THE SUCCINATE-GLYCINE CYCLE*

II. METABOLISM OF δ-AMINOLEVULINIC ACID

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A mechanism by which the α-carbon atom of glycine is metabolically detached from its carboxyl group has been postulated (1-3). “Active” succinate condenses on the α-carbon atom of glycine to yield α-amino-β-keto adipic acid which on decarboxylation yields δ-aminolevulinic acid (Reactions 1 and 2).

\[
\begin{align*}
(1) \quad & \text{HOOC} - \text{CH}_2 - \text{CH}_2 - \text{COOH} + \text{NH}_2 - \text{CH}_2 - \text{COOH} \\
& \quad \rightarrow \text{HOOC} - \text{CH}_2 - \text{CH}_2 - \text{CO} - \text{CHNH}_2 \text{COOH}
\end{align*}
\]

\[
\begin{align*}
(2) \quad & \text{HOOC} - \text{CH}_2 - \text{CH}_2 - \text{CO} - \text{CHNH}_2 \text{COOH} \\
& \quad \rightarrow \text{HOOC} - \text{CH}_2 - \text{CH}_2 - \text{CO} - \text{CH}_2 \text{NH}_2 + \text{CO}_2
\end{align*}
\]

This aminoketonic acid is the source of all the carbon atoms of protoporphyrin (2-4). In this series of reactions, the α-carbon atom of glycine becomes the δ-carbon atom of δ-aminolevulinic acid. Since the α-carbon atom of glycine, detached from its carboxyl group, is not only utilized for porphyrin synthesis but also conforms to the metabolic pattern of the “C1” compounds (3), this carbon acylation of glycine may be a mechanism by which the α-carbon atom of glycine is utilized in the synthesis of various compounds. If so, the δ carbon atom of δ aminolevulinic acid should follow the same metabolic course as the α-carbon atom of glycine and the remaining carbon atoms should be reconverted to succinate. The series of reactions would thus be cyclic in nature, succinate acting as the metabolic catalyst for the metabolism of glycine. This cyclic series of reactions, the succinate-glycine cycle, has been postulated in order to explain not only the formation of δ-aminolevulinic acid, the precursor of porphyrin, but other features of the metabolic behavior of glycine as well.

In this communication, evidence is presented which supports the postulated cyclic series of reactions. The experiments demonstrate that the

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\(\delta\)-carbon atom of \(\delta\)-aminolevulinic acid is utilized for the ureido groups of purines and is converted to formic acid. Further, it has been found that the succinyl moiety of \(\delta\) aminolevulinic acid gives rise to succinate.

In order to study the metabolic fate of the \(\delta\)-carbon atom, we have administered \(\delta\)-aminolevulinic acid-5-C\(^{14}\) to birds and compared its utilization for purine and hemin formation to that of glycine-2-C\(^{14}\).

In the experiment in Table I, ducks were injected with these two isotopic compounds and subsequently the radioactivities of the heme and of the ureido groups of guanine from the red blood cells were determined. It can be seen (Table I) that both the heme and the ureido groups of the guanine synthesized from \(\delta\)-aminolevulinic acid-5-C\(^{14}\) were about 2 times more radioactive than the compounds produced from glycine-2-C\(^{14}\). This

<table>
<thead>
<tr>
<th>Radioactive product</th>
<th>(\delta)-Aminolevulinic acid-5-C(^{14})</th>
<th>Glycine-2-C(^{14})</th>
<th>Ratio of activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C(^{14}) activity</td>
<td>molar activity</td>
<td>C(^{14}) activity</td>
</tr>
<tr>
<td>Hemin</td>
<td>3900</td>
<td>209,000</td>
<td>1835</td>
</tr>
<tr>
<td>Guanine sulfate</td>
<td>214</td>
<td>3,900</td>
<td>56</td>
</tr>
<tr>
<td>Carbon 2 of guanine as guanidine picrate</td>
<td>100</td>
<td>2,380</td>
<td></td>
</tr>
</tbody>
</table>

ratio of 2 may be minimal in view of the excretion of \(\delta\) aminolevulinic acid and of the ease with which this compound may condense with itself to form pyrazine derivatives.

In Table II, the results are given for the utilization of \(\delta\)-aminolevulinic acid-5-C\(^{14}\) for the synthesis of uric acid in the pigeon. The C\(^{14}\) distribution among the carbon atoms of the uric acid resembles the distribution known for the \(\alpha\)-carbon atom of glycine and for formate. The radioactivity of carbon atom \(\delta\) of uric acid is the same as that of the respiratory carbon dioxide.

The production of formate and carbon dioxide from both \(\delta\)-aminolevulinic acid-5-C\(^{14}\) and glycine-2-C\(^{14}\) was studied in the rat. These compounds were injected into rats, and, with the aid of carrier formate, the radioactivity of the urinary formate was determined. During the experimental period, the respiratory carbon dioxide was collected and its radioactivity measured. Table III shows that the radioactivity of the formate formed...
from δ-aminolevulinic acid-5-C\textsuperscript{14} is equal to or greater than that of the sample formed from glycine-2-C\textsuperscript{14}, whereas the carbon dioxide produced from glycine-2-C\textsuperscript{14} is far more radioactive than that formed from δ-aminolevulinic acid-5-C\textsuperscript{14}.

In order to assess the cyclic nature of the postulated series of reactions, the possible formation of succinate from δ-aminolevulinic acid was studied. Rats were injected with δ-aminolevulinic acid-1,4-C\textsuperscript{14} together with

<table>
<thead>
<tr>
<th>Compound (duration of experiment)</th>
<th>Activities</th>
<th>C\textsuperscript{14} activities in Uric acid carbon No.\textsuperscript{*}</th>
<th>Respiratory CO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ-Aminolevulinic acid-5-C\textsuperscript{14} (8 hrs.)</td>
<td>C.p.m.</td>
<td>600</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>Molar activity, % total in uric acid</td>
<td>8,400</td>
<td>5,700</td>
</tr>
<tr>
<td>δ-Aminolevulinic acid-5-C\textsuperscript{14} (24 hrs.)</td>
<td>C.p.m.</td>
<td>1,090</td>
<td>374</td>
</tr>
<tr>
<td></td>
<td>Molar activity, % total in uric acid</td>
<td>15,300</td>
<td>10,200</td>
</tr>
<tr>
<td>δ-Aminolevulinic acid-5-C\textsuperscript{14} (72 hrs.)</td>
<td>C.p.m.</td>
<td>1,030</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>Molar activity, % total in uric acid</td>
<td>14,500</td>
<td>7,000</td>
</tr>
<tr>
<td>γ-Ketoglutaraldehyde-5-C\textsuperscript{14} (72 hrs.)</td>
<td>C.p.m.</td>
<td>164</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Molar activity, % total in uric acid</td>
<td>2,300</td>
<td>1,200</td>
</tr>
</tbody>
</table>

\textsuperscript{*} Carbon 5 counted as dimedon formaldehyde; the others as BaCO\textsubscript{3}.

malonate, and succinic acid was isolated from the urine. It was found that the excreted succinic acid was highly radioactive and that all of the radioactivity resided in the carboxyl groups (Table IV). The dilution was merely 180-fold.

γ-Ketoglutaraldehyde, presumably derived by oxidative deamination of δ-aminolevulinic acid (Reaction 3), had been postulated previously (3) as an intermediate in the formation of succinate from δ-aminolevulinic acid.

(3) \text{HOOC—CH\textsubscript{2}—CH\textsubscript{2}—CO—CH\textsubscript{2}NH\textsubscript{2}→HOOC—CH\textsubscript{2}—CH\textsubscript{2}—CO—CHO}  

If this were indeed the case, the aldehydic carbon atom of γ-ketoglutaralde-
hyde would be utilized for compounds other than heme, in the same manner as the δ-carbon atom of δ-aminolevulinic acid.

The production of formate and carbon dioxide from γ-ketoglutaraldehyde-5-Cl4 was studied in the rat. The data in Table III show that the radioactivity of the formate formed from γ-ketoglutaraldehyde-5-Cl4 is a number of times greater than the samples from glycine-2-Cl4 or δ-aminolevulinic acid.

**Table III**

Comparison of Radioactivities in Formic Acid and Respiratory CO₂ Synthesized from δ-Aminolevulinic Acid-5-Cl4 (0.06 Mc. per Mmole), from Glycine-2-Cl4 (0.05 Mc. per Mmole), and from γ-Ketoglutaraldehyde-5-Cl4 (0.05 Mc. per Mmole) in Rat

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Radioactivity of Formate*</th>
<th>Respiratory CO₂*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mmoles excreted</td>
<td>No. of mmoles excreted</td>
</tr>
<tr>
<td>δ-Aminolevulinic acid-5-Cl4</td>
<td>620</td>
<td>0.66</td>
</tr>
<tr>
<td>Glycine-2-Cl4</td>
<td>569</td>
<td>0.63</td>
</tr>
<tr>
<td>γ-Ketoglutaraldehyde-5-Cl4</td>
<td>1732</td>
<td>0.61</td>
</tr>
</tbody>
</table>

* Counted as BaCO₃.

**Table IV**

Distribution of Radioactivity of Succinic Acid Synthesized from δ-Aminolevulinic Acid-1,4-Cl4 (0.025 Mc. per Mmole) in Rat

<table>
<thead>
<tr>
<th>Compound analyzed for C₁⁴</th>
<th>C¹⁴ activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c.p.m.</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>910</td>
</tr>
<tr>
<td>Anilic acid*</td>
<td>362</td>
</tr>
<tr>
<td>Carboxyl groups of succinic acid as BaCO₃</td>
<td>319</td>
</tr>
</tbody>
</table>

* Succino-p-phenylazoanilic acid.

levulinic acid-5-Cl₄. Studied also was the utilization of γ-ketoglutaraldehyde-5-Cl₄ for the synthesis of uric acid in the pigeon. As shown in Table II, the C¹⁴ distribution among the carbon atoms of the uric acid closely resembles the distribution found for δ-aminolevulinic acid-5-Cl₄.

Further, it was found that γ-ketoglutaraldehyde cannot be converted back into δ-aminolevulinic acid. This is evidenced by the failure of hemolyzed duck erythrocytes to utilize γ-ketoglutaraldehyde-5-Cl₄ for heme synthesis as well as by the non-utilization of this compound for heme synthesis in the pigeon in vivo. Although γ-ketoglutaraldehyde...
was metabolized somewhat as expected, these experiments, of course, cannot establish it as an intermediate.

**EXPERIMENTAL**

**Radioactive Compounds**

$\delta$-Aminolevulinic acid-5-C$^{14}$ was made from $\beta$-ketoadipic acid-2-C$^{14}$ as previously described (3). $\delta$-Aminolevulinic acid-1,4-C$^{14}$ was prepared by Dr. Elliott Schiffmann (4). Glycine-2-C$^{14}$ was purchased from Tracer Laboratories, Inc.

Ethyl levulinate-5-C$^{14}$ was obtained as a byproduct in the preparation of diethyl $\beta$-ketoadipate-2-C$^{14}$. After hydrolysis of the ester in 2 N HCl, the levulinic acid-5-C$^{14}$ was brominated to form 3,5-dibromolevulinic acid-5-C$^{14}$ in 80 per cent yield. This compound was hydrolyzed to give $\gamma$-ketoglutaraldehyde-5-C$^{14}$ as described by Wolff (5). The aldehyde was further characterized by preparing a number of derivatives previously described by Veibel (6): the p-nitrophenyl osazone, the 2,4-dinitrophenyl osazone, and its pyridine salt. These compounds were shown to have the same specific radioactivity as the dibromolevulinic acid-5-C$^{14}$. Mixture of these derivatives with samples prepared from authentic levulinic acid resulted in no depression of melting points.

An alternative synthesis of levulinic acid from methyl iodide and $\beta$-carbomethoxypropionyl chloride as described by Cason (7) was found to be satisfactory and is adaptable for making levulinic acid-5-C$^{14}$. Starting with 25 mmoles of methyl iodide, methyl levulinate was obtained in a yield of 67 per cent. Hydrolysis of the ester in this case was carried out in 0.3 N KOH at room temperatures for 24 hours. The hydrolysate was neutralized with 1 N HCl, the methanol removed from the solution by aeration, and the water removed by vacuum distillation and desiccation in vacuo over KOH. The residue was extracted several times with diethyl ether. The ether solution was fractionally distilled. The yield of levulinic acid from the methyl levulinate was 88 per cent.

**Measurement of Radioactivity**—Samples were deposited as an “infinitely thick” layer on a standard dish and assayed for their radioactivity with an end window counter. The results are reported as counts per minute above background. The use of the term molar activity is as defined earlier (8, 9).

\[
\text{Molar activity} = \frac{\text{activity found}}{((\text{mol. wt. in gm.})/12 \text{ gm.})} \times \text{number of moles of compound in parent molecule}
\]

**Hemin Synthesis in Pigeon and by Hemolyzed Duck Erythrocytes**

1 mmole of $\gamma$-ketoglutaraldehyde-5-C$^{14}$ (0.05 me. per mmole) was injected intraperitoneally in the pigeon in four divided doses over a period of 1 Weliky, I., and Shemin, D., manuscript in preparation.
48 hours. 24 hours after the last injection, the pigeon was exsanguinated by decapitation and hemin was prepared from whole blood (10).

Hemolyzed duck erythrocytes were incubated as previously described (3, 8) with 4 mg. of \( \gamma \)-ketoglutaraldehyde-5-C\(^{14} \) (0.05 mc. per mmole) in a volume of 30 ml. Hemin was prepared as described previously (10).

**Hemin and Purine Synthesis in Duck Blood**—A duck was injected intraperitoneally with 5 mmoles of \( \delta \)-aminolevulinic acid-5-C\(^{14} \) (0.054 mc. per mmole) or glycine-2-C\(^{14} \) (0.1 mc. per mmole) divided into four doses over a period of 2 days. After 5 days the duck was exsanguinated. Hemolysis was effected with saponin, and the nuclei sedimented by centrifugation and extracted with \( 1 \text{M} \) sodium chloride. The nucleic acids were precipitated with ethanol. After acid hydrolysis, guanine was isolated from a Dowex 50 column, recrystallized as guanine sulfate, and carbon 2 was obtained as guanidine picrate by the method described by Brown et al. (11). Hemin was prepared from the supernatant solution as described previously (10).

**Uric Acid Synthesis in Pigeon**—Pigeons were fasted for 24 hours and injected with 1 mmole of \( \delta \)-aminolevulinic acid-5-C\(^{14} \) (0.05 mc. per mmole) intraperitoneally. Droppings and respiratory CO\(_2\) were collected in one experiment for 8 hours, in another for 24 hours. In a third experiment the 1 mmole of \( \delta \)-aminolevulinic acid-5-C\(^{14} \) (0.05 mc. per mmole) was divided into four doses over a period of 2 days and the droppings collected for 3 days. The latter experiment was repeated by using \( \gamma \)-ketoglutaraldehyde-5-C\(^{14} \) (0.05 mc. per mmole). The uric acid was isolated from the droppings and degraded by a combination of the methods of Buchanan et al. (12) and Cavalieri et al. (13). Carbons 2 and 8 and 6 and 4 were counted as BaCO\(_3\), carbon 5 as dimedon formaldehyde.

**Formate and Respiratory CO\(_2\) Synthesis in Rat**—A 100 gm. rat was fasted for 12 hours and then injected with 2 mmoles of sodium formate and 0.25 mmole of \( \delta \)-aminolevulinic acid-5-C\(^{14} \) (0.05 mc. per mmole), glycine-2-C\(^{14} \) (0.05 mc. per mmole), or \( \gamma \)-ketoglutaraldehyde-5-C\(^{14} \) (0.05 mc. per mmole), intraperitoneally. Respiratory CO\(_2\) and urine were collected for 6 hours. Urinary formate was isolated and oxidized to CO\(_2\) as described by Piria (14). Formate and CO\(_2\) were counted as BaCO\(_3\).

**Succinate Synthesis in Rat**—Two rats totaling 340 gm. in weight received 1.2 mmoles of sodium malonate per 100 gm. subcutaneously (15). 0.5 hour thereafter they were injected with 0.34 mmole of \( \delta \)-aminolevulinic acid-1, 4-C\(^{14} \) (0.025 mc. per mmole) per 100 gm. intraperitoneally. Urine was collected for 8 hours, neutralized with dilute sodium hydroxide solution, and made 5 per cent in NaHSO\(_3\). The urine was then brought to pH 4 with HCl and continuously extracted with diethyl ether for 48 hours. The ether was removed by distillation and the solid residue taken up in
10 ml. of water and heated on a steam bath for 2 hours, treated with charcoal, and filtered. The filtrate was evaporated to a volume of 0.5 ml. and the succinic acid crystallized. 32 mg. of succinic acid were obtained. The succinic acid was recrystallized several times from hot water until the radioactivity of a number of successive samples remained unchanged. Succino-p-phenylazoanilic acid was made by the method of Henbest and Owen (16) and recrystallized from ethanol. The degradation of succinic acid was carried out by the method of Schmidt as described by Phares (17).

A control of this experiment was made by repeating the procedure as described except that the δ-aminolevulinic acid-1, 4-C14 was placed directly into the receiver collecting the urine. The isolated succinic acid in this case was not radioactive, indicating that δ-aminolevulinic acid did not spontaneously give rise to succinic acid in the urine during collection or during isolation.

DISCUSSION

The findings reported in this communication demonstrate that the δ-carbon atom of δ-aminolevulinic acid, which arises from the α-carbon atom of glycine, has a similar metabolic pattern to that known for the α-carbon atom of glycine. The present results also show that the series of reactions, initiated by the condensation of succinate and glycine, may be cyclic in nature and provides a pathway for glycine metabolism since the succinyl moiety of the δ-aminolevulinic acid is reconverted to succinate.

It is difficult to estimate, at present, the quantitative aspects of this series of reactions in reference to all of the metabolic reactions of glycine in the whole animal. However, in duck erythrocytes (Table I) the δ-carbon atom of δ-aminolevulinic acid was incorporated into hemin twice as well as the α-carbon atom of glycine. That the ratio of incorporation into the ureido carbon atom of guanine in the same cell population was also about 2, suggests that δ-aminolevulinic acid is an intermediate in the utilization of the α-carbon atom of glycine in purine synthesis as well as for hemin. It should be emphasized that similar conclusions could have been drawn even if the conversions studied with δ-aminolevulinic acid had been lower than those found with glycine, for they merely require qualitative evidence that the δ-carbon atom of the aminoketonic acid has the same metabolic spectrum as the α-carbon atom of glycine in the whole animal.

The observation in the experiment performed with the rat (Table III) that the carbon dioxide formed from δ-aminolevulinic acid-5-C14 was considerably less radioactive than that formed from glycine-2-C14 or γ-ketoglutaraldehyde-5-C14 indicates the existence of other pathways of metabolism for these compounds. It appears that the condensation of
succinate and glycine may not be unique but represents a more general reaction. It seems that "active" acetate likewise acylates the α-carbon atom of glycine, with subsequent formation of aminoacetone, a metabolic analogue of δ-aminolevulinic acid. It has been found that the carbon atom in aminoacetone which arose from glycine is more efficiently oxidized to carbon dioxide than is the α-carbon atom of glycine, as measured by respiratory carbon dioxide formation.

**SUMMARY**

We have found that the δ-carbon atom of δ-aminolevulinic acid is converted to the ureido groups of guanine in the red blood cell and to those of uric acid and that it is also converted to formate. Since the δ-carbon atom of δ-aminolevulinic acid originated from the α-carbon atom of glycine and since both these carbon atoms show the same metabolic pattern, it is suggested that this is a pathway for the synthesis of "Cl" compounds from the α-carbon atom of glycine as well as the pathway for the synthesis of porphyrins from succinate and glycine.

It has also been found that the succinyl moiety of δ-aminolevulinic acid is converted to succinate. These findings suggest that the series of reactions initiated by the condensation of glycine and succinate is cyclic in nature, the succinate being regenerated and the glycine being metabolized.

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