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# Correlation between Fetal and Maternal Serum Bile Acid Concentrations

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**ABSTRACT. Serum concentrations of different bile acids (BA) were determined by radioimmunoassay in 56 human fetuses and mothers. Serum was obtained immediately after legal abortion, performed between the 14th and the 21st wk of gestation. Conjugated cholic (CCA) and chenodeoxycholic acid (CCDCA) concentrations were determined in 33 cases, conjugated lithocholic (CLCA) and deoxycholic acid (CDCA) in 20, and sulfolithocholyglycine (SLCG) in 15. In fetal blood, mean concentrations of CCA (0.80 ± 0.40 μmol/liter), CCDCA (4.50 ± 2.70 μmol/liter), and CLCA (1.70 ± 1.04 μmol/liter) were significantly higher than those in the mother (CCA 0.34 ± 0.17 μmol/liter; CCDCA 0.79 ± 0.34 μmol/liter; CLCA: 0.70 ± 0.30 μmol/liter; $p < 0.001$); fetal serum levels of CDCA (0.46 ± 0.32 μmol/liter) and SLCG (0.15 ± 0.09 μmol/liter) were lower than in the mothers (CDCA 1.20 ± 0.80 μmol/liter, $p < 0.001$; SLCG 0.40 ± 0.30 μmol/liter, $p < 0.01$). There was no correlation between levels of BA and gestational age. Serum total protein and albumin concentrations were both reduced in 10 fetuses as compared with the mothers. These data support the concept of a state of physiologic cholestasis during development and suggest that placental transfer of primary BA occurs mostly in the fetal to maternal direction. This transfer could be facilitated by the reduced fetal plasma albumin concentration, since BA in free solution diffuse more easily through the placenta. There is evidence of lithocholic acid synthesis in the fetal liver, while deoxycholic acid appears to be mostly of maternal origin. Finally, sulfation of BA is poorly developed at this age of gestation. (*Pediatr Res* 19: 227–231, 1985)**

**Abbreviations**

**BA, bile acids**
**CCA, conjugated cholic acid**
**CCDCA, conjugated chenodeoxycholic acid**
**CDCA, conjugated deoxycholic acid**
**CLCA, conjugated lithocholic acid**
**SLCG, conjugated sulfolithocholic acid**

We recently reported that the fasting serum concentrations of the two primary BA conjugates, CCA and CCDCA, are significantly elevated during the first few months of life (3). However, during the 1st yr of age, concentrations decrease to values similar to those of adults. This state of hypercholanemia, reflecting an immaturity of the enterohepatic circulation, has since been corroborated by other investigators using similar radioimmunoassays (4, 25, 27), and "physiologic cholestasis" is now recognized as a normal developmental condition. The factors responsible for physiologic cholestasis have not yet been completely defined in the human infant; data obtained in animal models have documented the immaturity of several of the processes involved in the enterohepatic circulation of BA (hepatic uptake, conjugation, excretion, and intestinal absorption) (5, 10, 23, 24, 26). However, the presence of marked species differences, as has been well demonstrated for other fetal hepatic excretory mechanisms, makes it difficult to extrapolate these data to the human infant. Data concerning metabolism and placental transfer of BA in the human fetus are extremely limited.

The aim of this study was to determine the relationship between fetal and maternal serum BA concentration and compo-

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sition. By the analysis of these data, we hope to elucidate some aspects of fetal BA metabolism in early gestation.

## MATERIALS AND METHODS

Fifty-six fasting maternal peripheral and fetal umbilical blood samples were simultaneously collected immediately after legal voluntary abortion, performed through suction, between the 14th and the 21st wk of gestation, because of psychosocial indications. Fetal age was estimated by clinical history, as well as by means of ultrasound. All the pregnancies were uncomplicated until the moment of termination; none of the mothers had evidence of gastrointestinal or hepatic disease. The serum concentrations of CCA, CCDCA, and CLCA were determined using specific radioimmunoassays, as described previously (18, 19). Due to limitations in fetal sample size, CCA and CCDCA levels were determined in only 33 cases and CLCA levels in 20 cases.

These assays proved to be accurate and precise; the sensitivity was of the order of 1 pmol/tube, *i.e.* 0.05 μmol/liter for all of them. The antisera for primary BA were specific for both glycine and taurine conjugated forms (100% cross-reactivity), showing minimal cross-reaction with the unconjugated compounds (less than 2%) and no cross-reaction with other BA. The antiserum for conjugated lithocholic acid also was specific for both glycine and taurine conjugates (100% cross-reactivity) and showed a 25% cross-reactivity with unconjugated lithocholate; neither sulfated lithocolate, nor other BA, were seen to cross-react significantly with the same antiserum (less than 2%).

Serum concentration of SLCG was measured in 15 cases, using a commercial Kit (SLCG-RIA, Abbott Laboratories, North Chicago, IL). This radioimmunoassay is highly sensitive (0.04 μmol/liter) and specific for sulfated conjugates of lithocholate (sulfolithocholytaurine and sulfolithocholyglycine); there is no cross-reactivity with other sulfated or nonsulfated BA (such as 3 β-hydroxy-5 cholenoic acid) or with other steroidal compounds (6).

Serum levels of CDCA were measured in 20 maternal and fetal sera, using a special preparation of a previously available Kit (Nordic Lab., Oulo, Finland) (15). Its sensitivity is of the order of 2 pm/tube, *i.e.* 0.1 μmol/liter. The antiserum recognizes not only deoxycholic acid conjugates and unconjugated deoxycholic acid (100% cross-reactivity), but also, to some extent, unconjugated and CLCA (30–50% cross-reactivity). Thus, serum concentrations of deoxycholic acid may be somewhat overestimated.

In 10 paired samples (fetal and maternal) we also determined total serum protein and protein electrophoresis, with conventional techniques.

Statistical evaluations were done using the Student's *t* test for paired data.

## RESULTS

The levels of the five BA (CCA, CCDCA, CDCA, CLCA, SLCG) in maternal and fetal serum are shown in Figure 1. Mean concentrations of CCA (0.80 ± 0.40 μmol/liter), CCDA (4.50 ± 2.70 μmol/liter), and CLCA (1.70 ± 1.04 μmol/liter) in fetal blood were significantly higher than those in maternal blood (CCA 0.34 ± 0.17 μmol/liter; CCDCA 0.79 ± 0.34 μmol/liter; CLCA 0.70 ± 0.30 μmol/liter; $p < 0.001$). CCDCA was the predominant BA in umbilical blood and its concentration showed the greatest difference between fetus and mother. In six of 20 fetuses, CDCA was not detectable in serum; in the other 14, the mean concentration of CDCA (0.46 ± 0.32 μmol/liter) was significantly lower than that observed in the mothers (1.20 ± 0.80 μmol/liter; $p < 0.001$). SLCG mean serum concentration was also lower in the fetuses (0.15 ± 0.09 μmol/liter) as compared with the mothers (0.40 ± 0.30 μmol/liter; $p < 0.01$). Sulfated lithocholate accounted for only 8% of total lithocholate (sulfated plus nonsulfated) present in umbilical blood; this is in contrast with almost 40% of the lithocholate being sulfated in maternal serum.

Total and fractional serum protein concentrations in fetuses and mothers are shown in Table 1. Total protein levels were significantly lower in the fetuses compared with the mothers ($p < 0.001$). The mean plasma albumin concentration was also significantly reduced in the fetus, compared with the mother ($p < 0.001$). Albumin accounted for about 75% of total fetal protein in serum.

None of the BA showed any significant correlation with gestational age, at least in the narrow range of gestation, which has been considered in this study (Fig. 2).

## DISCUSSION

The results of our study support the concept of physiologic cholestasis during fetal and neonatal life: in fact, we have found that between the 14th and the 21st wk of gestation, serum concentrations of CCA, CCDCA, and CLCA in umbilical blood are somewhat higher than those in maternal blood. Previous studies, made in premature and term neonates, have shown that at birth, cord blood primary BA levels are either slightly higher or equal to maternal levels, but immediately following birth they increase markedly (3, 4, 9). The comparison between serum primary BA concentrations *in utero* and those found postnatally would suggest that, in the human fetus, there is a rather minimal enterohepatic circulation of BA, probably until stimulated by feeding postnatally.

The higher serum concentrations of primary BA in the fetus than in the mother also suggest that their placental transfer occurs mostly in the direction of fetus to mother. Quantitative information concerning placental exchange of BA has been obtained in the fetal lamb (8–20) and dog (12): also in these species, the placental transfer of CCA and CCDCA occurs from fetus to mother, with a very limited mother to fetus exchange. Therefore, primary BA in fetal serum may represent at least in part fetal BA synthesis. These synthesis mechanisms must be reasonably well developed in the period of gestation considered in this study.

Levels of a secondary BA, CDCA, were found to be lower in the fetus than in the mother. It has been demonstrated, in the fetal animal, that there is no synthesis of deoxycholic acid from cholesterol or cholic acid (14). This BA is a bacterial metabolite of cholic acid: the fetal steril gut may not produce it and therefore CDCA found in fetal serum is presumably of maternal origin. Our results are in agreement with those recently obtained from the analysis of serum BA pattern in the newborn and mother at birth: CDCA was found in maternal blood, but it was absent or present in very low amount in cord blood (11). Moreover, the fetal to maternal exchange of this BA has been demonstrated to be minimal in the sheep (21).

Lithocholic acid is generally considered to be a secondary BA (and would be expected to show a similar behavior to CDCA), but under certain conditions, it may be a primary BA. During fetal and neonatal life, synthesis of BA has been postulated to occur via an alternate pathway, which has lithocholic acid and 3 β-hydroxy-5 cholenoic acid as intermediates (16–28). There appears to be synthesis via the usually accepted pathway as well, however, the alternate pathway seems to be of major importance in fetal life (13). Therefore, lithocholic acid should be synthesized in the fetal liver. Our results support this speculation, showing that serum concentrations of CLCA are higher in the fetus than in the mother, as it has been shown for the two primary BA. This suggestion is also supported by the fact that serum concentrations of CCDCA tend to be much higher than CCA in the fetus: in fact, the alternate pathway for BA synthesis leads predominantly to the formation of chenodeoxycholic acid.

SLCG was found to be in lower concentrations in the fetus, compared with the mother. This finding and the elevated levels of unsulfated lithocholic acid may indicate that sulfation capacity is not sufficiently developed in fetuses of the gestational age

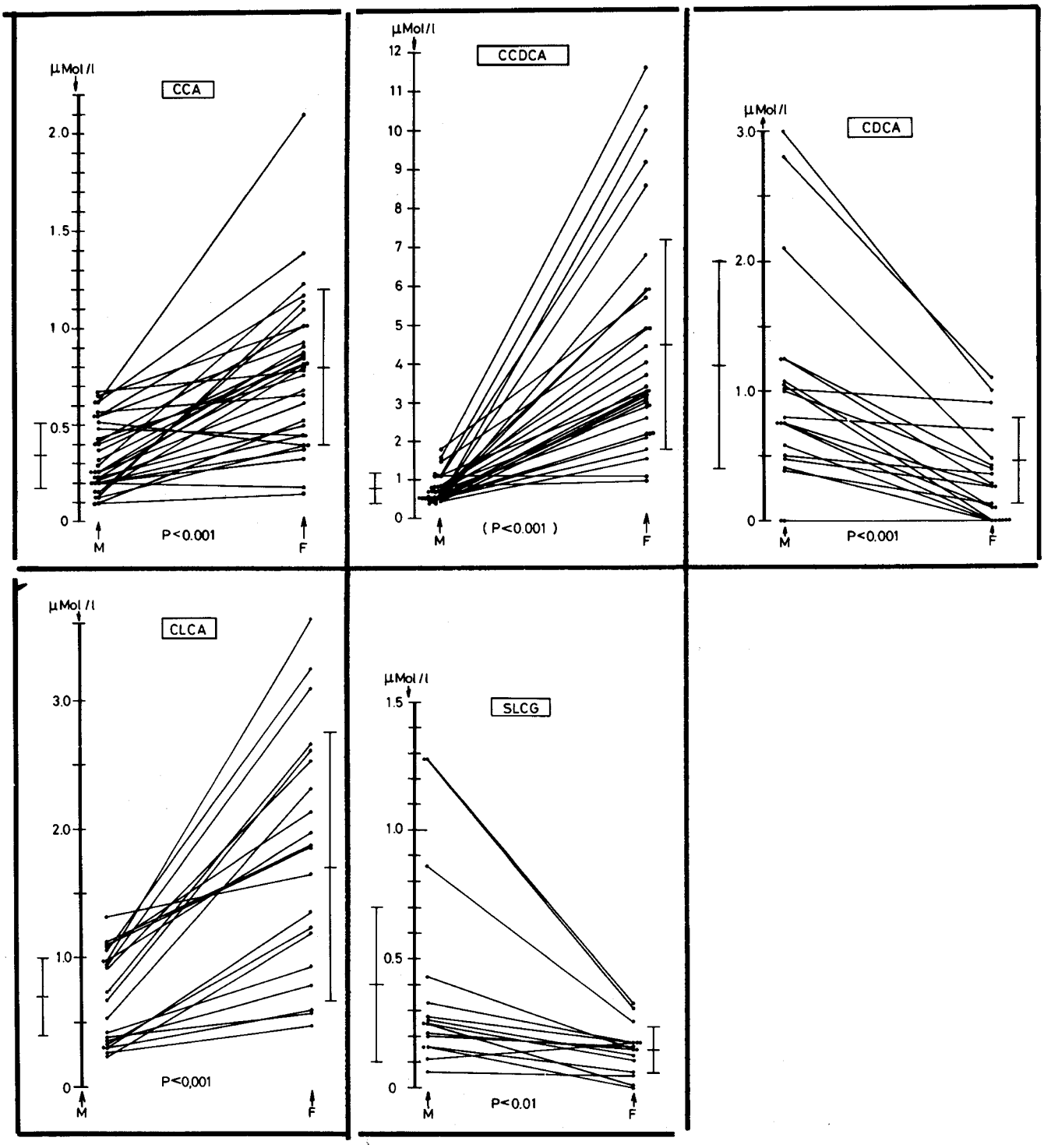


Fig. 1. Serum BA concentrations in mother (*M*) and fetus (*F*). For abbreviations, see text. Mean value ± 2 SD are shown.

Table 1. *Protein concentration (mean values ± SD) in fetal and maternal serum*

| $n = 10$ | Total protein (g/100 ml) | Albumin (g/100 ml) | Globulins (g/100 ml) | | | |
|---|---|---|---|---|---|---|
| | | | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ |
| Fetuses | 2.53 ± 0.09 | 1.88 ± 0.02 | 0.14 ± 0.02 | 0.09 ± 0.05 | 0.23 ± 0.07 | 0.15 ± 0.05 |
| Mothers | 6.31 ± 0.29 | 3.00 ± 0.34 | 0.34 ± 0.005 | 0.69 ± 0.01 | 0.86 ± 0.05 | 1.35 ± 0.08 |
| | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ |

considered in our study. Watkins *et al.* (29) found that taurolithocholate sulfotransferase is present in low amounts in the liver of fetal guinea pigs, lambs, rabbits, and humans. More recently, fetal levels of hepatic sulfotransferase have been shown to be very low in rats and a progressive increase in its activity during the first 3 wk of life has been documented (2). The very low levels of SLCG in the fetus also could be explained by the physical chemical characteristics of this compound; it is very polar and

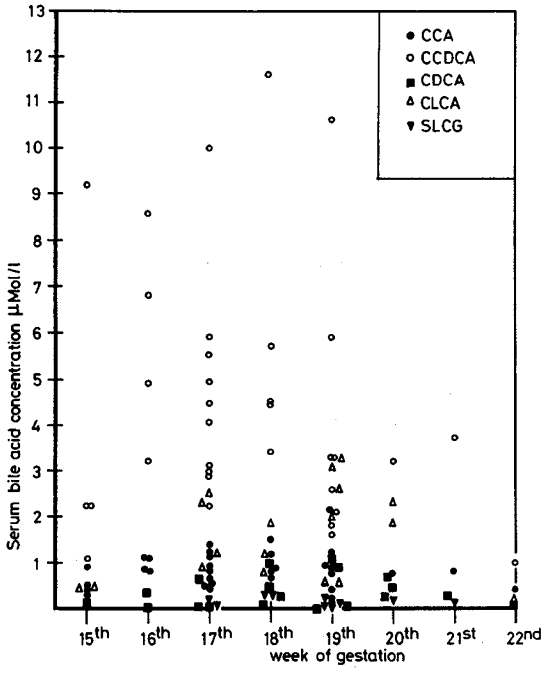


Fig. 2. Relationship between serum levels of different BA and gestational age.

in ionized form, and placental transfer (which is assumed to be by passive nonionic diffusion) of SLCG would likely be impaired (30).

Several other points are to be considered in the elucidation of fetal serum BA homeostasis. First, the anatomical differences in the enterohepatic circulation of the fetus (as compared to the adult) could contribute in determining elevated serum BA levels. Shunting of fetal blood via the ductus venosus may result in a less efficient perfusion of hepatocytes by portal blood and in BA spill over into the systemic circulation.

Second, an immaturity of hepatic BA transport mechanisms has been postulated to be present during development. A progressive maturation has been well demonstrated for hepatic BA uptake *in vitro*: the uptake of taurocholate by isolated hepatocytes of the rat is impaired in the first days of life (24) and increases progressively. Constant affinity of liver membrane binding sites with increased uptake suggests a higher number of transport or binding sites with age. Thus, also in fetal life, the immaturity of BA hepatic uptake may in part explain the higher fetal (as compared to maternal) serum BA concentrations.

Third, the qualitative and quantitative interaction of BA with serum albumin in both fetal and maternal compartments must be taken into account. The different degree of affinity of each BA to albumin, for example, could facilitate placental exchange for a specific BA, as compared to others. chenodeoxycholic acid has a greater affinity for albumin as compared to cholic acid, which is the weakest bound BA (17). This would explain a better equilibrium of cholic acid concentrations between the fetal and maternal serum compartments. Conversely, CCDCA would tend to accumulate in fetal serum, as it would be exchanged less easily.

The relative availability of albumin in both fetal and maternal serum is likely to affect the entity of the placental exchange of BA, as protein unbound BA would diffuse more easily through the placenta. A greater proportion of glycocholate has been found to be free in solution in fetal blood than in the adult (22). This finding can be at least in part explained by the reduced fetal concentration of serum albumin found in our study. Therefore, fetal hypoalbuminemia would appear to be a factor facilitating placental excretion of BA and may be advantageous to the fetus.

In the same way, hypoalbuminemia may facilitate fetal hepatic uptake of BA, since the uptake rate is assumed to be directly determined by the plasma concentration of the free BA. On the other hand, it has been recently hypothesized that BA hepatic uptake may involve binding of complex to a specific cell surface membrane receptor for albumin; the association with protein would in fact enhance the presentation of BA to their carrier on the cell surface. Therefore, uptake would be at least in part determined by the concentration of albumin bound, rather than free BA (7). In this way, hypoalbuminemia may hinder binding and hepatocyte uptake in the fetus. However, whether this mechanism is of some importance on BA uptake by the immature hepatocyte, remains to be established. A more general deficiency of receptors and/or carriers for BA on the liver cell membrane surface, could probably better explain the immaturity of hepatic uptake in fetal and neonatal life.

Since we did not find a significant correlation between fetal serum levels of the different BA and gestational age, a significant maturation of BA hepatic uptake and secretion does not seem to occur between 14 and 21 wk gestation. Therefore, the event or events modulating this maturation must occur later in gestation and/or postnatally, with the beginning of oral and enteral feeding.

This hypothesis is supported by recent observations that only in the newborn at term, but not in the premature infant, does the presence of milk in the gut initiate the release of several gastrointestinal and pituitary hormones. These hormones, in turn, would stimulate developmental changes in the intestinal, pancreatic, and hepatic structure and function (1). Therefore, future studies must be carried out to define the influence of diet and different hormones on the development of enterohepatic circulation of BA after birth and in early life.

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# Effect of Postnatal Anoxia on Bilirubin Levels in Rat Brain

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**ABSTRACT. The effects of a period of anoxia 18–24 h after birth on bilirubin levels in rat brain were investigated during anoxia, recovery, and development. Postnatal anoxia induces a significant, temporary increase (up to 200% with respect to control values) in newborn rat brain bilirubin levels during anoxia and short-term recovery. Pretreatment of the newborn rats with a single dose of the drug sulfixosazole markedly enhances bilirubin accumulation in the brain of the anoxic rats. A second rise in brain bilirubin concentration is detected in a group of the newborn rats 3–6 days after oxygen deprivation. Autoradiographic localization of radiolabeled bilirubin following *in vivo* experiments suggests that this substance is preferentially accumulated in some areas of the newborn rat brain as a consequence of postnatal anoxia, and indicates, together with the effect of sulfixosazole, that as a result of anoxia, a displacement of unbound bilirubin from blood to the nervous tissue occurs. Our results confirm the importance of anoxia as a risk factor for the development of bilirubin-induced encephalopathy. The possible relevance of intracerebral hemorrhages caused by perinatal asphyxia producing delayed bilirubin accumulation in the newborn rat brain is suggested. (*Pediatr Res* 19: 231–236, 1985)**

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Despite the current development of neonatology and the use of exchange transfusion and phototherapy, kernicterus continues to occur, specially in sick, premature, or low birth weight babies, as has been recently pointed out by different reports (20, 31, 36). Despite the various diagnostic and therapeutic measures intended to prevent bilirubin encephalopathy, at present no blood chemistry value (including bilirubin concentration) or clinical factors can completely predict the risk of kernicterus.

Perinatal asphyxia, together with prematurity, acidosis, respiratory distress syndrome, and the use of certain drugs have been reported to be the major predisposing risk factors for the occurrence of bilirubin encephalopathy in newborn infants with low levels of serum bilirubin (1, 6, 15, 20, 43). These clinical reports, together with the fact that the neurological damage associated with perinatal asphyxia or anoxia shows brain regional distribution and clinical manifestations somewhat similar to those observed in bilirubin encephalopathy (9, 27, 29), have prompted us to investigate the relationship between episodes of lack of oxygen in the postnatal period and the accumulation of bilirubin in the brain.

## MATERIALS AND METHODS

*Chemicals.* 1′, 2, 3′, 4, 5, 6′, 7′, 8 [$^{14}$C]bilirubin (62.5 mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks, UK. Sulfixosazole and bilirubin were purchased from Sigma Chemical Co., St. Louis, MO. All other reagents used were of the highest purity available.

*Animals.* Dated pregnant rats of the Wistar strain weighing