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Shunt Bilirubin: Evidence for Two Components

Abstract. *Studies with C^{14}-labeled glycine and δ-aminolevulinic acid as heme-bilirubin precursors in man indicate that the early labeled or shunt bilirubin consists of two fractions. Fraction 1 requires 1 to 24 hours for maximum synthesis, is not dependent on marrow erythropoietic heme synthesis, and is possibly of anabolic origin (formed by a direct pathway from heme precursors). Fraction 2 requires 3 to 4 days for maximum production, is dependent on heme synthesis, and probably has its origin in the bone marrow, as a degradation product of red-cell heme.*

The observations of London, West, Shemin, and Rittenberg (*1*) and Gray, Neuberger, and Sneath (*2*) indicated that 10 to 20 percent of the bile pigment excreted by humans is derived from sources other than circulating red cells. This fraction was recognized by the appearance of N^{15}-labeled stercobilin within 5 to 10 days of the administration of N^{15}-glycine. This pigment, which appears much in advance of that derived from the heme of circulating red cells, has been termed the "early labeled" or "shunt fraction." It occurs at elevated levels in persons with certain diseases, among them pernicious anemia, congenital porphyria, thalassemia, refractory anemia, bleeding, and some cases of congenital hyperbilirubinemia due to primary overproduction (*3*). In these circumstances, this early labeled fraction may account for as much as 70 percent of the total bile pigment. It has been suggested that this early labeled stercobilin arises from one or more of the following sources: (i) hemoglobin of newly formed red cells destroyed shortly after formation; (ii) intracorpuscular degradation of hemoglobin in erythrocyte precursors in the marrow; (iii) heme formed in excess of globin and rapidly converted to bile pigment; (iv) other heme compounds such as myoglobin, catalase, peroxidase, or the cytochromes; (v) a direct synthetic pathway from a common precursor pool that does not require the synthesis of heme as an intermediate compound.

In a recent study reported from this laboratory, we were able to quantitate and time the appearance of this early labeled or shunt bilirubin in dogs in which a biliary fistula had been made ("bile fistula" dogs). The dogs were given glycine-2-C^{14} intravenously (*4*). In normal dogs this shunt accounted for 5 to 16 percent of a proposed common heme-bilirubin precursor pool, and labeled bilirubin appeared in the bile of all dogs within 4 to 8 hours of the injection of glycine. The formation of labeled bilirubin observed in some animals prior to the labeling of heme in the red blood cells or the marrow buffy coat (including red cell precursors) suggested that the heme in these sites was not an obligatory precursor of this bilirubin. Thus the possibility that there is a direct synthetic pathway of bilirubin from heme precursors was raised. We now report evidence that the early labeled fraction of bilirubin consists of two components which arise through different pathways.

Bilirubin was isolated from plasma by precipitation of the plasma proteins with ammonium sulfate and alcohol by a modification of the method described by Cole and Lathe (*5*). The bilirubin was then taken up in chloroform and purified by column chromatography to constant specific activity (*4*). Hemin was prepared and assayed for radioactivity (*4*). Fifty microcuries of glycine-2-C^{14} (Picker Nuclear) was given intravenously to a 112-kg man. Venous blood was drawn at intervals, and the bilirubin and hemin were isolated and assayed for radioactivity. The results are shown in Fig. 1. Radioactivity appeared in circulating heme within 24 hours and reached a plateau of 10 counts per minute per milligram on the 8th day. Labeled bilirubin was first detected at 3 hours and exhibited an initial peak at 24 hours; the activity then declined to a minimum at 48 to 60 hours, and a second peak was observed on the 3rd and 4th days. The same pattern was observed in two additional subjects.

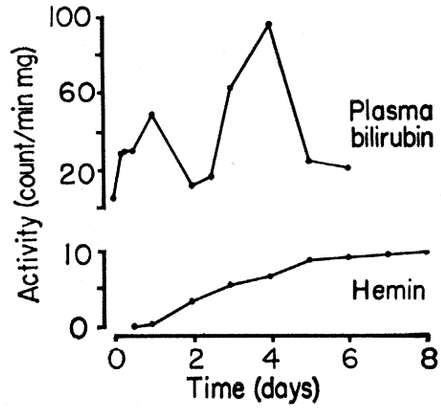

Fig. 1. Specific activity of bilirubin isolated from plasma and of hemin from circulating erythrocytes in man given 50 μc of glycine-2-C^{14} intravenously.

In a parallel experiment 12.5 μc of δ-aminolevulinic acid-4-C^{14} (California Biochemicals) was given intravenously to a 75-kg man. The appearance of labeled bilirubin in his plasma and of labeled heme in his red cells is shown in Fig. 2. Although δ-aminolevulinic acid was a poor heme precursor, labeled bilirubin appeared within 30 minutes, reached its peak at 90 minutes, and then rapidly decreased. There was no second peak. The same pattern was observed in two other subjects.

The first component of labeled bilirubin found after administration of glycine is comparable to, although slower to appear than, that found when δ-aminolevulinic acid is used as the precursor. Glycine as a precursor of

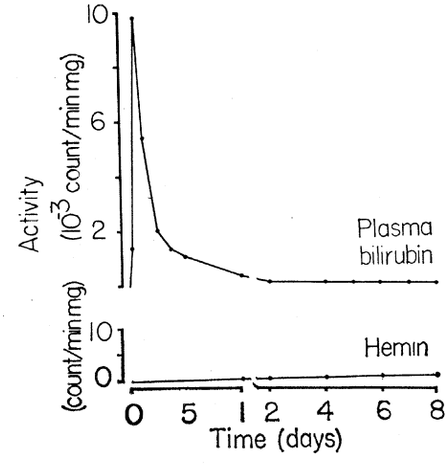

Fig. 2. Specific activity of bilirubin isolated from plasma and of hemin from circulating erythrocytes in man given 12.5 μc of δ-aminolevulinic acid-4-C^{14} intravenously.

δ-aminolevulinic acid follows the common pathway into the pyrolle rings which are common to the structure of porphyrin and bilirubin. However, the two compounds differ in that glycine is a good heme precursor in vivo whereas δ-aminolevulinic acid is not (*6*). The second component is seen only in those individuals who are given glycine.

Previously we reported that, in dogs given 700 r of total body radiation with resultant marrow aplasia and depressed heme synthesis, labeled bilirubin appeared in the bile with peak activity at 4 hours after administration of glycine-2-C^{14} (*4*). The peak is analogous to the first peak for human plasma. Therefore the first component is present when red cell production is depressed and does not appear to be dependent on erythropoietic heme synthesis. Its appearance at times before the appearance of labeled heme indicates that the heme of hemoglobin is not an obligatory precursor. Its rapid appearance in the plasma in man within 30 to 90 minutes after administration of C^{14}-labeled δ-aminolevulinic acid also suggests that it may represent an anabolic bilirubin formed by a direct pathway from heme precursors.

The second peak seen in human plasma after administration of C^{14}-labeled glycine is analogous to a secondary plateau noted in "bile fistula" dogs with active erythropoiesis. In those dogs with active heme formation following venesection, labeled bilirubin appeared rapidly in the bile with an initial peak at 24 hours; then it fell to a plateau at 48 hours, with plateau activity remaining to the 5th day (*4*). The second component in human plasma and the plateau in the bile of dogs are absent under those circumstances in which heme synthesis from the labeled precursor is minimal or absent, as in man given C^{14}-labeled δ-aminolevulinic acid and in the radiated dog. This suggests that this second component is dependent on the synthesis of labeled heme, and its abolition in the marrow-depleted irradiated dog indicates that it originates in the bone marrow. Its presence on the 3rd or 4th day suggests that it may arise from newly-formed erythrocytes or late normoblasts that took up the pulse of radioactivity as early precursors of red cells (*7*).

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Aluminum Silicate System: Experimental Determination of the Triple Point

Abstract. *The kyanite-sillimanite-andalusite triple point exists in the pressure-temperature plane at 8 ± 0.5 kb and 300 ± 50°C. Reactions are accomplished experimentally with a Bridgman opposed-anvil press (with an external furnace), modified to provide shearing of the sample charges. All three equilibrium boundaries are proved by reversed reactions.*

Problems of the existence and location of the kyanite-sillimanite-andalusite triple point on the pressure-temperature plane have been of considerable interest to petrologists and geophysicists (*1*, *2*). Unfortunately, thermochemical data in this system are not easily obtained, and experimental reactions are difficult.

Kennedy (*3*) reviewed experimental results in the high pressure-temperature range in the system Al_2O_3-SiO_2-H_2O. Clark (*4*) redetermined the equilibrium boundary for kyanite-sillimanite with reversed reactions in the range 900°C, 16 kb to 1500°C, 24 kb with a solid medium press. The other two boundaries, kyanite-andalusite, and andalusite-sillimanite, have not been previously determined because of the difficulties of synthesizing andalusite. Nevertheless, phases closely resembling andalusite have been found in the products of hydrothermal runs (*5*).

The apparatus used in the present study is a Bridgman opposed-anvil press (with external furnace), modified to provide alternating shear action to the specimen. The press was designed by F. Birch and is similar to the one described by Dachille and Roy (*6*). Heat-treated tool steel anvils of ¼-inch working diameter and 1⅛-inch outside diameter were used. The pressure on the specimen was calculated from the ratio of areas and the pressure measured on the large piston with a bourdon gauge; the temperature was measured with a chromel-alumel thermocouple located axially at the base of the lower anvil.

Synthetic gels of composition $Al_2SiO_5 \cdot xH_2O$ fired at 600°C for 6 hours were used to define the stability fields, and all three polymorphs were synthesized. Three naturally occurring materials were used in seeded runs to reverse reactions near the equilibrium boundaries. They are andalusite from Laws, Calif., and Mount Washington, N.H., sillimanite from Monroe, N.Y., and kyanite from Minas Gerais, Brazil. Phases were identified with a Norelco x-ray diffractometer. X-ray patterns of the products were compared with those of the seeded reactants in order to determine whether the seed had grown at the expense of the starting phase. Unless distinct growth of the seed material could be observed, the results were judged inconclusive.

Experimental results are presented in Table 1 and shown diagramatically in Fig. 1. Good agreement is found with the extrapolated extension of Clark's (*4*) kyanite-sillimanite curve. The kyanite-andalusite and andalusite-sillimanite boundaries are located in zones where the experimental results are inconclusive. Reactions are sluggish in these

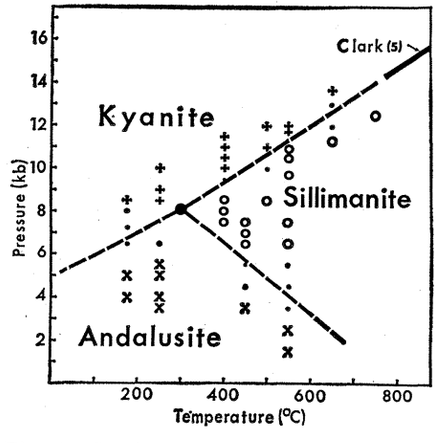

Fig. 1. Syntheses of kyanite, sillimanite, and andalusite. Pluses stand for runs in which kyanite was produced, circles for runs in which sillimanite was produced, and crosses for runs in which andalusite was produced. Dots stand for runs in which the results were inconclusive. The solid line (upper right) is Clark's (*4*) curve.