ISOTOPIC STUDIES OF PORPHYRIN AND HEMOGLOBIN METABOLISM

I. BIOSYNTHESIS OF COPROPORPHYRIN I AND ITS RELATIONSHIP TO HEMOGLOBIN METABOLISM*

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Coproporphyrins I and III have been identified in plant and animal tissues, but comparatively little is known about their biologic significance and their relation to hemoglobin protoporphyrin. Abnormally large amounts of coproporphyrin I are excreted both by patients with porphyria (particulary of the light-sensitive type) and by those with accelerated rates of erythropoiesis. Acute porphyria, chemical poisoning, some types of infections, and alcoholic cirrhosis are associated with increased excretion of coproporphyrin III (1, 2). Coproporphyrins I and III can be synthesized by certain unicellular organisms, i.e. yeast cells (3, 4) and Corynebacterium diphtheriae (5, 6); composition of the culture medium as well as the type of organism has been shown to influence the synthesis.

A new and promising approach to the study of porphyrin metabolism is provided by the recent demonstrations: (1) that the nitrogen (7) and α-carbon (8) of glycine are direct precursors of hemoglobin protoporphyrin, and (2) that hemoglobin protoporphyrin can consequently be tagged by feeding animals glycine labeled either with N¹⁵ or with C¹³ or C¹⁴ in the α position. If one assumes that the pyrrole nucleus is the primary unit involved in the biosynthesis of porphyrins, then other pyrrole compounds formed during the synthesis of protoporphyrin should contain these same isotopic precursors. Similarly, the degradation of hemoglobin containing tagged protoporphyrin should give rise to pyrrole compounds also labeled with N¹⁵, C¹³, or C¹⁴.

The above concept forms the basis of the method used in these studies

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715
of porphyrin and hemoglobin metabolism. The present paper deals particularly with the biosynthesis of coproporphyrin I in dogs.

EXPERIMENTAL

Duplicate experiments were done in healthy mongrel dogs. The first part of each experiment (that involving Dogs I and III) was designed to confirm the demonstration that glycine nitrogen is incorporated into hemoglobin protoporphyrin, and to determine whether coproporphyrin I and stercobilin are formed as by-products in the synthesis of hemoglobin protoporphyrin. In the last half of each experiment, red blood cells with hemoglobin containing protoporphyrin tagged with N\textsuperscript{15} were transfused into animals (Dogs II and IV); consequently, in the recipient dog the increased excess N\textsuperscript{15} was present only in the hemoglobin. Hemolysis was then induced in order to determine whether excreted coproporphyrin I, protoporphyrin IX, and stercobilin are derived from hemoglobin breakdown. Protocols are given below; results are tabulated in Table I.

Experiment 1—Dog I, weight 12 kilos, was bled at intervals for 6 days to reduce its erythrocyte volume from 54 to 24 ml. of packed cells per 100 ml. of blood. At the time of the greatest anemia, 5.6 gm. of glycine containing 27.7 atom per cent excess N\textsuperscript{15} were given by stomach tube in six divided doses over a 3 day period. While the glycine was being administered, no food was allowed; thereafter, the animal was fed Purina dog chow ad libitum. On the 12th and 21st days following the first dose of glycine, 10 ml. samples of blood were obtained for the isolation of protoporphyrin IX dimethyl ester (9) and globin (10). Porphyrins were isolated from the urine and feces collected during the first 21 days. The initial extractions were made according to Dobriner's method (11); final purification and isolation were accomplished by chromatography on a CaCO\textsubscript{3} column with crystallization (12).

By the 34th day, the packed red cell volume had returned to 51 per cent. Blood was obtained for the isolation of hemoglobin protoporphyrin and for the determination of free erythrocyte protoporphyrin (free EP) (13). An ordinary transfusion set containing acid citrate-dextrose solution was then connected through an 18 gage needle to one of the femoral arteries, and the animal was bled to death. The collected cells, equivalent to 350 ml. of packed cells, were transfused into Dog II.

Dog II, weight 10 kilos, was made acutely anemic by two bleedings, 5 hours apart, of 250 ml. each. Immediately after the second hemorrhage, the animal was transfused with the cells from Dog I. The following day, a 10 ml. sample of blood was obtained; thus the N\textsuperscript{15} content of isolated crystalline protoporphyrin IX dimethyl ester could be determined. Phenylhydrazine anemia was then induced by the oral administration
of 0.8 gm. of phenylhydrazine hydrochloride in divided daily doses of 0.1 gm. over a 12 day period. The packed red cell volume decreased from 55 to 22.5 per cent on the 12th day. On the 21st day after the transfusion (cell volume 34 per cent, reticulocytes 15 per cent), blood was again drawn for the preparation of crystalline protoporphyrin IX dimethyl ester. From

Table I

Atom Per Cent Excess N\textsuperscript{15} in Various Prophyrins (and Globin) during Synthesis and Breakdown of Hemoglobin\textsuperscript{*}

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Blood</th>
<th>Urine and feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time in days after</td>
<td>H\textsubscript{b} protoporphyrin IX dimethyl ester</td>
</tr>
<tr>
<td>1. Dog I</td>
<td>N\textsuperscript{15}-Glycine 12</td>
<td>0.960</td>
</tr>
<tr>
<td></td>
<td>“ “ 21</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td>“ “ 34</td>
<td>0.720</td>
</tr>
<tr>
<td>Dog II</td>
<td>Transfusion 1</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Phenylhydrazine 20</td>
<td>0.132</td>
</tr>
<tr>
<td>2. Dog III</td>
<td>N\textsuperscript{15}-Glycine 12</td>
<td>0.710</td>
</tr>
<tr>
<td></td>
<td>“ “ 24</td>
<td>0.490</td>
</tr>
<tr>
<td></td>
<td>“ “ 36</td>
<td>0.320</td>
</tr>
<tr>
<td>Dog IV</td>
<td>Transfusion 1</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>Phenylhydrazine 15</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{*} No coproporphyrin III or deuteroporphyrin IX could be isolated from any of the dogs with present methods.

the urine and feces quantitatively collected during the first 20 days, porphyrins were also isolated by the methods mentioned above.

Experiment 2—Dog III, weight 10 kilos, was bled repeatedly for 9 days. The cell volume decreased from 45 to 26 per cent, and the reticulocytes rose to 14 per cent. 5.15 gm. of glycine containing 31.9 atom per cent excess N\textsuperscript{15} were then given by stomach tube in six divided doses during a 3 day period. No food was allowed during these 3 days; a meat-free diet was fed thereafter. Crystalline protoporphyrin IX dimethyl ester
and globin were prepared from samples of blood drawn on the 12th and 36th days. Urine and feces were collected separately during the first 24 days in two equal periods of 12 days each. Feces were extracted (14) for the simultaneous isolation of porphyrins and stercobilin; final purification and isolation of the porphyrins were then accomplished (12). On the 36th day, the animal was bled to death. It had not completely recovered from the induced anemia and the red cells had the characteristics of a hypochromic anemia: red blood cells, 6,200,000 per cmm.; Hb, 9.8 gm. per 100 ml.; cell volume, 34.2 per cent; mean corpuscular hemoglobin concentration, 29 per cent; free EP, 120 γ per 100 ml. of packed red blood cells. The blood collected was centrifuged and the plasma was removed. The remaining packed cells, 296 ml., were transfused into Dog IV.

Dog IV weighed 11 kilos. After an acute anemia had been induced by the withdrawal of 500 ml. of blood in two bleedings separated by an interval of approximately 5 hours, the packed red blood cells from Dog III were injected intravenously. On the following day, the first of seven daily 0.1 gm. doses of phenylhydrazine hydrochloride was given by mouth. By the end of this period, the red cell volume had fallen from 57 to 20 per cent. Porphyrins and stercobilin were isolated from the urine and feces collected during the first 15 days following the first dose of phenylhydrazine. Blood was also collected on the 15th day for the preparation of crystalline protoporphyrin IX dimethyl ester. N₁⁵ concentrations in the various samples described in the above protocols were determined by the mass spectrometer.¹

Comments

The results of these experiments indicate that glycine serves in dogs as a direct nitrogenous precursor for coproporphyrin I and stercobilin, as well as for hemoglobin protoporphyrin (Table I). The fact that the N₁⁵ concentration of hemoglobin protoporphyrin in Dogs I and III was greater than that previously reported for rats (15) and humans (16) was probably due to the increased rate of hemoglobin formation stimulated by the bleedings. The subsequent decrease which occurred in the N₁⁵ concentration was probably caused by dilution with untagged hemoglobin formed during recovery from the induced anemia.

The high N₁⁵ concentration of the coproporphyrin excreted by Dogs I and III, at a time when hemoglobin (protoporphyrin IX) was being regenerated rapidly, indicates that coproporphyrin I is probably a product

¹ Determinations of the N₁⁵ concentration in samples from Dogs III and IV were made in Dr. Alfred O. C. Nier's laboratory, Department of Physics, University of Minnesota. We also wish to record our indebtedness to Dr. H. S. Anker, Department of Biochemistry, University of Chicago, whose cooperation made possible the isotopic analysis of samples from Dogs I and II.
of the same biosynthesis and is derived, most likely, from a common pyrrole precursor. Conversely, the very small $^{15}N$ concentration of the coproporphyrin isolated from Dogs II and IV during periods of great hemolysis indicates that this porphyrin is not a product of hemoglobin breakdown. The small amount of $^{15}N$ found probably resulted from the uptake from a "metabolic pool" introduced with the transfused cells.

The increased $^{15}N$ concentration of stercobilin in Dog III during rapid hemoglobin synthesis confirms the observations of London et al. (17) in humans, and probably means that some stercobilin is formed from a source other than the degradation of circulating hemoglobin.

Finally, the increased $^{15}N$ concentration of the protoporphyrin IX excreted by Dogs II and IV, at a time when hemolysis was greatly accelerated, suggests that at least a portion of fecal protoporphyrin may be derived from hemoglobin breakdown. The possibility exists, however, that it may have come from free EP in tagged, hemolyzed cells. The total amount of free EP transfused into Dogs III and IV was 268 and 356 $\gamma$ respectively. The $^{15}N$ concentration of the free EP fraction was not determined; thus this possibility cannot be excluded.

Rimington (18) and Dobriner and Rhoads (19) have postulated that coproporphyrin I is a by-product of the synthesis of a Type III porphyrin. The results reported are in accord with that hypothesis. The chemical nature of this Type III porphyrin is not known, although ultimately it becomes protoporphyrin IX. The in vitro synthesis of coproporphyrin III by the method of Fischer and Hierneis (20) leads to a mixture of coproporphyrin I and III. The same phenomenon may well take place in nature, and the ratio of the isomers may be regulated by an enzyme mechanism as suggested by Rimington (18). In that case, coproporphyrin III would be an intermediate metabolite, a precursor of protoporphyrin IX. There is no experimental evidence which excludes this possibility, and the following observations tend to support it:

1. An increase in coproporphyrin III excretion is frequently associated with a disturbance of hemoglobin metabolism, as in metal intoxication, sulfonamide therapy, etc. These increases may well be explained by blockage of the transformation of coproporphyrin III to protoporphyrin IX, or by a decrease in the utilization of protoporphyrin IX for hemoglobin metabolism. The concept that the reverse is true, that protoporphyrin IX is converted to coproporphyrin III, has not been supported by several investigators (21–23).

2. The work of Kench and Wilkinson (4), of Pappenheimer (5), and Rawlison and Hale (24) with yeast cells and Corynebacterium diphtheriae, respectively, suggests that coproporphyrin III is incorporated into enzymes. Thus far, the only porphyrin identified in enzymes is protoporphyrin IX.

If Granick's conclusion that protoporphyrin IX is a precursor of chloro-
phyll (25) is correct, one could explain the presence of the coproporphyrins in plants according to the following scheme.

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\text{Pyrrole precursors} \quad \xrightarrow{\text{Enzymes}} \quad \text{coproporphyrin III} \rightarrow \text{protoporphyrin IX}
\]

**SUMMARY**

1. Glycine is a specific precursor, in dogs, of hemoglobin protoporphyrin, coproporphyrin I, and stercobilin.
2. Coproporphyrin I is formed during the biosynthesis of protoporphyrin IX and does not represent a hemoglobin derivative.
3. It is postulated that coproporphyrin I is a by-product of the synthesis of coproporphyrin III and that coproporphyrin III gives rise to protoporphyrin IX.
4. Protoporphyrin IX, excreted in the feces of dogs, is apparently a hemoglobin derivative.
5. Stercobilin is derived in part from sources other than circulating hemoglobin breakdown.

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**BIBLIOGRAPHY**
