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Bile Acid Secretion and Pool Size during Phenobarbital Induced Hypercholeresis (42655)

HIROAKI OKUDA, DARIO SORRENTINO, GIANFRANCO ALPINI, NICOLA TAVOLONI,[1] MARY JANE T. JONES, CHI-LI KIANG, AND PAUL D. BERK

The Polly Annenberg Levee Hematology Center and the Hepatic Research Group (Department of Medicine), Mount Sinai School of Medicine, New York, New York 10029

Abstract. An increase in bile flow after phenobarbital administration occurs in the rat and other species; however, the mechanism(s) of the choleretic effect is incompletely understood and the role of the increase in liver weight is controversial. We therefore measured bile flow, bile acid secretion and pool size in male Sprague–Dawley rats pretreated with phenobarbital (75 mg/kg/day) for 6 days; liver weight, liver cell volume and DNA content were also evaluated. Phenobarbital treatment increased liver weight and mean hepatocyte volume by 39 and 26%, respectively, while total DNA content did not change, thus indicating that the hepatomegaly results principally from hypertrophy rather than hyperplasia. Bile flow was significantly higher in treated rats when expressed per unit of body weight (64.6 ± 2.4 (S.E.) vs 53.3 ± 1.6 μl/min/kg; $P < 0.05$) but was unchanged when expressed per gram of liver (1.40 ± 0.04 vs 1.37 ± 0.06 μl/min/g; $P > 0.5$). The initial bile acid secretion rate and pool size were both significantly reduced in the phenobarbital group compared to controls (1224.2 ± 110.4 vs 1656.6 ± 163.2 nmol/kg/min and 562.8 ± 41.5 vs 814.3 ± 78.3 μmol/kg; both $P < 0.05$), whereas the basal synthetic rate was unchanged. These findings suggest that the enlarged, phenobarbital-treated hepatocyte produces more bile than the normal cell, despite the decreased secretion of bile acids. Therefore, the drug-induced choleresis involves a selective increase in the bile acid-independent fraction of bile flow.

More than 25 years have passed since Conney *et al.* (1) first described the induction of various microsomal enzymes by phenobarbital (PB). Since then we and others have reported a number of other effects of this drug on liver structure and function, including increased liver weight and hepatic blood flow (2, 3), enhanced uptake of endogenous and exogenous compounds (4, 5), increased tumorigenesis by other chemicals (6), changes in bilirubin metabolism, and increased bile flow (7–9). Despite the considerable number of papers dealing with the subject, the mechanisms by which PB affects hepatobiliary function are still incompletely understood. Although some of its effects are intimately interrelated, others appear completely independent. A most interesting event associated with the administration of the drug is the hypercholeresis, in which several factors, alone or in combination, appear to play a role. An increased bile acid-independent fraction of bile flow (10), increased liver weight (5), and changes in biliary lipid composition and metabolism (11) are most often claimed to be responsible. Although there is general agreement on the role of bile acid-independent flow in the drug-induced choleresis, involvements of bile acid dependent flow and increased liver weight are much more debated. The aim of the present study therefore was to establish whether PB affects bile acid secretion and pool size, and whether the choleresis is dependent upon the increase in liver mass.

Materials and Methods. *Animals and operative procedures.* Male Sprague–Dawley rats (300–400 g, Charles River Breeding Laboratories, Wilmington, MA) with free access to water and standard rat chow were used throughout. The animals were randomly assigned to receive intraperitoneal injections of either phenobarbital sodium (Abbott Laboratories, North Chicago, IL) at 75 mg/kg/day for 6 days or normal saline. On the seventh day, 20 hr after the last injection, polyethylene catheters (Clay Adams, Parsippany, NJ) were inserted into the jugular vein (PE-50)

[1] To whom reprint requests should be addressed at the Division of Hematology, Box 1079, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029.

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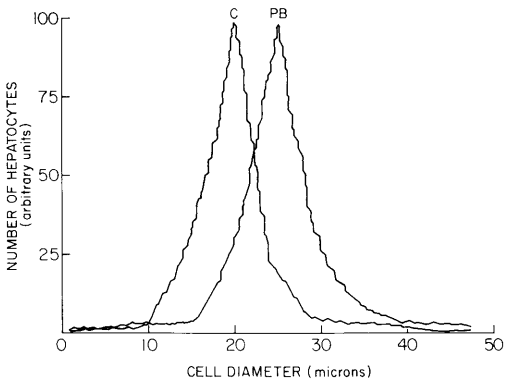

FIG. 1. Cell diameter distribution in representative populations of hepatocytes isolated from control (C) and PB-treated rats. Note that treatment with PB results in increasing average cell diameter by approximately 8%.

and common bile duct (PE-10) under pentobarbital sodium (Abbott Laboratories, North Chicago, IL) anesthesia (50 mg/kg). Postoperatively, Krebs–Henseleit bicarbonate solution was infused intravenously at a rate of 3–4 ml/hr via the jugular catheter in order to compensate for losses of water and electrolytes. The animals were allowed to recover from anesthesia and placed in modified Bollman restraining cages.

Bile collection. Bile was collected for up to 27 hr after cannulation of the bile duct in multiple aliquots in preweighed tubes kept on ice. Bile flow was assessed gravimetrically, assuming a specific weight of 1 g/ml. To avoid interfering effects of starvation and diurnal changes in bile acid metabolism and synthesis, all experiments were conducted in nonfasting animals and started at the same time of day (approximately 2:00 PM). Total bile acids in bile were measured by the hydroxysteroid procedure as previously reported (12). Basal bile acid synthetic rate and pool size were calculated according to Myant and Eder (13) as previously described (14).

Determination of liver weight, DNA content, and cell size. At the end of the bile collection period, the animals were sacrificed and the livers were removed and weighed. Cellular DNA content was determined by the diphenylamine assay (15) as modified by Giles and Myers (16). To assess the effects of PB on cell size, hepatocytes were isolated from treated and control animals by the method of Berry and Friend (17). After being tested for viability (which exceeded 90% by trypan blue exclusion), the hepatocytes were counted and sized in a Coulter ZM cell counter and chanalyzer (Coulter Electronics, Hialeah, FL). From the measured diameter, the cell volume was calculated, assuming that the hepatocyte is spherical. Statistically significant differences were established by Student's t test.

Results. *Liver weight, cell volume, and DNA content.* Figure 1 illustrates the distribution of average cell diameter in representative samples of control and PB-treated hepatocytes. PB treatment increased the mean cell diameter from 22.5 ± 0.2 [SE] to 24.3 ± 0.5 μm ($P < 0.001$). Body weight was the same in the two groups whereas liver weight and the liver weight/body weight ratio were significantly increased in treated animals, the latter by 39% (Table I). Mean hepatocyte cell volume, calculated from the measured hepatocyte diameter distribution, was increased by 26%, while hepatic DNA content was unchanged. These data suggest that phenobarbital-induced hepatomegaly is due principally to hypertrophic changes rather than hyperplasia.

TABLE I. BODY AND LIVER WEIGHT, DNA CONTENT, AND CELL SIZE IN CONTROL AND PHENOBARBITAL-TREATED RATS

	Body weight (g)	Liver weight (g/100 g body weight)	DNA (mg hepatic DNA/kg body weight)	Mean hepatocyte cell volume (μm^3)
Phenobarbital	343.3 ± 24.3	5.3 ± 0.2	164.2 ± 11.2	7555.8 ± 211.3
Control	349.8 ± 18.4	3.8 ± 0.1	153.6 ± 10.2	5994.8 ± 69.3
P	>0.5	<0.001	>0.1	<0.001

Note. Data are means ± SE of four to five experiments each.

TABLE II. EFFECT OF PHENOBARBITAL TREATMENT ON BILE FLOW AND BILE ACID CONCENTRATION IN BILE

Time (hr)	Bile flow				Bile acid concentration (m*M*)	
	Controls	PB	Controls	PB		
	(μL/min/kg)		(μL/min/g liver)		Controls	PB
0–1	66.31 ± 4.40	80.36 ± 5.66*	1.54 ± 0.09	1.51 ± 0.11	46.98 ± 2.85	31.35 ± 0.98*
1–2	77.81 ± 3.56	83.48 ± 5.65	1.81 ± 0.13	1.78 ± 0.09	49.85 ± 4.55	33.90 ± 2.87*
2–3	85.52 ± 3.05	83.84 ± 5.16	1.98 ± 0.10	1.58 ± 0.11*	48.85 ± 3.99	41.88 ± 4.91*
3–4	68.68 ± 2.19	79.15 ± 3.94*	1.60 ± 0.06	1.49 ± 0.09	28.77 ± 1.94	20.50 ± 2.64*
4–5	56.83 ± 2.78	73.95 ± 2.97*	1.32 ± 0.08	1.39 ± 0.06	16.85 ± 1.91	11.11 ± 1.33*
5–6	50.70 ± 1.44	61.30 ± 2.81*	1.18 ± 0.05	1.15 ± 0.06	9.40 ± 1.31	6.03 ± 0.73*
6–11	46.52 ± 1.13	58.88 ± 2.13*	1.22 ± 0.04	1.26 ± 0.05	3.75 ± 0.75	4.05 ± 0.61
11–27	46.06 ± 1.66	57.36 ± 2.31*	1.20 ± 0.04	1.21 ± 0.04	4.86 ± 0.29	5.12 ± 0.37
0–27	53.33 ± 1.56	64.62 ± 2.44*	1.40 ± 0.04	1.37 ± 0.04		

Note. Values are means ± SE (n = 24 for both controls and phenobarbital-treated rats).
* $P < 0.05$–0.001.

Bile flow. As expected, the bile flow rate was significantly increased in the PB group during the 27-hr study when expressed per kilogram of body weight (Table II). The difference, however, is no longer present when the bile flow is expressed per gram of liver weight.

Bile acid secretion and pool size. Figure 2 illustrates the bile acid secretion rate vs. time in control and PB-treated rats: after an early peak following cannulation of the bile duct, secretion decreases with time until a low point is reached; thereafter the secretion remains fairly constant. The last portion of the curve represents basal bile acid synthesis, which was unchanged by PB treatment (220.1 ± 25.2 vs 230.6 ± 26.1 nmol/min/kg body weight, $P > 0.5$). The area under the initial portion of the curve (0–6 hr) minus the basal secretion represents the bile acid pool size, which is decreased in the PB group (562.8 ± 41.5 vs 814.3 ± 78.3 μmol/kg body weight, $P < 0.05$). Biliary bile acid concentration was significantly decreased by PB treatment at all time points during the study (Table II).

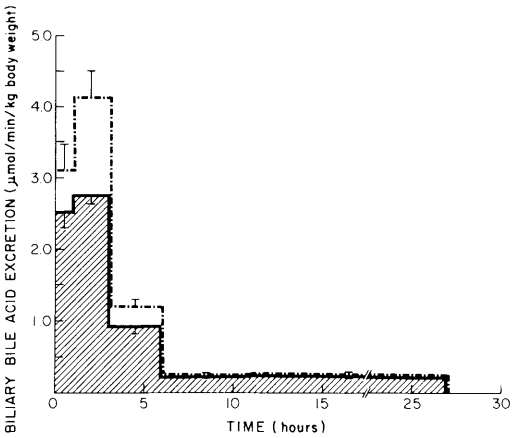

FIG. 2. Biliary bile acid excretion in PB-treated (continuous line) and control rats (dotted line). Values represent means ± SE (bars). The area under the initial portion of the curve (0–6 h), which estimates the bile acid pool size, is decreased by PB treatment.

Discussion. PB increases bile flow in the rat (4, 5, 18, 19), monkey (11), and human (20) but not in the hamster (21). As much as 30% of administered PB (and/or its metabolites) is excreted in bile (22, 23); however, the choleresis cannot be explained by an osmotic effect of the drug itself (24). Microsomal induction, increased liver weight, and changes in the bile acid-independent and -dependent fractions of bile flow are the mechanisms advocated to explain the choleresis. The increase in bile flow does not correlate with microsomal enzyme induction: it occurs earlier than the rise in P_{450} and blocking the latter does not affect choleresis (25, 26). Also, among several microsomal enzyme inducers in the rat, only a few increase bile flow (27).

PB causes a clear increase in liver weight in the rat (1, 2, 28–31), in the hamster (21), and in the human (32). Although some authors

have reported enhanced DNA content and number of nuclei (29, 30), our research and other studies (31, 33) suggest that the hepatomegaly is largely due to hypertrophic rather than hyperplastic changes. In fact, we found that hepatic DNA content is unchanged by PB treatment, while cell size is increased (Table I). The mean cell volume in control hepatocytes in the present study, determined by electronic particle-sizing technology, agrees closely with values derived from more conventional morphometric techniques (34).

The increased liver weight was the mechanism first proposed to explain the choleretic effect of PB (5), based on the fact that bile flow, expressed per unit of liver weight (rather than body weight), often did not change. In keeping with this observation, we found (Table II) that each gram of liver in the PB group produces the same amount of bile as the control group. However, bile flow and liver weight do not increase at the same rate following initiation of PB administration (26, 27, 35–37). Moreover, they do not always increase to the same extent; thus, even when expressed per unit of liver weight, an increase in bile flow has been observed (27, 31, 38). Finally, other microsomal enzyme inducers (3-methylcholanthrene and 3,4-benzopyrene) increase liver weight but not bile flow (9, 27) and in the hamster, PB increases liver weight but not bile flow (21). Thus, even though our own data reveal equivalent increases in bile flow and liver weight, it is reasonable to conclude that these increases are not related.

Since PB decreases the bile acid pool size and, as a result, the initial output of endogenous bile acids, the hypercholeresis could be explained either by an increased osmotic activity of bile acids (39, 40) or by a stimulatory effect on bile acid-indepedent flow. The first explanation seems unlikely, for if bile flow is plotted against bile acid excretion, the slope of the regression line (which estimates the osmotic effect of excreted bile acids) is not changed by PB treatment (8.5 ± 1.6 vs 8.2 ± 1.2 μl of water excreted for each μmole of bile acid, $P > 0.5$), a finding consistent with previous reports (10, 24). Thus, it appears that PB-induced choleresis is due to an increase in bile acid independent flow, and that the flow of bile associated with bile acid excretion may actually be decreased by the drug, due to the depressed secretion of endogenous bile acids, which may in turn be secondary to the diminished bile acid pool size.

The effect of PB on bile acid pool size is interesting and deserves further comment. The bile acid pool is the amount of bile acids present at any time in the enterohepatic circulation and its size is essentially a function of hepatic synthesis and intestinal and renal loss and reabsorption. Our data clearly suggest that the synthetic rate is unchanged by PB and therefore the decrease in pool size appears to be due to increased loss. In this respect, a significant role could be played by a change in the enzymatic activities responsible for sulfation and glucuronidation of bile acids. Sulfate and glucuronide derivatives of bile acids, in fact, tend to be less effectively absorbed from the intestine and kidney than other bile acids (41, 42). While PB treatment does not appear to affect sulfate conjugation (43), it does induce liver microsomal bile salt glucuronyltransferase (44) and, as a result, increases bile acid glucuronides in serum, bile, and urine (45). Thus, the reduced pool size observed in our studies could be explained in terms of an increased urinary loss of bile acid glucuronides.

However, two studies have reported an increased synthesis (14) and intestinal reabsorption (46) of bile acids in PB-treated rats with consequent increase in pool size. Since these previous studies were conducted in female rats, whereas we used males, one explanation for the disparate results is that PB effect on the bile acid pool is sex dependent. This is not inconceivable since several PB effects appear to depend upon the dose, species, and sex. For example, in Wistar but not Sprague–Dawley rats, the enzyme 7α-hydroxylase is increased by the drug (47), and in the rat, man, and monkey, but not in the hamster, PB increases bile flow (21). Sex differences have also been reported in the effects of PB on hepatic phase I drug metabolism in the rat (48). Hence, in view of these observations and the finding reported by Klaassen (24) that the initial biliary excretion of endogenous bile acids is decreased in PB-treated male rats, as in our studies, it is reasonable to conclude that the drug effect on

bile acid pool size is sex dependent. In the female rat, it has a stimulatory effect while in the male, it produces inhibition.

As to the mechanism of the choleretic effect of PB, our results add little to present information. The increase in bile acid-independent bile flow could be secondary to a change in Na^+K^+-ATPase activity (36, 49), possibly mediated by *de novo* synthesis of the enzyme (49, 50). Alternatively, the effect could be brought about by a change in the membrane lipid microenvironment to which the enzyme is known to be sensitive (51). Although one study did not show a change in fluidity (52), other reports suggest that PB affects lipid composition and membrane microviscosity (29, 53, 54). The issue of whether an increase in Na^+K^+ATPase activity is involved in PB-induced choleresis is, however, complicated by the fact that some authors have not found a change in the specific and total activity of the enzyme (52, 55–57). More importantly, the role of this enzyme in bile formation is presently unclear.

In conclusion, our study shows that PB administered for 6 days to male rats causes hypercholeresis, hypertrophic changes which are responsible for the increased liver weight, and decreases in bile acid pool size and secretion. Our results support the concept that PB-associated choleresis is due to an effect of the drug on the bile acid-independent fraction of bile flow.

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