Biliary excretion of injected conjugated and unconjugated bilirubin by normal and Gunn rats

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—Conjugated and unconjugated bilirubin were injected intravenously at different times into normal rats and homozygous, jaundiced Gunn rats. As was expected, Gunn rats did not excrete injected unconjugated bilirubin in the bile. After the intravenous injection of conjugated bilirubin into normal rats, approximately twice as much bilirubin appeared in the bile per 100 g of body wt within 10 min than after the injection of comparable amounts of unconjugated bilirubin. This difference probably reflects the time required for cellular uptake and conjugation of bilirubin prior to excretion. The maximal biliary bilirubin excretory rate in Gunn rats following the administration of conjugated bilirubin was 56±8.4 (S.D.) µg of bilirubin excreted/100 g body wt/min. This does not differ significantly from the maximal biliary bilirubin excretory rate observed in normal rats after infusions of either conjugated or unconjugated bilirubin. This demonstrates that conjugation alone does not limit metabolism of bilirubin by normal rat liver. These studies, when considered in the light of other investigations, suggest that the ability to excrete conjugated bilirubin is the limiting factor in metabolism of bilirubin by normal rat liver.

IN RECENT YEARS it has been demonstrated that bilirubin is conjugated in the liver mainly with glucuronic acid (1-3) but also with sulfate and perhaps other substances (4) and that conjugation is required for the excretion of bilirubin into the bile and urine (5). The complete mechanism by which bilirubin is metabolized by the liver may be considered to involve at least three processes (Fig. 1): a) the uptake of bilirubin from plasma by the liver cell; b) the conjugation of bilirubin, primarily with glucuronic acid; and c) the excretion of conjugated bilirubin into the bile. The physiological limitations of each of these processes in effecting the normal metabolism of bilirubin by the liver are not known. It seemed important to estimate such limitations by determining the actual quantity of bilirubin excreted into the bile after intravenous infusion of conjugated and unconjugated bilirubin into normal (JJ) rats and homozygous (jj) Gunn rats. Homozygous Gunn rats have nonhemolytic acholuric jaundice and are virtually unable to conjugate bilirubin with glucuronic acid due to a deficiency of glucuronyl transferase activity (5-7).

METHODS

In general, the technique of Weinbren and Billing (8) was used in these experiments. Male Wistar and homozygous (jj) Gunn rats weighing 185-410 g were kept on a Rockland rat diet (complete) and were not fasted before any of the experiments. Veterinary pentobarbital sodium (Ethical) was injected intraperitoneally in doses of 3 mg/100 g of body wt with a maximum of 12 mg in 0.2 ml. Supplemental ether was administered by inhalation, and the animal was kept under light anesthesia throughout the experiment. The common bile duct was ligated at its junction with the duodenum and cannulated approximately 1 cm proximal to the duodenum with polyethylene tubing. The collecting tube was changed at intervals that varied with the particular experiment. Blood samples were obtained from a tail vein in heparinized capillary tubes. The right jugular vein was cannulated with polyethylene tubing, and a 24-gauge needle was inserted in the free end. The needle was connected to a delivery syringe that, in the second group of experiments, was driven by a motor at a speed of delivery of approximately 1 ml of solution/3 min.

Unconjugated bilirubin was prepared by dissolving 100 mg of recrystallized bilirubin (Eastman) in 50 ml of isotonic solution containing 0.52 g Na2CO3 and 0.52 g NaCl/100 ml (1 ml contained 2 mg bilirubin). The solution was prepared freshly for each experiment and was kept in the dark at 4 C. The bilirubin concentration was estimated by the method of Malloy and Evelyn (9). The bilirubin for infusion was prepared by diluting the
priming solution with either a half or equal volume of 0.85% NaCl.

Conjugated bilirubin was obtained by several methods. Initially, fresh, sterile human bile was obtained from T-tube drainage, centrifuged at 25,000 rev/min for 15 min at 4°C, and the concentration of conjugated bilirubin in the clear supernate was estimated by the method of Malloy and Evelyn (9) and by paper chromatography of the pigments after diazotization (3). Subsequently, bile was collected from the cannulated common bile ducts of normal rats receiving infusions of unconjugated bilirubin. This bile was treated in a similar manner.

The "direct-reacting" and "indirect-reacting" bile pigments in serum were estimated by a micromodification of the method of Weinbren and Billing (8) and by paper chromatography of the pigments after diazotization (3). Subsequently, bile was collected from the cannulated common bile duct of normal rats receiving infusions of unconjugated bilirubin. This bile was treated in a similar manner.

The "direct-reacting" and "indirect-reacting" bile pigments in serum were estimated by a micromodification of the method of Malloy and Evelyn (9). The method described by Weinbren and Billing (8) was used for the estimation of pigments in bile.

**PROCEDURE**

**Effect of single intravenous injections of unconjugated and conjugated bilirubin on serum bilirubin levels and pigment excretion in bile in normal and Gunn (JJ) rats.** Single doses of 0.1 and 2.5 mg of unconjugated bilirubin/100 g of body wt were each given to six normal Wistar rats and to two Gunn rats. Blood samples were obtained for estimation of the concentration of bilirubin in the serum before the injection and at approximately 4-min intervals. The bile was collected continuously, and the collecting tube was changed every 3–5 min. Fig. 2 shows the disappearance of injected unconjugated bilirubin from the plasma in a representative normal rat and the rate of bile pigment excretion in micrograms per 100 g of body wt/min in each of the collecting periods. Similar observations are shown for a representative Gunn rat. As expected, the Gunn rat was unable to clear injected unconjugated bilirubin from the serum at a normal rate (10), and the rate of pigment excretion in the cannulated common bile duct did not exceed 14 μg/100 g of body wt/min during the experiment.

Single doses of approximately 1.0 and 3.0 mg of conjugated bilirubin/100 g of body wt (calculated for bilirubin diglucuronide) were similarly given to four normal and four Gunn rats. Fig. 3 indicates the disappearance of injected conjugated bilirubin from the serum and the rate of pigment excretion in the cannulated common bile duct in a representative normal and Gunn rat. Both the rate of disappearance from the serum and the rate of biliary excretion of injected conjugated bilirubin appeared to be similar in the normal and in the Gunn rats.

Although the data in Fig. 3 suggest that normal rats may excrete the larger dose of conjugated bilirubin faster than Gunn rats, the differences indicated are not statistically significant when the results obtained in the group of eight animals are compared.

In the normal rats excretion of injected conjugated bilirubin was more rapid than unconjugated bilirubin. Fig. 4 presents the mean excretion of bilirubin in the first three 5-min bile-collection periods following the separate intravenous administration of 10 mg of conjugated and unconjugated bilirubin per kilogram of body weight to six normal rats. The P values indicated in Fig. 4 were determined using the t test. In each instance, following the intravenous injection of conjugated bilirubin into normal rats approximately twice as much bilirubin appeared in the bile per 100 g of body wt within 10 min than following the injection of comparable amounts of unconjugated bilirubin.

**Effect of saturation of hepatic excretory mechanism by constant intravenous infusions of conjugated and unconjugated bilirubin in normal and Gunn (JJ) rats.** The technique of Weinbren and Billing (8) was modified and applied to the study of five normal and six Gunn rats, each of which received infusions of conjugated bilirubin, and ten normal and two Gunn rats, each of which received infusions of unconjugated bilirubin. A priming dose of approximately 2.3 mg of conjugated or unconjugated bilirubin/100 g of body wt was injected via the jugular venous cannula, and a continuous intravenous infusion was then delivered at an approximate rate of 1 mg of unconjugated bilirubin/3 min or 2.5 mg of conjugated bilirubin/3 min, respectively. Bile collections were made over 5-min intervals from 25–45 min after the priming dose was given. The serum bilirubin concentrations at the end of the infusion of unconjugated bilirubin into normal rats ranged from 16 to 23 mg %. In Gunn rats the range was 29–48 mg %. At the end of the infusion of conjugated bilirubin, the total serum bilirubin concentration in normal rats ranged from 13 to 19.5 mg % and in Gunn rats the direct-reacting serum bilirubin concentration ranged from 14.8 to 21.6 mg %.

Fig. 5 presents the maximal pigment excretion in the bile following the intravenous infusion of unconjugated and conjugated bilirubin in normal and Gunn rats. The final maximal pigment excretion represents the mean of three collections. The standard deviation of the mean for these determinations was not more than 10 %.

The maximal biliary excretion in normal rats following infusion of unconjugated bilirubin was 69 μg of bilirubin excreted/100 g of body wt/min ± 9.2 S.D., and in two Gunn rats 0.2 and 6.4 μg of bilirubin were excreted/100 g of body wt/min. The maximal biliary excretion in normal rats following the administration of conjugated bilirubin was 58 ± 10.0 S.D. and in Gunn rats was 56 ± 8.4 S.D. The maximal biliary excretion following the administration of conjugated bilirubin to Gunn rats was similar to that observed in the normal rats after infusions.
of either unconjugated or conjugated bilirubin. The differences between the maximal biliary excretion under these three circumstances are not statistically significant.

**DISCUSSION**

Following the administration of conjugated bilirubin to Gunn rats, the maximal pigment excretion in the cannulated bile duct was similar to that observed in the normal rat after administration of unconjugated bilirubin. These observations provide further evidence that conjugation is required for bilirubin to be excreted in the bile (5). Following the administration of conjugated bilirubin to Gunn rats, the maximal biliary excretion was similar to that following the administration of either conjugated or unconjugated bilirubin to normal rats. The observations indicate that conjugation alone does not limit the metabolism of bilirubin by normal rat liver. These experiments do not differentiate between limitations in uptake of bilirubin from plasma by the liver cell and the ability of the liver cell to excrete conjugated bilirubin in normal rats. If hepatic cellular uptake is considered to limit the over-all ability of the normal liver to excrete bilirubin, then the capacity for uptake of conjugated and unconjugated bilirubin must be the same in normal rats. Tisdale et al. (11) have demonstrated that intravenous infusions of large quantities of unconjugated bilirubin in man regularly led to a rise in the serum concentration of conjugated bilirubin about 1/2 hr after maximal concentration of unconjugated bilirubin was attained in the serum. This observation suggests that conjugated bilirubin may have entered the plasma from the liver cell due to a limitation in the ability of the liver cell to excrete conjugated bilirubin. The present studies support the concept that the ability to excrete conjugated bilirubin is the limiting factor in the metabolism of bilirubin by the normal liver.

Following the injection of conjugated bilirubin in normal rats, the initial excretion of conjugated bilirubin in the cannulated bile duct was more rapid than after infusion of the same amount of unconjugated bilirubin (Fig. 4). The entire curve of biliary excretion of injected bilirubin in 6 normal rats.

**FIG. 2.** Effect of injection of unconjugated bilirubin on serum bilirubin levels and pigment excretion in bile of normal and Gunn rats.

**FIG. 3.** Effect of injection of conjugated bilirubin on serum bilirubin levels and pigment excretion in bile of normal and Gunn rats.

**FIG. 4.** Mean rates of biliary excretion of injected bilirubin in 6 normal rats.

**FIG. 5.** Maximal pigment excretion in bile following intravenous infusion of unconjugated and conjugated bilirubin in normal and Gunn rats.
These studies focus attention on the transport of bilirubin from plasma into the liver cell and the process of excretion of conjugated bilirubin. Although little is known of the mechanisms involved in the excretion of conjugated bilirubin, the mechanisms appear to be affected by disease (12), drugs (13 and unpublished observations of Arias), and perhaps familial functional defects (14, 15).

REFERENCES