THE FORMATION OF BILE PIGMENT IN PERNICIOUS ANEMIA*

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Studies on the origin of bile pigment in normal man have revealed that a significant portion of bile pigment is derived from one or more sources other than the hemoglobin of mature, circulating erythrocytes (3). This finding suggested the possibility that the markedly increased quantities of bile pigment excreted in untreated pernicious anemia might also be derived in part from sources other than the hemoglobin of mature, circulating erythrocytes. Such a possibility is not in accord with the assumption, made by most investigators in the past, that the increased quantities of bile pigment which are excreted are the result solely of an abnormally increased rate of hemolysis (4). This assumption has been challenged by others (5, 6) who have held that the rate of turnover of hemoglobin and circulating erythrocytes which would be required to produce such large quantities of bile pigment was improbably high. Conclusive evidence in support of either position, however, was not available. The study described in this report was performed to provide information which might help to elucidate the relationship of hemoglobin metabolism to the formation of bile pigment in pernicious anemia.

The administration of glycine labeled with N\textsuperscript{15} affords a method for the study of the life span and pattern of destruction of the human erythrocyte in normal and pathologic states (7, 8). This method was used to study heme synthesis and dynamics of the red blood cell in a patient with the characteristic symptoms of pernicious anemia. The curve of N\textsuperscript{15} concentration in the hemin of this subject has been described previously (8). At the start of the experiment the patient had received no antianemia therapy. Glycine labeled with N\textsuperscript{15} was fed for 2 days. After 15 days, when the isotope concentration in the hemin appeared to be approaching its maximum value, it was considered inadvisable to withhold treatment longer, and liver extract in large dosage was administered. Analysis of the data re-

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revealed that the erythrocyte population was mixed, some of the cells enjoying a true life span and others being destroyed at random; the mean survival time of the erythrocytes was approximately 85 days. The rate of production of circulating red cell hemoglobin was about four-fifths the normal; the rate of production of circulating red cells about half the normal rate.

Stercobilin was isolated from 4 day collections of stools at intervals during the course of the same study and the N\(^{15}\) concentrations of the sterco- bilin samples determined. The isotope concentrations in the hemin and the sterco- bilin are shown in Fig. 1. The curve of N\(^{15}\) concentrations in sterco- bilin reveals strikingly high values from the 5th to the 12th day. The low value during the first 4 days is most likely a reflection of the lag in excretion of bile pigment after its formation, with isotopically labeled sterco- bilin diluted by unlabeled sterco- bilin formed prior to the administration of labeled glycine. The very high N\(^{15}\) concentrations attained between the 5th and 12th days indicate that a large fraction of the bile pigment is derived from a source other than the hemoglobin of mature, circulating erythrocytes. The magnitude of this fraction may be estimated from the

![Graph showing N\(^{15}\) concentrations in hemin and sterco-bilin after the start of feeding N\(^{14}\)-labeled glycine for 2 days.](http://www.jbc.org/)
isotope concentration in the bile pigment and the average isotope concentration in newly formed heme during an equivalent period. From the 5th to 12th day, the $^{15}N$ concentration in the stercobilin was approximately 2.75 atom per cent excess $^{15}N$. If one assumes a lag in this patient of about 3 days between the degradation of hemoglobin of circulating erythrocytes and the excretion of the resultant bile pigment in the feces, the equivalent 8 day period for the estimation of the average isotope concentration of newly formed heme is from the 2nd to the 9th day. The average isotope concentration in the newly formed heme during this period is approximately 6.3 atom per cent excess $^{15}N$ (for this type of calculation see the previous paper (3)). During this period, a portion of the newly formed circulating cells containing labeled heme undergoes destruction. The average $^{15}N$ concentration of the heme in the circulation between the 2nd and 9th days is 0.3 atom per cent excess $^{15}N$. If one assumes completely random destruction of the cells, the isotope concentration in bile pigment derived from the destruction of cells containing labeled heme during this period would have the same value. Inasmuch as a portion of the newly formed labeled cells may not be destroyed at random, the isotope concentration in the bile pigment derived from the destruction of circulating cells is probably somewhat less than 0.3 atom per cent excess $^{15}N$. Let us accept this as an upper limiting value, however.

The magnitude of the fraction of bile pigment which is not derived from the hemoglobin of circulating erythrocytes may be estimated as follows: If $\alpha$ is the fraction which is derived from the hemoglobin of circulating erythrocytes, then $0.3\alpha$ is the contribution of heme of circulating erythrocytes to the isotope content of the bile pigment. The remainder of the bile pigment $(1 - \alpha)$ is derived from sources which have, as an upper limit, an average isotope concentration similar to that of newly formed heme. The average isotope concentration of newly formed heme is approximately 6.3 atom per cent excess $^{15}N$. The contribution of these other sources to the isotope content of the bile pigment is $6.3 \times (1 - \alpha)$. Then $0.3\alpha + 6.3(1 - \alpha) = 2.75$. Solving for $\alpha$ yields a value of 0.59; i.e., at most about 60 per cent of the bile pigment is derived from the hemoglobin of circulating erythrocytes and at least about 40 per cent is derived from other sources.

These findings provide an explanation for the discrepancy in untreated pernicious anemia between the very high levels of bile pigment production and the relatively moderate rates of destruction of circulating red blood cells. The possible sources for that portion of bile pigment (at least 40 per cent) which is not derived from the hemoglobin of mature, circulating erythrocytes are similar to those which have been considered in normal man.
Bile pigment may be formed from the hemoglobin of erythrocytes which are destroyed in the bone marrow before they reach the peripheral blood or from the hemoglobin of newly formed erythrocytes which are destroyed shortly after entering the circulation. The studies of Peabody and Broun on the phagocytosis of erythrocytes in specimens of bone marrow obtained post mortem indicate that, whereas phagocytosis occurs to a very limited extent in normal marrow, it is quite marked in the active stages of pernicious anemia (9). This finding, however, was observed by Watson et al. (10) to be only an irregular occurrence. It seems reasonable that a portion of the abnormal cells in the bone marrow may be particularly vulnerable to destruction and may not even survive the transition to the circulating blood. In addition, of those cells which do reach the circulation a portion may be destroyed shortly after release from the bone marrow. Abnormal reticulocytes which survive apparently for a very short time in the peripheral blood have been observed by Heilmeyer and Eilers (11). The destruction of young cells may result from structural abnormality and the ordinary trauma to which erythrocytes are exposed normally, or it may result from unusual susceptibility of these abnormal cells to hemolytic agents which are thought by some workers (12–15) to be present in the blood of some patients with untreated pernicious anemia. In any case, destruction of very young cells is probably a more prominent factor in the production of bile pigment in this disease than in normal man.

The contribution of myoglobin and the respiratory heme pigments to bile pigment formation in this disease is unknown, as is the rate of turnover of myoglobin. It seems unlikely, in the light of the considerations presented in the previous paper (3), that myoglobin accounts for a large part of this portion of bile pigment.

Heme which has not been utilized for hemoglobin production may serve as a source of bile pigment in this disease. It is possible that inadequate amounts of globin are produced or that the binding of available heme and globin is incomplete. In either case, the excess or unbound heme could be converted to bile pigment. Hematinemia has been reported to occur in pernicious anemia (16). That hematin which is not bound to globin can be converted to bile pigment is indicated by the finding that the intravenous administration of isotopic hematin results in the formation of isotopic stercobilin (17).

Pernicious anemia is characterized by the excretion of increased amounts of coproporphyrin I; following treatment with liver extract, the excretion declines to normal levels (18). Increased excretion of coproporphyrin I, which may be a by-product in the formation of porphyrins related to the etioporphyrin III configuration, may reflect increased formation of porphyrins of the III type. If there is a partial block in the conversion of
iron-free porphyrin to heme, the accumulated porphyrin may be directly converted to bile pigment. With the administration of antianemia therapy, the block in the conversion of porphyrin to heme would disappear and a simultaneous decline in bile pigment formation and rise in heme production would occur.

Finally, it remains to be determined whether bile pigment can be formed from pyrroles or pyrrole precursors directly, without prior conversion to a porphyrin ring.

As yet it is not possible to determine the sources which are responsible for the production of the large fraction of the bile pigment which is not derived from the hemoglobin of circulating erythrocytes. However, destruction of defective cells in the bone marrow probably plays a more important rôle in the formation of bile pigment in pernicious anemia than it does in normal man.

EXPERIMENTAL

The subject was a 51 year-old man whose history, physical findings, and hematologic data were characteristic of untreated pernicious anemia. The data have been presented in detail previously (8).

The subject received 48 gm. of glycine containing 31.7 atom per cent excess N\textsuperscript{15} over the course of 48 hours. The glycine was fed hourly in equal doses, except for triple doses at 12 midnight and 3 a.m., with no other doses between 12 midnight and 6 a.m.

Hemin and stercobilin were isolated by the methods described in the previous paper (3). Optical rotations of some of the stercobilin samples were performed on CHCl\textsubscript{3} solutions containing 20 to 30 mg. per 100 ml. The results were as follows: days 0 to 4, \([\alpha]_b^{20} = -3040^\circ\); days 5 to 8, \([\alpha]_b^{10} = -3500^\circ\); days 9 to 12, \([\alpha]_b^{10} = -3600^\circ\); days 21 to 24, \([\alpha]_b^{10} = -3580^\circ\); days 25 to 34, \([\alpha]_b^{20} = -3620^\circ\); days 35–38, \([\alpha]_b^{10} = -3560^\circ\).

SUMMARY

In untreated pernicious anemia a large part of bile pigment is derived from one or more sources other than the hemoglobin of mature, circulating erythrocytes.

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