THE UTILIZATION OF GLYCINE IN THE BIOSYNTHESIS OF HEMOGLOBIN*

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(Received for publication, December 27, 1948)

Isotopic studies have demonstrated that glycine is used in the biosynthesis of hemoglobin. Glycine has been tagged in one of three ways: (a) with N¹⁵ in the amino group (1-3), (b) with C¹⁴ in the α-methylene carbon position (4), and (c) with C¹⁴ in the carboxyl group (5). When any of these three forms of glycine is fed to animals, the isotope is incorporated into hemoglobin, but interesting differences have been observed in the distribution of the isotope between the pigment and protein portions of the molecule. For instance, Shemin and Rittenberg, using glycine labeled with N¹⁵, found greater concentrations of the heavy nitrogen in hemin than in red blood cell protein (2). Similarly, Altman and his associates observed that when the methylene carbon of glycine is tagged 7 to nearly 10 times as much C¹⁴ appear in hemoglobin protoporphyrin as in globin (4). On the other hand, recent observations in this laboratory have shown that when glycine containing C¹⁴ in the carboxyl group is administered the radioactive carbon is synthesized into globin but cannot be demonstrated in protoporphyrin (5).

The experiments to be described in this report were designed (1) to confirm the demonstration that the carboxyl carbon of glycine is used for the biosynthesis of globin but not protoporphyrin, (2) to determine whether globin within the red cell participates in protein interchange, and (3) to discover whether coproporphyrin I isolated from the urine and feces after the tagged glycine was fed would contain C¹⁴ even if the hemoglobin protoporphyrin did not.

Materials and Methods

Two healthy dogs and one rat were fed glycine tagged with C¹⁴ in the carboxyl group.¹

Dog I, male, weighed 12 kilos. By removing 150 to 200 ml. of blood from the femoral artery on each of several days, this animal’s packed

* Supported by a grant from Mr. John Mosby and one established in memory of Mr. Nathan Greenberg.

¹ The authors are indebted to Dr. R. B. Loftfield of the Massachusetts Institute of Technology for the synthesis of the labeled glycine.
erythrocyte volume was reduced from 51 to 29 per cent. 100 mg. of the labeled glycine were then given by stomach tube in divided doses on 3 successive days; the total radioactivity administered amounted to $5 \times 10^7$ counts per minute, assayed by a procedure described elsewhere (5, 6). During these 3 days, water was allowed ad libitum but all food was withheld. Thereafter, the stock ration of Purina dog chow was again fed. At intervals until the experiment was terminated on the 153rd day, 10 ml. samples of blood were obtained for analysis. Crystalline protoporphyrin IX dimethyl ester (7) and globin (8) were isolated for determination of radioactivity (5, 6).

Dog II, female, weighed 10 kilos. The packed red blood cell volume was reduced from 53 to 24 per cent by serial bleeding. The labeled glycine was again given by stomach tube in three divided doses within a period of 27 hours; the total dose was 50 mg. and the total radioactivity amounted to $1.7 \times 10^7$ counts per minute. Food was withheld during the period of administration. Blood was collected at intervals in 10 ml. samples as in the previous experiment for the same determinations, but observations were stopped at the end of 110 days. In addition, urine and feces were collected during the first 15 days following administration of the glycine and extracted for coproporphyrins (9). After purification and identification (10) coproporphyrin I was tested for radioactivity with a nucleometer.2

One white rat weighing 100 gm. was kept on a protein-free diet (2) for several days and then given by stomach tube, in four divided doses over 3 days, 200 $\gamma$ of the labeled glycine (total radioactivity $2.1 \times 10^6$ counts per minute). 20 days later the animal was bled to death. From the 4.6 ml. of blood obtained, crystalline protoporphyrin IX dimethyl ester was isolated for determination of its radioactivity. Measurement was also made of the radioactivity in the remaining cell protein.

All samples were assayed without resorting to conversion to carbonate. Satisfactory checks were obtained in pilot experiments in which samples were assayed both as carbonate and as untreated material. All samples were measured in triplicate and values reported are averages, the spread of which is included in the errors assigned.

Results

No radioactivity could be demonstrated in the protoporphyrin isolated from the blood of any of the three animals. The activity in the globin

2 The authors are indebted to Professor A. I. Lansing, Department of Anatomy, Washington University School of Medicine, for permission to use the nucleometer, a product of the Radiation Counter Laboratories, Chicago, Illinois. The nucleometer was calibrated with standard samples of labeled glycine assayed previously with the standard, thin-walled Geiger-Müller tube (5).
obtained from the dogs was at a maximum on the 1st day determinations were performed, the 23rd and 13th days, respectively (Fig. 1 and Table I). The concentration remained relatively constant for 77 days in Dog I and

![Graph](image)

**Fig. 1.** Radioactivity in globin prepared from blood of Dog I after oral administration of glycine tagged with C\(^{14}\) in the carboxyl position. \(\Delta\) represents half the period required for half the cells to die.

**TABLE I**

*Radioactivity in Hemoglobin Protoporphyrin and Globin of Dog II after Oral Administration of Glycine Containing C\(^{14}\) in Carboxyl Group*

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Protoporphyrin IX dimethyl ester</th>
<th>Globin</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>&lt;0.1</td>
<td>11.2 ± 0.6</td>
</tr>
<tr>
<td>33</td>
<td>&lt;0.1</td>
<td>10.1 ± 0.5</td>
</tr>
<tr>
<td>47</td>
<td>&lt;0.1</td>
<td>11.7 ± 0.4</td>
</tr>
<tr>
<td>66</td>
<td>&lt;0.1</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>80</td>
<td>&lt;0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>93</td>
<td>&lt;0.1</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>110</td>
<td>&lt;0.1</td>
<td>3.2 ± 0.2</td>
</tr>
</tbody>
</table>

until about the 66th day in Dog II. Thereafter, the radioactivity fell rather abruptly to low levels. In Fig. 1 are plotted the results obtained for Dog I. When mathematical analysis of this curve was made in the manner described by Shemin and Rittenberg (2) to estimate the average
survival time of red blood cells of the dog, a figure of 95 ± 5 days was obtained. The period in which half of the red cells was destroyed extended from the 85th to the 105th day, as shown in Fig. 1. The average survival time of the red blood cells for Dog II, determined in the same manner, was 75 ± 5 days. The cell protein from 4.6 ml. of the rat's blood contained 1.5 ± 0.5 counts per minute per mg., a value reasonably close to that to be expected from the initial dose of glycine administered, in light of the experience with uptake into globin in the dogs.

From the urine and feces collected for 15 days following the last administration of glycine to Dog II, 310 γ of crystalline coproporphyrin I tetramethyl ester were isolated. No radioactivity was observed.

**DISCUSSION**

These results confirm the previous demonstration that in the biosynthesis of hemoglobin the carboxyl carbon of glycine is incorporated into globin but not into protoporphyrin. Since other work has shown that both the amino nitrogen (1–3) and the methylene carbon (4) of glycine are introduced into protoporphyrin during its formation, it would appear that only the —CH₂·NH₂ group of glycine becomes a part of protoporphyrin. If the whole of the glycine molecule is involved in the synthesis of protoporphyrin, CO₂ from the carboxyl group must be split off in the process.

On the other hand, since the amino nitrogen (2) and the methylene carbon (4) atoms as well as the carboxyl carbon are incorporated into globin, it seems likely that glycine as a whole is utilized for globin synthesis. The data available do not permit any conclusion as to whether CO₂ detached from glycine can be used for globin formation (11).

When the radioactivity of globin is plotted against time, the shape of the resulting curve is very similar to that obtained for protoporphyrin when the concentration of N¹⁵ is plotted against time (2). This result indicates that the globin of intact erythrocytes remains in the red cells without participating in protein interchange until the erythrocyte is destroyed.

The radioactivity of the globin fraction did not fall off with time to as low a value as might be expected on the basis that C¹⁴ from degraded globin was not reutilized for globin synthesis. Insufficient data are at hand to decide to what extent such reutilization occurred. It seems unlikely, however, that simple reutilization of glycine from degraded globin can be reconciled with results such as those shown in Fig. 1. Miller, Robscheit-Robbins, and Whipple have reported evidence indicating that globin contributes to the “protein pool” of the body, and that this “protein pool” in turn is used for the formation of new hemoglobin (12). When
hemoglobin is injected intraperitoneally into dogs made both anemic and hypoproteinemic, new hemoglobin is formed. The conditions of these experiments, however, were quite different from those reported in the present paper. The two dogs used were not anemic at the conclusion of the experiment and had been fed a stock ration known to be nutritionally adequate.

The average times of survival of the red cells in Dogs I and II, as measured by the curves of radioactivity in globin, were 95 ± 5 days and 75 ± 5 days, respectively. Hawkins and Whipple estimated the life span of dog red cells to be about 124 days (13). These workers forced great numbers of new red cells into the circulation of four bile fistula dogs by blood destruction or blood withdrawal; as a consequence, the bile pigment output fell and did not rise again for 112 to 133 days. This interval was regarded as an indication of the survival time for the red cells. No conclusion is drawn regarding the difference between these figures and the ones calculated from the globin curves. More observations would be necessary to establish a more accurate average figure and the expected limits of variation.

Since available evidence indicates that coproporphyrin I is formed as a by-product during the synthesis of protoporphyrin IX (type III) for hemoglobin formation, and since hemoglobin protoporphyrin did not assimilate any radioactivity from the C\textsuperscript{14} of the carboxyl-tagged glycine, it is not surprising that the coproporphyrin I isolated from Dog II likewise possessed no radioactivity. Other experiments, furthermore, have shown that when coproporphyrin I is isolated from the feces of an animal fed glycine tagged with N\textsuperscript{15} the fecal coproporphyrin I as well as hemoglobin protoporphyrin IX (type III) contains heavy nitrogen in relatively high concentration (14). These results provide additional evidence in favor of the hypothesis that coproporphyrin I is a by-product of protoporphyrin IX formation.

**SUMMARY**

1. In the biosynthesis of hemoglobin, the carboxyl carbon is incorporated into globin but not into protoporphyrin.

2. Globin within the intact erythrocyte does not participate in protein interchange. No evidence was obtained to indicate that the C\textsuperscript{14} derived from the carboxyl carbon of glycine and synthesized into globin was reutilized for hemoglobin formation.

3. Coproporphyrin I isolated from the feces of one animal during a 15 day period, following the administration of carboxyl-tagged glycine, contained no radioactivity. Reference was made to other experiments in which coproporphyrin I was isolated from the feces of an animal fed N\textsuperscript{15}-tagged glycine; both the fecal coproporphyrin I and the hemoglobin proto-
porphyrin IX (type III) contained the isotope. These observations support the concept that coproporphyrin I is a by-product of protoporphyrin IX formation.

BIBLIOGRAPHY

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