The Measurement of the Synthetic Rate of Bilirubin from Hepatic Hemes in Patients with Acute Intermittent Porphyria

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ABSTRACT A new method for the direct measurement in vivo of the synthetic rate of bilirubin from hepatic hemes is proposed. This method depends on the application of the labeled precursor-product relationship to the hepatic pool of porphobilinogen, which is a common precursor of both urinary porphobilinogen and hepatic-synthesized bilirubin. The hepatic pool of porphobilinogen is labeled by means of an intravenous injection of δ-aminolevulinic acid-4-^{14}C. The proportion of total bilirubin production which is derived from hepatic hemes is calculated from the ratio of the mean ^{14}C specific activities of stercobilin and porphobilinogen estimated in pooled specimens of feces and urine, respectively. The method can be most readily applied to patients with acute intermittent porphyria, as the appreciable quantities of prophobilinogen in the urine of these patients greatly facilitate the measurement of porphobilinogen-^{14}C specific activity. In three patients with acute intermittent porphyria, values obtained for the synthetic rate of bilirubin from hepatic hemes were 20.7, 15.8, and 13.3% of total bilirubin production.

INTRODUCTION

In this paper a new method is described for directly measuring the synthetic rate of bilirubin from hepatic hemes, which complements previous methods which have depended on administering a labeled precursor and then quantitating the "early labeled peak" in bile pigments in either blood or feces (1–8). The method involves the biosynthetic labeling of porphobilinogen and bilirubin by means of an intravenous injection of δ-aminolevulinic acid-4-^{14}C (ALA-4-^{14}C).[1] The method depends on the following two conditions being true: (*a*) ^{14}C administered as ALA-^{14}C is not incorporated to any appreciable extent into the developing erythrocyte (4, 9, 10). Hence ^{14}C-labeled bilirubin and other bile pigments arising within the first few days of the administration of ALA-^{14}C reflect the incorporation of the label into bilirubin derived from hepatic hemes; (*b*) hepatic porphobilinogen (PBG) is a common precursor of both urinary PBG and hepatic-synthesized bilirubin. These two conditions are essential prerequisites for applying the labeled precursor-product relationship (11, 12) to the hepatic pool of PBG. According to this relationship, during a given period of time after the administration of ALA-4-^{14}C the mean specific activity of urinary PBG would be equal to the mean specific activity of hepatic-synthesized bilirubin. Thus a simple equation for the synthetic rate of bilirubin from hepatic hemes can be written in terms of the total mass of PBG excreted in urine and the total ^{14}C radioactivity incorporated into both bilirubin and urinary PBG. A derivation from this equation indicates that the proportion of total bilirubin production, which is due to the turnover of hepatic hemes, can be expressed in terms of the ratio of the mean ^{14}C specific activities of stercobilin and PBG in pooled specimens of feces and urine, respectively. In practice the ^{14}C specific activity of PGB in urine can be measured most readily and accurately in patients with acute intermittent porphyria (AIP), on account of the

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[1] *Abbreviations used in this paper:* AIP, acute intermittent porphyria; ALA, aminolevulinic acid; PBG, porphobilinogen.

high urinary concentrations of PBG in these patients. Because of this, initial studies using this method have been made on patients with AIP. We report the results of measurements of bilirubin production from the turnover of hepatic hemes in three patients with AIP.

METHODS

Patients

Three patients with acute intermittent porphyria were studied. There were two males and one female. Their ages varied between 23 and 42 yr. The average daily urinary porphobilinogen excretions, measured by the method of Mauzerall and Granick (13), were 16, 93, and 116 mg/day, respectively. At the time of study all were considered to be in a metabolically steady state with particular reference to body weight, diet, hematocrit, plasma unconjugated bilirubin concentration, and urinary excretion of PBG. The studies were conducted with the fully informed consent and cooperation of each patient.

Materials

Bilirubin-^3H. Bilirubin-^{3}H was prepared biosynthetically from δ-aminolevulinic acid-2,3-^{3}H in bile fistula dogs (14).

δ-Aminolevulinic acid-4-^{14}C. δ-Aminolevulinic acid-4-^{14}C having a specific activity of 37.4 mCi/mmole, obtained from New England Nuclear Corp. Boston, Mass., was dispensed in ampoules containing 47 μCi in 5 ml of 1 N saline and stored in − 20°C.

Design of studies

At zero time known volumes of bilirubin-^{3}H (15–30 μCi) dissolved in an albumin solution (15, 16) and δ-aminolevulinic acid-4-^{14}C (30–40 μCi) were injected simultaneously through the tubing of an intravenous infusion. Appropriate standard solutions for counting were made from portions of both of the injected solutions.

A base line blood specimen was taken immediately before the administration of the labeled ALA and bilirubin, and subsequently specimens were taken at intervals of approximately 10 min for the 1st hr, 15 min for the next 2 hr and 20 min for the subsequent 2 hr. A further four to eight specimens were taken at increasing intervals during the ensuing 36–72 hr.

Feces was collected in 24-hr lots for 7–10 days (patient 1, 7 days; patients 2 and 3, 10 days). None of the patients were constipated. The weight of each lot was measured, and the specimen was stored at − 20°C.

Successive 24-hr specimens of urine were collected for 7 days. These urines were kept in the dark at 4°C. The volume of each specimen was measured. To determine approximate values for urinary excretion of ALA-^{14}C and PBG-^{14}C, 2-ml portions of each 24 hr urine specimen were passed through a small column of appropriately primed Dowex 1 resin, followed by 10 ml water and 10 ml sodium acetate buffer 0.01 M, pH 4.6 (13, 17). This first eluate was considered to contain most of the ^{14}C radioactivity in δ-ALA in the original portion (13, 17). 10 ml of 1 M acetic acid was then passed through the column, to give a second eluate which should contain most of the ^{14}C radioactivity in PBG in the original portion (13, 17). 1 ml portions of both eluates were counted for ^{14}C, and figures for total ^{14}C radioactivity in δ-ALA and PBG in each urine collection were calculated. In each study the rough estimates of ^{14}C radioactivity in both δ-ALA and PBG, determined in this way, had fallen to negligible quantities by the 5th day. Hence it appeared that virtually all urinary excretion of ALA-^{14}C and biosynthesized ^{14}C-labeled PBG was complete by the end of the 4th day. Pools of urine each comprising 25% of the volume of the first four 24-hr specimens were made (patients 1 and 2, three pools; patient 3, two pools). To minimize any loss of PBG, before the end of the 1st wk the PBG in these pools was precipitated with mercuric acetate, and the relatively stable PBG-mercury complex was stored at 4°C. These precipitates were processed further within 3 wk.

Laboratory procedure

Plasma unconjugated bilirubin-^3H specific activity (disintegrations per minute/milliliter plasma). Plasma specimens were processed by the methods previously described (15, 16). Portions of the lower layer of the Weber and Schalm (18) plasma separation were counted for both ^{3}H and ^{14}C radioactivity, and the derived figures for bilirubin-^{3}H and -^{14}C specific activity were expressed as disintegrations per minute/milliliter plasma.

Plasma unconjugated bilirubin concentration (milligrams/100 milliliters). At least 10 determinations of the plasma bilirubin concentration were made during each study by the method of Weber and Schalm (18), and the mean plasma bilirubin concentration was determined.

Fecal stercobilin-^{14}C specific activity (disintegrations per minute/milligram stercobilin). Each individual stool specimen was homogenized, and 10% portions were pooled. Stercobilin was crystallized from the pooled homogenate by the method of Watson et al. (19) and recrystallized until constant specific activity was obtained. Crystals obtained had optical rotations which varied between $[\alpha]_D$ − 2800 and − 4000. Portions of the crystals were weighed, dissolved in chloroform, bleached under ultraviolet light, dried under a nitrogen stream, dissolved directly into a liquid scintillation counting solution, and counted for ^{14}C.

Urinary PBG-^{14}C specific activity (disintegrations per minute/milligram PBG). The method employed for measuring PBG-^{14}C specific activity was based on Cookson and Rimington's (20) method for isolating PBG from urine. Determinations were made on each of the pooled urine specimens (see above). The second mercury-PBG precipitate of Cookson and Rimington's (20) method was agitated in dilute acetic acid and centrifuged, and the supernatant was discarded. This procedure was carried out at least five times to remove from the precipitate traces of contaminating ^{14}C in δ-ALA and other compounds in the supernatant. The precipitate was then decomposed with hydrogen sulfide. After centrifuging, the supernatant was filtered and aerated. The pH of this solution (solution P) was brought to 4.6 with 1 N sodium hydroxide. Four 1 ml portions of solution P were diluted either 10 or 100 times with sodium acetate buffer, 0.01 M, pH 4.6. 2-ml portions of each of these dilutions of solution P were passed through columns of Dowex 1 resin (4 cm in height, 1 cm in diameter, primed with 15 ml sodium acetate buffer, 0.1 M, pH 4.6) followed by 10 ml water and 10 ml sodium acetate buffer, 0.01 M, pH 4.6 (eluate 1, 22 ml). 10 ml of 1 M acetic acid was then passed through each column (eluate 2, 10 ml). 2-ml portions of eluate 2 were reacted with equal volumes of modified Ehrlich's reagent (containing 2 N perchloric acid), and the optical density (OD) was read after 15–20 min at 556 mμ on a spectrophotometer (13, 17). The dilutions of solution P were such that the OD's obtained fell in the range 0.1–1.0. The concentration of PBG in eluate 2 (milligrams

PBG/milliliter) was calculated using the molar extinction coefficient of 6.1×10^4 obtained by Mauzerall and Granick (13). 1 ml aliquots of eluate 2 were counted for ^{14}C. PBG-^{14}C specific activity was calculated by dividing values for ^{14}C disintegrations per minute/milliliter of eluate 2 by appropriate values for milligrams PBG/milliliter of eluate 2. The mean values of the separate estimates (patients 1 and 2, 12 estimates; patient 3; 8 estimates) were determined. The standard errors of the means were 1.27%, 0.71%, and 0.47% of each mean, respectively.

Measurement of ^3H and ^{14}C radioactivity. All counting was carried out in a three-channel liquid scintillation counter.[2] Channel A was peaked for ^3H, channel B for ^{14}C, and channel C for ^{14}C excluding ^3H. The counting fluids used were Turner's solution (700 ml toluene, 200 ml methanol, 1 g 2,5-diphenzyloxazole, 0.1 g *p*-bis[2-(5-phenyloxazolyl)]benzene) for the bilirubin and stercobilin measurements and Waggs solution (416 ml toluene, 500 ml methyl Cellusolve, 84 ml Liquifluor[3]) for the PBG measurements. Counting was continued until a minimum of 10,000 counts had been collected in appropriate channels. All samples were recounted after the addition of ^{14}C internal standard and, in the case of plasma samples, counted again after the addition of ^3H internal standard to enable appropriate corrections for quenching to be made.

Validity of methods

Because the method of measuring urinary PBG-^{14}C specific activity has not been previously described, the following further experiments were conducted to test the validity of the method employed.

(*a*) 1 ml portions of eluate 1 obtained from patients 2 and 3, were counted for ^{14}C, and the partitioning between eluates 1 and 2 of ^{14}C radioactivity in the diluted solution P was determined. Less than 4% of the ^{14}C radioactivity applied to the column appeared in eluate 1. When δ-ALA-4-^{14}C was added directly to a solution P, obtained from the urine of a porphyric patient who had not received δ-ALA-4-^{14}C intravenously, and when this solution P, after dilution, was applied to a Dowex 1 column, less than 0.5% of the applied ^{14}C radioactivity appeared in eluate 2. It follows than even if, under the experimental conditions of the studies reported here, all of the ^{14}C radioactivity appearing in eluate 1 represents δ-ALA-4-^{14}C and not PBG-^{14}C, then no more than 0.02% of the ^{14}C radioactivity applied to the Dowex 1 column as diluted solution P would appear in eluate 2 as δ-ALA-4-^{14}C.

(*b*) In one patient with AIP after the intravenous injection of δ-ALA-4-^{14}C, the specific activity of urinary PBG-^{14}C was determined in two 24-hr urine collections by both (*i*) the method described above and (*ii*) directly from crystals of PBG monohydrate, obtained by the method of Cookson and Rimington (20), which had been washed repeatedly with dilute acetic acid. The values obtained were 1.33×10^5 and 3.10×10^4 dpm/mg PBG using method (*i*) and 1.16×10^5 and 3.33×10^4 dpm/mg PBG using method (*ii*), respectively.

(*c*) The absorbance spectra of portions of solution P which had been reacted with equal volumes of modified Ehrlich's solution, were determined using a Cary recording spectrophotometer between 350 and 700 mμ. The spectra obtained were found to be identical with those given by aqueous solutions of a commercial preparation of PBG[4] between the same wavelengths. These observations provide no evidence for the presence of any Ehrlich positive substance other than PBG in solution P.

(*d*) Thin-layer chromatograms[5] of solution P using butanol–acetic acid (13) revealed a single Ehrlich positive spot in the same position as that on a chromatogram of a commercial preparation of PBG run in parallel. ^{14}C counting of strips of unstained chromatograms of solution P also run in parallel, revealed that the region corresponding to the Ehrlich positive spot was associated with a peak of ^{14}C radioactivity. This was the only peak of ^{14}C radioactivity found in chromatograms of solution P. In particular, no peak of radioactivity was found in the position corresponding to the peak observed in chromatograms of δ-ALA-4-^{14}C.

CALCULATIONS

After intravenous δ-ALA--4-^{14}C, the ^{14}C label becomes incorporated into positions 2 and 4 of the PBG molecule and positions 1′, 2, 3′, 4, 5, 6′, 7′, and 8 of the bilirubin molecule (21). If the labeled precursor-product relationship (11, 12) is then applied to the hepatic pool of PBG, it follows that during a given period of time (t_1–t_2) the mean specific activity of carbon atoms 2 or 4 of PBG in this pool, or in urinary PBG derived from it, will be equal to the mean specific activity of any one of carbon atoms 1′, 2, 3′, 4, 5, 6′, 7′, and 8 in hepatic-synthesized bilirubin which is derived from the precursor pool of hepatic PBG. Let, as follows:

m_b = Total mass of carbon incorporated into any one of positions 1′, 2, 3′, 4, 5, 6′, 7′, and 8 in hepatic-synthesized bilirubin (mg C).

r_b = Total radioactivity incorporated into that particular carbon atom in hepatic synthesized bilirubin (dpm).

m_p = Total mass of carbon incorporated into position 2 or 4 in urinary PBG (mg C).

r_p = Total radioactivity incorporated into that particular carbon atom in urinary PBG (dpm).

R_p = Total ^{14}C radioactivity incorporated into urinary PBG (dpm).

M_p = Total mass of PBG excreted in urine (mg PBG).

R_b = Total ^{14}C radioactivity incorporated into bilirubin (dpm).

M_b = Total mass of bilirubin synthesized from hepatic hemes (mg bilirubin).

2 = Number of labeled carbon atoms/molecule PBG.

8 = Number of labeled carbon atoms/molecule bilirubin.

12 = Atomic weight of carbon.

225 = Molecular weight of PBG.

584.7 = Molecular weight of bilirubin (22).

[2] Nuclear-Chicago Corporation, Des Plaines, Ill.

[3] New England Nuclear Corp.,

[4] Portex, Montreal, Canada.

[5] 6060 silica gel; Eastman Kodak Co., Rochester, N. Y.

Then from t_1 to t_2

$$\frac{r_b}{m_b} = \frac{r_p}{m_p}, \tag{1a}$$

or

$$m_b = \frac{r_b}{r_p} \times m_p, \tag{1b}$$

or

$$\frac{m_b}{m_p} = \frac{r_b}{r_p}. \tag{1c}$$

It follows that

$$r_p = \frac{R_p}{2}, \tag{2}$$

$$m_p = M_p \times \frac{12}{225}, \tag{3}$$

$$r_b = \frac{R_b}{8}, \tag{4}$$

$$m_b = M_b \times \frac{12}{584.7}. \tag{5}$$

Substituting equations 2, 3, 4, and 5 in equation 1*b* and simplifying, during the time interval t_1–t_2,

$$M_b = \frac{R_b}{R_p} \times M_p \times 0.650. \tag{6}$$

From equation 1*c* it follows that under steady-state conditions the ratio m_b/m_p over one particular time interval would be the same as the ratio r_b/r_p over any other time interval. Thus,

$$M_b(t_0 - t_{1\,day}) = \frac{R_p(t_0 - t_\infty)}{R_p(t_0 - t_\infty)} \times M_p(t_0 - t_{1\,day}) \times 0.650. \tag{7}$$

Since virtually all urinary PBG-^{14}C has been excreted by 4 days (see above),

$$R_p(t_0 - t_\infty) = 4 \times M_p(t_0 - t_{1\,day}) \times \bar{p}, \tag{8}$$

where $\bar{p}$ = mean ^{14}C specific activity of PBG in a pool of the first 4 days urine (dpm/mg PBG).

The data of Berk, Howe, Bloomer, and Berlin (16) and measurements of total ^{14}C radioactivity in serial stool specimens in these studies indicated that virtually all fecal excretion of ^{14}C radioactivity has occurred by the end of the period of the stool collection. Hence,

$$R_b(t_0 - t_\infty) = N \times M_B \times \bar{s} \times \frac{594}{584.7}, \tag{9}$$

where

N = Duration of stool collection (days).
M_B = Total bilirubin production rate (mg/day) (uncorrected).
$\bar{s}$ = Mean ^{14}C specific activity of stercobilin in the pooled specimen of feces (dpm/mg stercobilin).
594 = Molecular weight of stercobilin (22).
584.7 = Molecular weight of bilirubin (22).

Values for M_B were determined from the plasma disappearance curve of unconjugated bilirubin-^{3}H and the mean plasma unconjugated bilirubin concentration using the methods described by Berk et al. (16). Substituting equations 8 and 9 in equation 7 and simplifying, the following expression for the synthetic rate of bilirubin from hepatic hemes (S_h mg/day) is obtained:

$$S_h = N \times M_B \times \frac{\bar{s}}{\bar{p}} \times 0.1651. \tag{10}$$

Dividing both sides of equation 10 by M_B, an equation for the proportion of total bilirubin production, which is due to the turnover of hepatic hemes (K_h), is given

$$K_h = N \times \frac{\bar{s}}{\bar{p}} \times 0.1651. \tag{11}$$

The proportion of S_h which passes directly to the plasma in the unconjugated form (S_p) and hence the proportion which passes into bile without prior passage through the plasma in the unconjugated form (S_b) have been determined elsewhere[6] for the three patients reported here. Estimates of total bilirubin production derived using the plasma disappearance curve of intravenously injected bilirubin-^{3}H (M_B) do not take into account hepatic-synthesized bilirubin which does not appear in the plasma in the unconjugated form. Consequently, to obtain valid absolute values, estimates of M_B, S_h, S_p, and S_b derived by the analysis described above should each be corrected by multiplying by a constant. It is shown in the Appendix that this constant (K) is given by the following expression:

$$K = \frac{1}{1 - \frac{S_b}{M_B}}. \tag{12}$$

RESULTS

The results are given in Table I. Mean plasma unconjugated bilirubin concentrations varied between 0.25

[6] Jones, E. A., R. Shrager, J. R. Bloomer, P. D. Berk, R. B. Howe, and N. I. Berlin. Quantitative studies of the delivery of hepatic synthesized bilirubin to plasma utilizing δ-aminolevulinic acid-4-^{14}C and bilirubin-^{3}H in man. Manuscript in preparation.

TABLE I

Hepatic Synthesis of Bilirubin

Patient	Body weight	Plasma unconjugated bilirubin concentration	Total bilirubin synthesis (corrected; M_B*)	Hepatic synthesis: Total (corrected; S_h*)	Hepatic synthesis: Appearing as plasma unconjugated bilirubin (corrected; S_p*)	Hepatic synthesis: Not appearing as plasma unconjugated bilirubin (corrected; S_b*)	(KS_h/KM_B)	(M_B/KM_B)
	kg	*mg/100 ml*	*mg/kg per day*		*mg/kg per day*			
1	87.8	0.44	4.75	0.98	0.52	0.46	0.207	0.904
2	58.1	0.25	2.69	0.42	0.28	0.14	0.158	0.948
3	59.6	0.35	3.56	0.47	0.30	0.17	0.133	0.952

$$KS_h/KM_B = \frac{\text{hepatic bilirubin synthesis}}{\text{total bilirubin synthesis}}.$$

$$M_B/KM_B = \frac{\text{uncorrected total bilirubin synthesis}}{\text{corrected total bilirubin synthesis}}.$$

mg/100 ml and 0.44 mg/100 ml, and the uncorrected value for total synthesis of bilirubin (i.e. plasma unconjugated bilirubin turnover) varied between 2.55 and 4.29 mg/kg per day. These figures are in general agreement with similar data obtained in other patients with AIP (23).

The availability of values for the proportion of hepatic-synthesized bilirubin not appearing as plasma unconjugated bilirubin obtained in these patients[6] (patient 1, 0.465; patient 2, 0.332; patient 3, 0.361), enables the calculation of uncorrected absolute values for bilirubin synthesized in the liver which does not enter the plasma as unconjugated bilirubin. These values were used to calculate a correction factor for each patient to make due allowance in the estimates of absolute values (milligrams/day or milligrams/kilogram per day) based on M_B for the proportion of total bilirubin production which does not appear as plasma unconjugated bilirubin (see Appendix). The corrected total bilirubin synthesis varied between 2.69 and 4.75 mg/kg per day. The corrected hepatic-synthesized bilirubin which appears as plasma unconjugated bilirubin varied between 0.28 and 0.52 mg/kg per day, and the corrected hepatic-synthesized bilirubin which does not appear as plasma unconjugated bilirubin varied between 0.14 and 0.46 mg/kg per day. Corrected values of total hepatic bilirubin synthesis for the three subjects were 0.98, 0.42, and 0.47 mg/kg per day or 20.7, 15.8, and 13.3% of the total bilirubin synthetic rate, respectively. Values for M_B underestimated the corrected total bilirubin synthetic rate by 4.8–9.6% (mean 6.5%).

DISCUSSION

The validity of values for the synthetic rate of bilirubin derived from hepatic hemes obtained in these studies depends on the applicability of the labeled precursor-product relationship to the hepatic pool of PBG in patients with AIP. The conditions governing the use of this principle have been discussed with reference to the hepatic pool of guanidine carbon of arginine by Reeve, Pearson, and Martz (11) and Tavill, Craigie, and Rosenoer (12). The main assumptions inherent in the present application of this relationship are as follows:

(*a*) The synthetic rate of bilirubin from all sources and of PBG from the liver remain constant during the relatively short period of a study.

(*b*) Hepatic PBG can be regarded as the immediate precursor of both urinary PBG and hepatic-synthesized bilirubin. There are several intermediary metabolic steps in the biosynthesis of bilirubin from PBG. However, bilirubin becomes labeled rapidly after the administration of labeled δALA (4–7, 24) suggesting that the net effect of the existence of these intermediary steps in this context is merely to constitute a short delay in the synthesis of bilirubin from hepatic PBG. It seems likely, therefore, that the ^{14}C specific activity of newly formed hepatic-synthesized bilirubin at a particular instant of time reflects that of hepatic PBG a short time earlier. No allowance for this delay is necesary in making the calculations described in this paper.

(*c*) The contribution of any extrahepatic source to urinary PBG is negligible. The proportion of the total PBG excreted daily in the urine of nonporphyric subjects, which is derived from extrahepatic sources is unknown, but probably small. However, as a consequence of the biochemical lesion in AIP, the PBG excreted in the urine of patients with this disease, in excess of normal quantities, is probably derived exclusively from the liver. Thus in patients with AIP the proportion of urinary PBG arising from extrahepatic sources is likely to be exceedingly small.

(*d*) After the administration of ALA-^{14}C, no ^{14}C radioactivity incorporated into urinary PBG, bilirubin, and stercobilin during the period of a study is derived from erythroid sources. This assumption is supported by experimental data in both man and rats, which indicate that, after the administration of labeled ALA, the labeling of hemin in circulating red cells and hence the incorporation of labeled ALA into the developing erythrocyte is very small (4–7, 9, 10, 24).

(*e*) After the administration of ALA-^{14}C, ^{14}C-labeled bilirubin is derived almost entirely from the turnover of ^{14}C-labeled hepatic hemes. There is some evidence that when ALA-^{14}C is used as a labeled precursor, there is ^{14}C labeling of nonhemoglobin hemes in tissues other than the liver, in particular the kidney (3, 7, 25). However, there is no evidence that the turnover of either these hemes or myoglobin gives rise to bilirubin under physiological conditions in vivo. In addition it has been shown that the relative incorporation of a dose of ^{14}C, administered as δ-ALA-^{14}C, into bilirubin in rats with bile fistulae is not reduced by nephrectomy (8) and is similar in the isolated perfused rat liver (26). These observations suggest that the ratio of bilirubin synthesized from extrahepatic nonhemoglobin hemes to that from hepatic hemes is probably very small.

This method of measuring hepatic-synthesized bilirubin could be applied to normal subjects and patients who do not excrete increased quantities of PBG in the urine, when more refined methods of isolating small quantities of PBG from urine specimens become available.

The figures obtained for hepatic synthesis of bilirubin in the studies reported here may well be representative, not only of the population of patients with AIP, but also of normal individuals. It has been shown in both nonporphyric subjects and patients with AIP that the factors relating the circulating red cell mass and total bilirubin production (23) are similar. Further in one patient with AIP, the relative size of the "early labeled peak" of bilirubin after the administration of glycine-2-^{14}C was similar to that found in normal subjects (23). These findings suggest that hepatic production of bilirubin is unlikely to be appreciably abnormal in patients with AIP.

Landlaw, Callahan, and Schmid (27) have demonstrated that in allylisopropylacetamide (AIA) induced experimental porphyria, relatively more carbon monoxide than bilirubin is produced, implying that not all hepatic heme is degraded to bilirubin. It follows that the measurements of hepatic bilirubin synthesis made in these studies will not necessarily reflect total hepatic heme turnover if there is an alternate pathway for the catabolism of hepatic heme that does not yield bilirubin.

The finding that some hepatic-synthesized bilirubin is conjugated and secreted into bile without prior passage through the plasma in the unconjugated form is consistent with the data of Yamamoto, Skanderbeg, Zipursky, and Israels (4). Because of this phenomenon, estimates of total bilirubin production derived from plasma disappearance curves of labeled bilirubin (15, 16) will underestimate true total bilirubin production. Such underestimation of total bilirubin production will tend to be relatively less in patients with increased bilirubin production secondary to hemolysis. This underestimation would not apply to estimates of total bilirubin production based on carbon monoxide production. Although a good correlation has been found between estimates of total bilirubin production derived from carbon monoxide production data and from plasma disappearance curves of labeled bilirubin measured simultaneously in the same subjects (16, 28), it seems unlikely that the precision of the two methods is sufficient to detect differences of less than 10% with confidence.

The magnitude of the absolute synthetic rate of bilirubin from hepatic hemes and the proportion of this hepatic-synthesized bilirubin, which appears as plasma unconjugated bilirubin found in these studies, raises the possibility that increased turnover of hepatic hemes may be associated with unconjugated hyperbilirubinemia.

APPENDIX

Because the estimate of the total bilirubin synthetic rate derived from the plasma disappearance curve of bilirubin-^{3}H (M_B) does not take into account hepatic-synthesized bilirubin, which does not appear as plasma unconjugated bilirubin (S_b), to obtain the true total bilirubin synthetic rate (M_B^*) it is necessary to correct M_B as follows

$$M_B' = M_B + S_b, \tag{1}$$

where M_B' = the first approximation of M_B^*. But since M_B was used to calculate S_b, a first approximation (S_b') to the true value of hepatic-synthesized bilirubin which does not appear as plasma unconjugated bilirubin (S_b^*) can be derived from M_B'.

$$S_b' = S_b \frac{M_B'}{M_B} = S_b + \frac{S_b^2}{M_B}. \tag{2}$$

The corresponding second and third approximations to M_B^* and S_b^* are

$$M_B'' = M_B + S_b' = M_B + S_b + \frac{S_b^2}{M_B}. \tag{3}$$

$$S_b'' = S_b \frac{M_B''}{M_B} = S_b + \frac{S_b^2}{M_B} + \frac{S_b^3}{M_B^2}. \tag{4}$$

$$M_B''' = M_B + S_b'' = M_B + S_b + \frac{S_b^2}{M_B} + \frac{S_b^3}{M_B^2}. \tag{5}$$

$$S_b''' = S_b \frac{M_B'''}{M_B} = S_b + \frac{S_b^2}{M_B} + \frac{S_b^3}{M_B^2} + \frac{S_b^4}{M_B^3}. \tag{6}$$

It follows that

$$M_B^* = M_B + S_b + \frac{S_b^2}{M_B} + \frac{S_b^3}{M_B^2} + \cdots + \frac{S_b^\infty}{M_B^{\infty-1}}, \quad (7)$$

and

$$S_b^* = S_b + \frac{S_b^2}{M_B} + \frac{S_b^3}{M_B^2} + \frac{S_b^4}{M_B^3} + \cdots + \frac{S_b^\infty}{M_B^{\infty-1}}. \quad (8)$$

M_B^* and S_b^* can be calculated most easily by multiplying M_B and S_b respectively by a constant. This constant (K) is given by the infinite series obtained by dividing either equation 7 by M_B or equation 8 by S_b. Thus

$$K = 1 + \frac{S_b}{M_B} + \frac{S_b^2}{M_B^2} + \frac{S_b^3}{M_B^3} + \cdots + \frac{S_b^\infty}{M_B^\infty}. \quad (9)$$

By multiplying both sides of equation 9 by $1 - \frac{S_b}{M_B}$, it can readily be shown that

$$K = \frac{1}{1 - \frac{S_b}{M_B}}. \quad (10)$$

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