THE METABOLISM OF 2-CARBON COMPOUNDS RELATED TO GLYCINE

II. ETHANOLAMINE*

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In a continuation of the search for intermediates involved in the condensation of the α-carbon atoms of 2 glycine molecules (1), investigations were carried out with ethanolamine-1-C¹³, 2-C¹⁴ (C¹³H₂OH—C¹⁴H₂NH₂). It was considered possible that the serine synthesized from administered glycine-2-C¹⁴ is, at first, labeled only in the α-carbon atom, the β-carbon atom being derived from unlabeled, endogenous sources of "formate." If the ethanolamine formed from this serine (2) were capable of yielding a labeled 1-carbon fragment, α,β-labeled serine would be produced. The results described below failed to support such a reaction scheme. It was shown, however, that a major reaction in the metabolism of ethanolamine is deamination, oxidation, and reamination to glycine.

C¹³H₂OH—C¹⁴H₂NH₂ → [C¹³H₂O—C¹⁴HO] → C¹³H₂NH₂—C¹⁴OOH (1)

With aspartic acid labeled with N¹⁵ as a source of labeled nitrogen, indirect evidence was also obtained for the conversion of glycolaldehyde to glycine.

EXPERIMENTAL

Preparation of Ethanolamine-1-C¹³, D-2-C¹⁴ (C¹³D₂OH—C¹⁴H₂NH₂)—A mixture of 76 mg. of C¹³H₂COONa² and 556 mg. of CH₃C¹⁴OONa was converted to acetyl bromide (3), and the product was used to synthesize

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¹ Deuterium was introduced at carbon 1 of the ethanolamine in connection with another investigation.

² The BaC⁴O₃ used for the synthesis of the methyl-labeled acetate was obtained from the United States Atomic Energy Commission.
glycine (4). Repeated esterification with absolute alcoholic HCl gave 615 mg. of glycine ethyl ester hydrochloride, m.p. 142–143°. To a suspension of 600 mg. of ester hydrochloride in 0.8 cc. of chloroform, cooled in an ice bath, were added dropwise with stirring 7 cc. of 2 per cent NH₃ in chloroform (5). The mixture was stirred for an additional 15 minutes and centrifuged in the cold. The precipitate was stirred with additional cold chloroform, and the combined supernatant solutions were taken to dryness in vacuo.

The residual glycine ethyl ester was dissolved in a few cc. of absolute ether and added dropwise to 420 mg. of lithium aluminum deuteride in 5 cc. of absolute ether, in a flask equipped with a magnetic stirrer, reflux condenser, and dropping funnel (6). 15 minutes after the last addition of ester, 3 cc. of D₂O were added dropwise. The resulting mixture of water, ether, and lithium and aluminum hydroxides was transferred to a continuous extractor with the aid of 15 cc. of water and extracted for 48 hours with ether containing 3 cc. of 20 per cent HCl. The ether was taken to dryness, and the residual ethanolamine hydrochloride was crystallized, and recrystallized, from a small volume of hot absolute alcohol by the addition of dry ether. Yield 200 mg. (50 per cent); m.p. 74–76°; activity, 3.59 \times 10⁸ c.p.m. per dish of labeled carbon under standard conditions; C¹³ 38.4 and D 98 atom per cent excess on the hydroxyl carbon.

Other Compounds—The L-aspartic acid-N¹⁵ and the CH₃C⁰OONa were the kind gifts of Dr. D. Rittenberg. Glycolic acid was a commercial product, while glycolaldehyde, as the dimer, was prepared from dihydroxymaleic acid (7). We are indebted to Dr. E. Pollaczek and Dr. Z. Dische for the glycolaldehyde phosphate.

Administration of Compounds, Isolation and Degradation of Products—Materials were administered and products isolated essentially as described in Paper I (1). Ethanolamine hydrochloride was incorporated into the same stock diet for feeding to a rat. Uric acid was degraded by a modification (8) of the usual method (9). Glycogen was degraded by the Lactobacillus casei method (10).

In the investigation of various 2-carbon compounds as acceptors of nitrogen for the formation of glycine, a solution of 0.5 mM of benzoate, 0.37 ±
0.03 mM of L-aspartic acid-N\textsuperscript{15}, and 0.5 mM of the compound was neutralized with NaHCO\textsubscript{3} and injected intraperitoneally into rats, in four portions, at 2 hour intervals. Food was withheld from the animals during the 24 hour experimental period.

Determination and calculation of C\textsuperscript{14} activity were as reported in Paper I. Analyses for N\textsuperscript{15}, C\textsuperscript{13}, and D were performed by the usual procedures (11, 12).

**RESULTS AND DISCUSSION**

*Conversion of Ethanolamine to Glycine*—Following the administration of C\textsuperscript{13}H\textsubscript{2}OH—C\textsuperscript{14}H\textsubscript{2}NH\textsubscript{2} to a pigeon, 75 per cent of the activity of the excreted uric acid was found in carbon atom 4 (Table I). The only appreciable C\textsuperscript{13} concentration was in carbon atom 5. Since positions 4 and 5 of uric acid are derived from the carboxyl and \(\alpha\)-carbon atoms, respectively, of glycine, the amino carbon of ethanolamine must have been oxidized to the carboxyl, while the carbinol carbon was converted to the methylene, group of glycine.

Similar results were obtained after administration of the labeled ethanolamine, together with benzoate, to a rat (Table II). The carboxyl group of the glycine in the excreted hippuric acid contained most of the activity. Glycogen, isolated from the liver, showed the highest activity in carbon atoms 3 and 4 of the glucose, with small activities in the 1, 6 and 2, 5 positions. This is the isotope distribution to be expected if the administered ethanolamine were converted *predominantly* to glycine-1-C\textsuperscript{14} and the latter utilized for glycogen formation by way of serine-1-C\textsuperscript{14} (13).

The most reasonable way to explain these results is indicated briefly in Equation 1. The facile deamination of ethanolamine is known from an investigation of this compound labeled with N\textsuperscript{15} (14). Glycolaldehyde, the

<table>
<thead>
<tr>
<th>Carbon atom No.</th>
<th>C\textsuperscript{14}</th>
<th>C\textsuperscript{13}</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(\times 10^{-6})</td>
<td>atom per cent excess</td>
</tr>
<tr>
<td>5</td>
<td>8.13</td>
<td>0.080</td>
</tr>
<tr>
<td>4</td>
<td>48.0</td>
<td>0.00</td>
</tr>
<tr>
<td>2 + 8</td>
<td>1.73</td>
<td>0.010</td>
</tr>
<tr>
<td>6</td>
<td>4.72</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* 0.044 mM of labeled ethanolamine hydrochloride per 100 gm. injected. Activity, \(3.59 \times 10^7\) c.p.m. per dish of labeled carbon under standard conditions. The C\textsuperscript{13} was 38.4 atom per cent excess in carbon 1.
expected product, could be oxidized, first at the aldehyde carbon and then at the carbinol carbon, to glycolic and glyoxylic acids, respectively. An enzyme system, present in liver, has recently been described which is specific for the oxidation of glycolic to glyoxylic acid (15). The latter is rapidly aminated to glycine (1, 16).5

The ability of glycolaldehyde to become available as an acceptor of nitrogen for glycine formation is further illustrated in Table III. 1-Aspartic acid labeled with N15 was administered to rats, together with benzoate and several compounds metabolically related to glycolaldehyde. In the presence of glycolic acid, glycolaldehyde, glycolaldehyde phosphate, and glyoxylic acid, the N15 of the excreted hippurate was appreciably higher than in the controls. Since glycolic (16) and glyoxylic (1, 16) acids labeled with C14 have been shown to be effective precursors of glycine, the similarity of glycolaldehyde to these compounds as an acceptor of nitrogen supports the view that glycolaldehyde is also capable of becoming converted to glycine.

The results presented in this paper suggest that glycolaldehyde can be converted to glycine and that it occurs as an intermediate in the metabolism of ethanolamine. Several recent investigations in the metabolism of glucose and ribose have indicated the occurrence of an active form of glycolaldehyde (17–19). Such a derivative might be an important source for the carbon chain of glycine. These considerations imply that at least some glycine is formed from carbohydrate directly, rather than from serine.

* It should be noted that the postulated intermediates as well as the ethanolamine itself may be involved as phosphorylated or other derivatives.
The conversion of ethanolamine to glycine indicated in Equation 1 provides for a cycle of reactions in which the carbon atoms of a serine molecule are successively removed as CO₂, while "formate" carbon is converted to serine. In one turn of this cycle the carboxyl group of glycine is lost as CO₂ when serine is decarboxylated to ethanolamine. The α-carbon of glycine, now the amino carbon of ethanolamine, forms the carboxyl group of the new glycine. This would then be lost as CO₂ in the next turn of the cycle. The source of the carbon atom which replenishes that lost as CO₂ is the "formate," which is the precursor of the β-carbon atom of serine. This may arise from the α-carbon of glycine itself or from other sources. After three turns of the cycle, a molecule of serine is lost as CO₂, and a new one is produced which is derived from "formate."

**Metabolism of Ethanolamine via Choline—**The results in Tables I and II show that, although Equation 1 may represent the predominant pathway for the conversion of dietary ethanolamine to glycine, another pathway exists by which the amino carbon of the base, rather than the carbonyl carbon, is the precursor of the methylene carbon atom of glycine. It can be observed that carbon 5 of uric acid has one-sixth of the activity of carbon 4, and the α-carbon of hippurate has one-fourth of the activity of the carboxyl carbon. Similarly, carbons 1, 6 and 2, 5 of the glucose from liver

### TABLE III

*Effect of Glycolaldehyde and Related Compounds on Utilization of L-Aspartate-N¹⁵ for Hippuric Acid Formation in Rats*

<table>
<thead>
<tr>
<th>Compound tested*</th>
<th>N¹⁵ in hippuric acid atom per cent excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Aspartic acid-N¹⁵</td>
<td>0.424</td>
</tr>
<tr>
<td>&quot; &quot; + glyoxylic acid†</td>
<td>0.725</td>
</tr>
<tr>
<td>&quot; &quot; + glycolic acid</td>
<td>0.618</td>
</tr>
<tr>
<td>&quot; &quot; + glycolaldehyde</td>
<td>0.686</td>
</tr>
<tr>
<td>&quot; &quot; + glycolaldehyde phosphate</td>
<td>0.631</td>
</tr>
</tbody>
</table>

* 0.5 mM of 2-carbon compound, 0.37 ± 0.03 mM of L-aspartic acid (29.7 atom per cent excess N¹⁵), and 0.5 mM of sodium benzoate per 100 gm, were injected intraperitoneally in four portions at 2 hour intervals. The experiment was terminated 24 hours after the first injection.
† The dose of sodium glyoxylate was 0.39 mM per 100 gm.
glycogen had small but significant activity. It would appear that the methylation of ethanolamine to choline and oxidation of the latter to betaine, followed by demethylation to glycine (14, 22-24), are responsible for this type of labeling. The conversion of betaine (14) and sarcosine (24), labeled with N\textsuperscript{15}, to glycine has been shown to be extensive. The dilutions encountered in this pathway would make it difficult to show a direct utilization of ethanolamine-N\textsuperscript{15} for glycine formation in the intact animal (14, 25).

**SUMMARY**

Ethanolamine was synthesized labeled with C\textsuperscript{13} and deuterium in the carbinol carbon and with C\textsuperscript{14} in the amino carbon (C\textsuperscript{13}D\textsubscript{2}OH—C\textsuperscript{14}H\textsubscript{2}NH\textsubscript{2}). With the aid of this compound it could be shown that ethanolamine is converted to glycine in the rat and the pigeon. The amino carbon of ethanolamine is the precursor of the carboxyl group of glycine, and the alcohol carbon of ethanolamine appears as the methylene group of glycine. The possible rôle of glycolaldehyde in this process is discussed.

To a minor extent the amino carbon of the ethanolamine is also converted to that of glycine. The possible rôle of choline and betaine in this series of reactions is indicated.

With L-aspartic acid-N\textsuperscript{15} as a source of labeled nitrogen, glycolaldehyde was shown to resemble glyoxylic and glycolic acids as an effective acceptor of nitrogen for hippurate formation. The relation of these results to the origin of the carbon chain of glycine is briefly discussed.

We are indebted to Mr. I. Sucher for the mass spectrometric analyses.

**BIBLIOGRAPHY**


* The ratio of activities of carbons 3,4 to carbons 1,6,2,5 in the glucose is much greater than the ratio of carboxyl carbon to methylene carbon in the glycine (Table II). This may be attributed to the incorporation of respiratory CO\textsubscript{2} into carbons 3,4 of glucose (10, 20, 21). In general the distribution of isotope in the glucose is what would be expected from the administration of a mixture of glycine-1-C\textsuperscript{14} and glycine-2-C\textsuperscript{14} in which the former predominated (21).
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