Production and Excretion of Bilirubin in Gunn Rats Treated with Phenobarbital[1] (35879)

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Phenobarbital has been shown to decrease levels of serum bilirubin in some patients with jaundice (1–3). The mechanism is doubtless complex, although induction of the activity of the conjugating enzyme, bilirubin UDP-glucuronyl transferase, probably plays a major role (1, 3, 4). The observation that phenobarbital appears to be ineffective in both humans (3) and rats (5) with complete deficiency of this enzyme supports this explanation. However, this drug has an additional effect on bile pigment metabolism, and has been reported to induce striking increases in bilirubin production from nonerythroid sources (6). It therefore seemed possible that the failure to alter serum bilirubin levels in enzyme-deficient subjects (3, 5) might be due to offsetting increases in both the formation and excretion of bile pigment. Experiments were therefore performed to evaluate the effect of phenobarbital on the rate of bilirubin turnover and the production of early-labeled bilirubin in homozygous Gunn rats, mutant Wistar rats with complete inability to conjugate bilirubin (5, 7, 8).

Materials and Methods. Studies were performed in male rats weighing 350–450 g. Experimental animals received subcutaneous injections of phenobarbital,[3] 40–100 mg/kg/day 6 days of each week for 4–6 weeks. Controls received comparable injections of 2% benzyl alcohol in propylene glycol, the vehicle in the drug solution. Bilirubin turnover was measured by the method of Schmid and Hammaker (9); measurements were performed twice in each rat, before treatment with phenobarbital or control solution and then during the last 6 days of the treatment period. In each study the rat received an intravenous injection of 0.2–0.3 μCi (20-30 μg) of bilirubin-^{14}C,[4] and the specific activity of serum bilirubin was measured at frequent intervals over the next 6 days (9). The half-time of labeled bilirubin disappearance from plasma, the total miscible bilirubin pool, and the rate of bilirubin turnover were calculated from the decrease in specific activity and the dose of labeled pigment injected (9, 12). Serum bilirubin concentration was measured by the method of Malloy and Evelyn (13).

Production of early-labeled bilirubin was measured in one control and two drug-treated rats 1 month after completion of the second turnover study; these animals were maintained on their original treatment schedules during this interval which permitted hematocrits and reticulocyte counts to return to normal levels. Details of the methods and their experimental verification have been described previously (14). Briefly, 400 μCi of glycine-2-^{14}C[5] were injected intravenously and blood samples were removed by tail puncture at 1, 3, 7, 24, 32, and 72 hr for crystallization and radioassay of serum unconjugated bilirubin. Rates of labeled bilirubin formation (dpm in bilirubin-^{14}C produced per hour) were computed from the changes in

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[3] Sodium phenobarbital injection, William S. Merrell Co., Cincinnati, Ohio.

[4] Prepared in rats by the method of Ostrow *et al.* (10), as modified by Barrett *et al.* (11).

[5] Glycine-2-^{14}C, 18 mCi/mmole, New England Nuclear Corp., Boston, Mass.

specific activity of serum bilirubin on the basis of the rate of endogenous pigment turnover, measured 1 month earlier with bilirubin-^{14}C (14) This method expresses results as a step-function, yielding a constant rate of labeled bilirubin production for each consecutive interval between measurements of bilirubin specific activity. The computed rates were plotted at the midpoints of the corresponding measurement intervals, and smooth curves were drawn through these values.

Results. Serum bilirubin concentration and bilirubin turnover (*Table I*). There was no significant change in serum bilirubin concentration or in any of the indices of bilirubin turnover after treatment with phenobarbital. Moderate changes were observed in a few animals, but these showed no consistent trend and occurred in both control and drug-treated animals. Serum bilirubin levels were stable throughout each of the turnover studies.

Early-labeled bilirubin production (*Fig. 1*). The curve for the control animal was comparable to those observed previously in 4 normal Gunn rats (14). In both of the phenobarbital-treated animals there was a small rise in the initial sharp component of early-labeled bilirubin formation; in one of these rats, however, the increase was of questionable significance. After 10 hr, both curves fell to within the normal range. Further measurements were not performed in view of the conformity of these findings to the lack of change in total bilirubin turnover in 8 additional drug-treated rats (Table I).

Discussion. These experiments confirm the observation (5) that phenobarbital fails to alter serum bilirubin levels in animals totally unable to conjugate bilirubin. More important, they demonstrate that this apparent lack of change does not obscure a significant increase in bile pigment excretion, counterbalanced by a rise in early bilirubin production. This would have been reflected by an elevated rate of turnover and a shortened $T_{1/2}$ (or conceivably an increase in pool size) in the studies with bilirubin-^{14}C (Table I).

These findings are significant in two respects. The homozygous Gunn rat and its

TABLE I. Serum Bilirubin Concentration and Labeled Bilirubin Metabolism in Homozygous Gunn Rats Before and After Treatment with Phenobarbital.[a]

Treatment	No.	Serum bilirubin conc (mg/100 ml)		Labeled bilirubin metabolism					
				$T_{1/2}$ (hr)		Miscible pool (mg/100 g)		Turnover (mg/24 hr/100 g)	
		Before	After	Before	After	Before	After	Before	After
Control	8	6.7 ± 0.2	6.6 ± 0.4	29.1 ± 1.1	26.6 ± 1.8	1.03 ± 0.05	0.98 ± 0.07	0.59 ± 0.03	0.61 ± 0.03
				$p > .2$					
Phenobarbital[b]	10	7.0 ± 0.3	6.0 ± 0.4	28.3 ± 1.4	25.8 ± 1.4	1.10 ± 0.05	1.01 ± 0.08	0.66 ± 0.04	0.65 ± 0.06
		$p > .2$		$p > .2$		$p > .4$			

[a] Mean values ± SE (23) are shown. There were no significant differences between values before and after either control or drug treatment; p values derived from Student's t test (23) are shown where questionable differences were found.

[b] Forty to 100 mg/kg/day for 4–6 weeks. Individual dosage rates are not specified since no dose-related changes were found.

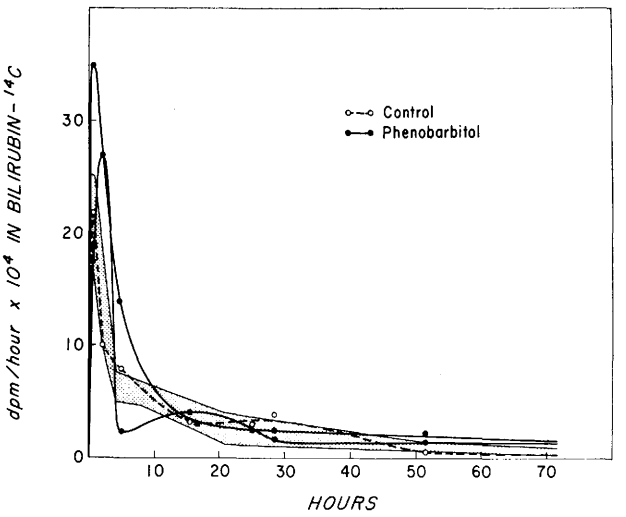

FIG. 1. Formation of early-labeled bilirubin from glycine-2-^{14}C in one control and 2 drug-treated homozygous Gunn rats. The highest curve is from a rat which received 40 mg of phenobarbital/kg/day; the next highest, from an animal given 60 mg/kg/day. Stripped area shows the mean ± SD for four untreated Gunn rats from a previous study (14). Animals received 400 μCi of glycine-^{14}C, but curves have been adjusted to correspond to a dose of 1 mCi for ease of comparison with other studies.

human counterpart eliminate bile pigment by diffusion of unconjugated bilirubin into the bile and across the bowel mucosa and, more important, by degradation of bilirubin to polar catabolites not requiring conjugation for excretion (9). The phenobarbital effect might be mediated in part through stimulation of these "alternate pathways" (15, 16). However, the failure to demonstrate any facilitation of pigment excretion in these animals (Table I) virtually eliminates this possibility.

The second point concerns the lack of a substantial effect on bile pigment production in the Gunn rats. Schmid *et al.* (6) have reported that phenobarbital induces a 5-fold increase in the early bilirubin fraction, with a consequent doubling of total pigment production, in normal rats. By contrast, drug treatment led to only minor changes in the early-labeled peak in two Gunn rats (Fig. 1), and correspondingly failed to alter total pigment turnover in a total of 10 animals (Table I). Similarly, little enhancement of the early bilirubin fraction was found in a human subject with congenital impairment of pigment conjugation (17). This discrepancy may well be due to variations in experimental conditions, since two groups of investigators have recently reported only mild–moderate changes in early bilirubin production in normal animals (18, 19). However, other possibilities merit brief consideration. The initial component of early bilirubin is derived from extra-erythroid sources, primarily in the liver (14, 18–22). It is possible that animals lacking the microsomal enzyme, bilirubin UDP-glucuronyl transferase, might also be partly deficient in the metabolic sources of the hepatic pigment fraction. Alternatively, more labeled bilirubin than was evident in the plasma might have been formed in the liver of the Gunn rats, but might have been degraded there and the products excreted directly into the bile. Final clarification of this problem awaits further studies of the early bilirubin fraction in both normal and enzyme-deficient subjects.

Summary. Phenobarbital causes no significant change in either serum bilirubin concentration or the rate of bilirubin turnover in homozygous Gunn rats. There is only minimal enhancement of bilirubin production from nonhemoglobin sources in the liver, in contrast to the large changes reported for normal animals. These studies indicate that phenobarbital does not stimulate the alter-

nate pathways by which animals unable to conjugate bilirubin excrete bile pigment. Moreover, they raise intriguing questions concerning the production and excretion of the hepatic bilirubin fraction in the Gunn rat.

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