PORPHYRIN FORMATION AND HEMOGLOBIN METABOLISM
IN CONGENITAL PORPHYRIA*

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The studies reported here were conducted on a patient with congenital
porphyria who excretes large amounts of uroporphyrin I and coproporphyrin I. They were directed to the following problems: (a) to determine
whether glycine is specifically utilized in the biologic synthesis of porphy-
rins related to the etioporphyrin I configuration as it is utilized in the
synthesis of protoporphyrin which is related to the etioporphyrin III
configuration (1); (b) to measure the rates of formation (and degradation)
or uroporphyrin I and coproporphyrin I; (c) to investigate the life span and
pattern of destruction of the erythrocyte and the origins of bile pigment
in this disease.

Material and Methods

The subject was a 15 year-old white girl previously reported by Do-
briner, Strain, and their associates (2, 3). She has enjoyed good health
except for the manifestations of congenital porphyria, namely photosen-
sitivity, brownish discoloration of the teeth, and excretion of large
amounts of uroporphyrin I and coproporphyrin I. Physical examination
in March, 1949, revealed no other abnormal findings. Laboratory data
at that time included the following: red blood cells, 4.54 million per c.mm.
of blood; hemoglobin, 12.5 gm. (99 per cent) per 100 ml. of blood; hemato-
crit, 41.0 per cent; reticulocytes, 5.9 per cent; white blood cells, 5800
per c.mm. of blood; and a normal differential white cell count. Examina-

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1 We are indebted to Dr. W. H. Strain, Strong Memorial Hospital, Rochester,
New York, who afforded us the opportunity of studying this patient; his continued
interest and help were invaluable in the performance of the study.

2 We are grateful to Dr. R. W. Davis, Dr. R. M. Christian, and Dr. L. E. Young
for permission to report these results of their physical and laboratory examinations,
which were performed at the Strong Memorial Hospital, Rochester, New York.

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tion of sternal bone marrow in January, 1949, revealed erythroid hyperplasia. A Coombs test for the presence of a circulating autoagglutinin was negative.

Glycine labeled with 32 atom per cent excess N\textsuperscript{15} (4) was given in doses of 4.0 gm. every 3 hours during the day for a total of five doses per day; 60 gm. of glycine were administered over the course of 3 days.

Uroporphyrin I was isolated from 4 day samples of urine and coproporphyrin I was isolated from 4 day stool collections. The isolation procedures were those described by Watson (5) and by Grinstein, Schwartz, and Watson (6). The methyl esters of uroporphyrin I and coproporphyrin I were prepared. The melting points of the various samples of uroporphyrin I methyl ester and of coproporphyrin I methyl ester are shown in Table I.

<table>
<thead>
<tr>
<th>Table I</th>
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<tbody>
<tr>
<td>Melting Points of Methyl Esters of Uroporphyrin I and Coproporphyrin I</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>Uroporphyrin I methyl ester °C.</th>
<th>Coproporphyrin I methyl ester °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>284-287</td>
<td>245-253</td>
</tr>
<tr>
<td>5-8</td>
<td>291-294</td>
<td>247-253</td>
</tr>
<tr>
<td>9-12</td>
<td>284-288</td>
<td>251-255</td>
</tr>
<tr>
<td>13-16</td>
<td>247-253</td>
<td>245-254</td>
</tr>
<tr>
<td>21-24</td>
<td>247-253</td>
<td>249-253</td>
</tr>
<tr>
<td>25-28</td>
<td>247-253</td>
<td>245-251</td>
</tr>
<tr>
<td>29-32</td>
<td>247-254</td>
<td>246-254</td>
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<tr>
<td>33-36</td>
<td>247-254</td>
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<td>50-53</td>
<td>247-254</td>
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</tr>
<tr>
<td>65-68</td>
<td>283-288</td>
<td>246-254</td>
</tr>
<tr>
<td>81-84</td>
<td>283-291</td>
<td></td>
</tr>
</tbody>
</table>

Stercobilin was isolated from the stools according to a method of Watson (7). Hemin was prepared from 20 to 30 ml. of venous blood by the usual procedure (8). N\textsuperscript{15} analyses were carried out in the mass spectrometer.

**RESULTS AND DISCUSSION**

The N\textsuperscript{15} concentrations in the uroporphyrin I and in the coproporphyrin I following the start of labeled glycine feeding are plotted in Fig. 1. The maximum isotope concentration in the uroporphyrin I (4.65 atom per cent excess N\textsuperscript{15}) is reached during the first 4 days, after which there is a rather rapid decline. The maximum isotope concentration in the coproporphyrin I (6.82 atom per cent excess N\textsuperscript{15}) is reached during the second 4 day collection period. This lag in attaining maximum concen-
tration in the coproporphyrin is most likely a reflection of delayed excretion by the intestine. These high $N^{15}$ values in uroporphyrin I and coproporphyrin I are of the same order of magnitude as the $N^{15}$ concentration in the newly formed heme. The isotope concentration in newly formed heme during the first 8 days may be determined by analysis of the curve of $N^{15}$ concentration in hemin (Fig. 2). The $N^{15}$ concentration in the total circulating heme on the 8th day is 0.53 atom per cent excess $N^{15}$. The average isotope concentration in the newly formed heme during this 8 day period is equal to $(0.53 \times \text{average erythrocyte life span (days)})/8$ days since the only circulating cells which contain labeled heme have been formed during these 8 days. The average life span of the erythrocytes in this subject is approximately 120 days. The average isotope concentration in the newly formed heme is accordingly about 8.0 atom per cent excess $N^{15}$. That lower isotope concentrations are reached in the uroporphyrin I and coproporphyrin I may be due to the
presence of pools of these substances with dilution of isotopically labeled molecules by non-isotopic molecules formed prior to the ingestion of labeled glycine. In the case of heme, the dilution of newly formed isotopic heme by previously formed non-isotopic heme can be calculated, since the total circulating heme can be measured. The dilution of the isotope concentrations in the newly formed uroporphyrin I and coproporphyrin I by previously formed non-isotopic molecules cannot be determined precisely, because the size of the pools of these substances is not known. Since glycine is specifically utilized in the biologic synthesis of protoporphyrin (1) and the maximum isotope concentrations in the uroporphyrin I and coproporphyrin I are of a magnitude similar to that in newly formed heme, it can be concluded that glycine is specifically utilized in the biologic synthesis of porphyrins related to the etioporphyrin I configuration.

The decline in the isotope concentrations in the uroporphyrin I and coproporphyrin I provides some indication of the rates at which these porphyrins are synthesized. From the declining curves one can estimate $t_1$, the half life time of these substances in the organism, i.e. the time in which half the molecules which are formed at one time have disappeared from the organism. It should be emphasized that these values for $t_1$ are maximum. As measured from the declining portion of each curve, the $t_1$ of coproporphyrin I is about 3 to 4 days, of uroporphyrin I about 6 to 7 days. These values represent rapid rates of turnover, and, since these values are maximum, the actual rates of synthesis of these porphyrins may be even more rapid. It can be concluded that with turnover times ($t_2 \times (1/\ln 2)$) of 5 and 9 days for coproporphyrin I and uroporphyrin I, respectively, the rates of synthesis (and degradation) for these substances are at least 20 and 11 per cent per day of their pools.

It is tempting to speculate on the relationship between uroporphyrin I and coproporphyrin I on the basis of their isotope concentrations. It is not as yet known whether coproporphyrin may be derived from uroporphyrin by decarboxylation of the acetic acid substituents, or whether the reverse reaction may occur by carboxylation of the methyl substituents of coproporphyrin. It has been suggested by Grinstein et al. (9), on the basis of findings in another case of congenital porphyria, that uroporphyrin may be derived from coproporphyrin. Their study, utilizing the same techniques, revealed a very high N$^{16}$ concentration in coproporphyrin I initially and its immediate rapid decrease accompanied by an increase of the N$^{16}$ concentration of the uroporphyrin I. The requirement that the maximum concentration in the product should never be greater than the maximum concentration of the precursor is a necessary but not sufficient condition in a homogeneous system only. In a heterogeneous system, the data are susceptible to an alternative explanation. If copro-
porphyrin were derived from uroporphyrin before either porphyrin enters its metabolic pool, the relative isotope concentrations in the excreted porphyrins would depend on the amounts of these porphyrins made per day and on the sizes of their metabolic pools. If the dilution of newly formed coproporphyrin were less than that of newly formed uroporphyrin, the isotope concentration in the coproporphyrin would be greater than that in the uroporphyrin even though the coproporphyrin were derived from uroporphyrin. Until further information is available on the routes of biologic synthesis of porphyrin compounds, definitive conclusions concerning the interrelations of uroporphyrin I and coproporphyrin I cannot be derived from such data.

Fig. 2 shows the N\textsuperscript{15} concentrations in hemin and stercobilin. The curve of N\textsuperscript{15} concentration in hemin reveals a normal pattern of red cell survival and a normal average erythrocyte life span of about 120 days. These findings are of interest in the light of the reticulocytosis (about 6 per cent) in the peripheral blood and the erythroid hyperplasia of the bone marrow which are generally considered to be associated with increased erythropoiesis. In the absence of anemia or polycythemia and in the presence of a normal pattern of erythrocyte survival and a normal aver-
age erythrocyte life span, there is no other evidence of increased erythropoiesis. The reticulocytosis may reflect an earlier release of reticulocytes from the bone marrow or a longer persistence of erythrocytes in their reticulated form than is usual.

The curve of N\textsuperscript{15} concentrations in the stercobilin reveals strikingly high values during the first 2 weeks of the experiment. Since there is no apparent destruction of labeled, mature, circulating erythrocytes at this time, the high isotope concentrations in the bile pigment indicate that a large portion of the bile pigment in this disease is derived from one or more sources other than the hemoglobin of mature, circulating erythrocytes. This finding is in confirmation of our earlier findings in normal man and in pernicious anemia (10, 11). By calculations similar to those employed in the previous publications (10, 11), it can be shown that at least 31 per cent of the stercobilin of this patient is derived from sources other than the hemoglobin of mature, circulating erythrocytes. (N\textsuperscript{15} concentration in stercobilin (2.5))/(N\textsuperscript{15} concentration in newly formed heme (8.0)) = 0.31.

Despite the normal pattern of red cell destruction and the normal average erythrocyte life span, it is possible that a component fraction of the erythrocyte population is destroyed while still in the bone marrow or shortly after entering the circulation. Such destruction of young erythrocytes might be reflected in increased erythropoietic activity, and the hemoglobin of these destroyed cells could be rapidly converted to bile pigment. The production of large amounts of porphyrin may require an active synthesis of large quantities of pyrroles. If bile pigment can be formed directly from pyrroles, the high N\textsuperscript{15} concentration in the stercobilin which is observed early in the experiment may be due to the formation of stercobilin from an active pool of pyrroles which may exist in this disease.

An additional possibility may be considered; namely, that uroporphyrin III and coproporphyrin III may also be made in increased quantities but that they are very rapidly degraded to bile pigment and hence do not accumulate and are not detected.\textsuperscript{3}

These possibilities are stressed, although the possible contributions of other sources which have been considered previously (10, 11) should not be overlooked. The other possible sources are myoglobin and the respiratory heme pigments, and heme which is not utilized for hemoglobin production.

\textsuperscript{3} It is conceivable that stercobilin may be derived from porphyrins of the etioporphyrin I isomer configuration as well as from porphyrins of the etioporphyrin III structure. If this is true (and, it should be stated, there is no evidence to support this speculative possibility), the labeled stercobilin in this subject might be derived in part from the isotopically labeled uroporphyrin I and coproporphyrin I.
SUMMARY

1. Glycine is specifically utilized in the biologic synthesis of porphyrins related to the etioporphyrin I configuration.

2. The rates of formation and degradation of uroporphyrin I and of coproporphyrin I in a subject with congenital porphyria are found to be rapid.

3. A normal average erythrocyte life span of about 120 days was observed in this subject with congenital porphyria.

4. A large part, at least 31 per cent, of the stercobilin in this subject is derived from sources other than the hemoglobin of mature, circulating erythrocytes. The nature of these possible sources is discussed.

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