Maximal Hepatic Excretion of Bilirubin in Sheep[1] (34716)

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Biochemical phenomena associated with the hepatic uptake of bilirubin, its intracellular transfer to the smooth endoplasmic reticulum for conjugation, storage and transport maxima, are not clearly defined (1, 2). The present investigation measured excretion of intravenously administered unconjugated bilirubin (UCB) into bile at various bile flow rates, to determine both the hepatic excretory maximum (E_m) of bilirubin in unanesthetized sheep and the rate-limiting step in the transport process. Other investigators have reported excretory maxima for bilirubin in the mouse, dog, and rat; their studies involved primarily anesthetized animals in various hypothermic states and at declining bile flow rates.

Materials and Methods. Native female sheep in this study were of mixed breeding, and weighed from 40 to 76 kg. Pentobarbital sodium was used intravenously as the anesthetic agent for all surgical procedures. Cholecystectomy was performed and an indwelling rubber T-tube[3] was inserted into the common bile duct with the free end exteriorized. The T-tube contained inflatable cuffs in its two arms which allowed closure of the distal portion for the quantitative collection of hepatic bile (3). The exteriorized free end was closed between trials allowing hepatic bile to enter the duodenum normally. All experiments were performed after a postoperative period of 3 to 4 weeks.

The concentrations of total bilirubin (TB) and conjugated bilirubin (CB) in bile, plasma, and urine were determined using a micro-modification of the method of Malloy and Evelyn (4). The optical density of direct-reacting bilirubin was recorded at 1 min. Determinations were made on a multiple sample spectrophotometer.[4]

Sheep were placed standing in a stanchion with minimal restraint. All experimental animals exhibited normal body temperatures, heart and respiratory rates, and periodic rumination. During an initial preinfusion drainage period of 90 to 120 min, bile was quantitatively collected. With distal cuff inflation, all hepatic bile was diverted for outside collection. Quantitative collection of bile using this technique has been radiographically tested by Alpert *et al.* (3). After the drainage period, an aqueous solution of taurocholic acid[5] was continuously infused to augment and stabilize bile flow. This was followed in 10 min by infusion of recrystallized UCB[5]. Bilirubin was dissolved in distilled water which was rendered alkaline (pH 8–8.5) by adding and constantly mixing a few drops of 50% NaOH. Taurocholic acid and bilirubin were separately infused into the left jugular vein via two indwelling polyethylene catheters (PE 100). Two rotary tubing pumps[6] were used in all infusion trials.

Infusion rates (mg/min/kg) were calculated according to body weight. Bile was continuously collected and divided into 10-min aliquots and volumes were recorded. Blood sam-

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[3] Bard Instrument Company, Murray Hill, N.J.

[4] Gilford Model 200, incorporating a Beckman DUR monchromator.

[5] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[6] Emdeco, I-V Injector.

ples were collected at 10-min intervals by venipuncture of the right jugular vein. Urine was continuously collected via urethral catheter and separated into 30-min samples. Blood, bile, and urine samples were stored at 3° in darkness and assayed in 1 hr. Bilirubin was infused for 3 hr in each trial to allow biliary concentration and excretion of bilirubin to become constant (plateau period). Mean values for bile flow, bile bilirubin concentrations, bilirubin excretion, and ranges of plasma bilirubin concentrations were calculated from at least six separate 10-min samples during the plateau period.

All data were compared by analysis of variance. Group means were compared by Student's t test (5). A significance level of $p<0.05$ was used as a basis for the rejection of the null hypothesis.

Results. Bile flow. Preliminary trials indicated that drainage of bile from sheep for 1.5 to 2 hr decreased the flow rate to a base plateau level of 7.4 μl/min/kg (av). At that plateau level, little taurocholic acid was excreted and flow rates agreed with previous observations (6, 7). Bile flow was adjusted to stable flow rates at various predetermined levels and maintained for several hours by the continuous intravenous infusion of taurocholic acid.

Endogenous bilirubin excretion. The biliary excretion of endogenous bilirubin was determined during the bile collection period prior to the start of UCB infusion. In 17 trials (4 samples/trial), bilirubin excretion averaged 1.88 μg/min/kg.

Submaximal hepatic bilirubin excretion. When UCB was infused at either 0.2 or 0.4 mg/min/kg (19 experiments on four sheep), excretion of bilirubin was *submaximal*. Submaximal excretion trials were characterized by having corresponding plateaus for bilirubin concentration and excretion rate in bile, and constant total bilirubin concentration in plasma. An initial 90-min equilibrium period was uniformly observed in all submaximal trials before a stable plateau was reached. As bile flow was increased by taurocholic acid infusion, the concentration of bilirubin decreased correspondingly. Bilirubin excretion, however, did not vary significantly (Fig. 1). During the plateau period, 77.7% of the infused UCB was excreted into bile.

The average TB plasma concentration during the plateau period of 0.4 mg of UCB/min/kg infusion trials was 25.5 mg/100 ml; during the plateau period of the 0.2 mg of UCB/min/kg infusion trials, 14.9 mg/100 ml. Direct-reacting bilirubin accounted for 9.6% of TB during plateau periods of all submaximal trials.

Maximal hepatic bilirubin excretion. When UCB was infused at either 0.60 or 0.65 mg/min/kg in 15 experiments on four sheep, mean excretory maximum (E_m) was 47.3 μg of bilirubin/100 g of body wt/min. The initial equilibrium time for maximal infusion trials was less than for submaximal trials. A constant biliary concentration and excretion of bilirubin (plateau) was reached within 60 min in maximal trials. Maximal bil-

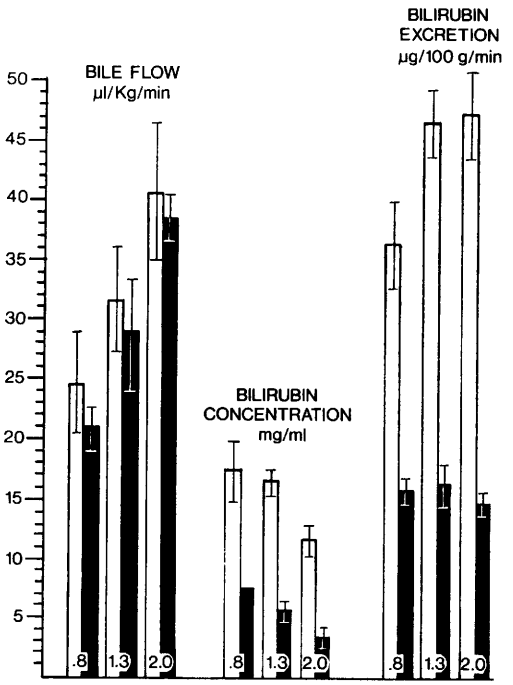

FIG. 1. Relationship of varying bile flow rates and bilirubin concentration and excretion in bile: E_m trials □ (bilirubin infused at 0.65 mg/kg/min); submax trials ■ (bilirubin infused at 0.2 mg/kg/min); values are means ±SD; taurocholic acid infusion rates: 0.8, 1.3, and 2.0 mg/min/kg.

irubin excretion into bile was confirmed by a continuous increase in the concentration of plasma TB during the excretion plateau period. Bile bilirubin concentration differed significantly when the rate of bile flow was varied by the infusion of taurocholic acid at the two higher rates. At the taurocholic acid infusion rate of 0.8 mg/min/kg, bilirubin concentrations in bile did not increase above that observed at taurocholic acid infusion rates of 1.3 mg/min/kg (Fig. 1). Average biliary concentrations of bilirubin observed in maximal trials were 11.8, 16.5, and 17.4 mg/ml in the three different bile-flow-rate groups (Fig. 1); bilirubin E_m was essentially the same at the two higher bile flow rates, but decreased significantly at the lowest bile flow rate.

The range of plasma bilirubin concentrations during the excretory plateau period of the E_m trials was 24 to 59 mg TB/100 ml. "Direct reacting" bilirubin accounted for 10.7% of TB during the excretory plateau period of maximal trials at the two higher bile flow rates. At the low bile flow rate, "direct reacting" bilirubin averaged 14.0%, significantly different from the 10.7% of the two higher bile flow rates. No variations in bilirubin E_m was observed in repeated trials with the same sheep.

Although bile pigments were excreted in the urine, the TB excretory rate into urine was less than 0.9% of the biliary excretion rates in all trials.

Toxicity of infused bilirubin. In two sheep, bilirubin was infused at 0.8 mg/min/kg and produced untoward clinical signs. Neurotoxicity was manifested as severe ataxia. Hepatotoxicity was evidenced by an abrupt drop in bile flow and a marked reduction in ability to concentrate bilirubin in bile. Clinical signs became apparent when plasma levels exceeded 60 mg/100 ml of TB. Bilirubin infusion was discontinued, and all sheep survived.

Discussion. In all submaximal trials, hepatic excretion of bilirubin was independent of the bile flow rate within the limits of the investigation. Bile flow increases were accompanied by corresponding decreases in bile bilirubin concentration, while excretion remained constant. Plasma bilirubin levels remained constant after an initial equilibrium period, indicating that a steady state existed with plasma cleared of bilirubin at a rate approximating the infusion rate. During the steady state of submaximal trials, approximately 22% of the infused UCB was not accounted for by hepatic and urinary excretion.

Since the infusion of UCB at either 0.6 or 0.65 mg/min/kg exceeded the excretory capacity of the liver without apparent toxic manifestations, the excretory maximum (E_m) was determined at these infusion rates. E_m's have been reported in other species: human, 38.9 μg/kg/min (8); mouse, 30.0 to 43.7 μg/100 g/min (9, 10); rat, 28.7 to 95.0 μg/100 g/min (10–16); and dog, 100.0 μg/g of liver/20 min (17). The average bilirubin E_m of 47.3 μg/100 g/min for sheep in this study falls well within the range of previously reported values for other animals.

Our data indicate that hepatic excretion of bilirubin in sheep is limited by the ability of the liver to concentrate CB and to transport CB across the canicular membrane. Supporting evidence is shown in (Fig. 1): (A) at the two higher bile flow rates, bilirubin excretion was constant with a proportional reciprocal change in concentration and flow rate; (B) comparing the two lower bile flow rates shows biliary bilirubin concentration remaining constant with excretion changing proportionally with flow; and (C) at the low bile flow rate, excretion of CB decreased with a subsequent regurgitation of CB into the plasma pool. The hepatic E_m for sulfobromophthalein (BSP) excretion has recently been observed in dog (18), and sheep (19) to be dependent upon a BSP concentration maximum in bile and the rate of bile flow.

At the levels of taurocholic acid and bilirubin infused in this study, no interference in the transfer or excretion of UCB or taurocholic acid was apparent. Similar bile flow rates at each taurocholic acid infusion rate were present regardless of bilirubin infusion rate. These observations would support the concept recently proposed by Alpert *et al.* (3) that bilirubin and taurocholic acid do not share a common hepatic excretory pathway.

Summary. The maximal excretory rate of

bilirubin from plasma into bile is similar in sheep to values reported for other species. The average hepatic excretory maximum for bilirubin was 47 μg/100 g/min. An average concentration maxima of 17 mg of bilirubin/ml of bile was observed. Hepatic excretion of bilirubin into ovine bile is limited by a concentration maximum and the rate at which conjugated bilirubin may be transported from the hepatocyte into bile.

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