Corrected for loss of G6 in the degradation procedure.

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The low rate of pyruvate decarboxylation is further evidenced by the small percentage of 14C in carbon 5 compared to carbon 4 of glutamate (Table I). Carbons 1 and 2 of acetyl-CoA are the precursors of carbons 5 and 4 of α-ketoglutarate. Therefore, there was minimal formation of [1-14C]acetyl-CoA from the [2-14C]acetate. Nevertheless, G1/G6 ratios were <2 (1.5–
obtained by dividing \( G_r \) into the average of \( G_1 \) and \( G_2 \). The glucose would be if isotopic equilibration were complete is that ratio to estimate carbon exchange in the Krebs cycle.

When \([3-14C]\)acetate was given to 60-h fasted humans, I\(^{14}C\) in blood glucose was 1.9 (Table II). When the distributions are corrected for \( ^{14}CO_2 \) fixation, the ratios increase to near 5 (Table V), in keeping with Krebs cycle metabolism and the occurrence of gluconeogenesis.

The \( G_1/G_3 \) ratios of near 2 that we have observed on administering [2-\(^{14}C\)]acetate are similar to ratios other investigators have found on administering carbon 2 labeled acetate or its equivalent. Ratios of between 1.6 and 3.0 were found in blood glucose from normal animals and in glucose from urine from diabetic animals given [2-\(^{14}C\)]acetate (9, 17, 18), [2-\(^{13}C\)]acetate (19), [2-\(^{14}C\)]palmitate (17), and [6-\(^{13}C\)]palmitate (20), the \( ^{14}C \)-labeled palmitates being converted to [2-\(^{13}C\)]acetyl-CoA in their oxidation. Ratios of 2.7–3.3 have been reported on administering [2-\(^{14}C\)]acetate to humans (10, 11). Hetenyi et al. (8) gave 19 fasted rats [2-\(^{14}C\)]acetate, and the average of the ratios in glucose from 12 of the rats was 3.9. They used that ratio to estimate carbon exchange in the Krebs cycle. However, the ratios in glucose from the other seven rats were not used because they were “unaccountably low, below 2.2.”

The ratios of the distributions of \(^{14}C\) in glutamate (\( K_2/K_1 \)) were also frequently <2 (1.3–2.3) (Table I), and as noted, \( K_2/K_1 \) would be expected to be 2 for a closed system. Also as noted, \( K_2/K_0 \) would be expected to be 1 rather than 2–3 (Table I). Similar distributions of label to those in Table I were found in glutamate from the liver of one rat given [2-\(^{14}C\)]acetate (19) and rats given [2-\(^{14}C\)]acetate (21). When the distributions in glutamate in Table I were corrected for \( ^{14}CO_2 \) fixation, the \( K_2/K_0 \) ratios increased to between 2.3 and 2.9 (Table IV). However, those ratios are still lower than the corrected \( G_1/G_3 \) ratios of 4.8–5.2 for glucose (Table V). In the 60-h fasted human, there is extensive equilibration between oxalacetate and fumarate (1). In this circumstance, if the glucose and glutamate were from a single oxalacetate pool, \( K_2/K_0 \) should have been similar to \( G_1/G_3 \).

Comparisons of Distributions to Those for [3-\(^{14}C\)]Lactate—When [3-\(^{14}C\)]lactate was given to 60-h fasted humans, \( ^{14}C \) in carbon 1 of glucose was \(~1.2\) times that in carbon 2. This reflected the extensive equilibration between oxalacetate and fumarate. A good approximation of what the \( G_1/G_3 \) ratio in glucose would be if isotopic equilibration were complete is obtained by dividing \( G_1 \) into the average of \( G_1 + G_2 \), i.e. \((G_1 + G_2)/2)G_3/(G_7) \).

If [3-\(^{14}C\)]lactate and [2-\(^{14}C\)]acetate were metabolized in the same site, as depicted in the model, with isotopic equilibration being extensive, the \((G_1 + G_3)/2)G_3 \) ratio observed with [3-\(^{14}C\)]lactate should then have been essentially the same as the \( G_1/G_3 \) ratio observed with [2-\(^{14}C\)]acetate. The ratios were markedly different. The ratios without correction for \( ^{14}CO_2 \) fixation were 1.5–1.9 from [2-\(^{14}C\)]acetate (Table II), compared to 5.1–7.4 from [3-\(^{14}C\)]lactate (calculated from Table II of Ref. 1). Ratios were 4.8–7.8 in subjects given [3-\(^{14}C\)]lactate after an overnight fast (22).

\( ^{14}CO_2 \) fixation contributed in a much greater proportion to the incorporations into carbons 3 and 4 of glucose when [2-\(^{14}C\)]acetate was substrate than when [3-\(^{13}C\)]lactate was substrate. This is apparent from the 3 times greater \( ^{14}C \) specific activity in urinary urea from acetate (Table III) than from lactate (Table IV of Ref. 1), whereas the specific activity of blood glucose was 3 times greater from lactate than from acetate. This is evidence that acetate is metabolized in a site other than lactate and that the site contributes in very significant measure to the formation of \( ^{14}CO_2 \). Fixation of \( ^{14}CO_2 \) from [2-\(^{14}C\)]acetate has been assumed to be insignificant (7, 9, 10). On correction for \( ^{14}CO_2 \) fixation, the ratios in glucose observed with [2-\(^{14}C\)]acetate (Table V) approached those observed with [3-\(^{13}C\)]lactate. Furthermore, when liver slices were incubated with [2-\(^{14}C\)]acetate (17, 23) and hepatocytes with [3-\(^{13}C\)]lactate (24) and the distribution of label in glucose was determined, the \( G_1/G_3 \) ratios were between 3.9 and 6.0, also similar to the ratios obtained on giving [3-\(^{13}C\)]lactate in vivo. This is evidence that if [2-\(^{14}C\)]acetate were metabolized solely in liver in vivo, the distributions in glucose would be similar to those observed with [3-\(^{13}C\)]lactate.

Rognstad (18) recently reported a comparison of the distribution in glucose on administering [2-\(^{14}C\)]acetate and [3-\(^{13}C\)] lactate to rats. Since the \( G_1/G_3 \) ratio in glucose from acetate was different from that from lactate, i.e. 2.5 as compared to 4.9, he concluded that the data obtained using [2-\(^{14}C\)]acetate are not applicable to the determination of gluconeogenesis in vivo and that acetate was metabolized in a site other than liver. Our results are then in agreement with those conclusions. However, he gave acetate and lactate in bolus injections and killed the rats 10 min later. Under those conditions, unless steady state was achieved or changes in the specific activity of pyruvate from lactate paralleled those from acetyl-CoA formed from the acetate, the distributions from acetate...
and lactate would probably have been different, even if acetate and lactate were metabolized at the same site.

**Explanation for Distributions Obtained with [2-14C]Acetate**—The distributions of 14C in glucose then represent the sum of the metabolism of [2-14C]acetate in one site, liver, and the fixation of 14CO2 formed from the [2-14C]acetate primarily in another site. The distributions in glucose cannot be explained by acetate’s conversion in tissues other than liver to labeled glutamine and glutamate and then their conversion in liver to glucose. If the labeled glutamate was the precursor of glucose, the relative 14C activities of Gl, G2, G3, and G4 would be the average of K2 and K3, and G4 the average of K2 and K5. Thus, for example, in subject U.E., where the percent 14C distributions in carbons 1–5 of glutamate were 10, 17, 17, 50, and 6 (Table I), respectively, the relative distributions would have been 34, 34, 12, 12, 34, and 34 in glucose carbons 1–6, respectively. G2/G4 would then have been 2.8 rather than 1.8 (Table II).2

The distributions in glutamate cannot be explained by acetate’s metabolism to glutamate in liver with fixation into carbon 1 of the glutamate of 14CO2 formed from the acetate in another site. If labeled glutamate, used in conjugation, were formed only in liver, where glucose was formed and CO2 fixation occurred, 14CO2 fixation should have contributed in a similar proportion to the 14C in carbon 1 of glutamate and in carbons 3 and 4 of glucose. This was the case for [3-14C] lactate: an ~30% contribution for both (1), but not for [2-14C] acetate; an ~60% contribution for glucose; and a 30% contribution for glutamate. This is reflected in similar (G1 + G2)/2/G4, and K2/K3, ratios with [3-14C] lactate, when corrected for 14CO2 fixation (Tables VI and VII of Ref. 1), but higher G1/G4, than K2/K3, ratios with [2-14C] acetate (Tables IV and V).

Incorporations of 14C from the [2-14C]acetate into glutamate in peripheral tissue(s) and into glucose and glutamate in liver, when combined with fixation of 14CO2 by pyruvate in liver, which 14CO2 formed in the oxidation of the [2-14C]acetate, provide a possible explanation for the distributions. Since acetate is extensively utilized and most glutamine is produced by muscle (25, 26), muscle is depicted as the peripheral tissue (Fig. 1). When [2-14C]acetate was administered to rats, muscle glutamate was labeled (27); and when [1-14C]acetate and [2-14C]acetate were administered to rats, glutamate from carcass protein was labeled (21, 28).

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1 However, as noted after correction for 14CO2 fixation, the G1/G4 ratio from [3-14C]acetate approaches, but is less than, the ratio from [3-14C]acetate. This difference could be due to incorporation into glucose of 14C from 14C-labeled glutamate formed in the periphery. Labeled lactate has also been reported to be formed from [2-14C] acetate (9).

2 Whereas a contribution by kidney to the incorporation of 14C from [2-14C]acetate into blood glucose, as has been suggested (18), cannot be excluded, we believe liver is the primary source of the labeled glucose (Fig. 1). This is so, although kidney slices were reported to oxidize acetate more readily than liver slices and incorporation of acetate carbon into glucose as well as glutamate was appreciable (29). Our reasons include: 1) the large net production of glucose by liver, but, at most, only a very small production by kidney in the 60-h fasted human (30); 2) the ratio G1/G4 = 2 in glucose from diabetic animals given [2-14C]palmitate (17) and [6-14C]palmitate (20); and 3) the increases in concentration and specific activity of glucose in an overnight fasted subject we gave glucagon after giving [2-14C]acetate and an oral glucose load. Concentration increased from 3.6 to 5.2 mM and specific activity from 11.4 to 16.6, presumably reflecting release of labeled glucose from liver of a higher specific activity than that from the circulation. The percent distribution of 14C in the glucose before glucagon was C1 = 19.0, C2 = 14.6, C6 = 6.2, C8 = 9.4, C12 = 23.2, and C7 = 27.6 (97.3% recovery). After glucagon, it was C1 = 16.6, C6 = 15.3, C8 = 8.9, C12 = 13.4, C1 = 22.9, and C7 = 22.9 (96.9% recovery), so G1/G4 is ~2.

3 Katz and Chaikoff (29) compared acetate’s metabolism in the intact animal and in liver slices. They concluded that in the intact animal, acetate is oxidized mainly in extrahepatic tissues; and therefore, acetate’s metabolism in vivo may show little resemblance to its metabolism in isolated liver.

4 Consoli et al. (10, 11) concluded that using [2-14C]acetate, as proposed by Katz (7), provides an accurate noninvasive measure of gluconeogenesis. They concluded this because their estimates of the rates of gluconeogenesis were those to be predicted from previous measurements of splanchnic substrate balance and hepatic glycogen content. The rates of gluconeogenesis were calculated by dividing the specific activities of glucose by the estimated specific activities of P-enolpyruvate. The specific activity of P-enolpyruvate was estimated by multiplying the specific activity of 1-3-14C-lactate, formed from [1-14C]ethanol, [1-14C]acetyl-CoA, formed from [1-14C] ethanol, would form [2-14C]acetone and then [2-14C]acetate by

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To the extent that the Krebs cycle functions as a “closed system” in muscle, glutamate formed from [2-14C]acetate would be expected to have about twice as much 14C in carbon 2 as in carbon 1, i.e. a K3/K1 ratio of 2. Glutamate formed in liver would be expected, with correction for 14CO2 fixation, to have a K2/K1 ratio similar to the G4/G1 ratio in glucose (~5) (Table V). Glutamate from the conjugate, formed from a mixture of the glutamate derived from liver and muscle, would then have a K3/K1 ratio intermediate between 5 and 2, as it does, i.e. 2.3–2.9 (Table IV).

In accord with our results, Kalderon et al. (27), on giving [2-14C]acetate to rats, found much less 14C enrichment in liver glucose than in glutamate carbons. They concluded this was incompatible with a pool of oxalacetate common to the Krebs cycle and gluconeogenesis. They offered, as an explanation, metabolic channeling of oxalacetate formed from pyruvate toward gluconeogenesis, resulting in incomplete mixing of mitochondrial oxalacetate (Scheme 1 of Ref. 27). The K3/K4 and K1/K2 ratios in glutamate on administering [3-14C]acetate should not then have been similar, as they were, to the G4/G1 and G2/G2 ratios. This is because the channeling of oxalacetate, used in gluconeogenesis, would not have experienced as much randomization in the Krebs cycle as the carbons of oxalacetate used in α-ketoglutarate formation.

An alternative explanation for our results is that lactate is converted to glucose and metabolized in the Krebs cycle in a cellular site different from that where acetate is primarily metabolized and gluconeogenesis does not occur, but that both sites are in the liver. The metabolic picture in Fig. 1 would be the same, except, rather than the peripheral site of metabolism, that site would be in liver.5

**Ethanol Metabolism**—Since it seemed possible that if [2-14C]acetate was formed in the liver, distributions in glutamate and glucose may better reflect liver metabolism, [1-14C] ethanol, which is converted to [2-14C]acetate in liver (34), was administered to one subject. However, [2-14C]ethanol gave a distribution similar to that of [2-14C] acetate (Tables I and II), in accord with the acetate, formed from ethanol, being metabolized primarily by peripheral tissues (35–38).


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5 Kalderon et al. (29) compared acetate’s metabolism in the intact animal and in liver slices. They concluded that in the intact animal, acetate is oxidized mainly in extrahepatic tissues; and therefore, acetate’s metabolism in vivo may show little resemblance to its metabolism in isolated liver.
Metabolism of Acetate

the pathway and hence α-[2,3-14C]ketoglutarate. Incorporation was only into carbons 1 and 5 of the glutamate (Table I), indicating that the pathway was not active and therefore did not contribute to the incorporation of 14C into K1 from [2-14C]acetate.

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