of choline to the deficient diet. 2. The hypothesis is advanced that these phenomena are reflections of an increased susceptibility to injury of the vessel wall, probably related to deposition of lipid, and that this condition may be an essential factor in the pathogenesis of hemorrhagic renal degeneration. 3. Blood pressure of formerly choline deficient rats tends to rise with time. It is suggested that this method of producing experimental hypertension be used more frequently, because of its simplicity and effectiveness.

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Enzymatic Defect in Metabolism of Bilirubin in Fetal and Newborn Rat. (23719)

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Hyperbilirubinemia and kernicterus can occur in the newborn in the absence of increased hemolysis of the erythrocytes(1-4). The incidence of this nonhemolytic hyperbilirubinemia is particularly high in the premature infant. A low rate of clearance of bilirubin in the liver due to some functional immaturity of that tissue may be responsible for the condition(5). Since bilirubin apparently cannot be cleared by the fetal liver, it has been suggested that this pigment may pass through the placenta and be carried to the mother's liver(6) or may be metabolized by the placental tissue itself(7). Bilirubin is excreted in bile as a conjugate with glucuronic acid (8,9). This conjugation takes place in the liver, to a lesser degree in the kidney(10), and possibly in the stomach and intestine(11). A scheme of the conjugation mechanism for glucuronides has been proposed(12): glucose-1-phosphate + uridine triphosphate ⟶ uridine diphosphoglucose $\xrightarrow{DPN}$ uridine diphosphoglucuronic acid (UDPGA) + aglycone ⟶ aglycone glucuronide. Although conjugation of *o*-aminophenol with glucuronic acid could be demonstrated in slices of adult rat liver(13,14), no appreciable conjugation was found in slices of fetal rat liver.

In a previous study using tissue homogenates, we demonstrated a defect in the conjugation of bilirubin in adult rats with constitutional nonhemolytic hyperbilirubinemia(15, 16). In the present study homogenates were again used to investigate the nature of the mechanism for synthesis of bilirubin glucuronide in tissue from prepartum and postpartum rats of various ages.

Methods. The homogenate system used was similar to that described previously(10). Forty micrograms of bilirubin suspended in an albumin solution were added to 100 mg of tissue homogenate in phosphate buffer. One ml of boiled extract of adult liver(17) (extract A) or of fetal liver from animals 5 to 7 days prepartum (extract B) was also added as a source of UDPGA. The mixture was incubated for 45 min. at 37°, then centrifuged in a Servall refrigerated centrifuge

TABLE I. Amounts of Conjugated Bilirubin Produced by Homogenates of Tissue from Adult and Fetal Rats (μg/100 mg Wet Wt Tissue).

	Adult				Fetal*		
Exp. No.	Liver	Kidney	Stomach and intes.	Placenta	Liver	Kidney	Stomach and intes.
1	6.3	2.4	.1	.0	.3	.0	.0
2	7.2	3.0	.0	.0	.0	.1	.0
3	6.7	2.2	.0	.0	.2	.1	.1

* Age of fetuses varied from 5 to 7 days prepartum.

for 5 min. at 10.000 g to remove excess proteins and to stop further enzyme action. "Zero-time" incubations served as the experimental controls. The amount of bilirubin glucuronide produced was determined after centrifugation by measuring the increase in "direct" reacting azo pigment in the supernatant, using the method of Ducci and Watson(18). All determinations were made in duplicate. For assay of β-glucuronidase 2.0×10^{-5} M phenolphthalein glucuronide was substituted for bilirubin as a substrate and incubated under the conditions described above. The hydrolysis of the phenolphthalein glucuronide was followed by measuring, in alkaline medium at 550 mμ, the free phenolphthalein released. Homogenates were prepared from the liver of Wistar rats, ranging in age from 10 days prepartum to 16 weeks postpartum. Similar homogenates were prepared of kidney, stomach and gastrointestinal tract from fetal animals 5 to 7 days prepartum, and of placenta from the mother. (A 21-day gestation period was assumed for the purpose of estimating the prepartum age of the animals.) Since the production of bilirubin glucuronide in homogenates is not entirely reproducible from day to day, parallel determinations of the conjugative activity in tissues from 4-month-old male rats were used as standards for each experiment. These values were taken to represent 100% synthesis.

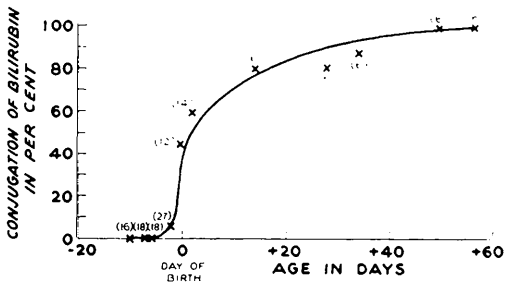

FIG. 1. Conjugation of bilirubin in liver homogenates from rats of various prepartum and postpartum ages. Conjugation of bilirubin in tissue from 4-mo-old adult rats was taken to represent 100%. Figures in parentheses indicate No. of animals.

Results. As seen in Fig. 1, no conjugation of bilirubin with glucuronic acid was detected in the liver from fetal rats up to 4 days prepartum. At about this time conjugative activity was first observed, and by the day of birth it had reached approximately 50% of that found in the liver of adult rats. The conjugative activity continued to increase at a slower rate until it reached the control level by 5 to 6 weeks. The shape of the curve was not markedly affected whether the activity was expressed on the basis of mg wet weight of the tissue or on the basis of μg of nitrogen.

The amounts of conjugated bilirubin produced by homogenates of various tissues from adult and fetal rats are compared in Table I. The conjugative activity in the kidney tissue of the adult rats was about $\frac{1}{3}$ that found in the liver. No activity could be detected in the kidney tissue of the fetal animals. Regardless of the age of the rats, homogenates of stomach, intestine, or placenta* showed no apparent synthesis of bilirubin glucuronide.

Fig. 2 shows the results obtained when fetal liver was used as a source of UDPGA. Enhancement of bilirubin glucuronide synthesis by fetal liver extracts was only about 10% that obtained with extracts from a corresponding amount of adult liver. Addition of fetal liver homogenates from animals 5 to 7 days prepartum to an incubation mixture containing adult liver did not inhibit the bilirubin conjugative activity of the latter tissues (Fig. 2).

* Identical results were obtained with homogenates of human placenta.

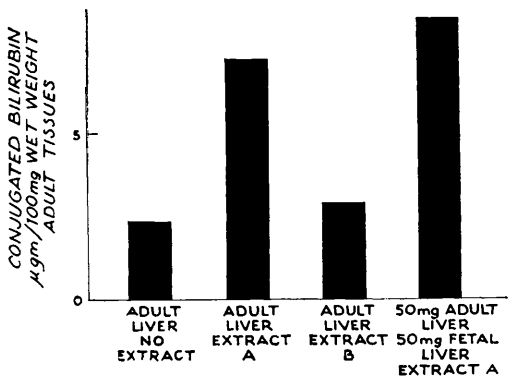

FIG. 2. Effect of homogenates and extracts of fetal liver on conjugation of bilirubin in homogenates of adult liver. Extract A: boiled adult liver, a source of uridine diphosphoglucuronic acid; Extract B: boiled fetal liver.

The addition of 10^{-3} M potassium saccharate to inhibit β-glucuronidase activity failed to influence the conjugative activity in the tissues studied. No hydrolysis of phenolphthalein glucuronide was observed under the conditions of the experiment.

Discussion. Despite the addition of adequate amounts of both bilirubin and UDPGA, no conjugation of bilirubin could be observed in homogenates of liver from fetal rats.† This finding supports the hypothesis that the activity of the enzyme responsible for the transfer of the glucuronic moiety to bilirubin (glucuronyl transferase) is defective in the fetal liver. The failure to observe conjugation of bilirubin in this homogenate system does not necessarily establish that glucuronyl transferase itself is absent. Nonetheless, the presence of inhibitors or absence of cofactors in the fetal tissue could not be demonstrated by the mixed homogenate technic.

Not only is glucuronyl transferase activity impaired, but apparently an earlier defect in glucuronide metabolism exists since the levels of UDPGA found in the fetus were only 1/10 that in the adult tissue. By contrast, in constitutional hyperbilirubinemia, another condition in which a defect in glucuronyl transferase activity has been demonstrated(16), UDPGA levels were normal(19). A number of defects early in the metabolic chain of glucuronide synthesis may exist in the immature liver of the fetus.

The presence of excessive amounts of β-glucuronidase could account for the failure to observe glucuronide production in fetal tissue. This seems improbable since potassium saccharate in amounts capable of inhibiting this enzyme(17) did not influence conjugation. Furthermore, no hydrolysis of phenolphthalein glucuronide was demonstrable.

The defects observed were not specific to fetal liver since conjugation of bilirubin could not be detected in homogenates of fetal kidney. Although Hartiala(14) reported that *o*-aminophenol is rapidly conjugated in slices of stomach and intestine from adult rabbits, we could not demonstrate bilirubin conjugative activity in homogenates of these tissues from adult or fetal rats. It seems unlikely that bilirubin is conjugated by the placenta (7) since no conjugative activity could be detected in homogenates of this tissue.

The finding that glucuronyl transferase activity is impaired in the fetal and newborn rat suggests that a similar lack may be responsible for the hyperbilirubinemia and kernicterus often observed in the premature infant. Although there is good evidence that bilirubin interferes with oxidative phosphorylation *in vitro*(20,21), the toxic effect of elevated bilirubin levels has not been definitely established(22). Steroids, hormones and various phenols(23,24) are normally inactivated and excreted as glucuronides. In the premature infant elevated levels of these substances resulting from impaired glucuronyl transferase activity might be responsible for some of the toxic symptoms observed in association with kernicterus.

Summary. 1. Homogenate preparations of liver and kidney from fetal rats failed to synthesize bilirubin glucuronides. The conjugative activity did not reach normal levels until the animal was 5 to 6 weeks old. 2. The levels of uridine diphosphoglucuronic acid were only 10% of those found in adult tissue. 3. Inhibition of beta glucuronidase with potassium saccharate did not stimulate the conjugation of bilirubin. Phenolphthalein glu-

† In an abstract which appeared during preparation of this manuscript, Lathe and Walker reported impairment of glucuronyl transferase activity for bilirubin conjugation in homogenates of human fetal liver (*Biochem. J.*, 1957, v67, 9P).

curonide was not hydrolyzed. 4. No conjugation was observed in homogenates of stomach, intestine or placental tissue. 5. The evidence presented suggests that glucuronyl transferase activity and the production of uridine diphosphoglucuronic acid are impaired in the newborn and the premature animal.

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Renal Clearance of Citric Acid in the Dog.* (23720)

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In alkalosis there is an increased excretion of citric acid(1). This is a report of the effect of administration of bicarbonate and citric acid or citrate upon renal clearance of citric acid.

Methods. All clearances were determined on trained conscious female dogs at least 15 hours after removal of food. With one exception the dogs weighed 16 to 31 kg. Catheterization and bladder washing were used. Generally, clearance periods began about 45 minutes after oral administration of Na or $KHCO_3$ and creatinine. In other cases, the periods were concurrent with intravenous injection of these compounds. Citric acid was determined by the method of Perlman *et al.* (2). Creatinine was determined with alkaline picrate. A total of 126 clearance periods were done on 42 different days on 10 dogs. Typical data are in the tables. Citric acid concentration in an ultrafiltrate of plasma, which was in equilibrium with 5.5% CO_2, was identical with that in plasma water. The amount of citric acid filtered was calculated as the product of creatinine clearance (C_{cr}) and plasma concentration of citric acid.

Results. Clearance of citric acid (C_{ca}) in the controls was less than 1 ml/min. in 28 of 40 determinations on 9 dogs. Of the 12 clearances greater than 1 ml/min., 7 were from dog C. The maximum for this dog was 15.1 ml/

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