THE METABOLISM OF L-LYSINE-6-C\textsuperscript{14}\textdagger* 

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The intermediary metabolism of lysine has been the subject of considerable speculation, but no definitive picture of its catabolism has been obtained. Dakin (1) in 1913 found that lysine is not a glucose or ketone body former in phlorizinized dogs, and this has been supported by more recent findings (2, 3). Ringer et al. (4), in 1913, proposed glutaric acid as a lysine metabolite, as it too did not form acetoadacetate or glucose. In 1944 Neuberger and Sanger (5) again proposed glutaric acid as a product of lysine metabolism, suggesting a-aminoadipic acid or \( \delta \)-aminovaleric acid as a probable intermediate. More recently, Borsook and coworkers (6) using lysine-6-C\textsuperscript{14} indicated that lysine forms a-aminoadipic acid in guinea pig liver homogenate and that this in turn forms glutaric acid (7).

Miller and Bale (8)' using lysine-6-C\textsuperscript{14} described evidence for the direct conversion of lysine to glutamic acid. This reaction had been missed by the previous workers, who had assumed that such a conversion would involve the loss of the radioactive \( \epsilon \)-carbon during transformation to a 5-carbon intermediate. Further evidence by Altman et al. (9) on the probable conversion of lysine to \( \alpha \)-ketoglutaric acid added weight to the contention that lysine may in part be directly converted to glutamic acid.

Other work has shown that lysine does not undergo reversible deamination (10), but, under certain dietary conditions, forms an unidentified keto acid (11), possibly \( \epsilon \)-amino-\( \alpha \)-ketocaproic acid.

It is the purpose of this paper to clarify some aspects of the main pathways by which lysine is metabolized. In this respect, evidence will be presented to show the conversion of lysine in rats to glutaric acid, \( \alpha \)-keto-glutaric acid, and glucose, and the pathways involved will be discussed.

EXPERIMENTAL

DL-Lysine-6-C\textsuperscript{14} and DL-\( \alpha \)-Aminoadipic Acid-6-C\textsuperscript{14}—These compounds were synthesized by the method of Rothstein and Claus (12), and had an activity of 0.87 mc. per gm.

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' Miller, L. L., and Bale, W. F., in preparation.
Glutaric Acid-1,5-C\textsubscript{14}—This compound was prepared as previously described (13).

L-Lysine-6-C\textsubscript{14}—DL-Lysine-6-C\textsubscript{14} was resolved by modifying the procedure of Kearley and Ingersoll (14) to adapt it to small scale preparation and to minimize the handling of radioactive compounds.

2.00 gm. of 3,5-dibromo-N-acetyl-L-tyrosine were dissolved in a hot mixture of 5 ml. of water, 5 ml. of methanol, and 0.32 ml. of concentrated NH\textsubscript{4}OH in a 30 ml. beaker. To this hot solution 1.87 gm. of DL-lysine-6-C\textsubscript{14} monohydrochloride were carefully added with stirring until solution was complete. The mixture was then placed in a refrigerator for 4 hours, and the resulting white crystalline L-lysine-L-tyrosine salt was filtered with the aid of a little cold methanol. Once filtration had begun, the filtrate was successively used to complete the transfer. The solid was washed finally with cold methanol, and the combined washings and filtrate were saved for subsequent racemization of the n-lysine and unchanged L-lysine. It was found unnecessary to purify the salt further; it was then washed through the filter with water. The filtrate was acidified with hydrochloric acid (total volume 40 ml.), and the resulting mixture was allowed to stand in the refrigerator for 2 days and then filtered. The filtrate was distilled \textit{in vacuo} to about 5 ml. and filtered, the flask being rinsed with a little ice water. This procedure allows the removal of a small residuum of dibromoacetyltyrosine.

The filtrate was distilled to dryness \textit{in vacuo} and the residue converted to L-lysine monohydrochloride by the usual methods (15). The yield was 0.564 gm. or 60 per cent. The material was recrystallized once and had \([\alpha]_{D}^{28} +18.8^\circ \text{ in 0.6 N HCl (c = 1); reported } +18.7^\circ \text{ (14).}\)

Isolation of Glutarate Derived from L-Lysine-6-C\textsubscript{14}—Male Sprague-Dawley or Wistar rats, weighing about 350 gm., were fasted 24 hours and then given intraperitoneally a solution of 500 mg. of glutaric acid and 6.4 mg. of L-lysine-6-C\textsubscript{14} monohydrochloride. The solution was neutralized with sodium hydroxide and filtered before injection, the total volume being made up to 6 to 7 ml. Urine was collected for 10 hours and filtered and the alkaline solution extracted continuously with ether for 8 hours after dilution to about 50 ml. The aqueous solution was then acidified with H\textsubscript{2}SO\textsubscript{4} and extraction continued for 24 hours with fresh ether. The ether extract of the acid solution was dried with anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered, and evaporated. The residue was steam-distilled to remove volatile impurities, treated with Norit, and distilled to dryness. Two recrystallizations of the residue from benzene yielded 53 mg. of white crystals; m.p. 90–91\(^\circ\). The material was converted to the piperazine salt, which was recrystallized from ethanol-acetone, and the free glutaric acid recovered (13). Recrystallized from benzene, it melted at 93–94.5\(^\circ\). A portion of this material was assayed
for C\textsuperscript{14} activity. The remainder was diluted with a known amount of inert glutaric acid, recrystallized, and assayed; m.p. 96–97°. Both assays showed the same specific activity.

**Isolation of Glutarate Derived from DL-\(\alpha\)-Aminoadipic Acid-6-C\textsuperscript{14}**—The procedure was carried out as described above, except that DL-\(\alpha\)-aminoadipic acid-6-C\textsuperscript{14} (9.45 mg.) was injected in place of the lysine and the piperase-zine salt procedure was eliminated. The diluted glutaric acid melted at 97.5°.

**Isolation of \(\alpha\)-Ketoglutarate and Acetate Derived from DL-Lysine-6-C\textsuperscript{14}**—A male Sprague-Dawley rat, fasted for 24 hours, was given 300 mg. of phenylaminobutyric acid as the sodium salt by stomach tube. Then an aqueous solution of 500 mg. of \(\alpha\)-ketoglutaric acid and 11.5 mg. of DL-lysine-6-C\textsuperscript{14} monohydrochloride was neutralized with sodium hydroxide, filtered, and injected intraperitoneally. The total volume was 6 to 7 ml. The urine was collected for 10 hours, filtered, diluted, made basic with sodium hydroxide, and continuously extracted with ether for 8 hours. The aqueous solution was then acidified with sulfuric acid and extracted continuously for 24 hours with ether. The ether extract was dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was dissolved in 5 ml. of water, treated with Norit, and filtered. The solution was seeded with authentic L-acetamidophenylbutyric acid and placed in a refrigerator overnight. The resulting fine needles were filtered and recrystallized twice from water containing 4 to 5 mg. of inert \(\alpha\)-ketoglutaric acid. The material melted at 179–180° and was assayed for radioactivity.

The supernatant fluid from the original acetamidophenylbutyric acid crystallization was treated with an excess of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid, and the resulting 2,4-dinitrophenylhydrazone was recrystallized repeatedly from 10 per cent ethanol and ethyl acetate-chloroform until constant specific activity was obtained; m.p. 223–224°.

**Isolation of \(\alpha\)-Ketoglutarate and Acetate Derived from Glutaric Acid-1,5-C\textsuperscript{14}**—The procedure was the same as that outlined above, except that 29.7 mg. of glutaric acid-1,5-C\textsuperscript{14} were used in place of DL-lysine-6-C\textsuperscript{14}. A paper chromatogram (16) of the dinitrophenylhydrazone of one sample prepared in this way showed only one spot, corresponding exactly with the same derivative of authentic \(\alpha\)-ketoglutaric acid.

**Isolation of \(\delta\)-Aminovaleric Acid Derived from \(\lambda\)-Lysine-6-C\textsuperscript{14}**—A male Sprague-Dawley or Wistar rat, fasted for 24 hours, was injected intraperitoneally with a neutralized solution containing 500 mg. of \(\delta\)-aminovaleric acid hydrochloride and 6.4 mg. of \(\lambda\)-lysine-6-C\textsuperscript{14} monohydrochloride. In one case, 50 mg. of inert \(\lambda\)-lysine were added along with the lysine-6-C\textsuperscript{14}. The 16 hour urine sample was filtered and diluted to 100 ml. About 4
mg. of inert L-lysine monohydrochloride were added to the diluted urine, and the urine was then passed consecutively through Amberlite IR-4 and IRC-50. The effluent was vacuum-distilled to about 40 ml. A paper chromatogram (collidine-lutidine-water) indicated the presence of δ-aminovaleric acid and a trace of one other ninhydrin-positive material with an RF value twice that of the aminovalerate. The solution was further evaporated to about 25 ml. and treated with sodium hydroxide and benzoyl chloride. After acidification the mixture was allowed to stand in the cold overnight and filtered. The white crystalline residue was dried and dissolved in hot benzene. Low boiling petroleum ether (b.p. 35–60°) was added to precipitate δ-benzamidovalerate, and the resulting mixture was cooled for 2 hours, filtered, and the residue washed with low boiling petroleum ether. The residue was purified by recrystallization from hot benzene or benzene and petroleum ether. The yield varied in the various experiments between 36 and 72 mg. of pure material; m.p. 107°; reported, 105–106° (17). In the experiment in which extra L-lysine was administered (Table II, Experiment 10), a sample of the benzamidovalerate was saved, and the remainder was recrystallized once more from benzene. Assay of both samples showed that they had the same specific activity.

δ-Benzenesulfonamidovaleric Acid—Part of one of the ion exchange effluents above was made alkaline with sodium hydroxide and warmed for 1 hour with benzenesulfonyl fluoride2 with occasional shaking. On acidification and cooling for 3 hours, colorless platelets were obtained. This material was recrystallized successively from water, benzene, and then water; m.p. 96.5–97°. A mixed melting point with material similarly prepared from authentic δ-aminovaleric acid showed no depression. Both this and the benzamido derivative from the same urine showed a net activity not exceeding 80 per cent of the background value.

Isolation of Formate Derived from L-Lysine-6-C14—The procedure used was that of Weinhouse and Friedmann (18), except that 7.2 mM of formate were injected and 2.9 mM were recovered from the urine.

Glucose Isolation and Degradation—Phlorizinized male Wistar rats, fasted for 24 hours, were given small amounts of DL-lysine-6-C14 by stomach tube, along with 250 mg. of inert L-lysine. Urines were collected for 24 hours. Glucose solutions were obtained by passing the filtered, diluted urines consecutively through Amberlite IR-4 and IRC-50 and concentrating the effluent. Degradations were carried out by the following procedures: carbons 1, 2, 3, and carbon 6, by periodate degradation of glucose phenyl-}

drazone derivatives. Both derivatives were purified by three recrystallizations. The latter showed only one spot on a paper chromatogram. Carbon 1 was obtained by hydrobromic acid degradation of glucose to formic acid and levulinic acid (21), followed by oxidation of the formate to carbon dioxide with mercuric sulfate.

**Acetoacetic Acid**—This was present in the urine of phlorizinized rats after administration of DL-lysine-6-C\(^{14}\) and 250 mg. of inert L-lysine, and was degraded without isolation by appropriate techniques (22).

**Hippuric Acid**—DL-Lysine-6-C\(^{14}\) and sodium benzoate (350 mg.) were added to the food of a rat fasted 24 hours. The hippuric acid was isolated by standard procedures (23) and assayed for radioactivity.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expiration of C(^{14})O(_2) after Administration of DL-Lysine-6-C(^{14}) to Phlorizinized Rats</strong> Fasted 24 Hours</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Per cent dose of L-lysine-6-C(^{14}) expired†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2 hrs.</td>
</tr>
<tr>
<td>1</td>
<td>4.96</td>
</tr>
<tr>
<td>2</td>
<td>4.56</td>
</tr>
</tbody>
</table>

* Substrate, 1.5 mg. of DL-lysine-6-C\(^{14}\) + 300 mg. of L-lysine.
† Based on L-lysine-6-C\(^{14}\), the dose contained 920 \(\times\) 10\(^3\) disintegrations per minute per millimole. DL-Lysine-6-C\(^{14}\) does not contribute significantly to expired C\(^{14}\)O\(_2\) (Rothstein, M., Bly, C. G., and Miller, L. L., unpublished results).

**Expired Carbon Dioxide**—This was collected and assayed as described previously (13) after administration of DL-lysine-6-C\(^{14}\) plus 300 mg. of inert L-lysine by stomach tube to male Wistar rats fasted 24 hours.

**Radioactivity Assays**—These were carried out after wet combustion by the procedure previously reported (13).

**RESULTS AND DISCUSSION**

From Table I it can be seen that the maximal rate of C\(^{14}\)O\(_2\) expiration is reached between 4 and 6 hours. This is relatively slow and is probably due to the large amount of L-lysine administered. By comparison, the maximal C\(^{14}\)O\(_2\) output by the isolated perfused liver is attained in 1 to 2 hours (24).

Table II summarizes the principal results of the experiments performed to evaluate the radioactivity of specific catabolites of various radioactive precursors.

Some discussion of the technique used in most of the experiments in Table II is in order. The procedure is an extension of the "trapping technique" as exemplified by Weinhouse and Friedmann (18) and makes use of...
several isolated observations that many metabolites, when administered to animals in large doses, are excreted in significant amount in the urine unchanged. The assumption that the injected compound is equilibrated

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Radioactive compound administered</th>
<th>Amount of catabolite administered (mg.)</th>
<th>Catabolite isolated</th>
<th>Specific activity of isolated catabolite (Disintegrations per minute per millimole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>dl-Lysine-6-C(^{14})</td>
<td>8,990 (\dagger) 500</td>
<td>(\alpha)-Ketoglutarate $$</td>
<td>15,000 (\dagger)</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>8,990 $\dagger$ 350 $|$</td>
<td>&quot;Acetate&quot; $|$</td>
<td>12,700 $|$</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>8,990 $\dagger$ 500</td>
<td>(\alpha)-Ketoglutarate $$</td>
<td>4,640 $|$</td>
</tr>
<tr>
<td>6</td>
<td>Glutaric acid-1,5-C(^{14})</td>
<td>10,200 500</td>
<td>(\alpha)-Ketoglutarate $$</td>
<td>110,000 $|$</td>
</tr>
<tr>
<td>7</td>
<td>dl-Lysine-6-C(^{14})</td>
<td>9,750 500</td>
<td>Glutarate</td>
<td>64,500 $|$</td>
</tr>
<tr>
<td>8</td>
<td>dl-(\alpha)-Aminoadipic acid-6-C(^{14})</td>
<td>14,400 500</td>
<td>&quot;</td>
<td>201,000 $|$</td>
</tr>
<tr>
<td>9</td>
<td>L-Lysine-6-C(^{14})</td>
<td>9,750 500</td>
<td>(\delta)-Aminovalerate $|</td>
<td>$</td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>9,750 500</td>
<td>&quot;</td>
<td>1,000 $|</td>
</tr>
<tr>
<td>11</td>
<td>dl-Lysine-6-C(^{14})</td>
<td>7,930 300 $|$</td>
<td>Acetate $|$</td>
<td>3,140 (\dagger)</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>8,990 $\dagger$ 332</td>
<td>Formate</td>
<td>339</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>7,930 $\dagger$ 350 $|$</td>
<td>Glycine $|$</td>
<td>(530) $|$</td>
</tr>
</tbody>
</table>

In Experiment 10, 50 mg. of inert L-lysine monohydrochloride were added. In Experiments 11 and 13, dl-lysine-6-C\(^{14}\) was administered in the food. This method of administration always led to reduced activities in isolated glucose and acetate.

* Disintegrations per minute \(\times 10^{-3}\).
† Disintegrations per minute per millimole.
‡ Based on L-lysine-6-C\(^{14}\). D-Lysine is known not to form metabolites convertible to C\(^{14}\)O\(_2\) in the rat (Rothstein, M., Bly, C. G., and Miller, L. L., unpublished results).
§ Isolated as the 2,4-dinitrophenylhydrazone.
|| \(\gamma\)-Phenylaminobutyric acid.
¶ Isolated as phenylacetamidobutyric acid.
** Isolated as the \(\text{N}\)-benzoyl or \(\text{N}\)-benzenesulfonyl derivative.
†† Benzoic acid fed.
‡‡ Isolated as hippuric acid.
§§ Too low for accurate measurement. The actual measurement observed for the sample was 40 per cent of background.

in some measure with similar biologically formed material is borne out by the finding that large doses of some non radioactive metabolites, after being partly excreted in the urine, were found to be radioactive. It is
therefore reasonable to assume that some "sampling" of the biologically formed material does take place. In practice, because the related compounds in question are distributed throughout a heterogeneous system of numerous compartments by processes with a variety of unknown equilibrium and reaction constants, it is impossible to draw more than presumptive qualitative conclusions concerning the directness of conversion of a radioactive compound to another metabolite. It is possible that some compounds do not equilibrate well, and hence negative results (i.e., no or little activity in the isolated metabolite) are not necessarily significant.

Table II presents evidence for the conversion of lysine-6-C$^{14}$ to radioactive $\alpha$-ketoglutaric acid. Because of the limitations imposed by the unknown factors mentioned above, it can be concluded only presumptively that the carbon chain of lysine-6-C$^{14}$ is converted to radioactive $\alpha$-ketoglutaric acid more directly than via conversion to "acetate." Furthermore, the carboxyl-labeled ketoglutaric acid formed here should not lead to carboxyl-labeled radioactive acetate in significant amount. Thus it becomes apparent that lysine may yield both acetate and $\alpha$-ketoglutarate by independent pathways. Examination of the metabolism of glutaric acid-1,5-C$^{14}$ has previously (13) indicated that this compound forms acetate, and that it also probably formed some pyruvate-1-C$^{14}$, possibly by way of carboxyl-labeled $\alpha$-ketoglutarate. Experiment 6 (Table II) strongly supports the view that this is indeed the case, glutaric acid being directly converted to $\alpha$-ketoglutaric acid, and, as in the case of lysine, also forming an acetate fragment with the same order of activity as that of the keto acid.

It should be pointed out that the conversion of glutarate to $\alpha$-ketoglutarate is a newly discovered biochemical oxidation. It may well involve $\alpha,\beta$ dehydrogenation to form glutaric acid, with subsequent addition of the elements of water to form $\alpha$-hydroxyglutarate. This latter compound has been shown to be oxidized to $\alpha$-ketoglutarate in many animal tissues (25). This oxidation as a whole may thus be analogous to the succinate-fumarate-malate system.

Glutaric acid is very likely the common precursor of both the $\alpha$-ketoglutarate and acetate derived from lysine, a view in keeping with the finding of Borsook and coworkers that glutarate is a product of lysine metabolism in guinea pig liver homogenate (7). Although the radioactivity and conversion figures obtained with their preparations were exceedingly low, the metabolism in vivo of both lysine-6-C$^{14}$ and $\alpha$-aminoadipic acid-6-C$^{14}$ in our experiments results in the isolation of highly radioactive glutarate in spite of the large dilution of the latter compound with injected material. Thus, while we have not yet demonstrated the conversion of lysine to $\alpha$-aminoadipic acid, it seems safe to assume that this too probably occurs in vivo.
Our experiments favor the pathway, lysine → α-aminoadipate → glutarate → α-ketoglutarate + acetate, the α-aminoadipic acid going through α-ketoacid to glutarate, as indicated by Borsook et al. (7). Additional indirect evidence for the conversion of lysine to α-ketoglutarate and acetate by pathways such as those outlined above is found in the fact that, as was the case with glutaric acid-1,5-Cl4 (13), the isolated radioacetate could not alone account for the specific activity of the isolated glucose (Table II, Experiment 11). The second source of glucose activity is probably carboxy-labeled α-ketoglutarate directly derived from glutarate.

The above evidence for the conversion of lysine to α-ketoglutarate confirms the observation of Altman et al. (9) that lysine in its conversion to hemin probably passes through a 5-carbon intermediate identical with α-ketoglutarate. It also confirms the findings of Miller and Bale (8) that lysine is, at least in part, converted directly to glutamic acid.

The suggestion of Neuberger and Sanger (5) that lysine might form δ-aminovaleric acid warranted investigation in view of the finding of Waelsch and Miller (11) that excess lysine fed to rats resulted in urinary excretion of an unidentified keto acid. If this acid were the α-keto analogue of lysine, the compound would not be reaminated (10), but one might expect it to be decarboxylated to produce δ-aminovaleric acid. Further evidence indicating that δ-aminovalerate might play a part in the intermediary metabolism of lysine is found in the fact that it is transaminated with α-ketoglutaric acid by mouse liver acetone powders and brain preparations at about the same rate as β-alanine and γ-aminobutyric acid (26). δ-Aminovalerate could form glutaric acid in this way. However, our experiments with large doses did not yield significantly radioactive δ-aminovalerate from lysine, except when a large excess of L-lysine was given concomitantly (Table II, Experiment 10). In this experiment, a small but significant activity was obtained in the isolated δ-aminovalerate. This suggests that the lysine → keto acid → δ-aminovalerate reaction may be of a secondary nature, being brought into play only if there is a large excess of lysine present. On the basis of the limited observations available, the role of δ-aminovaleric acid in lysine metabolism cannot yet be clearly defined.

Although lysine is not glucogenic in the classical sense, Table III shows that there is some conversion of lysine-6-C14 to glucose. The actual percentage conversion is not large and appears to vary with the degree of glucose excretion. This conversion, and that to acetoacetate (Table III), is to be expected from the biological conversion to glutaric acid and the known metabolism of the latter (13).

It is to be expected that the radioactivity of the glucose would be in carbon atoms 3 and 4, since it would be derived from carboxy-labeled glutarate

3 Dr. Eugene Roberts, private communication.
Examination of Table IV shows that 85 per cent of the isotope is indeed in these carbons.

The remaining 15 per cent of the C\textsuperscript{14} is not distributed equally between carbons 1, 6 and 2, 5. From Table IV it can be seen that the values for Table III

**Conversion of Lysine-6-C\textsuperscript{14} to Glucose and Acetoacetate in Phlorizinized Rats**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Dose\textsuperscript{*}</th>
<th>Acetoacetate (COOH)\textsuperscript{†}</th>
<th>Glucose isolated</th>
<th>Glucose activity\textsuperscript{†}</th>
<th>Per cent conversion of lysine to glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>917</td>
<td>1520</td>
<td>550</td>
<td>8,000</td>
<td>1.33</td>
</tr>
<tr>
<td>15</td>
<td>917</td>
<td>250</td>
<td>6,750</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>6030</td>
<td>2370</td>
<td>1250</td>
<td>30,600</td>
<td>1.77</td>
</tr>
</tbody>
</table>

\textsuperscript{*} Disintegrations per minute $\times 10^{-3}$; based on L-lysine-6-C\textsuperscript{14}. 250 mg. of inert L-lysine monohydrochloride were mixed with the radioactive DL-lysine (1.5 mg. in Experiments 14 and 15, and 98.8 mg. in Experiment 16).

\textsuperscript{†} Disintegrations per minute per millimole.

**Table IV**

**Distribution of C\textsuperscript{14} in Carbon Atoms of Glucose Derived from Lysine-6-C\textsuperscript{14}**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Per cent activity of entire glucose molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbons 1, 2, 3</td>
</tr>
<tr>
<td>16</td>
<td>51.0</td>
</tr>
<tr>
<td>17</td>
<td>48.4</td>
</tr>
<tr>
<td>18</td>
<td>14.6</td>
</tr>
<tr>
<td>19</td>
<td>14.9</td>
</tr>
</tbody>
</table>

\textsuperscript{*} The values for carbons 1, 2, 5, and 6 were obtained from the dimedon and 2,4-dinitrophenylhydrazone derivatives of the acetaldehyde derived from the permanganate oxidation of lactic acid. The values found for carbons 3 and 4 by this procedure were approximately 10 per cent lower than the calculated values, indicating that some dilution of the carbon dioxide had occurred.

\textsuperscript{†} By subtraction of carbons 1, 2, 5, and 6 from the total activity of the glucose.
similar to the labeling found in glucose obtained from rats injected intraperitoneally with formate-C14 (28). For this reason an experiment was performed in which formate was isolated from the urine of a rat after administration of lysine-6-C14 (Table II). The activity found was 339 disintegrations per minute per millimole. The usual activity of glucose isolated from the urine of a phlorizinized rat fasted 24 hours, given similar doses of lysine-6-C14 by stomach tube, is in the vicinity of 30,000 to 45,000 disintegrations per minute per millimole. Since we have estimated that 13 per cent of this may be derived from formate, and carbons 1, 2, 5, and 6 are equivalent to four formates, the estimated activity of a glucose carbon atom derivable from formate becomes 975 to 1460 disintegrations per minute per millimole. This could be provided by formate having a radioactivity in the range of that isolated, when allowance is made for the large dilution of activity caused by the injection of 7.2 mM of extra formate.

The experimental evidence described in this and other reports indicates the metabolic pathways for lysine-6-C14 illustrated in the accompanying scheme.

**SUMMARY**

1. An isotope-trapping technique has been used in rats which permits the identification of specific catabolites from radioactive precursors.

2. By this technique, evidence for the following conversions in vivo has been obtained in the rat: lysine to glutaric acid, α-amino adipic acid to glutaric acid, glutaric acid to α-ketoglutaric acid plus acetate, and lysine to α-ketoglutaric acid plus acetate. In addition, a lesser conversion of lysine-6-C14 to formate takes place.

3. Interpretation of these and other experimental results supports the
conclusion that lysine is metabolized in vivo as follows: lysine → α-amino-adipate → glutarate → α-ketoglutarate + acetate. The latter compounds yield 3,4-labeled glucose.

4. Formate-C\textsuperscript{14} derived from lysine-6-C\textsuperscript{14} is probably responsible for the small amount of isotope found in carbons 1, 2, 5, and 6 of glucose derived from lysine-6-C\textsuperscript{14} in phlorizinized rats.

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Morton Rothstein and Leon L. Miller


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