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FAILURE OF PHENOBARBITAL TO INCREASE BILIRUBIN PRODUCTION IN THE RAT

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Abstract—Bilirubin production, as measured by bile bilirubin excretion in 16 untreated Sprague–Dawley rats, averaged 0·71 ± 0·05 (mean ± S.E.M.) mg/100 g/day in the 24 hr after surgical creation of an external biliary fistula. Despite the wide range (0·48 to 1·10 mg/100 g/day) in the group, values for each individual animal were highly consistent, averaging 101, 103, 93 and 92 per cent of the baseline value during the subsequent four 24-hr collection periods. Bile bilirubin excretion in 15 rats pretreated with phenobarbital (70 mg/kg) for 5 days prior to surgery averaged 0·86 ± 0·06 mg/100 g/day in the initial collection period, which did not represent a significant increase when compared to the untreated controls ($0{\cdot}1 > P > 0{\cdot}05$). Values in these animals likewise remained constant during the subsequent 3 days, during which phenobarbital therapy was continued. In an additional six animals, phenobarbital was begun 24 hr after surgery. During the 4 days of treatment, a period of time sufficient to produce a 2- to 3-fold increase in "early labeling" of bile bilirubin and a 3- to 4-fold increase in hepatic cytochrome P-450, bile bilirubin excretion averaged 105, 92, 80 and 100 per cent of the baseline pretreatment value. Hence phenobarbital-associated increases in "early labeling" of bile pigment and hepatic cytochrome P-450 content are not accompanied by an increase in total bilirubin excretion.

THE ADMINISTRATION of phenobarbital to rats in doses of 60–120 mg/kg/day has been reported to increase the hepatic content of heme[1] and heme-containing enzymes,[2] and to augment the incorporation of glycine-2-^{14}C and δ-aminolevulinic acid-^{14}C into both hepatic heme and into early labeled bilirubin and carbon monoxide.[2–5] These data have been interpreted as indicating that phenobarbital administration is associated with an increase in total heme turnover and, by implication, a consequent increase in the molar production of bilirubin and carbon monoxide.[3] Support for this interpretation was provided by the data of Schmid *et al.*,[2] which indicated that there was a 2-fold increase in bile bilirubin excretion in phenobarbital-treated bile fistula rats. Subsequently, Coburn[6] reported an approximately 2-fold increase in carbon monoxide (CO) production in normal human volunteers taking phenobarbital. Other studies of bilirubin excretion in rats,[4] of bilirubin turnover as measured isotopically in rats[7] and in man,[8] and of carbon monoxide production in man[8] have failed to confirm that phenobarbital administration is associated with an increased production of either bilirubin or CO. The present study was carried out in an effort to define more precisely the effects of phenobarbital on bilirubin production in the rat.

MATERIALS AND METHODS

Collection of bile samples

All studies were performed in male Sprague–Dawley rats weighing 270–600 g. The largest available animals were used because of our observation that larger rats tolerated the chronic biliary diversion required for these studies for longer periods than did smaller, younger animals. For measurement of bile bilirubin excretion, the common bile duct was cannulated with PE-10 tubing (Clay Adams, Parsippany, N.J.) under either ether or pentobarbital (45 mg/kg) anesthesia. At the completion of surgery, animals were placed in a restraining cage which permitted free access to standard laboratory chow and water. Starting 30 min after surgery, bile was collected on ice in the dark in 24-hr aliquots in tubes containing 50 mg ascorbic acid. At the completion of each 24-hr period, sample volumes were recorded and the samples promptly frozen and stored at $-20°$ in the dark until analyzed. Daily collections were continued until the study was terminated by either cholestasis—most often due to a clot or other mechanical obstruction at the junction of the bile duct and cannula—or death. Where experiments were terminated by cholestasis, data for all completed 24-hr collection periods preceding the one in which bile flow ceased were included in the analysis. In those experiments terminated by death of the animal, data in the 24-hr period preceding the one in which the animal died were not included in the subsequent analysis. In some of these experiments, as noted below, an intravenous infusion of sodium taurocholate approximating the physiologic excretion rate of bile salt in the rat[9] was administered through a jugular vein catheter.

Measurement of bile bilirubin concentration

The total bilirubin concentration in 24-hr bile samples was measured by two methods: Weinbren and Billing's[10] modification of the diazotization procedure of Malloy and Evelyn,[11] and the oxidative method of Malloy and Evelyn.[12] Standard curves for both methods were prepared from the same set of solutions of thrice-recrystallized bilirubin (E = 60,000) in chloroform. Despite standardization of both methods from the same stock solutions, values obtained for bile bilirubin by the diazo method were consistently lower than those obtained by the oxidative method. The values were proportional for all samples, and the average ratio of diazo values to oxidative values was $0{\cdot}72 \pm 0{\cdot}01$ (mean $\pm$ S.E.M.). Although values for mean daily bile bilirubin excretion in untreated rats ($0{\cdot}52 \pm 0{\cdot}04$ mg/day/100 g body weight) using the diazo method were virtually identical to values repeatedly reported by others using this method,[2,4] we believe this value is appreciably less than the theoretical estimate of bilirubin production in the rat,* possibly because of degradation of some bilirubin to compounds which are diazo negative but nevertheless can be oxidized to biliverdin. Accordingly, results presented below are those obtained with the oxidative technique. Because of the narrow range of proportionality observed between the two methods, the fundamental conclusions of the study were the same, irrespective of which method was used for the bile bilirubin measurements.

* Based on data indicating that the average circulating red cell volume in the normal rat is 2·5 ml/100 g[13] and the overall mean red blood cell (RBC) lifespan is 55 days,[14] then the rat destroys 0·045 ml of red cells/day/100 g, which is converted to 0·55 mg bilirubin (12 mg/ml of RBC's). Since, in the rat, nonerythropoietic bilirubin production appears to contribute approximately 25 per cent of the total,[15] total bilirubin production would be expected to be approximately 0·73 mg/100 g/day.

Experimental design

Daily bile flow and daily bile bilirubin excretion were measured in six groups of rats. Group I consisted of 16 untreated control animals who had free access to water but received no supplemental intravenous bile salts or electrolytes. Groups II–IV received 51 μmoles/day of sodium taurocholate (Schwarz/Mann, Orangeburg, N.Y.) by continuous intravenous infusion. In group II (10 animals), this was administered in 60 ml isotonic saline containing 1·2 m-equiv KCl; in group III (9 animals), in 30 ml isotonic saline containing 1·2 m-equiv KCl; and in group IV (8 animals), in 30 ml of a hypotonic solution (1:1 normal saline and 5% dextrose plus 1·2 m-equiv KCl). The 15 rats comprising group V were given daily intraperitoneal injections of phenobarbital (Merck & Co., Rahway, N.J.), 70 mg/kg, dissolved in polyethylene glycol 400 solvent (Ciba, Summit, N.J.), starting 5 days before and continuing daily after the creation of the biliary fistula. Like group I, they had free access to drinking water, but received no supplemental bile salts or electrolytes.

Fourteen of the 43 animals in groups I–IV had their surgery performed under pentobarbital anesthesia. The remaining animals in these groups and all of the phenobarbital-treated animals received ether. There was no difference in the biliary excretion of bilirubin in the animals of groups I–IV who had received pentobarbital as compared with those receiving ether (Student's t-test, $P > 4$).

In group VI, biliary fistulae were constructed in untreated animals, and the subsequent 24-hr bile bilirubin output was measured. At the end of the first post-operative day, animals were begun on phenobarbital, 70 mg/kg, and bile collections were continued in 24-hr aliquots. Although survival of animals in apparent good health for 4 days after surgery was common in untreated control animals and in animals pretreated with phenobarbital, initiation of phenobarbital therapy 24 hr after surgery resulted in a very high mortality in the subsequent 2 days, associated with a virtual cessation of food and water intake. Hence, data are available in only 6 animals for group VI of the 14 initially operated on and begun on therapy.

Other studies

Early labeled peak studies. Ten male Sprague–Dawley rats weighing 400–540 g received daily intraperitoneal injections of phenobarbital at a dose of 70 mg/kg. Biliary fistulae were constructed on days 1, 3, 5 or 7 of treatment. Three hr after surgery, 20 μCi glycine-2-^{14}C (New England Nuclear, Boston, Mass; sp. act., 38 mCi/m-mole) was injected intravenously through a jugular cannula, and bile was collected over the subsequent 24 hr. Bile bilirubin content was measured by the oxidative method, and bilirubin was crystallized from the 24-hr bile sample by a modification of the method of Ostrow *et al.*,[16] and then recrystallized from methanol. The crystals were then dissolved in chloroform and their specific activity was determined by liquid scintillation counting, after first bleaching under u.v. light to reduce quenching. The total dis./min excreted in the bile as bilirubin during the 24-hr collection period, calculated from the bile bilirubin content and its specific activity, was considered to represent the "early labeled" bilirubin. Data were obtained in one rat after 1 day of treatment, and in three rats at each of the other time points. Results in each animal (per cent of injected dose) were expressed as the per cent of the mean value in three untreated control rats.

Measurements of cytochrome P-450 and Y protein. Twelve male Sprague–Dawley rats weighing 280–320 g were begun on daily intraperitoneal injections of phenobarbital, 70 mg/kg. After 1, 3, 5 and 7 days of treatment, three treated animals and one control animal were killed by decapitation. The liver was perfused *in situ* with cold isotonic saline, excised, and carefully blotted prior to weighing. A portion of the liver was then homogenized with 4 vol. of 1·15% KCl by a motor-driven homogenizer. After initial centrifugation for 25 min at 12,000 g, the microsomes were sedimented from the supernatant by centrifugation at 105,000 g for 90 min. The resulting pellet was resuspended in KCl and the protein concentration determined by the biuret reaction.[17] The solution was then diluted with 0·1 M phosphate buffer, pH 7·4, to obtain a microsomal protein concentration of 4 mg/ml. The CO difference spectrum of a dithionite-reduced microsome suspension was determined by the method of Omura and Sato[18] using a Beckman Acta III twin-beam spectrophotometer. Cytochrome P-450 content was calculated using the previously reported molar extinction coefficient of 91 cm^{-1} mM^{-1}.[18] The concentration of Y protein in hepatic cytosol was determined on portions of livers from three of the same control animals, and the three animals treated for 3 days, by the BSP binding method of Levi *et al.*[19]

RESULTS

Initial bile bilirubin excretion

Values for bile bilirubin content in the initial 24-hr collection after surgery are presented in Fig. 1. In 16 untreated controls (group I) the mean value was 0·71 ± 0·05 mg/100 g/day (mean ± S.E.M.). In the 27 animals who received supplemental intravenous bile salt infusions, the mean was 0·79 ± 0·04 mg/100 g/day. This was not significantly different ($P > 0·05$) from the values in group I, nor were there significant differences between groups II, III or IV. The mean for all four groups not receiving phenobarbital was 0·76 ± 0·03 mg/100 g/day. In 15 animals who received 5 days of

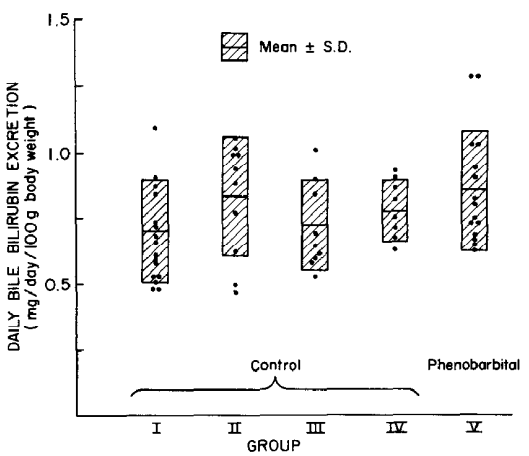

FIG. 1. Bile bilirubin excretion in the initial 24 hr after creation of a biliary fistula in untreated (groups I–IV) and phenobarbital-treated rats. In this study all animals had free access to water and laboratory chow, while groups II–IV received, in addition, 51 μmoles/day of sodium taurocholate administered intravenously with varying amounts of water and electrolytes. See text for details.

pretreatment with phenobarbital, the mean 24-hr bilirubin excretion in the initial 24 hr was 0·86 ± 0·06 mg/100 g/day. Although this represents a small increase of approximately 20 per cent from untreated animals (group I), this result is not significantly different from that of either group I ($0·1 > P > 0·05$) or the total population of animals who did not receive phenobarbital (groups I--IV, $P > 0·1$). The biological significance of even this 20 per cent increase observed in group V, compared to group I, is rendered questionable by the observation of the wide 2-fold range of values observed in each of the groups studied (Fig. 1). It is of particular interest that, when data from the untreated animals (group I) were averaged in consecutive sets of four animals, the means ranged from 0·59 ± 0·04 to 0·86 ± 0·04 mg/100 g/day. Hence, there was a statistically significant difference ($P < 0·01$) between two of the subgroups of control animals, one of which had a mean daily bilirubin excretion identical to that in the phenobarbital-treated group.

Serial measurements of bile bilirubin excretion

The results of consecutive 24-hr measurements of bile bilirubin excretion are presented in Fig. 2 for the animals who received no phenobarbital (groups I–IV) and for the animals who received phenobarbital for 5 days before and daily after the creation of the biliary fistula. Results for each rat were expressed as a percentage of the value obtained in the first 24 hr postoperatively. Although there was a wide range of bile bilirubin excretion for the various groups studied (Fig. 1), the value in any given rat was highly reproducible from day to day (Fig. 2). At no time in either the untreated (groups I–IV) or phenobarbital-treated (group V) animals was a mean daily value obtained which was significantly greater than the baseline value. Putting aside any possible effects of phenobarbital on bile bilirubin excretion during the initial 5 days

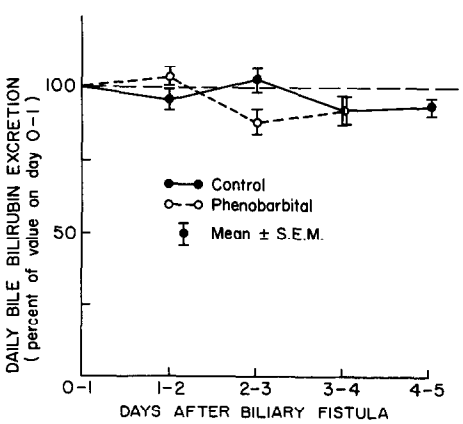

FIG. 2. Daily excretion of bilirubin in the bile during consecutive 24-hr collection periods after creation of a biliary fistula in control and phenobarbital-treated rats. For each rat, consecutive daily outputs were expressed as a percentage of the value obtained during the initial 24 hr (day 0–1). Of the animals initially entered into the study, data suitable for analysis (see text) were obtained in 100 per cent during the initial 2 days, in 80 per cent for 3 days and in 40 per cent for 4 days. Only 25 per cent of the control animals and none of the phenobarbital-treated animals survived the fifth day in good health and without the development of cholestasis.

TABLE 1. MEASUREMENTS OF EARLY LABELED BILIRUBIN EXCRETION, CYTOCHROME P-450 ACTIVITY, Y PROTEIN CONTENT AND LIVER WEIGHT DURING THE INITIAL 7 DAYS OF PHENOBARBITAL TREATMENT*

	Control	Days of phenobarbital treatment			
		1	3	5	7
Early labeled bile bilirubin					
% of injected dose	0·010 ± 0·0022	0·025	0·028 ± 0·0098	0·020 ± 0·0015†	0·020 ± 0·0016†
% of control	100 ± 22	237	271 ± 95	197 ± 14†	192 ± 15†
Hepatic cytochrome P-450					
nmoles/g liver	43 ± 6	104 ± 10‡	172 ± 13‡	147 ± 28†	198 ± 28‡
% of control	100 ± 13	242 ± 23‡	400 ± 30‡	341 ± 65†	461 ± 64‡
Hepatic Y protein					
μmoles/BSP bound/g liver	0·26 ± 0·04		0·53 ± 0·05‡		
% of control	100 ± 14		207 ± 19‡		
Liver weight§					
g/100 g body wt	3·4 ± 0·23	2·9 ± 0·01	3·7 ± 0·12	4·7 ± 0·04‡	4·3 ± 0·08‡

* All values represent mean ± S.E.M.
† Student's *t*-test, $P < 0.05$.
‡ Student's *t*-test, $P < 0.01$
§ Determined in animals whose livers were excised for cytochrome P-450 determinations.

of therapy, these data can be further interpreted as indicating that continuing phenobarbital therapy produces no change in bilirubin excretion after the fifth day of drug administration.

Because of the constancy of bile bilirubin excretion in each animal, we attempted to study the effects of phenobarbital on bile bilirubin excretion using each animal as his own control. As noted above, the initiation of phenobarbital therapy 24 hr after biliary surgery was associated with a high mortality. Nevertheless, adequate data were obtained in six animals, who constituted group VI. In this group, mean daily bilirubin excretion during each of the first 4 days of phenobarbital therapy averaged 105, 92, 80 and 100 per cent, respectively, of control. None of the animals in group VI survived the sixth day of treatment so that, as indicated above (Methods), no reliable data are available for the fifth day of treatment. Nevertheless, the data available for the first 4 days, combined with the serial measurements of bile bilirubin excretion in animals pretreated for 5 days, strongly suggest that phenobarbital administration is not associated with an increased excretion of bilirubin in the bile.

Measurements of early labeled bilirubin excretion, cytochrome P-450 activity, Y protein content and liver weight (as per cent of body weight) during the initial 7 days of phenobarbital therapy are presented in Table 1. Increases in both cytochrome P-450 and early labeled bilirubin excretion were observed after as little as 1 day of treatment, and highly significant increases in both of these parameters were observed by the third to fifth day of treatment. On the third day of phenobarbital treatment, Y protein levels had also increased to 207 $\pm$ 19 per cent of baseline ($P < 0.01$). Previous studies in our laboratory have indicated that as little as 4 days of treatment with either phenobarbital or glutethimide significantly augments bilirubin UDP-glucuronyl transferase activity to 144 per cent of baseline, an effect comparable to that achieved by 14 days of therapy.[20]

Measurements of bile flow

Bile flow during the initial 24 hr after surgery averaged 6·2 $\pm$ 0·30 ml/24 hr/100 g of body weight in the 46 animals receiving no phenobarbital. There were no differences in bile flow as a function of intravenous fluid and bile salt administration for the first 24 hr after operation. In contrast, bile flow during the first postoperative day was 8·8 $\pm$ 0·68 ml/24 hr/100 g of body weight in the 15 animals pretreated with phenobarbital for 5 days prior to surgery. This represents a statistically significant increase in total bile flow as a result of phenobarbital pretreatment ($P < 0.05$). However, when corrected for the phenobarbital-associated increase in liver weight (Table 1), there was no difference in bile flow per g of liver in the phenobarbital-pretreated animals (1·95 $\pm$ 0·16 ml/24 hr/g of liver) and those animals receiving no phenobarbital (1·90 $\pm$ 0·19 ml/24 hr/g of liver)($P > 0.9$). Bile flow increased in all groups during the subsequent two 24-hr collection periods. During the 48- to 72-hr collection period, the greatest increase, to 199 $\pm$ 19 per cent of baseline (12·2 $\pm$ 0·4 ml/day/100 g), was observed in the animals receiving intravenous sodium taurocholate in a total volume of 60 ml/day of isotonic NaCl–KCl. Essentially identical increases in bile flow, to 134 $\pm$ 13 per cent of baseline (7·8 $\pm$ 0·7 ml/24 hr/100 g of body weight), were observed in the remaining three groups of control animals. In the phenobarbital group, bile flow had increased to 132 $\pm$ 8 per cent of baseline (11·6 $\pm$ 0·82 ml/24 hr/

100 g of body weight) by the third day. As noted above, bile bilirubin excretion remained essentially constant in all groups, independent of the changes in bile flow.

DISCUSSION

The administration of phenobarbital and other agents metabolized by the microsomal enzyme systems of the liver has been shown to be associated with a wide range of alterations in hepatic physiology[21] and ultrastructure.[22] Clinical interest has focused on the ability of phenobarbital and other "enzyme inducers" such as glutethimide and dicophane (DDT) to cause a reduction in the plasma concentrations of both unconjugated[8,23–27] and conjugated bilirubin,[28,29] often producing marked amelioration of jaundice. While the reduction of hyperbilirubinemia during phenobarbital administration was initially attributed principally to induction of the enzyme, bilirubin UDP-glucuronyl transferase,[30–32] administration of phenobarbital at high doses to experimental animals increases total liver weight,[21] bile flow,[9,33] hepatic bilirubin uptake,[34] the level of the Y intrahepatic organic anion binding protein[35] and the relative storage capacity of the liver for a variety of organic anions.[35] Each of these factors may also contribute to the reduction of bilirubin levels observed during drug administration.

Of particular interest to pharmacologists have been the effects of phenobarbital on the hepatic microsomal drug-metabolizing enzyme systems and on hepatic heme metabolism. Administration of phenobarbital to rats in doses similar to those employed in the current study has been shown to produce an induction of hepatic δ-aminolevulinic acid (δ-ALA) synthetase (36–38), the first and rate-limiting step in heme biosynthesis.[36] This is followed sequentially by increased incorporation of heme precursors such as glycine-2-^{14}C, δ-ALA-^{14}C and ^{59}Fe into hepatic microsomal heme and heme proteins,[1,2,4,5] increased levels of cytochrome P-450,[2,4,37] and stimulation of certain hepatic microsomal drug oxidations.[21] The mitochondrial enzyme, ferrochelatase, representing the final step in heme biosynthesis, is also known to be increased by phenobarbital administration.[39] There has been conflicting information about whether the augmented levels of heme and cytochrome P-450 result from increased synthesis with a normal fractional catabolic rate,[40] a situation which would imply an increase in both heme synthesis and heme turnover, or result from a prolongation of the half-life of the compounds in question.[41,42] In the latter case, increased levels of heme and heme proteins would not necessarily imply increased heme turnover.

Under most physiologic circumstances, bilirubin and carbon monoxide are formed in equimolar quantities as the products of heme catabolism both *in vitro* and *in vivo*.[3,43,44] Hence the finding that phenobarbital administration produces a significant and equivalent increase in the incorporation of glycine-2-^{14}C into both early labeled bilirubin and carbon monoxide was interpreted as indicating an increased turnover rate of hepatic heme.[2,3] This interpretation appeared to be confirmed by the data of Schmid *et al.*,[2] who reported a 2-fold increase in the biliary excretion of bilirubin in phenobarbital-treated rats, and of Coburn,[6] who described a small increase in the carbon monoxide distribution space, a measure of total body heme, and a 74 per cent increase in CO production, a measure of heme turnover, in normal human volunteers taking phenobarbital at a much lower dose (*ca.* 2 mg/kg/day). As a result of these two studies, it has been widely accepted that phenobarbital administration is associa-

ted with a large, approximately 2-fold increase in CO and bilirubin production, derived from increased catabolism of microsomal heme and, in particular, cytochrome P-450.[2]

It appears unlikely, nevertheless, that this conclusion is correct. If, as is widely believed, hepatic heme turnover accounts for no more than 15–25 per cent of basal bilirubin production, a 4- to 6-fold increase in hepatic heme turnover would be required to produce a doubling of total bilirubin production. However, the increases in early labeled bilirubin and CO production associated with phenobarbital therapy have usually been reported as being approximately 2-fold.[2–4] To the extent that one can extrapolate at all from isotopic studies of "early labeling" to bilirubin production rates, the isotopic data might be interpreted as indicating a 2-fold increase in hepatic heme turnover, representing a 15–25 per cent increase in total bilirubin synthesis. However, because possible drug-induced changes in precursor pool sizes or in other pathways of precursor utilization have not been excluded, extrapolation from isotope incorporation to bilirubin production requires a number of critical, and as yet unverified, assumptions.

Although increases in bile bilirubin excretion of a few per cent, such as might result from a 30–50 per cent increase in hepatic heme turnover, cannot be ruled out by the methods employed, the present study demonstrates that there is, in fact, no large increase in total bilirubin synthesis as a result of phenobarbital administration. The conclusion is derived both from a comparison of data in populations of treated and untreated animals, and from studies in which each animal served as his own control. The data are consistent in this regard with other published studies of bile bilirubin excretion in the rat[4] and of isotopic measurements of bilirubin turnover in rats[7] and in man.[8] The data of Schmid *et al.*,[2] which are in fact the only published data to the contrary, are based on measurements in two control and two treated animals. However, in view of the more than 2-fold range of values for bilirubin excretion in untreated rats noted in this and other studies,[4] consistent with the correspondingly wide range of bilirubin turnover rates in normal man,[4,5] the use of such small groups carries a large risk of measuring a biologic artifact. Even with groups of four consecutive animals, we found statistically significant differences in rates of bilirubin excretion between some groups of untreated animals. There is no equally apparent flaw in the CO studies of Coburn.[6] However, a more recent study in a larger group of human subjects was unable to confirm a phenobarbital-induced increase in either CO space or CO production.[8]

In the present study, we have confirmed that phenobarbital produces a 2- to 3-fold increase in the early labeling of bile bilirubin, yet little or no increase in total bilirubin excretion. Some explanation of these superficially discordant observations is required. One obvious explanation is that an increase in hepatic heme turnover, early labeling and total synthesis of bilirubin occurs simultaneously with a corresponding decrease in bilirubin production derived from erythropoietic sources. While data relevant to this point were not obtained in the current study, it has been shown that phenobarbital administration produces no changes in either total red cell mass or red cell lifespan in man.* A second possibility—that the apparently increased incorporation of glycine-2-^{14}C into hepatic heme and early labeled bilirubin and CO reflects drug-related

* P. D. Berk, unpublished data.

changes in precursor pool sizes, not increased heme turnover—cannot be ruled out without more extensive data than those currently available.[3] Finally, it is possible that the increased early labeling of bilirubin and CO produced by phenobarbital reflects increased turnover of a highly specific heme characterized by a very small pool size and rapid turnover rate. Under these conditions, such an increased turnover of heme might appreciably increase early labeling of bilirubin while contributing little to total bilirubin synthesis. These and other possibilities have recently been reviewed by Schmid.[4-6]

The present study should serve to emphasize the possible problems in extrapolating from measurements of isotope incorporation or specific activity to rates of synthesis in the absence of other essential data, such as measurements of precursor pool sizes. As such, it may have relevance beyond the immediate question to which it was directed.

REFERENCES

1. T. R. TEPHLY, E. HASEGAWA and J. BARON, *Metabolism* **20,** 200 (1971).
2. R. SCHMID, H. S. MARVER and L. HAMMAKER, *Biochem. biophys. Res. Commun.* **24,** 319 (1966).
3. S. A. LANDAW, E. W. CALLAHAN, JR. and R. SCHMID, *J. clin. Invest.* **49,** 914 (1970).
4. M. LEVITT, B. A. SCHACTER, A. ZIPURSKY and L. G. ISRAELS, *J. clin. Invest.* **47,** 1281 (1968).
5. H. S. MARVER, in *Microsomes and Drug Oxidations* (Eds. J. R. GILLETTE, A. H. CONNEY, G. J. COSMIDES, R. W. ESTABROOK, J. R. FOUTS and G. J. MANNERING), p. 495. Academic Press, New York (1969).
6. R. F. COBURN, *N. Engl. J. Med.* **283,** 512 (1970).
7. S. H. ROBINSON, *Proc. Soc. exp. Biol. Med.* **138,** 281 (1971).
8. P. D. BERK, T. F. BLASCHKE and F. L. RODKEY, in *The Liver, Quantitative Aspects of Structure and Function* (Eds. G. PAUMGARTNER and R. PREISIG), p. 184. Karger, Basel (1973).
9. P. BERTHELOT, S. ERLINGER, D. DHUMEAUX and A.-M. PREAUX, *Am. J. Physiol.* **219,** 809 (1970).
10. K. WEINBREN and B. H. BILLING, *Br. J. exp. Path.* **37,** 199 (1956).
11. H. T. MALLOY and K. A. EVELYN, *J. biol. Chem.* **119,** 481 (1937).
12. H. T. MALLOY and K. A. EVELYN, *J. biol. Chem.* **122,** 597 (1938).
13. T. A. WALDMANN, W. F. ROSSE and R. L. SWARM, *Ann. N. Y. Acad. Sci.* **149,** 509 (1968).
14. S. A. LANDAW and H. S. WINCHELL, *Blood* **36,** 642 (1970).
15. S. A. LANDAW, *Ann. N. Y. Acad. Sci.* **174,** 32 (1970).
16. J. D. OSTROW, L. HAMMAKER and R. SCHMID, *J. clin. Invest.* **40,** 1442 (1961).
17. A. G. GORNALL, C. S. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1949).
18. T. OMURA and R. SATO, *J. biol. Chem.* **239,** 2370 (1964).
19. A. J. LEVI, Z. GATMAITAN and I. M. ARIAS, *J. clin. Invest.* **48,** 2156 (1969).
20. T. F. BLASCHKE and P. D. BERK, *Proc. Soc. exp. Biol. Med.* **140,** 1315 (1972).
21. A. H. CONNEY, *Pharmac. Rev.* **19,** 317 (1967).
22. H. REMMER and H. J. MERKER, *Ann. N. Y. Acad. Sci.* **123,** 79 (1965).
23. I. M. ARIAS, L. M. GARTNER, M. COHEN, J. BEN EZZER and A. J. LEVI, *Am. J. Med.* **47,** 395 (1969).
24. M. BLACK and S. SHERLOCK, *Lancet* **1,** 1359 (1970).
25. D. TROLLE, *Lancet* **2,** 705 (1968).
26. R. P. H. THOMPSON, C. W. T. PILCHER, J. ROBINSON, G. M. STATHERS, A. E. M. MCLEAN and R. WILLIAMS, *Lancet* **2,** 4 (1969).
27. J. HUNTER, R. P. H. THOMPSON, M. O. RAKE and R. WILLIAMS, *Br. Med. J.* **ii,** 497 (1971).
28. M. M. THALER, *Pediat. Res.* **3,** 355 (1969).
29. A. STIEHL, M. M. THALER and W. H. ADMIRAND, *N. Engl. J. Med.* **286,** 858 (1972).
30. S. J. YAFFE, G. LEVY, T. MATSUZAWA and T. BALIAH, *N. Engl. J. Med.* **275,** 1461 (1966).
31. C. CATZ and S. J. YAFFE, *Pediat. Res.* **2,** 361 (1968).
32. A. DELEON, L. M. GARTNER and I. M. ARIAS, *J. Lab. clin. Med.* **70,** 273 (1967).
33. C. D. KLAASSEN, *J. Pharmac. exp. Ther.* **168,** 218 (1969).
34. R. J. ROBERTS and G. L. PLAA, *Biochem. Pharmac.* **16,** 827 (1967).
35. H. REYES, A. J. LEVI, Z. GATMAITAN and I. M. ARIAS, *J. clin. Invest.* **50,** 2242 (1971).
36. S. GRANICK, *J. biol. Chem.* **241,** 1359 (1966).
37. J. BARON and T. R. TEPHLY, *Biochem. biophys. Res. Commun.* **36,** 526 (1969).
38. H. S. MARVER, R. SCHMID and H. SCHÜTZEL, *Biochem. biophys. Res. Commun.* **33,** 969 (1968).
39. E. HASEGAWA, C. SMITH and T. R. TEPHLY, *Biochem. biophys. Res. Commun.* **40,** 517 (1970).

40. H. GREIM, J. B. SCHENKMAN, M. KLOTZBÜCHER and H. REMMER, *Biochim. biophys. Acta* **201,** 20 (1970).
41. H. GREIM, *Naunyn-Schmiedebergs Arch. exp. Path Pharmak.* **266,** 261 (1970).
42. L. SHUSTER and H. JICK, *J. biol. Chem.* **241,** 5361 (1966).
43. R. TENHUNEN, H. S. MARVER and R. SCHMID, *Proc. natn. Acad. Sci. U.S.A.* **61,** 748 (1968).
44. P. D. BERK, F. L. RODKEY, T. F. BLASCHKE, H. A. COLLISON and J. G. WAGGONER, *J. Lab. clin. Med.*, **83,** 29 (1974).
45. P. D. BERK, R. B. HOWE, J. R. BLOOMER and N. I. BERLIN, *J. clin. Invest.* **48,** 2176 (1969).
46. R. SCHMID, *Drug Metab. Dispos.* **1,** 256 (1973).