THE CONVERSION OF HEMATIN TO BILE PIGMENT*

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The rôle of hematin in hemoglobin and bile pigment metabolism is not clearly defined. Opinion has been divided over the questions of whether or not hematin is a normal degradation product of hemoglobin and whether or not it is converted to bile pigment. Bingold observed that the occurrence of hematin in the plasma is associated with a variety of pathologic conditions (1). The conditions in which hematinemia has been observed by him and others include hemolytic anemias, hemoglobinuria, pernicious anemia, severe liver disease, and large blood extravasations. It has been noted in the fetus during the second half of gestation and in umbilical cord blood (2). Duesberg (3) claimed that under normal conditions hemoglobin is quantitatively converted to bile pigment and that hematin appears only in pathologic conditions. The studies of Fairley (4) indicate that hematin in the plasma unites immediately with albumin to form methemalbumin. This substance is not found normally; it occurs, according to Fairley, as a result of intravascular hemolysis, whereas normally hemoglobin degradation occurs in the reticulo-endothelial system and is converted to bile pigment without an intermediate stage of methemalbumin.

Attempts to determine whether hematin is converted to bile pigment have consisted for the most part in injections of hematin and measurements of bile pigment excretion. Brugsch and Kawashima (5) and Bénard et al. (6) found increased excretion of bilirubin in dogs, and Pass, Schwartz, and Watson (7) found increased excretion of urobilinogen in man following intravenous injection of hematin. On the other hand, no increase in bilirubin excretion in man was found by Duesberg (3), and Gitter and Heilmeyer (8) observed an increase in urobilinogen excretion in but one of two men following injection of hematin intravenously. The conclusion, on the basis of this type of experiment, that bile pigment has been derived from hematin is subject to the criticism that the increased bile pigment excretion may not represent actual conversion of hematin to bile pigment but may be an indirect effect of the injection of hematin. Studies of

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CONVERSION OF HEMATIN TO BILE PIGMENT

Lemberg (2) revealed that the injection of mesohematin in rabbits is followed by an increased excretion of bile pigment which, however, is not derived from mesohematin. Furthermore, early histologic studies by Brown (9) and more recently by Anderson et al. (10) have been interpreted as indicating that hematin is very slowly metabolized; it would consequently be difficult to ascribe the rapid increases in bile pigment excretion which follow injection of hematin to actual conversion of hematin to bile pigment.

This report is concerned with a study which attempts to provide direct evidence relating to the problem of the conversion of hematin to bile pigment.

Material and Methods

A normal dog, weighing approximately 17 kilos, was injected intravenously with 800 mg. of N¹⁶-labeled hematin in divided doses on 3 consecutive days and stools were collected for 9 days. Stercobilin was isolated from the stools and its N¹⁶ concentration was analyzed.

The labeled hematin was prepared as follows: 1.0 gm. of glycine labeled with 32 atom per cent excess N¹⁶ was injected into a duck subcutaneously each day for 10 days. The duck was exsanguinated on the 14th day and hemin was prepared from the erythrocytes by the usual procedure (11) and recrystallized (12). The isotope concentration in the hemin was 1.425 atom per cent excess N¹⁶. On the 1st day, 200 mg. of hemin were dissolved in 2 ml. of 5 per cent Na₂CO₃; the solution was diluted with 25 ml. of distilled water and added to about 95 ml. of 0.9 per cent sodium chloride solution to make a total volume of 120 ml. The dog was anesthetized with nembutal given intravenously in a dose of 0.065 gm. per kilo. The hematin solution was injected by intravenous drip over the course of an hour.

A similar procedure was followed on the 2 successive days. On the 2nd and 3rd days 300 mg. of hemin were dissolved in 3 ml. of 5 per cent Na₂CO₃; 27 ml. of distilled water and 120 ml. of 0.9 per cent saline were added for a total volume of 150 ml. The anesthesia, which lasted about 5 hours, and the infusions were well tolerated. The dog ate well and suffered no apparent toxic reactions.

Stercobilin was isolated in crystalline form by a method of Watson (13). 36 mg. were obtained from the 9 day stool specimen. The optical rotation, performed on a CHCl₃ solution containing 25 mg. per 100 ml., was [α]₅⁰° = -3550°.

RESULTS AND DISCUSSION

The isotope concentration in the stercobilin was found to be 0.325 atom per cent excess N¹⁵. This represents conversion of hematin to bile pig-
The extent to which the injected hematin was converted to bile pigment can be estimated approximately. In a normal dog of 17 kilos, the total circulating hemoglobin should be about 180 gm., and the total circulating heme about 7 gm. With a normal average erythrocyte life span of about 120 days (14), the daily turnover of circulating hemoglobin is about 0.8 per cent per day. The daily synthesis and degradation of circulating heme should therefore be about 56 mg. (7.0 gm. × 0.008). In 9 days a total of about 500 mg. of circulating heme should be degraded on the assumption of quantitative conversion of heme to bile pigment. The amount of labeled hematin which has been converted to bile pigment may be estimated as follows: if α represents this amount, then $1.425 \times (\text{atom per cent excess N}^{16}) \times \frac{\text{α(mg.))}}{\text{α + 500 mg.}}$ is equal to 0.325 atom per cent excess N$^{16}$. Solving for α yields a value of 148 mg. Of the total amount of labeled hematin which has been injected, 18 per cent (148/800) has been converted to bile pigment during the 9 day period. It is quite possible that the ultimate extent of conversion is much greater, for further degradation of hematin to bile pigment may occur after the first 9 days.

The objection might be raised that the conversion of hematin to stercobilin occurs through the utilization of the hematin for hemoglobin formation and subsequent degradation of the hemoglobin to bile pigment. Such a mechanism, however, would involve the dilution of isotopic hemoglobin by large amounts of non-isotopic hemoglobin. Bile pigment derived from isotopic hematin by such a route would have an isotope concentration far lower than that which is found. The rate of conversion of hematin to bile pigment indicates that at least a portion of hematin is metabolized much more rapidly than was suggested by earlier studies (9, 10). The demonstration of conversion of hematin to bile pigment lends support to the view that that portion of bile pigment which is not derived from the hemoglobin of mature circulating erythrocytes may originate, at least in part, from heme or hematin which is not utilized for hemoglobin formation (15, 16).

**SUMMARY**

Hematin can be readily converted to bile pigment in the dog.

**BIBLIOGRAPHY**

THE CONVERSION OF HEMATIN TO
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