PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 370-373 (1976)

Effect of Microsomal Enzyme Inducers on the Biliary Excretion of an Exogenous Load of Bilirubin in Newborn Rats[1] (39548)

CURTIS D. KLAASSEN[2]

Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas 66103

Neonatal physiological jaundice is a condition seen in some newborn children which is characterized by high plasma levels of unconjugated bilirubin. If unconjugated bilirubin reaches high plasma levels or if the bilirubin is displaced from the plasma proteins to which it is bound, bilirubin will enter the central nervous system and produce a type of brain damage termed kernicterus. It has been demonstrated that when phenobarbital is given to pregnant women before delivery, there is a reduction in the plasma bilirubin levels of the infants (1-3). However, if the neonates were not exposed to phenobarbital until after birth, the treatment was less effective (3-5) and the best treatment appeared to be when the neonate was exposed to the drug both *in utero* and during the first few days of life.

While most laboratory animals do not develop neonatal physiological jaundice, many have been shown to have an impaired ability to excrete an exogenous load of bilirubin in comparison to the adult (6, 7). This effect appears to be due both to an impaired conjugation and excretion.

We have demonstrated that the biliary excretion of a number of drugs is impaired in newborn rats (8-10). The biliary excretion of one of these drugs, ouabain, can be stimulated to develop at an earlier age by the administration of microsomal enzyme inducers (11). Spironolactone and pregnenolone-16α-carbonitrile were more effective in this regard than phenobarbital. Therefore, it was of interest to determine (i) if the biliary excretion of an exogenous load of billirubin is impaired in the newborn rat as has been shown in other species and (ii) if there is a difference in the ability of various microsomal enzyme inducers to enhance the development of the processes responsible for its excretion.

Materials and methods. Rats of various ages were used throughout the study. The rats were born in our laboratory and were produced by mating untreated Simonsen Sprague-Dawley 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.

Rats of 1, 6, 11, 16, 21, or 26 days of age were pretreated with the various microsomal enzyme inducers for 4 days. Phenobarbital sodium (PB; Merck and Company, Rahway, N. J.) was administered ip (50 mg/kg) in propylene glycol (5 ml/kg) to rats over 15 days of age. Rats less than 15 days of age were given 33 mg/kg of phenobarbital (3.3 ml/kg), because the higher dose decreased growth rate. Rats were also pretreated with spironolactone (S, 75 mg/kg; G.D. Searle and Company, San Juan, P. R.), pregnenolone-16α-carbonitrile (PCN, 75 mg/kg; The Upjohn Company, Kalamazoo, Mich.) or 3-methylcholanthrene (3-MC, 20 mg/kg; Eastman Kodak Company, Rochester, N. Y. in propylene glycol (5 ml/kg). Control rats were given propylene glycol (5 ml/kg). On the fifth day, [^{3}H]bilirubin was administered (30 mg/kg) into the distal portion of the femoral vein.

[^{3}H]bilirubin (unconjugated) was prepared by a modification of the method of Ostrow *et al.* (12). δ-Aminolevulinic acid hydrochloride (3,5-^{3}H (N)) (New England Nuclear, Boston, Mass.) was administered to anesthetized rats (urethane, 800 mg/kg ip) and bile was collected overnight in a test tube covered with aluminum foil and immersed in an ice bath. The [^{3}H]bilirubin was isolated from the bile as previously described (12). To determine if the ^{3}H excreted into bile after iv administration of

[1] This work was supported by funds from U.S. Public Health Service Grant AM 14513.

[2] The author was supported by U.S. Public Health Service Research Career Development Award GM 30996.

[^{3}H]bilirubin is representative of the amount of bilirubin excreted into bile, [^{3}H]bilirubin was administered to adult, anesthetized, bile-duct-cannulated rats. Bile was collected at 10, 20, 30, and 45 min after administration to control rats and rats that had been pretreated with microsomal enzyme inducers. The amount of bilirubin excreted into the bile quantitated by radiotracer techniques was found to be 99.8 ± 1.9% of that quantitated by the diazo colorimetric method (13).

Twenty-four hours after the last pretreatment with a microsomal enzyme inducer, [^{3}H]bilirubin was mixed with nonlabeled bilirubin (30 mg/kg) (K & K Laboratories, Plainview, N. Y.) and was prepared in an isotonic solution containing 0.5 g of Na_2CO_3 and 0.52 g of NaCl/100 ml of distilled water (10 ml/kg) and injected into the distal portion of the femoral vein. Twenty minutes later the rats were sacrificed and the small intestine was removed. As a measure of the biliary excretion of bilirubin, the small intestine was homogenized with 3 ml of saline in a Brinkman 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 ^{3}H in the small intestine of bile-duct-ligated and control rats 20 min after iv administration of [^{3}H]bilirubin; the concentration in bile-duct-ligated rats was 9.3% of control.

In one study, the amount of [^{3}H]bilirubin excreted into the small intestine was quantitated not only at 20 min after administration but also at 5, 10, and 30 min. In this study a blood sample was also taken by cardiac puncture under light ether anesthesia. Heparin was used as the anticoagulant. The rats were sacrificed and both the liver and small intestine were removed.

After centrifugation, aliquots of plasma (50–250 μl), intestinal homogenate (500 μl), and liver (approx 500 mg) were oxidized with a Packard Model 306 Tri-Carb sample oxidizer, and radioactivity was estimated with a Packard Model 3330 Tri-Carb liquid scintillation spectrometer (Packard Industries, Downers Grove, Ill.). Quenching was determined by using automatic external standardization. All values are expressed as milligram equivalents of bilirubin.

The data were compared by an analysis of variance. When the analysis indicated that a significant difference existed, the means of the treated groups were compared to the control mean by Student's *t* test (14).

Results and discussion. Figure 1 demonstrates the amount of ^{3}H excreted into the bile within 20 min after administration of [^{3}H]bilirubin to control and phenobarbital treated rats. Rats 15 days of age and younger were less efficient in excreting the bilirubin into the bile than were the older rats. Thus, the hepatic processes responsible for the biliary excretion of bilirubin are not mature in the newborn rat, similar to what has previously been shown for the guinea pig (6) and rabbit (7).

Pretreatment with phenobarbital tended to increase the biliary excretion of bilirubin (Fig. 1) but this was not statistically significant at any age. The ineffectiveness of phenobarbital in increasing the biliary excretion of bilirubin may be due to the relatively low dose administered due to its pharmacological and toxicological effects; rats less than 15 days of age were given 33 mg/kg and from 15 to 30 days of age, 50 mg/kg. In studies with adult rats, 75 mg/kg of phenobarbital is usually given.

Figure 2 depicts the effect of spironolactone and the hepatic disposition of bilirubin in rats of various ages. Pretreatment with spironolactone increased the biliary excretion of bilirubin in rats of all ages examined except the 30-day-old rats.

The effect of pregnenolone-16α-carbonitrile (PCN) on the development of hepatic excretory process is shown in Fig. 3. Rats at all ages pretreated with PCN excreted significantly more bilirubin into the bile than controls. Pretreatment of rats with 3-methylcholanthrene had no effect on the biliary excretion of bilirubin (Fig. 4).

While the first four figures demonstrate the effect of the four microsomal enzyme inducers on the amount of bilirubin excreted into the bile within 20 min of its administration, Fig. 5 depicts the plasma concentra-

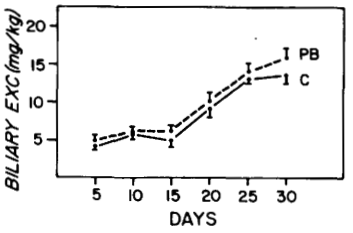

FIG. 1. The effect of pretreatment with phenobarbital (33 or 50 mg/kg) once a day for 4 days on the amount of bilirubin excreted into the intestine 20 min after administration (30 mg/kg iv) in rats of various ages. Each point represents the mean ± SE of three to eight rats.

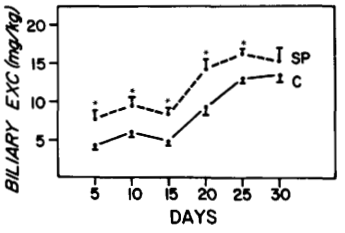

FIG. 2. The effect of pretreatment with spironolactone (SP, 75 mg/kg) once a day for 4 days on the amount of bilirubin excreted into the intestine 20 min after administration (30 mg/kg iv) in rats of various ages. Each point represents the mean ± SE of three to eight rats. Asterisks indicate the values that are significantly different from controls (C).

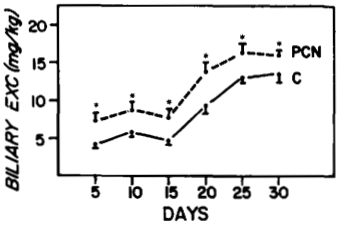

FIG. 3. The effect of pretreatment with pregnenolone-16α-carbonitrile (PCN, 75 mg/kg) once a day for 4 days on the amount of bilirubin excreted into the intestine 20 min after administration (30 mg/kg iv) in rats of various ages. Each point represents the mean ± SE of three to eight rats. Asterisks indicate the values that are significantly different from controls (C).

tion, the amount in the liver, and the amount excreted into the bile at various times after administration of the [^{3}H]bilirubin in 15-day-old control and PCN-pretreated rats. PCN increased the disappearance of bilirubin from the plasma. It also increased the amount in the liver at 5 and 10 min after administration. At the later time intervals no difference was observed, probably due to the larger amount excreted into the bile of the PCN-treated rats. No difference in the biliary excretion of bilirubin was observed at 5 min, but at the 10-, 20-, and 30-min time intervals the PCN-treated rats excreted more bilirubin into the bile than controls.

A definite difference in the ability of various microsomal enzyme inducers to enhance the plasma clearance and biliary excretion of bilirubin has been demonstrated. Why this difference exists and why the newborn is less efficient in clearing bilirubin is

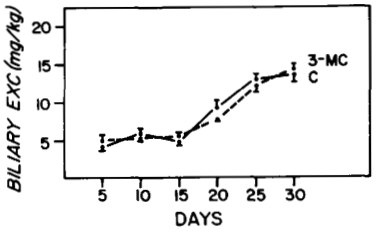

FIG. 4. The effect of pretreatment with 3-methylcholanthrene (3-MC, 20 mg/kg) once a day for 4 days on the amount of bilirubin excreted into the intestine 20 min after administration (30 mg/kg iv) in rats of various ages. Each point represents the mean ± SE of three to eight rats. C, control.

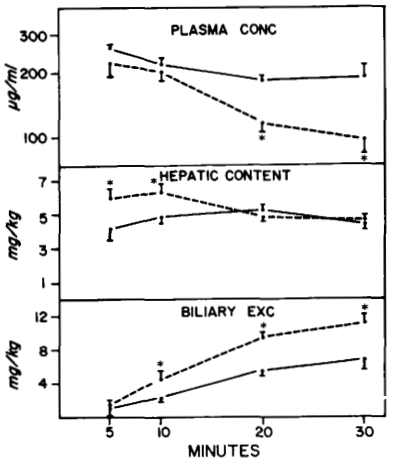

FIG. 5. Effect of PCN pretreatment (once a day for 4 days) on the hepatic disposition of bilirubin in 20-day-old rats. [^{3}H]bilirubin was administered iv (30 mg/kg). Each value represnets the mean ± SE of six to eight rats. Solid lines represent the controls and dashed lines represent the PCN-pretreated rats. Asterisks indicate the values that are significantly different from controls.

not clear. The low amount of ligandin in the newborn (15) has been suggested as an explanation. However, phenobarbital is more effective in increasing the amount of ligandin in the liver than is spironolactone and PCN (16) but it is less efficient in enhancing bilirubin clearance in the newborn. 3-MC has previously been shown to be a better inducer of hepatic bilirubin glucuronyltransferase activity than other microsomal enzyme inducers (17) yet it had no effect on the clearance of bilirubin in the newborn rat. However, spironolactone and PCN stimulate the excretion of exogenous chemicals to a greater extent than does phenobarbital, and 3-MC is without effect (18–20), which parallels the effect seen in newborn rats to bilirubin. Thus, indirectly, it would appear that the increased clearance of bilirubin after spironolactone and PCN in the newborn rat is probably more dependent on the increased excretion of bilirubin than it is on the amount of ligandin or glucuronyltransferase. Possibly the rate-limiting step in the clearance of bilirubin in the newborn rat is the transfer from liver to bile, as has been reported for the adult rat (21, 22).

Summary. The biliary excretion of administered unconjugated bilirubin in the newborn rat was demonstrated to be immature. Newborn rats were treated with microsomal enzyme inducers to determine whether they would enhance the overall hepatic excretory system responsible for the biliary excretion of bilirubin. 1-, 6-, 11-, 16-, 21-, or 26-day-old rats were pretreated for 4 days with phenobarbital, pregnenolone-16α-carbonitrile, spironolactone, or 3-methylcholanthrene. On the fifth day, [^{3}H]bilirubin was administered (30 mg/kg). Twenty minutes later the amount of bilirubin excreted into the intestine was determined. Spironolactone and pregnenolone-16α-carbonitrile enhanced the biliary excretion of bilirubin but phenobarbital and 3-methylcholanthrene were without effect.

The author wishes to acknowledge the technical assistance of Judson Maillie, Celeste Soutee, and Jean Fahrbach.

1. Trolle, D., Lancet 251 (1968).
2. Maurer, H. M., Wolff, J. A., Poppers, P. J., Finster, M., Pantuck, E., Conney, A. H., Lancet 122 (1968).
3. Trolle, D., Lancet 705 (1968).
4. Cunningham, M. D., Mace, J. W., Peters, E. R., Lancet 550 (1969).
5. Ramboer, C., Thompson, R. P. H., and Williams, R., Lancet 966 (1969).
6. Schenker, S., and Schmid, R., Proc. Soc. Exp. Biol. Med. **115,** 446 (1964).
7. Catz, C., and Yaffee, S. J., Pediat. Res. **2,** 361 (1968).
8. Klaassen, C. D., J. Pharmacol. Exp. Ther. **183,** 520 (1972).
9. Klaassen, C. D., J. Pharmacol. Exp. Ther. **184,** 721 (1973).
10. Hunter, A., and Klaassen, C. D., Drug Metab. Dispos. **3,** 530 (1975).
11. Klaassen, C. D., J. Pharmacol. Exp. Ther. **191,** 212 (1974).
12. Ostrow, J. D., Hammaker, L., and Schmid, R., J. Clin. Invest. **40,** 1442 (1961).
13. Weber, A. P., and Schalm, L., Clin. Chim. Acta **1,** 805 (1962).
14. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures in Statistics." McGraw-Hill, New York (1960).
15. Levi, A. J., Gatmaitan, Z., and Arias, I. M., N. Engl. J. Med. **283,** 1136 (1970).
16. Klaassen, C. D., J. Pharmacol. Exp. Ther. **195,** 311 (1975).
17. Potrepka, R. F., and Spratt, J. L., Biochem. Pharmacol. **20,** 861 (1971).
18. Klaassen, C. D., Biochem. Pharmacol. **19,** 1241 (1970).
19. Klaassen, C. D., J. Pharmacol. Exp. Ther. **191,** 201 (1974).
20. Klaassen, C. D., J. Pharmacol. Exp. Ther. **191,** 212 (1974).
21. Arias, I. M., Johnson, L., and Wolfson, S., Amer. J. Physiol. **200,** 1091 (1961).
22. Billing, B. H., Maggiore, Q., and Carter, M. A., Ann. N. Y. Acad. Sci. **111,** 319 (1963).

Received March 1, 1976. P.S.E.B.M. 1976, Vol. 153.