THE METABOLISM OF THE METHIONINE CARBON CHAIN IN THE INTACT RAT*

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In the present experiments the metabolism of the carbon chain of methionine has been studied in the intact rat by the administration of 2-, 3-, and 4-C\textsubscript{14}-DL-methionine. The distribution of C\textsubscript{14} in liver glycogen, serine, choline, and aspartic acid has been determined. The results are consistent with the metabolism of d- and l-methionine largely over the pathway of propionic acid catabolism and exclude the significant participation of certain other mechanisms.

EXPERIMENTAL

Methods and Materials

2-C\textsubscript{14}-DL-Methionine was purchased from Tracerlab, Inc., and the California Foundation for Biochemical Research and purified by chromatography on a Dowex 50 column (2).

3- and 4-C\textsubscript{14}-DL-methionine were synthesized from 1- and 2-C\textsubscript{14}-acetate, respectively, by the following series of reactions

\[
\begin{align*}
\text{CH}_3\text{COONa} & \underset{(3)}{\text{C}_6\text{H}_5\text{COCl}} \rightarrow \text{CH}_3\text{COCl} \underset{\text{Br}_2}{\rightarrow} \text{CH}_3\text{BrCOCl} \underset{\text{NaHCO}_3}{\rightarrow} \\
\text{CH}_3\text{BrCOONa} & \\
\text{CH}_3\text{SNa} & \underset{\text{LiAlH}_4}{\rightarrow} \text{CH}_3\text{SCH}_2\text{CH}_2\text{COOH} \rightarrow \text{CH}_3\text{SCH}_2\text{CH}_2\text{OH} \underset{\text{SOCl}_2}{\rightarrow} \text{CH}_3\text{SCH}_2\text{CH}_2\text{Cl} \\
\text{NHCOCH}_3 & \underset{\text{NaC}(\text{COOEt})_2}{\rightarrow} \text{CH}_3\text{SCH}_2\text{CH}_2\text{CHCOOH}
\end{align*}
\]

and purified by chromatography on Dowex 50 (2). The yield of methionine by this procedure was about 40 per cent. Paper chromatography of the amino acid with butanol-acetic acid solvent gave a single ninhydrin-...
positive and radioactive spot with an $R_f$ identical with that of DL-methionine.

A detailed description of the synthetic procedure will be published elsewhere.

CH$_3$-$C^{14}$-DL-Methionine was prepared by the method of Melville et al. (6).

Determination of $C^{14}$ Activity—The $C^{14}$ activity of all the samples of Experiments 1 to 4 (Table I), with the exception of tetramethylammonium iodide, was determined by converting the carbon to BaCO$_3$ and counting with an end window Geiger-Müller counter. Tetramethylammonium iodide was plated and counted on stainless steel planchets, but for purposes of comparison the results have been expressed as the activity determined on barium carbonate. The activities of all the samples in Experiment 5 were determined by gas phase proportional counting (7).

Biological Experiments

$C^{14}$-labeled methionine was administered to rats subcutaneously together with oral doses of glycine or glucose. The latter substances were given to produce glycogen deposition and the glycine to stimulate serine synthesis as well. The general experimental data are summarized in Table I. Two animals from each group were kept in a metabolism cage for the collection of respiratory CO$_2$. At the end of the experiments the rats were anesthetized by intraperitoneal doses of 0.1 ml. of 10 per cent sodium Amytal solution per 100 gm. In each experiment the appropriate viscera were removed and pooled for the isolation of glycogen and other substances, as described below.

$4-C^{14}$-DL-Methionine and CH$_3$-$C^{14}$-DL-Methionine Experiments

Choline reineckate was isolated from the abdominal viscera as previously described (8) and degraded to trimethylamine (9, 10). The latter substance was converted to tetramethylammonium iodide for the determination of radioactivity (iodine, found, 63.5 per cent; calculated, 63.1 per cent). After the extraction of phospholipides, glycogen was isolated from the liver by the procedure of Ostern and Hubl (11) and purified by precipitating it twice from 10 per cent trichloroacetic acid solution with alcohol. Glycogen was hydrolyzed and degraded by the procedure of Wood et al. (12) by utilizing Lactobacillus casei.

The neutral amino acid fraction of the liver hydrolysate, obtained as previously described (8), was chromatographed on Dowex 50 (2). Serine was separated from the fraction containing serine and threonine by precipitation as the $p$-hydroxyazobenzene-$p'$-sulfonate (13). After recrystallization from water, the product decomposed at 202–204$\degree$ simultaneously with a sample of the authentic L-serine derivative and with a mixture of the two...
samples. The distribution of the small amount of activity in serine was determined by degradation with periodate (8).

### Table I
General Experimental Data on Rats

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Weight of fasted rats*</th>
<th>Methionine administered</th>
<th>Other substances administered†</th>
<th>Length of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150-170 (Average 161)</td>
<td>4-C¹⁴</td>
<td>7.5 x 10⁴</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.25 ml. of 0.3 M solution at 0, 3, 6, and 10 hrs.</td>
<td>Glycine, 2.0 ml. of 2.5 M solution per 100 gm.</td>
</tr>
<tr>
<td>2</td>
<td>155-170 (Average 163)</td>
<td>CH₃-C¹⁴</td>
<td>7.5 x 10⁴</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.25 ml. of 0.3 M solution at 0, 3, 6, and 10 hrs.</td>
<td>Glycine, 2.0 ml. of 2.5 M solution per 100 gm.</td>
</tr>
<tr>
<td>3</td>
<td>174-190 (Average 183)</td>
<td>3-C¹⁴</td>
<td>1.75 x 10⁵</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.18 ml. of 0.3 M solution at 0 hrs.</td>
<td>Glucose, 2.5 ml. of 1.35 M solution per 100 gm.</td>
</tr>
<tr>
<td>4</td>
<td>150-158 (Average 154)</td>
<td>2-C¹⁴</td>
<td>1.43 x 10⁵</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.18 ml. of 0.3 M solution at 0 hrs.</td>
<td>Glucose, 2.5 ml. of 1.35 M solution per 100 gm.</td>
</tr>
<tr>
<td>5</td>
<td>149-151 (Average 150)</td>
<td>2-C¹⁴</td>
<td>1.09 x 10⁷</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.30 ml. of 0.15 M solution at 0 hrs.</td>
<td>Glucose, 2.5 ml. of 1.35 M solution per 100 gm.</td>
</tr>
</tbody>
</table>

* Six rats were used in Experiments 1, 2, and 5 and four in Experiments 3 and 4. The rats used in Experiments 1 and 2 were fasted for 48 hours, the others for 24 hours.
† Subcutaneous.
‡ By stomach tube.
§ Warmed before administration, supersaturated at room temperature.
|| In 0.08 M NaCl.

37 per cent of the C¹⁴ of 4-C¹⁴-methionine and 28 per cent of that of the Me-C¹⁴ compound were recovered in the respiratory CO₂. The labeling of the serine, choline, and glycogen is shown in Tables II and III. Very
little of the 4-C\textsuperscript{14}-methionine activity was incorporated into the serine-\(\beta\)-carbon or choline methyl groups. Glycogen was labeled in all carbons, with equal activity in the 1,6 and 2,5 positions. CH\textsubscript{3}-C\textsuperscript{14}-Methionine produced high labeling of the serine-\(\beta\)-carbon and of all of the glycogen carbon atoms

### Table II

**Distribution of Isotope in Liver Glycogen after Administration of C\textsuperscript{14}-Labeled Methionine**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Type of C\textsuperscript{14}-methionine administered</th>
<th>Average specific activity of glucose* carbons(\dagger)</th>
<th>Labeling of the carbon fractions of glucose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-C\textsuperscript{14}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CH\textsubscript{3}-C\textsuperscript{14}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3-C\textsuperscript{14}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2-C\textsuperscript{14}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2-C\textsuperscript{14}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* C.p.m. per mg. of carbon.
\(\dagger\) Positions of the carbon atoms in glucose.
\(\ddagger\) Determined on glucose phenylosazone.
\(\S\) Determined on glucose \(p\)-nitrophenylhydrazone.

### Table III

**Labeling of Serine, Choline, and Aspartic Acid by C\textsuperscript{14}-Methionine**

<table>
<thead>
<tr>
<th>Type of methionine administered</th>
<th>Serine</th>
<th>Choline</th>
<th>Aspartic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta)-C</td>
<td>(\alpha)-C</td>
<td>COOH-C</td>
</tr>
<tr>
<td>4-C\textsuperscript{14}</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>CH\textsubscript{3}-C\textsuperscript{14}</td>
<td>1700</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2-C\textsuperscript{14}</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In c.p.m. per mg. of carbon.
\(\dagger\) Calculated.

with significantly higher activity in the 1,6 than in the 2,5 position, as shown in Table II.

**3-C\textsuperscript{14}-DL-Methionine and 2-C\textsuperscript{14}-DL-Methionine Experiments**

In the 3-C\textsuperscript{14}-methionine and first 2-C\textsuperscript{14}-methionine experiments (Experiments 3 and 4), liver glycogen was isolated by the method of Good, Kramer, and Somogyi (14) and purified and degraded as described in the preceding section. The results are presented in Table II. 2-C\textsuperscript{14}-Methionine labeled the glycogen in the 3,4 carbon, whereas 3-C\textsuperscript{14}-methionine produced gly-
cogen labeled in all positions with approximately equal activity in the 1,6 and 2,5 carbons.

In the second 2-C\textsuperscript{14}-methionine experiment (Experiment 5) liver glycogen was isolated, and the liver protein was hydrolyzed as in Experiments 1 and 2. Portions of the glucose were converted to the phenyllosazone and \textit{p}-nitrophenylhydrazone derivatives which were recrystallized (m.p. 205\degree and 190\degree, respectively) and oxidized to CO\textsubscript{2} for the determination of the specific activity of the glucose carbon. A third portion of the glucose was partially degraded by fermentation with \textit{Leuconostoc mesenteroides}, which converts the 1 carbon to CO\textsubscript{2} (15). The dicarboxylic amino acids were separated from the hydrolysate by the procedure of Cannan (16). After removal of the glutamic acid as the hydrochloride, the aspartic acid was precipitated as the copper salt which was recrystallized and freed of copper by the usual procedure. Aspartic acid was purified by chromatography on a 70 cm. Dowex 50 column (2). Although the amino acid was pure, as indicated by a symmetrical elution curve of radioactivity and amino acid concentration (17), the sample was subjected to chromatography on a 40 cm. Amberlite IR-4B (acetate) column (18). The Amberlite introduced a colored impurity into the aspartic acid which was removed by rechromatographing on Dowex 50. Part of the aspartic acid was oxidized to CO\textsubscript{2} for determination of the specific activity of the carbon. The remainder was degraded with hypochlorite, which converted carbon atoms 2 and 3 to acetaldehyde (19). The acetaldehyde-2,4-dinitrophenylhydrazone was oxidized to CO\textsubscript{2} to determine the average C\textsuperscript{14} activity of the acetaldehyde carbon.

15 per cent of the C\textsuperscript{14} of 3-C\textsuperscript{14}-methionine was recovered in the respiratory CO\textsubscript{2}, and 32 and 42 per cent of the C\textsuperscript{14} were recovered after the administration of the 2-C\textsuperscript{14} compound in Experiments 4 and 5, respectively. The C\textsuperscript{14} activity of the liver glycogen and aspartic acid and the distribution of this activity are shown in Tables II and III. The glycogen and aspartic acid possessed considerable C\textsuperscript{14} activity, but very little was located in the 2 and 3 carbons of the aspartic acid or the 1 carbon of the glucose.

The conversion of \textit{D}- and \textit{L}-methionine to propionate or a propionic acid derivative, as shown in Scheme 1, is proposed as the principal pathway of methionine metabolism in animals. Previous studies have demonstrated the occurrence of the various steps of this scheme.

\[
\begin{align*}
\text{\textit{L}-Methionine} & \rightarrow \alpha\text{-ketobutyrate} \rightarrow \text{propionate} \\
\uparrow & \\
\alpha\text{-Keto-\gamma-methiolbutyric acid} & \\
\uparrow & \\
\text{\textit{D}-Methionine} &
\end{align*}
\]

\textbf{Scheme 1. Proposed principal mechanism of methionine metabolism}
The conversion of D- to L-methionine is indicated by the ability of D-methionine to support the growth of rats receiving a diet deficient in methionine and cystine (20) and by the existence of enzymes capable of deaminating D-methionine (21) and reaminating the corresponding keto acid to L-methionine (22-26).

α-Ketobutyric acid is formed from methionine by a number of mechanisms. Carroll et al. (27) showed that α-ketobutyrate is a product of L-cystathione cleavage by rat liver, and Binkley (28) observed that a purified cystathionase preparation degraded DL-homocysteine and DL-methionine with the formation of ammonia and an equivalent amount of \( \text{H}_2\text{S} \) and methyl mercaptan, respectively. α-Ketobutyrate was identified as a product of the desulfurization of homocysteine. Evidence for the formation of α-aminobutyric acid\(^1\) (29-31) and homoserine\(^1\) (31) from DL-methionine and for the conversion of D- (21) and L-α-aminobutyric acid (22, 23, 26) and L-homoserine (27, 32) to α-ketobutyrate has also been reported.

The conversion of α-ketobutyric acid to propionate is supported by reports that it is rapidly and oxidatively decarboxylated by pigeon brain (33, 34), and that DL-α-aminobutyrate is converted to α-ketobutyric and propionic acids by rat liver homogenate (35).

The results of the present experiment are in full accord with the postulate that carbon atoms 2, 3, and 4 of D- and L-methionine are metabolized in animals largely via propionic acid. The types of glycogen produced by the C\(^14\)-DL-methionines (Table II) were identical with those which would have been produced on conversion of the 2, 3, and 4 carbons to propionic acid. The 2 C\(^14\)-DL-methionine, like 1-C\(^13\)-propionate (36), introduced labeling only into the 3,4 position of the glycogen and 3-C\(^14\) and 4-C\(^14\)-DL-methionine, like 2-C\(^13\)- and 3-C\(^13\)-propionate (36), introduced labeling into all the carbons of the glucose with roughly equal labeling in carbon atoms 1,6 and 2,5.

It is recognized that the present data do not provide conclusive proof of the principal metabolism of methionine by the proposed scheme (Scheme 1). However, the occurrence in the rat of two other mechanisms is eliminated. One is the direct conversion of methionine to aspartic acid, a pathway suggested by the formation of homoserine from DL-methionine in rats (31) and the existence of a mechanism for the transformation of L-homoserine to L-aspartic acid in yeast (38-40). By this process, 2-C\(^14\)-methionine would label the 2 carbon of aspartic acid and the 1,6 and 2,5 positions of the liver glycogen. While the aspartic acid and glycogen were heavily labeled, negligible C\(^14\) was located in the 2 and 3 carbons of aspartic acid or in the 1 position of the glucose (Tables II and III). These results contrast with the report of Marshall and Friedberg (41) that the activity of 2-C\(^14\).

\(^1\) The optical identity of the amino acid was not reported.
methionine is primarily incorporated into the non-carboxyl carbons of aspartic acid in mice.

The metabolism of methionine via conversion of the carbon chain to pyruvate and formaldehyde (from the γ-carbon) has also been excluded by the present data.\(^2\) According to this pathway, 4-C\(^{14}\)-methionine would be expected to form C\(^{14}\)-formaldehyde and to label the serine-β-carbon and choline methyl groups (42–44). Little or no C\(^{14}\) was found in these moieties; the administration of an identical dose of methyl-C\(^{14}\)-methionine labeled the serine-β-carbon very heavily (Table III). In addition, this mechanism would produce 2-C\(^{14}\)-pyruvate from 2-C\(^{14}\)-methionine and would be expected therefore to introduce C\(^{14}\) into the 1,6 and 2,5 carbon atoms of the liver glycogen as is observed with 2-C\(^{14}\)-lactate (45). The results of Lactobacillus casei degradation of the glycogen indicated that there was no such labeling. A more exact determination of the C\(^{14}\) in the 1 carbon was made in Experiment 5 by gas phase proportional counting of the CO\(_2\) from this position. The specific activity of the 1 carbon of the glucose was very low (Table II).

The indication for the formation of propionic acid as a major intermediate of methionine catabolism contributes to the evidence that propionate is an amino acid metabolite of considerable quantitative importance. Coon et al. (46) have presented data indicating that propionic acid is formed from the 2,3- and 3'-methyl carbons of isoleucine in rats, and Fones et al. (47), Peterson et al. (48), and Kinnory et al. (49) have obtained evidence for the formation of propionate from the isopropyl group of L-valine in this animal. Lien and Greenberg (50) have also shown that α-ketobutyrate is formed from DL-threonine in rat liver homogenate. However, it appears from the studies of Meltzer and Sprinson (51) that this pathway is not of major quantitative significance in the metabolism of threonine in the normal rat.

The assistance of Dr. John R. Murphy with the gas phase proportional counting and of Dr. Murphy and Dr. J. A. Muntz in the L. mesenteroides degradation of glucose is gratefully acknowledged.

**SUMMARY**

2-C\(^{14}\), 3-C\(^{14}\), and 4-C\(^{14}\)-methionine have been administered to fasting rats, and the isotope distribution in the liver glycogen has been determined. 2-C\(^{14}\)-Methionine labels the glycogen solely in the 3,4 carbon, whereas 3-C\(^{14}\) and 4-C\(^{14}\)-methionine label the glycogen in all positions with equal labeling in the 1,6 and 2,5 carbons. It has also been found that 4-C\(^{14}\)-methionine does not label the serine-β-carbon and choline methyl groups.

\(^2\) This conversion was conceived to occur via α-keto-γ-hydroxybutyric acid which is formed from pyruvate and formaldehyde in liver (37).
and that 2-C\textsuperscript{14}-methionine labels aspartic acid principally in the carboxyl carbon. The implications of these findings are discussed.

BIBLIOGRAPHY

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