

Independence of Bile Acid and Ouabain Hepatic Uptake: Studies in the Newborn Rat¹ (39992)

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It is generally accepted that there are at least three separate active transport systems in the liver for the overall transfer of organic compounds from plasma into the bile (1). One transport system is responsible for the transfer of organic anions such as sulfobromophthalein and bilirubin, the second for organic cations such as procainamide ethobromide and *d*-tubocurarine, and the third for neutral organic compounds or steroids such as ouabain.

It is not known if bile acids are transported by one of the aforementioned processes or if yet another transport mechanism for bile acids exists. Sperber (2) originally suggested that a common mechanism existed for the hepatic excretion of all organic anions. However, it was later shown that Corridale sheep, which cannot excrete sulfobromophthalein and other organic acids into bile, can excrete bile acids normally into bile (3). This indicates that bile acids are excreted by a process different than that of most organic acids. Bile acids have been shown to inhibit the uptake and biliary excretion of ouabain (4), and thus it has been suggested that bile acids might be excreted by the steroid transport system.

We have recently shown that, while the liver of the adult rat has a marked ability to concentrate ouabain, the liver of the newborn rat has an absolute inability to concentrate ouabain (5). However, while the liver of the newborn rat is not as efficient in excreting organic anions as the adult, it does have the ability to concentrate them (6). Therefore, it was of interest to determine if bile acids are excreted as efficiently in the newborn as the adult rat. If the liver of the newborn rat was found to have the

ability to concentrate the bile acids, this would suggest that bile acids are not transported by the same process that transports ouabain.

Material and methods. Rats of various ages were used throughout the study. The rats were born in our laboratory and were produced by mating untreated Sprague-Dawley (Bio-Lab, White Bear, Minn.) rats. The mother and offspring were kept in "shoebox cages" for 1 month before separation. The rats had free access to food and water at all times.

[³H]Taurocholic acid (New England Nuclear, Boston, Mass.) and unlabeled taurocholic acid (Calbiochem, San Diego, Calif.) were mixed to give the appropriate specific activity. In the first study the [³H]taurocholic acid (30 mg/kg) was injected (10 mg/kg) into the distal portion of the femoral vein. Fifteen minutes later, a blood sample was obtained by cardiac puncture under ether anesthesia. Heparin was used as the anticoagulant. In the second study the [³H]taurocholic acid was injected similarly, but the blood sample was obtained at 2, 5, 10, 20, or 30 min thereafter. The rats were sacrificed, and the liver and small intestine were removed. As a measure of the biliary excretion of taurocholic acid, the small intestine was homogenized with 3 ml of saline in a Brinkmann Polytron homogenizer (Luzern, Switzerland). This method was used to estimate biliary excretion because it was not feasible to cannulate the bile duct of small rats and measure biliary excretion directly. This technique for estimating biliary excretion was verified by comparing the amount of ³H in the small intestine of bile-duct-ligated and control (adult) rats 2 min after iv administration of [³H]taurocholic acid; the concentration in bile-duct-ligated rats was 4.6% of the control value. Aliquots of plasma (50-250 μ l), intestinal homoge-

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nate (500 μ l), and liver (approximately 500 mg) were oxidized with a Packard Model 306 Tri-Carb sample oxidizer, and radioactivity was estimated with a Packard Tri-Carb liquid scintillation spectrometer (Packard Industries, Downers Grove, Ill.). Quenching was determined by using automatic external standardization.

The 7-day-old animals were compared to the 39-day-old animals by Student's *t* test as described by Steel and Torrie (7).

Results. The plasma concentration of taurocholic acid 15 min after iv administration (30 mg/kg) in rats of various ages is depicted in Fig. 1. In rats 20 days of age and older the plasma concentration of taurocholic acid is approximately 6 μ g/ml. The plasma concentration is more than fourfold higher in the 10-day-old rats. There is a marked decrease in the plasma concentration of taurocholic acid 15 min after administration as the rats increase in age from 5 to 20 days. The plasma concentration of taurocholate in the 5-day-old rats is significantly lower than that in the 10-day-old rats.

In an attempt to determine why the plasma taurocholate levels were higher in the newborn rats than in the adult rats 15 min after administration, the concentration of taurocholic acid in the plasma, the amount of taurocholic acid contained in the liver, and the amount excreted into the intestine were measured at 2, 5, 10, 20, and 30 min after administration to 7- and 39-day-old rats. Figure 2 illustrates that the plasma level of taurocholic acid was already significantly higher in the 7-day-old than in the 39-day-old rats at 2 min after administration, suggesting a larger volume of distri-

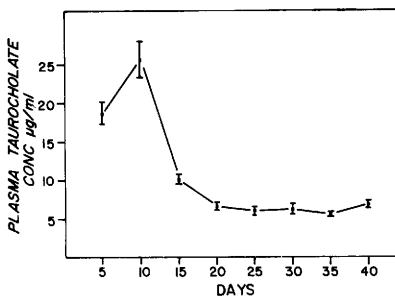


FIG. 1. Plasma taurocholic acid concentration 15 min after iv administration (30 mg/kg). Each value represents the mean \pm SE of eight rats.

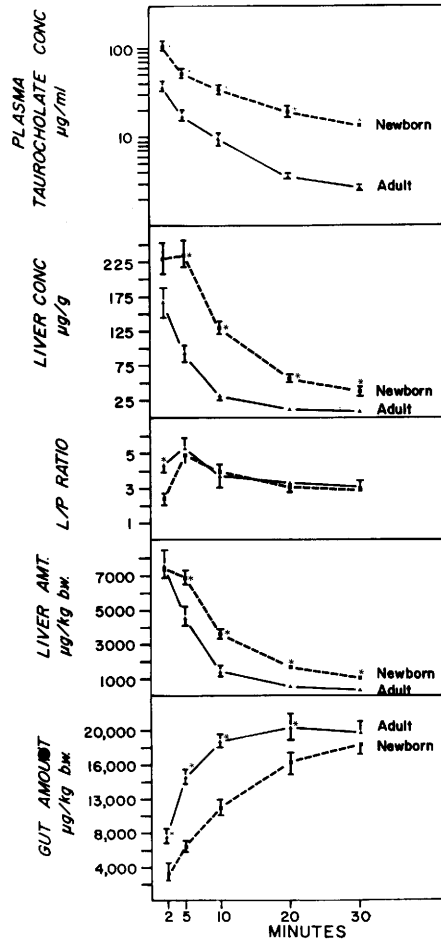


FIG. 2. Plasma disappearance of taurocholic acid (30 mg/kg), liver concentration, liver content, liver/plasma taurocholic acid concentration, and biliary excretion of taurocholic acid in 7- and 39-day-old rats. Each value represents the mean of 6-10 rats. Asterisk indicates the values are significantly different from controls, $P < 0.05$.

tribution. The difference between the newborn and adult became greater at the longer time intervals. The concentration of taurocholate in the liver of the newborn rat was significantly higher than that in the adult at 5 min after administration and at all times thereafter (Fig. 2). However, since the liver of the newborn rat comprises only 2.9% of its body weight while the liver of the older rat is 4.6% of its body weight, the difference between the quantity of taurocholic acid in the liver of the newborn and the adult is less than the difference in concentration.

However, there is significantly more taurocholic acid in the liver of the newborn than the adult rat at 5 min after administration and all times thereafter (Fig. 2). Another index of overall hepatic excretory function is the ratio of concentration of taurocholic acid in the liver to its concentration in the plasma. The value in the newborn rat was significantly lower than in the older animals at the 2-min interval but was similar to the adult rat at all times thereafter. The amount of taurocholic acid detected in the small intestine was used as a measure of its biliary excretion. The newborn rats excreted significantly less of the taurocholate into the intestine than the adults at all times after administration except at 30 min (Fig. 2).

Discussion. Similar to the neutral organic compound ouabain (5, 8) and the organic anions sulfobromophthalein (BSP) and indocyanine green (ICG) (6), taurocholic acid is not handled as efficiently by the liver of the newborn rat as it is by that of the adult. The time course for development of the systems responsible for the transfer of taurocholic acid is similar to that for ouabain (5), BSP, and ICG (6). The overall excretory capacity of the liver appears to be mature by 20 days of age. Interestingly, the liver of the 5-day-old rat appears to be more efficient in handling an exogenous load of taurocholic acid than the liver of the 10-day-old rat; similar results were obtained with ouabain (5, 8) as well as with BSP and ICG (6).

The mechanism for the delayed disappearance of taurocholic acid from the plasma of the newborn was examined by quantitating the amount of taurocholate in the plasma and liver and that excreted into the bile at various times after iv administration. At 2 minutes the concentration of taurocholate in the plasma was already higher in the newborn than in the adult. This difference in volume of distribution may be due to alteration in overall hepatic excretory function. The newborn excreted the taurocholate acid into the gut at a much slower rate than did the adult rats. This difference does not appear to be the result of the difference in availability of taurocholate to the transport system since the concentration of taurocholate in the liver of the

newborn is as high or higher than that of the adult. Thus it has been concluded that the system that transports bile acids from the liver is not mature in the newborn rat and is probably largely responsible for the delayed disappearance of taurocholic acid from the plasma. It is difficult to determine from the present experiments if the transport system responsible for transfer of taurocholate from the plasma to the liver operates at the same capacity in the newborn as the adult; however, the liver/plasma ratio of taurocholate is similar in the two groups.

While it appears that the major reason for the delayed disappearance of taurocholic acid from the plasma of the newborn rat is due to a lower capacity of the liver to transfer taurocholic acid from the liver into the bile, the major defect in the liver of the newborn to handle ouabain (5, 8), BSP, and ICG (6) is the inability to transfer the xenobiotic from the plasma into the liver. Both ouabain (9) and taurocholic acid (10) are transported into the liver by an active carrier-mediated system. With ouabain there appears to be a complete inability of the liver of the newborn rat to concentrate it, while the liver of the adult rat can concentrate it about 30-fold over the level in the plasma (5). Taurocholic acid is also more concentrated in the liver than in the plasma but only about five times more than in the plasma. In contrast to the results observed with ouabain, the liver of the newborn rat can concentrate taurocholic acid to the same extent as that seen in the adult. This would suggest that taurocholic acid is taken up by a process other than the one that transports ouabain. Thus it would appear that there are at least four processes in the liver responsible for the transfer of organic substances from the plasma into the liver; one for most organic anions, one for organic cations, one for neutral substances, and another for bile acids.

Summary. The plasma disappearance and biliary excretion of taurocholic acid was found to be slower in the newborn than the adult rat. This appears to be due largely to a less efficient transfer of taurocholic acid from the liver into the bile, while the liver/plasma concentration ratio of taurocholate in the newborn is similar to that in the

adult. Ouabain, in contrast, cannot be taken up and concentrated in the liver of the newborn rat. This suggests that taurocholic acid and ouabain are transported into the hepatocyte by different systems.

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