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Cholestasis liver
cytochrome C phenobarbital
kidney rose bengal

Effect of Phenobarbital on Hepatic Transport and Excretion of ^{131}I-Rose Bengal in Children with Cholestasis

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Extract

The effects of phenobarbital (PB) on plasma clearance, hepatic uptake and storage, and excretion in stool and urine of ^{131}I-rose bengal (RB) were studied in seven subjects, six with a variety of hepatobiliary disorders characterized by cholestasis, and one with normal hepatic functions. In three children with patent extrahepatic biliary passages but different degrees and types of cholestasis, plasma $t_{1/2}$ for RB was shortened from 61.3 to 44.5 hr, from 44.5 to 24.8 hr, and from 155.5 to 96.9 hr before and during PB administration (10 mg/kg/day), respectively. The corresponding quantitative 72-hr fecal excretion of RB increased from 3.2% to 9.2%, from 9.9% to 71.0%, and from 0.3% to 10.0% of the injected dose; the normal subject excreted 78.6% before and 94.9% during PB treatment. Plasma $t_{1/2}$ and fecal excretion of RB were unchanged in three infants with absence of extrahepatic bile ducts who were treated with PB. Hepatic uptake and storage capacity for RB and renal excretion of RB were not affected by PB.

The serum bilirubin concentration (total and direct-reacting) was lowered in patients with cholestatic jaundice not caused by biliary atresia. Interruption of drug therapy was followed by increase in the serum bilirubin to pretreatment concentrations in these patients. Phenobarbital treatment increased reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome C reductase activity 30–150% in four subjects, whereas activity remained unchanged in three others. The effect of PB on microsomal reductase activity was unrelated to its ability to stimulate hepatic excretion.

Phenobarbital in therapeutic dosages enhances biliary excretion of RB and conjugated bilirubin by stimulating hepatic excretory function in patients with intact extrahepatic bile ducts. Hepatic uptake, storage capacity, and NADPH-cytochrome C reductase induction do not seem to be directly involved in the acceleration of hepatic transport.

Speculation

Knowledge of the kinetics of ^{131}I-RB distribution in human plasma, tissue, and excreta can serve as a basis for further studies of normal and altered hepatic excretory functions in man. Phenobarbital-stimulated biliary excretion of anions such as RB and conjugated bilirubin reflects patency of extrahepatic bile ducts. For this reason, PB in conjunction with ^{131}I-RB may prove to be useful agents in the diagnosis and management of various cholestatic conditions.

Introduction

Phenobarbital (PB) stimulates bile flow [4, 7, 19, 20] and induces the microsomal enzymes which catalyze the transformation of compounds destined for biliary excretion [15, 18]. Although PB increases the conjugation of bilirubin in rats [2, 22], the excretion of conjugated bilirubin [21] and other organic anions, including those which are not transformed by the liver, is also enhanced [15, 16]. The relations among hepatic transport, biliary excretion, and microsomal enzyme induction have not been investigated in humans receiving PB.

We have studied the circulatory, hepatic, fecal, and urinary disposition of a cholephilic anion, ^{131}I-rose bengal (RB), in patients with various hepatobiliary disorders who were treated with PB. Subjects investigated were infants and young children not exposed to drugs other than occasional antibiotics prior to this study. Because RB is removed from the circulation largely by the liver and is excreted into bile without chemical modification [1, 8], this dye, isotopically labeled, is suitable for studies of biliary excretory efficiency independent of drug-inducible enzymatic transformations.

Methods

The study included seven subjects admitted to the Pediatric Clinical Research Center at the University of California, San Francisco, for investigation of hepatic status. Informed consent was obtained from the parents of all subjects in this study. Six patients were jaundiced at the time of hospitalization: four had complete biliary obstruction and two had lesser degrees of biliary deficiency. One anicteric infant had recovered from neonatal hepatitis and had normal liver function and cellular morphology at the time of this study.

Clinical Studies and Sample Collections

Every patient received routine diagnostic liver function tests and percutaneous liver biopsy at the beginning and termination of the study. Clinical and laboratory data are summarized in Table I. Lugol's solution (5 drops) was administered daily throughout the study period to block thyroid uptake of radioactive iodine. For collection of urine, subjects on a metabolic bed passed urine into a plastic bag securely affixed to the perineum. Once attached, the bag remained in place during the entire collection period. Its contents were aspirated frequently through catheters incorporated into the bag. Stool specimens were recovered after direct evacuation into preservative solution in preweighed plastic containers fitted to the mattress well. The exact time and date of excretion were noted on each container, and individual stools were processed separately.

Analysis of Samples

The contents of the sealed plastic vessels, each containing a single stool specimen in preservative solution of known weight and volume, were thoroughly homogenized in a Waring Blendor, and the volumes of each fecal specimen and each 12-hr pooled urine sample were measured. Aliquots of serum, made up to 2-ml volume, and 2-ml samples of fecal suspension or urine were analyzed for radioactivity in a Nuclear-Chicago Autogamma well counter. Appropriate standards, containing dilutions of the RB solution administered to each subject, were included with each run and samples were counted in duplicate to achieve 5% reproducibility.

Isotope Studies

The use of ^{131}I-RB as tracer for RB requires stability of the labeled compound during its study *in vivo* and resistance to transformation by processes inducible by PB. These properties were tested in two of the subjects with biliary atresia. One subject was given an injection of tracer at the beginning of each of the 2 consecutive weeks, the 1st week being a control period, and PB was administered during the 2nd week. The other subject was studied for 1 week only, with PB administered during the last 4 days. Serum samples taken 5 min, 4 hr, and daily for 7 days after the injection of ^{131}I-RB were used to determine the retention of isotope in stable configuration. Part of each sample was used to precipitate serum proteins with trichloroacetic acid. Whole serum, protein precipitates, and supernatant fractions were counted for radioactivity; the percentage of radioactivity present as free ^{131}I was calculated from the supernatant fraction.

Patients were positioned under a Nuclear-Chicago Pho/Gamma 3 scintillation camera with a divided field image which measured emissions simultaneously over the liver and lower abdominal area and provided a permanent scintigraphic record. After a 5-min background count, ^{131}I-Na RB solution [23] (3 μCi/kg body weight) was injected into the right antecubital vein. Counts were recorded continuously during the first 60 min after injection. Simultaneous scintiphotographs, obtained at 5-min intervals, were used to monitor positioning under the camera. Thereafter, scanning and counting were confined to 10-min periods at 4, 24, 48, and 72 hr after administration of the label.

Table I. Clinical and laboratory features of study subjects

Patient	Sex	Age, mo	Diagnosis	History of jaundice	Operative findings	Liver biopsy	Phenobarbital treatment	Average serum bilirubin, mg/100 ml[1]	Alkaline phosphatase, IU[2]/liter	Serum glutamic oxaloacetic transaminase, IU/liter	Lactic acid dehydrogenase, IU/liter	Serum cholesterol, mg/100 ml	Serum proteins, g/100 ml	Serum albumin, g/100 ml	Prothrombin time, sec
DC	M	24	Normal infant	1st 2 mo		Normal parenchyma	No	0.5/0.3	145	41	240	129	6.3	3.6	11.0
							Yes	0.3/0.2	140	40	265	136	6.1	3.3	11.0
DLC	F	24	Paucity of intrahepatic bile ducts	Continuous from 3 wk	Patent extrahepatic and major intrahepatic bile ducts	Few lobular bile ducts; cholestasis	No	21.5/15.7	1000	583	400	603	8.4	4.2	13.4
							Yes	9.5/9.0	1000	405		436	7.8	3.6	12.2
DQ	F	60	Idiopathic cholestasis	Intermittent severe episodes from 2 yr	Patent biliary system	Normal parenchyma; centrilobular cholestasis	No	14.8/10.7	247	45	267	223	6.9	3.0	11.0
							Yes	5.4/4.5	176	41	202	243	6.8	3.2	11.0
RB	M	1.5	Extrahepatic biliary obstruction	Continuous from 3 wk	Atretic extrahepatic bile ducts	Bile duct proliferation; cholestasis	No	8.8/6.4	313	162	599	247	6.4	3.7	12.0
							Yes	8.9/6.3	350	180	604	293	6.3	3.3	11.0
KC	F	2	Neonatal hepatitis. Extrahepatic biliary obstruction	Continuous from 3 wk	Sclerosis of biliary system	Giant cells; peribiliary inflammation and scarring	No	14.3/11.8	260	140	470	220	6.6	3.0	11.0
							Yes	13.7/12.1	230	160	480	180	6.8	3.1	
ER	F	4	Extrahepatic biliary obstruction	Continuous from 2 wk	Choledochal cyst	Periportal cirrhosis	No	11.0/6.3	925	194	624	272	6.3	3.0	12.0
							Yes	12.3/8.1	1300	235	620	265	6.1	3.0	
RZ	M	9	Extrahepatic biliary obstruction	Continuous from birth	Atretic extrahepatic bile ducts	Periportal cirrhosis	No	9.0/6.3	973	211	405	233	6.7	2.5	12.0
							Yes	9.3/6.4	1100	240	395	200	6.3	2.8	11.5
								Normal values for infants							
								0.3–1.3/ 0–0.3	80–150	5–40	80–330	110–300	6.5–8.4	3.5–5.0	9.0–12.0

[1] Total/direct.
[2] IU: international units.

Blood samples were obtained from the left antecubital vein 5 min after injection, and at 4, 24, 48, and 72 hr. Each subject was then treated with PB and the entire protocol was repeated with an identical dose of ^{131}I-RB so that patients served as their own controls.

Phenobarbital Therapy

Treatment was started when counts from the first RB injection were no longer detectable in excreta, and radioactive emissions over the liver and abdomen had returned to background levels, usually after 7–10 days in patients with complete biliary obstruction. Phenobarbital was administered intramuscularly in two divided doses, totalling 10 mg/kg/day for 8 days. This dose was found to be the most effective in controlling pruritus and reducing hyperbilirubinemia. Sedation did not interfere with normal food intake. The second injection of radioisotope was given on the 5th day of drug therapy, and the study period was completed on the 8th day.

Assay of NADPH-Cytochrome C Reductase Activity

Aspiration liver biopsies were performed with a size 18 Menghini needle, which usually provided approximately 20 mg liver tissue. A portion of this was immersed in Zenker's or Bouin's solution for light microscopy or fixed in 1% glutaraldehyde for electron microscopy, the remaining 5–10 mg were homogenized in 0.25 M sucrose, 1:100 w/v, and the homogenate was assayed directly for NADPH-cytochrome C reductase activity. Larger amounts of tissue from surgical biopsies were available for assay in three patients. The assay contained 0.05 M phosphate buffer (pH 7.5), 0.1 mM NADPH, 0.05 mM cytochrome C, and 0.33 mM potassium cyanide to a final volume of 1 ml. The reduction of cytochrome C was followed spectrophotometrically at 550 nm for 3 min. Rates were linear in all cases and samples were analyzed in triplicate. The extinction coefficient of 18.5 mM^{-1} cm^{-1} for reduced minus oxidized cytochrome C was used [13]. Results were expressed in terms of nanomoles cytochrome C reduced per milligram protein per minute. Protein was measured by the method of Lowry *et al.* [11].

Results

Stability of ^{131}I-Rose Bengal

Radioactivity was retained in readily measureable concentrations for at least 1 week in two infants with biliary atresia whose serum was used to determine the retention of isotope in stable configuration. The deproteinized serum supernatant fluids contained only 5% of the radioactivity of the corresponding protein precipitates 5 min after injection of ^{131}I-RB. Supernatant fluids from serum obtained at later intervals yielded background counts only, and indicated absence of significant breakdown of dye or release of labeled iodine before and during PB therapy. Total radioactivity in each supernatant fluid and precipitate pair was always within 5% of the radioactivity in the corresponding whole serum sample.

The radioactivity excreted in the stools by both infants over each 7-day period accounted for 5% of the injected dose, reflecting the absence of normal extrahepatic excretory pathways. Phenobarbital had no effect on the level of radioactivity delivered to the intestine. Radioactivity excreted in urine registered an approximate first order decline, the slope of which was unaltered by PB therapy. This indicated that the drug neither released free ^{131}I for clearance by the kidneys nor stimulated the excretion of ^{131}I-RB in the urine (Fig. 1). External monitors showed rapid concentration of radioactivity in the liver after injection of ^{131}I-RB. Radioactive dye persisted in the liver beyond 7 days before and during drug therapy.

Plasma Clearance Studies

Initial dilution of the total dose (t_0) of RB is not directly determinable from the serum, because considerable radioactivity disappears at variable rates from the blood before complete mixing, depending upon the type and severity of the liver disease. External counting demonstrated hepatic uptake of label within 2 min after injection of ^{131}I-RB. In the normal subject, approximately 53% of the injected radiodye had been cleared from the plasma within 5 min (t_5). (Percentage of injected dose cleared in 5 min (t_5) = (100/injected cpm) × (cpm/ml plasma at 5 min) × (plasma volume, ml). The latter was calculated by ^{51}Cr-labeled erythrocyte dilution [16].) Hence, plasma clearance rates could not be expressed as percentages of the dilution of the total dose at t_0 nor at equilibrium within the vascular compartment. Inasmuch as each patient acted as his own control, the problem of variable mixing phases was avoided and the effect of PB therapy on plasma clearance of RB in each subject was expressed as the ratio of the plasma radioactivity to the t_5 values before and during drug therapy. Calculations of relative plasma clearance rates were, therefore, free of assumptions about the distribution of RB during the rapid initial mixing phase.

As shown in Table II, plasma radioactivity de-

creased rapidly during the first 4 hr. Clearance was retarded in cholestatic subjects, indicating that initial plasma clearance was largely dependent upon hepatic uptake. Fewer than 4% of counts recorded at t_5 remained in the plasma of the normal infant 4 hr after injection of ^{131}I-RB. If the amount cleared prior to t_5 is taken into account (~53%), a minimum of 98% of the administered radiodye was extracted from the plasma during the first 4-hr period. Five–ten times as much radioactivity remained in the plasma of cholestatic subjects compared with the normal infant 4 hr after injection of label. Phenobarbital therapy had no effect on plasma clearance of ^{131}I-RB during this uptake phase.

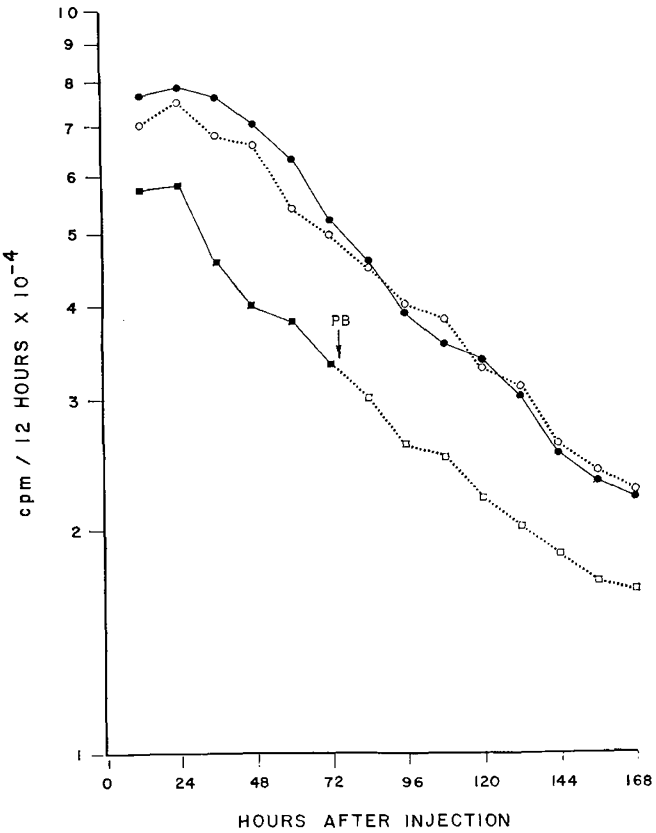

Fig. 1. Radioactivity from intravenous administration of ^{131}I-rose bengal in urine of two patients with complete biliary obstruction. *Patient 1* (*KC*) was studied for two consecutive 7-day periods (*circles*). *Patient 2* (*RZ*) was studied for 7 days; phenobarbital (PB) administration was begun after 3 days (*squares*). *Solid symbols* and *solid lines* represent pretreatment values, *open symbols* and *dotted lines* represent values obtained during PB administration.

The final exponential in the plasma pool of radioactivity was established more rapidly in the normal subject than in cholestatic children (Fig. 2). Half-time of ^{131}I-RB in plasma ($t_{1/2}$) calculated from terminal radioactivity curves by the method of fitting least squares, was longest in the presence of complete extrahepatic biliary obstruction (Table III). Phenobarbital had no appreciable effect on $t_{1/2}$ of the small fraction of radioactivity which remained in the plasma of the normal subject at equilibrium. In two children with cholestasis on the parenchymal level, $t_{1/2}$ was reduced from 61.3 to 44.5 hr, and from 40.7 to 24.8 hr, before and during PB therapy, respectively. Therapy did not alter the plasma $t_{1/2}$ in completely obstructed patients, with the exception of an infant in whom a choledochal cyst filled with freshly secreted bile was present at surgery on the 8th day of PB treatment.

Hepatic Uptake and Storage

The rapid transhepatic movement and excretion of radioactivity in the three patients with intact bile ducts (Fig. 3*a*) complicated the interpretation of hepatic uptake rates and storage capacity obtained by the external monitoring devices. However, in complete obstruction, hepatic uptake of ^{131}I-RB was followed until saturation levels of radioactivity were reached (Fig. 3*b*). Uptake by the liver was completed within 30 min; additional label accumulated extremely slowly (or not

Table II. Effect of phenobarbital on plasma radioactivity 5 min and 4 hr after ^{131}I-rose bengal injection

Patient	Biliary excretory defect	Plasma radioactivity					
		5 min (t_5) after injection		4 hr after injection			
		Control	Phenobarbital	Control		Phenobarbital	
		cpm/ml	cpm/ml	cpm/ml	$\%t_5$[1]	cpm/ml	$\%t_5$[1]
DC	None	6200	5900	200	3.2	220	3.8
DLC	Partial	14000	18000	4700	33.0	4800	27.0
DQ	Partial	9400	9000	1600	17.0	1700	19.0
RB	Complete	13000	14000	2500	19.0	2900	21.0
KC	Complete	12000	12000	3800	32.0	3700	31.0
ER	Complete	10000	12000	3700	36.0	4200	34.0
RZ	Complete	8000	9600	1700	21.0	2100	21.0

[1] $\%t_5$ indicates the percentage of radioactivity remaining, compared with the plasma level at 5 min as 100%.

at all) over the next 3–4 hr. Phenobarbital had no effect on rate or extent of uptake. The pool of radioactivity in the liver (*i.e.*, storage capacity) at saturation was also unchanged by drug therapy (Fig. 3*b*).

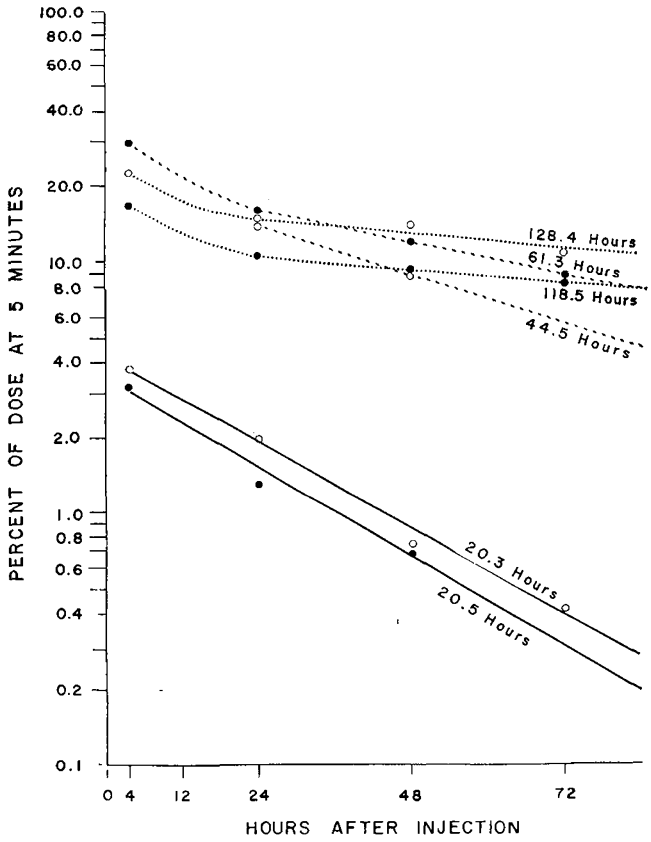

Fig. 2. Effect of phenobarbital (PB) on plasma disappearance of ^{131}I-RB in three representative subjects. Percentage of radioactivity remaining in plasma, compared with the plasma level at 5 min as 100%, is plotted on the abscissa. Half-time in hours is shown with the corresponding exponential. *Solid lines:* normal subject (*DC*); *dotted lines:* patient with complete biliary obstruction (*RB*); *cross-hatched lines:* patient with partial biliary deficiency (*DLC*). *Solid symbols:* control values; *open symbols:* PB-treated.

Table III. Effect of phenobarbital on half-time ($t_{\frac{1}{2}}$) of ^{131}I-rose bengal in plasma

Patient	Condition	Half-time, hr		
		Control	Phenobarbital	Change, %
DC	Normal	20.5	20.3	−1
DLC	Deficiency of intrahepatic bile ducts	61.3	44.5	−28
DQ	Idiopathic cholestasis	40.7	24.8	−40
RB	Extrahepatic biliary atresia	118.5	128.4	+8
KC	Complete biliary sclerosis	144.7	141.7	−2
ER	Blind choledochal cyst	155.5	96.9	−38
RZ	Extrahepatic biliary atresia	101.9	112.0	+9

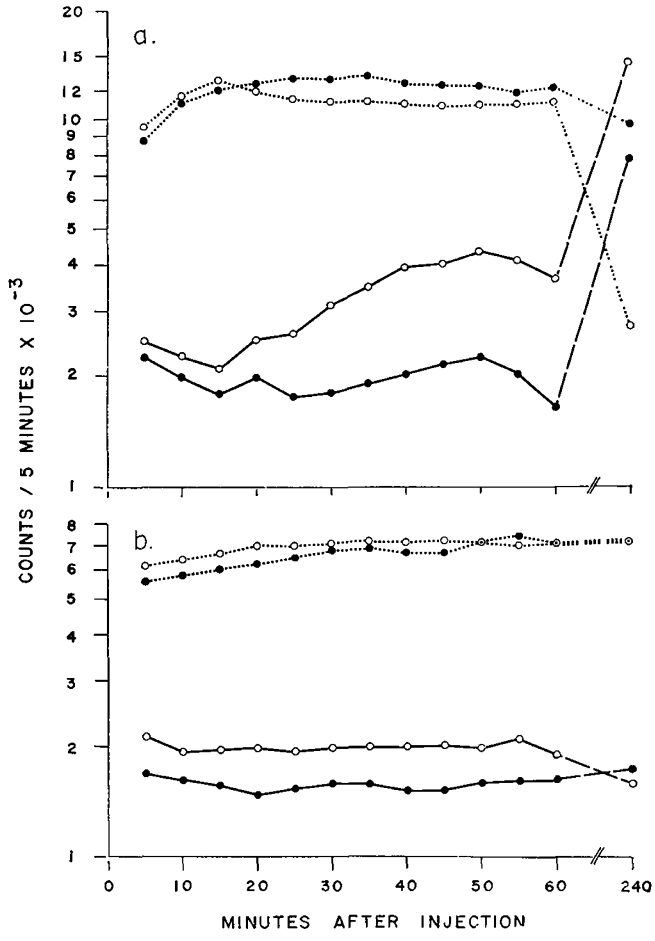

Fig. 3. Hepatic and intestinal accumulation of radioactivity. Cumulative counts (5 min) were recorded simultaneously over the liver and lower abdomen, a correction was applied for isotope decay, and background counts were substracted. *a*: normal subject (*DC*). *b*: patient with complete biliary obstruction (*RB*). *Dotted lines* represent hepatic radioactivity, *solid lines* represent intestinal radioactivity. Note changes in scale, denoted by broken lines, between 60 and 240 min. *Solid symbols:* control values; *open symbols:* phenobarbital-treated.

Hepatic Excretion

The disappearance of label from the liver was accelerated by PB in *DC*, an infant with normal hepatic functions, and in two cholestatic subjects with patent bile ducts. Hepatic radioactivity curves recorded in *DC* and in *DQ*, a patient with idiopathic cholestasis, are shown in Figure 4. In these patients, more radioactivity accumulated in the intestine during the first 60 min after PB therapy than before (Fig. 3*a*). Scintiphotographs revealed that radioactivity appeared in the gallbladder of the normal subject while on PB within 15 min after injection of ^{131}I-RB, and in the upper small intestine within 20 min, compared with 25 min and 40

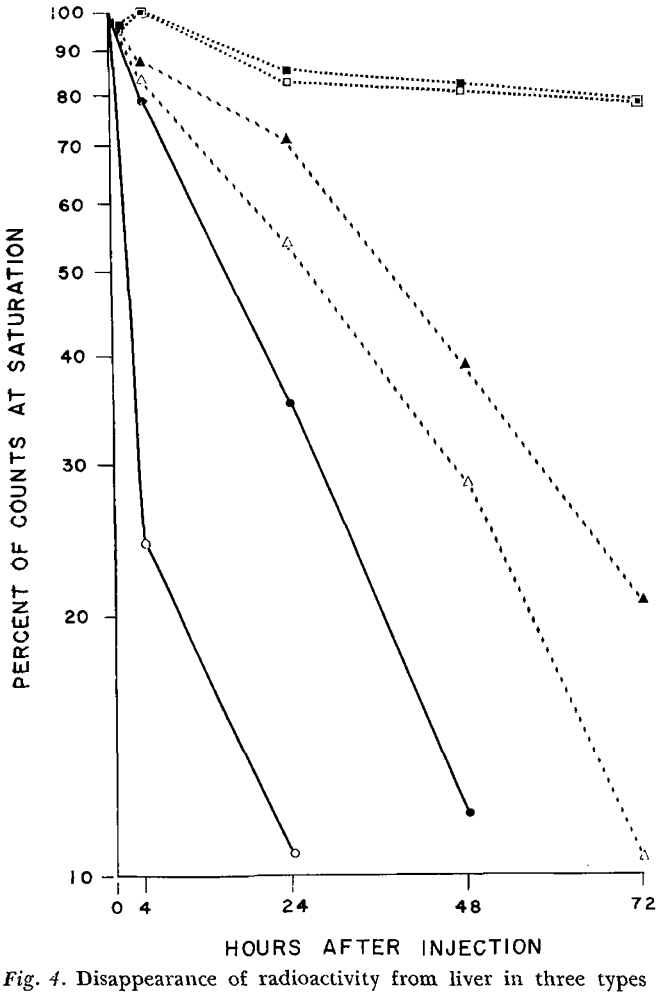

Fig. 4. Disappearance of radioactivity from liver in three types of subject. *Circles:* normal subjects (*DC*); *squares,* patient with complete biliary obstruction (*RB*); *triangles*: patient with partial biliary deficiency (*DQ*). *Solid symbols:* control values; *open symbols:* phenobarbital-treated.

min before treatment, respectively. Intestinal radioactivity in the normal subject reached maximal values at 24 hr during the control period and at 4 hr during the treatment period. In complete obstruction, hepatic and intestinal radioactivity curves paralleled each other, and hepatic disappearance rates were not affected by PB (Figs. 3*b* and 4).

Isotope Recovery in Feces and Bile

The radioactivity excreted with the stools during the 72-hr control period ranged from 78% of the injected dose in the normal subject to 1% in completely obstructed infants (Table IV). Phenobarbital increased total counts excreted in feces, and accelerated the initial appearance of radioactivity in stools of infants with intact major biliary passages (Fig. 5, *a* and *b*). In completely obstructed infants, fecal excretion accounted for less than 5% of the injected radioactivity. The percentage of label excreted, and the interval between its injection and appearance in stools was not changed by drug therapy in these infants.

The total radioactivity recovered from the stools of the infant (*ER*) with a blind choledochal cyst during each of two consecutive 72-hr periods accounted for less than 0.5% of the dose adminstered at the beginning of each period. Following 8 days of PB therapy, the cyst was drained at laparotomy: 60 ml *freshly* secreted green bile was recovered and yielded 3% of the injected tracer. An additional 6% of the label was excreted in stools during the first 48 hr after choledochojejunostomy.

Isotope Recovery in Urine

The urinary output of tracer reflected the plasma concentration of ^{131}I-RB (Fig. 6). Cholestatic subjects with higher circulating levels of radioactivity excreted proportionately more isotope in the urine (Table IV). Approximately 3% of the dose was recovered in the urine of the normal infant over 72 hr, compared with 17–38% in urine from cholestatic patients. Phenobarbital had no effect on the urinary excretion of label in normal and in completely blocked subjects; in those with partial cholestasis, relatively less radioactivity was excreted in the urine, and a larger amount in the feces, during the treatment period.

Plasma Bilirubin

The plasma concentration decreased sharply within 1 or 2 days following administration of PB to three jaundiced infants with intrahepatic biliary deficiency (Fig. 7, *a, b,* and *c*). Treatment was prolonged in two of these infants (one was not included in the current investigation) because every interruption of therapy

Table IV. Excretion of ^{131}I-rose bengal[1]

Patient	Stool		Urine	
	Control	Phenobarbital	Control	Phenobarbital
DC	78.6	94.9	3.1	2.4
DLC	3.2	9.2	35.0	29.4
DQ	9.9	71.0	29.3	21.0
RB	4.3	4.4	36.2	38.0
KC	3.5	3.0	24.0	22.8
ER[2]	0.3	0.3	17.0	19.6
RZ	0.6	0.9	19.2	18.4

[1] Expressed as percentage of the dose excreted in 72 hr.
[2] An additional 10% excreted in bile and stool after relief of biliary obstruction.

was followed by a prompt rise in plasma bilirubin levels (Fig. 7, *b* and *c*). The bilirubin concentration in infants with extrahepatic biliary atresia was not affected by PB.

NADPH-Cytochrome C Reductase Activity

Enzyme activity and biliary excretion of RB increased together during PB treatment in the normal control subject and in one patient with severe cholestasis owing to paucity of intrahepatic bile ducts (Table V). However, in two patients with idiopathic cholestasis and choledochal cyst, respectively, the drug stimulated biliary excretion of RB without a corresponding increase in enzymatic activity. The liver of two infants with complete biliary obstruction responded to PB with a 30% and 37% increase in enzyme activity, respectively, suggesting that bile flow is not essential for enzyme induction by this drug. Treatment with PB failed to alter enzymatic or excretory activity in the remaining patient with extrahepatic biliary atresia.

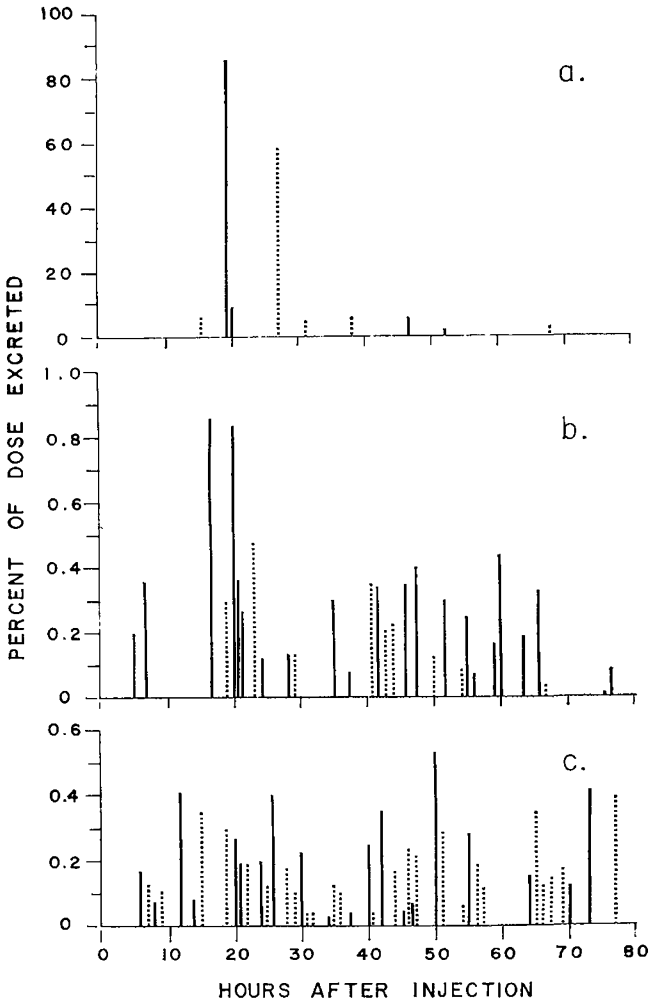

Fig. 5. Quantitative recovery of isotope from feces in three types of subject. *a*: normal subject (*DC*): total 72-hr recovery was 78% before and 95% during phenobarbital (PB) administration. *b*: patient with paucity of intrahepatic bile ducts (*DLC*): total 72-hr recovery was 3.2% before and 9.2% during PB administration. *c*: patient with complete biliary obstruction (*RB*): total 72-hr fecal excretion was 4.3% before and 4.4% during PB administration. Each bar represents radioactivity excreted per bowel movement. *Interrupted bars:* stools excreted during control periods; *solid bars:* stools excreted during PB administration.

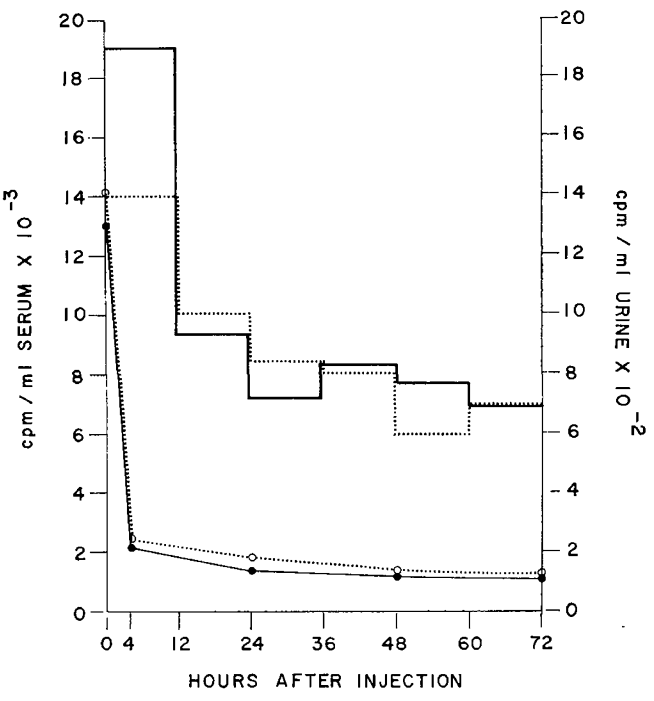

Fig. 6. Radioactivity in serum and urine in a representative patient with complete biliary obstruction (*RB*). *Heavy lines* (*top*) represent counts per minute excreted in urine during consecutive 12-hr periods; *thin lines* (*bottom*) represent counts per minute in serum; *solid lines* and *symbols:* control values; *broken lines* and *open symbols:* values obtained during phenobarbital administration.

Discussion

Phenobarbital in therapeutic doses seems to exert its stimulatory effect on hepatic excretion of RB principally by enhancing the secretion of bile, inasmuch as hepatic uptake and storage capacity of RB were not increased by exposure to the drug for several days. When hepatic excretion was blocked mechanically, as in extrahepatic biliary atresia, the plasma was cleared and the liver accumulated dye at similar rates and to the same extent before and during PB therapy. Either the induction of hepatic carrier proteins reported in

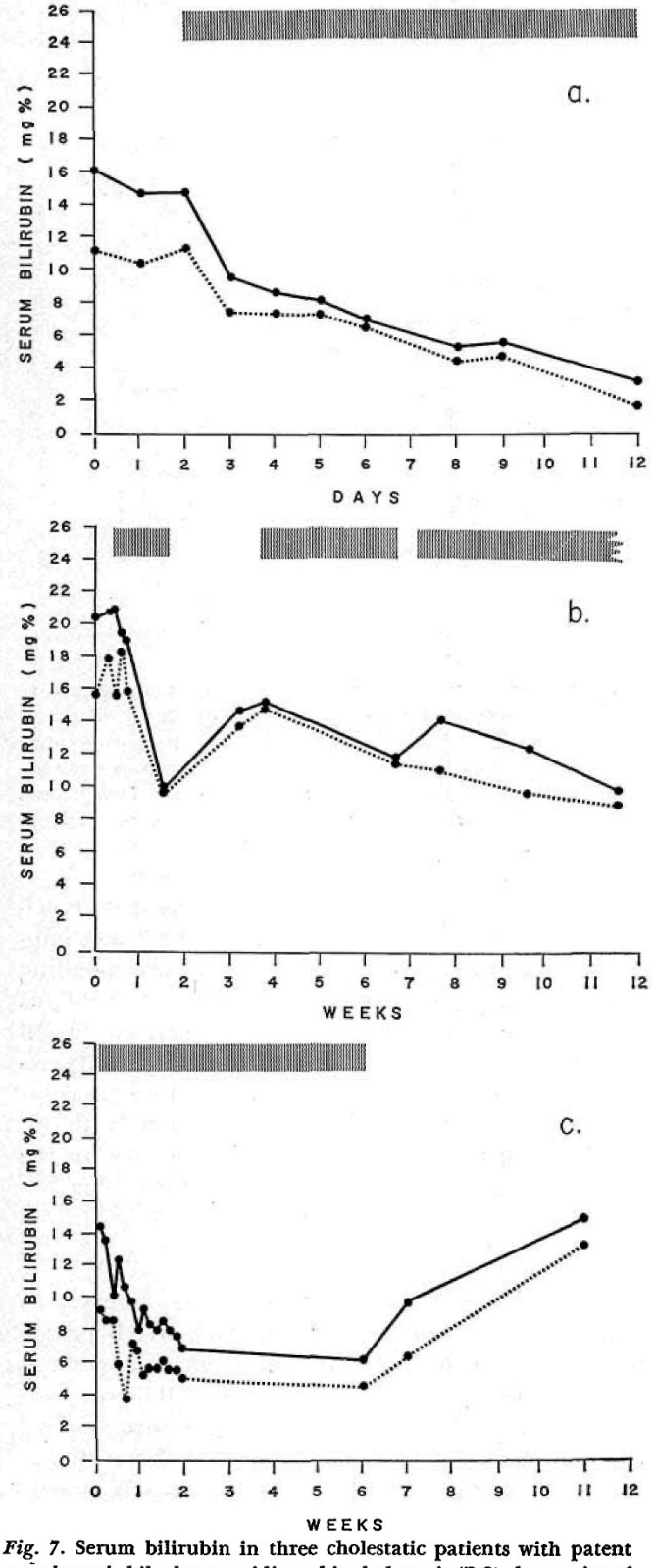

Fig. 7. Serum bilirubin in three cholestatic patients with patent extrahepatic bile ducts. *a:* idiopathic cholestasis (*DQ*). *b:* paucity of intrahepatic bile ducts (*DLC*). *c:* hypoplastic extrahepatic bile ducts (*KC*). *Horizontal bars:* duration of phenobarbital therapy. *Solid lines:* total bilirubin; *broken lines:* direct-reacting fraction of bilirubin.

PB-treated rats [9] does not occur in human liver, or these proteins are not the limiting factor in the hepatic uptake of RB.

Kinetic studies of the clearance and distribution of ^{131}I-RB indicate the existence of at least two distinct exponentials, corresponding to a rapid hepatic and a slower extrahepatic component [5, 14]; the latter exponential represents both the albumin-bound RB in the extravascular extrahepatic albumin pool and a non-albumin bound RB pool in the extrahepatic tissues which turns over much more slowly (for review, see reference 12). Radioactivity over thyroid, hepatic, and intestinal areas confirmed that most of the labeled RB was rapidly taken up by the liver, since only 2% of the dose remained in the plasma of the normal subject at 4 hr. The $t_{1/2}$ (approximately 20 hr) of the remaining minor plasma fraction of RB was unaltered during therapy, suggesting that exchange rates between the circulation and extrahepatic tissues are not influenced by PB. However, the possibility cannot be excluded that the residual radioactivity (approximately 2% of the dose) is due to breakdown products or contaminants. When biliary excretion is prevented by the absence of bile ducts, removal of RB becomes a function of renal mechanisms. The $t_{1/2}$ of RB in the plasma of infants with biliary atresia was usually in excess of 100 hr, and remained unchanged during drug therapy. The renal excretion of the label is, therefore, not appreciably affected by PB.

In contrast, the clearance of RB from plasma in patients with incomplete types of excretory deficiency was accelerated by PB. Excretion of the dye in such cases results from combined hepato-biliary and renal clearance. Slower plasma clearance of RB in patients with intrahepatic cholestasis is due both to deficient hepatic removal and disposal of the dye and to its slower transfer between plasma and extrahepatic compartments. It seems that, by relieving the excretory deficiency, PB accelerates removal of ^{131}I-RB from the circulation, and, secondarily, transfer from the extrahepatic compartment (mainly from the more labile extravascular albumin pool) into the plasma.

These results indicate that hepatic uptake and plasma clearance of RB in the normal subject are essentially completed within 30 min or less. In the partially deficient cholestatic subject, hepatic uptake and plasma clearance are limited primarily by the capacity of the hepatic excretory apparatus, and in the com-

Table V. Effect of phenobarbital on biliary excretion of ^{131}I-rose bengal and induction of NADPH-cytochrome C reductase

Patient and type	Excretion, %[1]			Enzyme activity[2]		
	Control	Phenobarbital	Change, %	Control	Phenobarbital	Change, %
Normal infant (*DC*)	78.6	94.9	+16.3	13.9	34.2	+146
Partial excretory block						
Paucity of intrahepatic bile ducts (*DLC*)	3.2	9.2	+6.0	13.2	33.0	+150
Idiopathic cholestasis (*DQ*)	9.9	71.0	+60.1	47.4	41.8	−12
Complete excretory block						
Biliary atresia (*RB*)	4.3	4.4	+0.1	38.0	49.2	+30
Sclerosis of major bile ducts (*KC*)	3.5	3.0	−0.5	18.8	25.7 (47.7)[3]	+37 (159)[3]
Choledochal cyst (*ER*)	0.3	10.0	+9.7	10.8	11.5	+6
Biliary atresia (*RZ*)	0.6	0.9	+0.3	27.6	24.1	−13

[1] Percentage dose of ^{131}I-RB excreted in stool in 72 hr.
[2] Nanomoles cytochrome C reduced per minute per milligram protein.
[3] Numbers in parentheses represent values after 6 weeks of phenobarbital therapy.

pletely obstructed subject, by renal tubular function. The first of these limiting factors (excretory function) is affected by PB therapy.

The mechanism whereby PB enhances the formation or excretion of bile is not understood. A relation may exist between the ability of PB to stimulate bile flow and the induction of enzymes which catalyze the transformation of compounds carried in bile, a process which usually increases the excretion of the modified substrate. Although RB is chemically unaltered during passage through the liver, increased biliary excretion of this inert dye in subjects treated with PB may be indirectly related to induction of microsomal enzymes [3]. The effect of PB on an essential component of microsomal oxidative activity, NADPH-cytochrome C reductase (P-450 reductase?), was, therefore, tested in liver biopsy tissue [13]. The biliary excretion studies and the reductase assays were performed concurrently on the same subjects, permitting direct comparison (Table V).

It is apparent from these data that the stimulatory effects of PB on human hepatic microsomal enzymes and biliary excretion can occur independently. Support for this conclusion comes from studies by Klaassen [6] on animals treated with a series of inducing agents; PB stimulated the secretion of bile and other drugs failed to do so; methylcholanthrene, a powerful inducer of microsomal enzymes, inhibited the secretory process. Thus, the mechanism for the cholegogic action of PB remains to be established.

Summary

In jaundiced patients with partial biliary deficiency, PB accelerated the elimination of radioactive RB dye as well as the removal of conjugated bilirubin from the plasma. In contrast, PB had no effect on the excretion of RB and conjugated bilirubin in patients with complete extrahepatic biliary obstruction. In patients with patent extrahepatic bile passages, drug therapy increased the rate at which RB was cleared from the liver and accumulated in the feces. Hepatic uptake and storage of ^{131}I-RB were not altered during PB treatment. The evidence presented indicates that PB used in dosages within the therapeutic range can stimulate human hepatic excretory function.

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