ISOTOPIC STUDIES OF PORPHYRIN AND HEMOGLOBIN METABOLISM

II. THE BIOSYNTHESIS OF COPROPORPHYRIN III IN EXPERIMENTAL LEAD POISONING*

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It is now well established that glycine is a direct nitrogenous precursor of hemoglobin protoporphyrin in rats (1), rabbits (2), dogs (3), and humans (4), of coproporphyrin I in dogs and humans (3, 5), of uroporphyrin I in humans (5), and of stercobilin in humans (5, 6). It is well known that lead intoxication in rabbits is associated with prompt and marked increases of urinary coproporphyrin III (7, 8). Three possibilities existed to explain these increases: (a) a derivation from destroyed hemoglobin (9), (b) new formation either in relation to a disturbed hemoglobin synthesis (10) or as an expression of some more obscure disturbance of pigment metabolism, (c) mobilization of free or bound coproporphyrin from tissue stores, with subsequent excretion in the urine.

The administration of glycine labelled with N\textsuperscript{15} to lead-poisoned rabbits offered a means of deciding which of these three possibilities is actually represented. The present paper describes the results of such a study.

Material and Methods

Rabbit 1, weighing 2.8 kilos, had received 200 mg. of lead acetate subcutaneously, as part of another study 6 months previously. The coproporphyrin III excretion was still high, 173 \( \gamma \) per day. 0.8 gm. of glycine containing 32 atom per cent excess N\textsuperscript{15} was administered by stomach tube in six doses over a 3 day period. On the 24th day thereafter, 10 ml. of blood were drawn and from this the protoporphyrin IX dimethyl ester was prepared in crystalline form, according to the usual method (11). The pooled urine for the entire 24 day period following the administration of glycine was subjected to the ether extraction-chromatography method of isolating coproporphyrin (12). The crystalline tetramethyl ester of coproporphyrin III was obtained for N\textsuperscript{15} analysis (see below).

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Rabbit 2, weight 2.4 kilos, received 100 mg. of lead acetate subcutaneously. On the 19th day, when the coproporphyrin III excretion had increased to 268 γ per day, 2.0 gm. of glycine containing 32 atom per cent excess N₁⁵ were administered intraperitoneally in fifteen doses and over a 3 day period. On the 10th and 29th days after the glycine had been injected, 10 ml. samples of blood were drawn and from each the protoporphyrin IX dimethyl ester was prepared in crystalline form.

The urine from Rabbit 2 was collected for two periods, i.e., the first 9 days and the subsequent 18 days following the glycine administration. Coproporphyrin III tetramethyl ester was crystallized from the pooled urine of each period. The entire feces for 22 days were extracted in the usual manner (11) with acetic acid and ether, and the porphyrin was re-extracted from the washed ether with 5 per cent HCl (prepared in the proportion of 5 ml. of concentrated or 37 per cent HCl diluted with distilled H₂O to 37 ml.). The acid solution was made red to Congo paper by addition of sodium acetate, and the porphyrin reextracted with ether. It was then returned to a small volume of 5 per cent HCl and esterified by addition of several volumes of methyl alcohol saturated in the cold with HCl gas. The methyl ester was taken into chloroform and purified in the usual manner (11), after which it was chromatographed on a CaCO₃ column from a mixture of benzene-petroleum ether (1:1) and developed with benzene. The chromatogram showed two porphyrin zones, the upper of which was much larger. This porphyrin, as yet unidentified, will be referred to again as porphyrin x. The lower porphyrin zone on the column was identified as protoporphyrin IX.

The experiment in Rabbit 3, weighing 3.13 kilos, differed only in that

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Days after glycine administration</th>
<th>Hemoglobin protoporphyrin</th>
<th>Coproporphyrin III</th>
<th>Feces porphyrin x</th>
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<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>0.066</td>
<td>0.076</td>
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</tr>
<tr>
<td></td>
<td>9</td>
<td>0.474</td>
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<td></td>
<td>21</td>
<td>0.097</td>
<td>0.147</td>
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</table>
this animal received ultraviolet irradiation on a 10 X 10 cm. shaved area of skin for 20 minutes on the 1st, 6th, and 14th days following the administration of 2 gm. of glycine containing 32 atom per cent excess N\textsuperscript{15}. The reason for the radiation was the recent finding of Pimenta de Mello that ultraviolet radiation greatly increases the coproporphyrinuria of lead-poisoned rabbits.\textsuperscript{1} Rabbit 3, in fact, showed a considerably greater increase of coproporphyrin in the urine than Rabbits 1 or 2. On the 10th day following the glycine a 10 ml. blood sample was drawn and the crystalline protoporphyrin methyl ester prepared. Urine and feces were collected for three consecutive periods of 1 week each for isolation of porphyrins as already mentioned.

The N\textsuperscript{16} concentration of the various porphyrins isolated was determined by the mass spectrometer.\textsuperscript{2} The data are given in Table I.

**Comments**

The results obtained indicate that the glycine nitrogen is incorporated directly into the coproporphyrin III as well as into the hemoglobin protoporphyrin. The early drop in the N\textsuperscript{15} concentration of the hemoglobin protoporphyrin of Rabbit 2 is probably explained by a relatively more rapid hemoglobin destruction, due to the more acute lead poisoning in this animal. It is interesting to note that this drop is not accompanied by an increase in N\textsuperscript{15} concentration of the coproporphyrin III as one would expect if the latter were derived from hemoglobin protoporphyrin. The high concentration of N\textsuperscript{15} at 6 days after the glycine administration in Rabbit 3 speaks against a preformation, storage, and mobilization as the mechanism by which the excessive urinary coproporphyrin is explained. This leaves only the second of the three possibilities mentioned at the outset as a tenable concept, \textit{i.e.,} a new formation of coproporphyrin III \textit{pari passu} with a hemoglobin synthesis which may be disturbed, or as an expression of some more obscure disturbance of pigment metabolism. The general trend of the values is in accord with but does not prove a close relationship to hemoglobin synthesis. The possibility of an entirely separate formation of the coproporphyrin III, even quite apart from sites of erythropoiesis, cannot be excluded.

The data obtained with respect to the fecal porphyrin \(x\) are insufficient to permit conclusions regarding its nature and possible significance. Its chloroform solubility and general behavior, and its association with protoporphyrin, point to a possible relationship analogous, perhaps, to the relationship of the pseudodeuteroporphyrins of human feces to proto-

\textsuperscript{1} To be published.

\textsuperscript{2} These determinations were carried out in Dr. Alfred O. C. Nier's laboratory, Department of Physics, University of Minnesota.
porphyrin. It is noteworthy that Fischer and Duesberg (8) described a porphyrin isolated from rabbit feces, similar to porphyrin x but differing in melting point of the methyl esters. Their porphyrin melted at 182-187°; the present porphyrin x at 210-215°. In so far as the N¹⁵ concentration noted in Table I is concerned, it is at least evident that the porphyrin x was not exogenous, since the glycine N¹⁵ was clearly utilized in its synthesis.

SUMMARY

Glycine is a direct nitrogenous precursor of the coproporphyrin III excreted in excess in the urine of lead-poisoned rabbits. The present data confirm the belief that this coproporphyrin III is not related to hemoglobin catabolism, and indicate that it is newly formed as a result of the lead poisoning, rather than a preformed porphyrin which had been stored and mobilized.

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