Bilirubin Metabolism in the Fetus

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A B S T R A C T Bilirubin metabolism was studied in dog
and monkey fetuses. Bilirubin-^{3}H was administered to
fetal animals *in utero* by prolonged intravenous infusion.
Fetal plasma disappearance, hepatic uptake, biliary ex-
cretion, and placental transfer of bilirubin-^{3}H were
measured.

Bilirubin metabolism and excretion in the fetus was
much less efficient than in the adult. Fetal plasma levels
of tritium were elevated for prolonged periods, and the
combined rate of placental and fetal hepatic excretion
was lower than normal values for adult hepatic excre-
tion. Species differences were noted. Hepatic conjuga-
tion and excretion appeared to be the primary mecha-
nism of fetal metabolism in the dog. In contrast, the
amounts of conjugated bilirubin-^{3}H excreted in fetal
monkey bile were negligible. Small amounts of ^{3}H-la-
beled bilirubin derivatives were excreted in fetal bile,
but 10 times as much of the administered material was
transferred intact across the placenta and excreted by
the maternal liver. The relationship of this functional
difference to known anatomic and biochemical species
differences is discussed. Preliminary observations on
alternate routes of fetal bilirubin metabolism were
obtained.

INTRODUCTION

The characteristics of bilirubin metabolism in the fetus
have been the subject of controversy. The importance of
the placenta as a site of fetal bilirubin excretion has been

emphasized by some but downgraded by others (1–6).
The possibility of major species differences in fetal bili-
rubin metabolism has been discussed briefly but largely
ignored. It is generally accepted that fetal liver has little
capacity to eliminate bilirubin as such, but the possi-
bility of alternate paths of bilirubin degradation in the
fetus is unexplored. Much of our present knowledge
about fetal bilirubin metabolism is based on short-term
studies performed on animals in the immediate post-
operative period. Almost invariably, the administration
of bilirubin and sampling of fetal plasma has involved
the manipulation of placental blood vessels. Most of the
previous investigations have been performed on a single
species of animal, with the use in each case of differing
surgical techniques. Comparisons between studies have,
therefore, proven difficult.

In the present investigation fetal bilirubin metabolism
was studied in both pregnant dogs and monkeys. New
intrauterine operative techniques were employed which
permitted the performance of prolonged studies 1–4 days
after the completion of experimental fetal surgery. De-
tailed information on the physiologic status of the fetus
was obtained. The disappearance of bilirubin from the
plasma, its transfer across the placenta, and its excretion
by fetal liver were measured over periods as long as 12
hr. Preliminary studies were performed on the existence
of potential alternate paths of fetal bilirubin metabolism.
The results establish that over a wide dosage range, the
fetus excretes bilirubin with much less efficiency than
the adult. Moreover, major species differences exist in
the mechanism of fetal bilirubin excretion.

METHODS

Surgical preparation. Experiments were conducted in two
species, dogs and monkeys. The pregnant dogs were pedigree
beagles and were bred specifically for research. They were
worm free and had been vaccinated against canine distemper
and hepatitis. They ranged in age from 1 to 3 yr. Experi-
ments were conducted in dogs between the 54th and 58th
days of pregnancy (total gestational period 63 days). Preg-
nant rhesus monkeys (*Macaca mulatta*) of recent capture
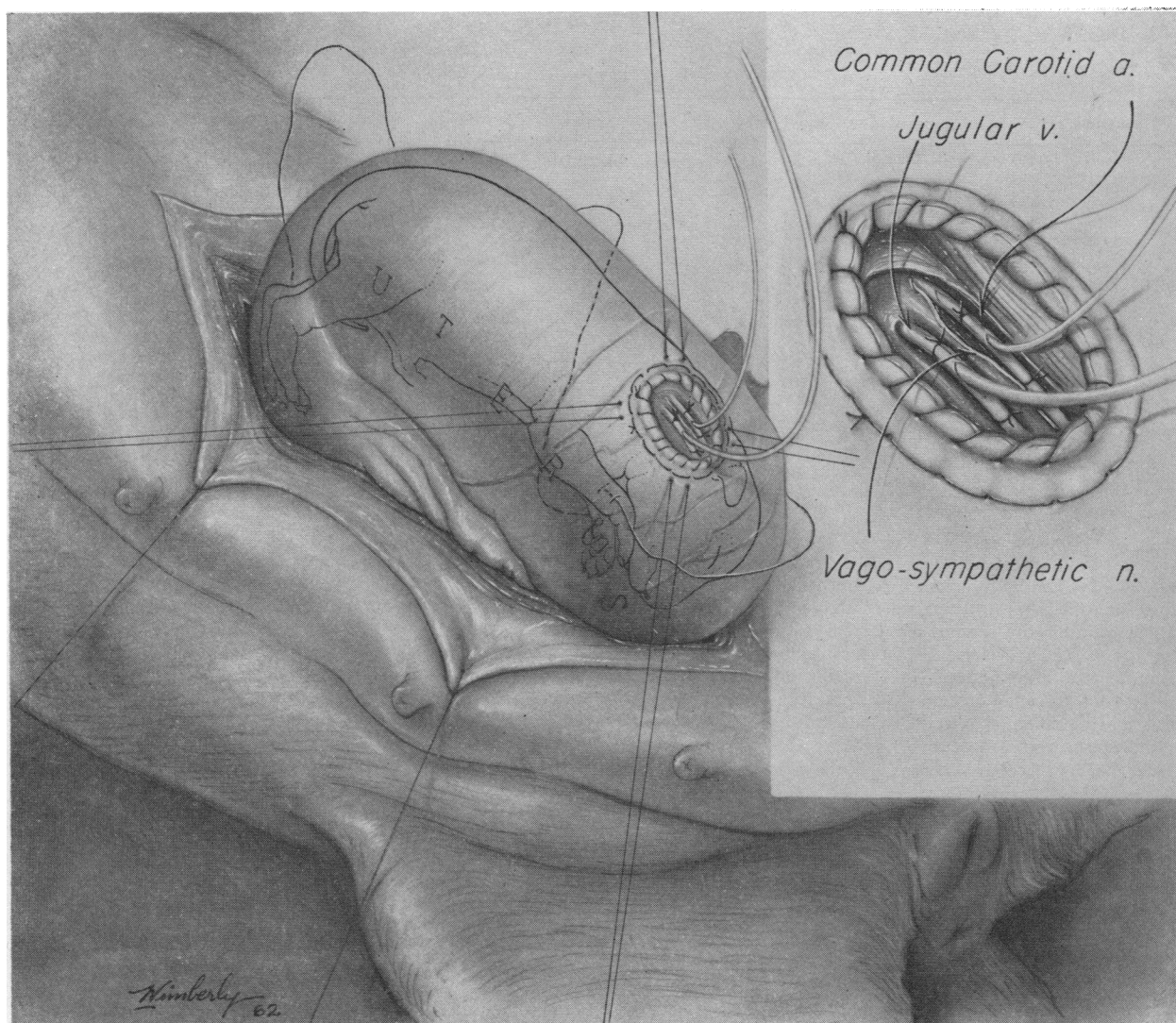were obtained through licensed dealers and were studied

FIGURE 1 Representation of the methodology of the experimental canine preparation. Infusions were administered through the jugular catheter, and blood samples were obtained through the carotid catheter. The fetus with catheters inserted floated free *in utero*, and the placental vessels were undisturbed.

during the last month of pregnancy (gestational period 165 days). The anesthetic halothane (2-bromo-2-chloro-1,1,1-tri-fluoroethane) was administered in a nonrebreathing system with an intermittent positive pressure respirator. Induction of anesthesia was obtained in both species with small intravenous doses of sodium thiamylal (Surital Sodium). The monkeys were initially sedated with intramuscular phencyclidine hydrochloride (Sernylan).

In dogs, a large midline laparotomy incision was performed, the maternal cystic duct was ligated, and a polyvinyl catheter (ID 0.053 inches, OD 0.085 inches) was placed in the common bile duct. Small, harpoon-like electrocardiogram (ECG) electrodes were implanted directly through the uterus, in the two forelegs and in one hind leg of a fetus (7). A marsupializing incision was made in the cervical region of the fetus, according to the method of Jackson and Egdahl

(8) (Fig. 1). This approach allowed the fetus to remain *in utero* throughout the operative procedure and prevented loss of amniotic fluid. The jugular vein and carotid artery were exposed, and a silicone rubber cannula (ID 0.025 inches, OD 0.047 inches) was inserted approximately 2 cm into the vein, and a molded polyethylene cannula (PE 50, ID 0.023 inches, OD 0.038 inches) was implanted in the artery. Fetal and uterine incisions were closed, and the fetus was released from its attachment to the uterus. Maternal biliary and fetal venous and arterial cannulas and fetal ECG electrodes were exteriorized through the maternal incision, which was then closed. The fetal ECG was monitored and heart rate noted, and all cannulas and wires were covered with a protective dressing. The biliary cannula was introduced into a collection tube attached to the dog.

Experiment	Animal	Fetal weight	Amount	Specific activity	Duration of infusion	Duration of study
		g	µg	dpm/µg	hr	hr
D-1	Dog	231	189	13,500	6	10
D-2	Dog	250	163	13,500	6	10
D-3	Dog	210	167	44,600	6	10
D-4	Dog	236	33	2,300	6	10
D-5	Dog	275	123	15,000	1/12	4
D-6	Dog	275	4300	2,000	6	10
D-7	Dog	208	2800	2,000	6	10
D-8	Dog	263	3450	2,100	6	10
D-9	Dog	323	156	2,300	1/12	9
M-1	Monkey	287	125	22,900	2	6
M-2	Monkey	269	239	22,900	6	10
M-3	Monkey	309	91	53,800	6	10

In monkeys, the fetal operations were performed in much the same fashion as in dogs. A modification of the marsupializing incision was utilized, and jugular vein and carotid artery cannulas of similar dimensions to those used in the dog fetus were inserted.[1] Placement of the maternal biliary cannula was accomplished on the day of the experiment (3–4 days after fetal surgery). A right subcostal incision was used for the common bile duct cannulation so that exposure of the uterus was avoided.

Experimental design in dogs. 24 hr after the completion of surgical preparation as described above, pregnant dogs were anesthetized lightly with halothane. (Ether anesthesia was used in one instance, fetus D-1.) Bilirubin-³H, prepared biosynthetically from Δ-aminolevulinic acid-3,5-³H (9) and dissolved in 2.5 ml of dog serum, was administered to the fetus via the indwelling jugular catheter with a calibrated Harvard infusion pump. Thereafter, fetal blood samples were obtained at intervals through the indwelling carotid catheter, and maternal bile was collected from the external biliary fistula. At the conclusion of each experiment the fetus and mother were autopsied, and it was ascertained that all catheters were appropriately positioned. Fetal plasma, organs, gal bladder bile, allantoic fluid, amniotic fluid, and maternal liver were collected. All specimens were protected from light during collection and stored at −15°C until analysis.

In four studies, 33–189 µg (specific activity 2300–44,600 dpm/µg) of bilirubin-³H were infused into a dog fetus at a constant rate over 6 hr, and sample collections were continued for an additional 4 hr (D-1 to D-4, Table I). The amounts of bilirubin infused represent from less than 1/18 to less than 1/3 the estimated values for fetal bilirubin production during the period of infusion (estimation based on established rates of production in adults [10]). Therefore, these studies are referred to as "low dose" investigations.

Between 2.8 and 4.3 mg (specific activity 2000–2100 dpm/µg) of bilirubin-³H were infused intravenously into a dog fetus in three additional experiments (D-6 to D-8, Table I). Bilirubin disposition in these experiments, there-

fore, occurred in the presence of an excessive pigment load, and they are referred to as "overload" investigations.

Bilirubin-³H was administered by rapid intravenous infusion to a fetus (D-5, Table I) prepared as described above. This experimental modification was introduced primarily to allow comparison with other reported investigations. Since the results did not differ substantially from those obtained with constant infusions, and since the latter more closely approximate physiologic conditions, studies with rapid injection were not repeated except as noted below.

The *rate* of fetal hepatic bilirubin-³H excretion was measured directly in one fetus (D-9, Table I). In a fetus *in utero* the cystic duct was ligated, and a cannula was inserted into the common bile duct and exteriorized. External fetal bile flow was maintained for 48 hr postoperatively and for an additional 9 hr during and after the rapid infusion of 156 µg of bilirubin-³H. Although this study has not been repeated, the results are reported since the surgical preparation and the direct measurement of rates of fetal bile flow and pigment excretion are unique.

Experimental design in monkeys. Bilirubin metabolism in the monkey fetus was studied in three experiments. One fetus received 125 µg of bilirubin-³H intravenously over 2 hr (M-1, Table I), while the other two received 91 and 239 µg over a 6 hr period. Investigations of bilirubin metabolism were performed 3–4 days after the completion of fetal surgery, but maternal bile duct catheterization was performed on the day of study as noted above. In other respects, the conditions of the studies and the methods of specimen collection were identical with those described above for pregnant dogs.

Analytical methodology. During experiments, standard limb lead, fetal ECG's were obtained intermittently, and a single lead was monitored continuously, utilizing the electrodes implanted in the fetus and a Sanborn direct-writing recorder. Fetal arterial blood pressure was determined with a Sanborn pressure transducer and recorder. Arterial blood samples were collected in capillary tubes and analyzed for pH, P_{CO_2}, and P_{O_2} using an Instrumentation Laboratory pH/Gas Analyzer, model 113. Plasma volume was determined in three fetal dogs using Evans blue dye (11).

Bilirubin concentrations in bile and plasma were determined by a micromethod modification of the technique of

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[1] Novy, M. J., G. J. Piasecki, and B. T. Jackson. Study of cardio-respiratory function in the intact monkey fetus using chronically implanted cannulas. Manuscript in preparation.

TABLE II

Physiologic Data Obtained from Fetal Preparations

Experiment	pH*		Pco₂*		Po₂*		Heart rate		Blood pressure	
	Start	End	Start	End	Start	End	Start	End	Start	End
			mm Hg		*mm Hg*				*mm Hg*	
D-1	—	—	—	—	—	—	195	165	—	—
D-2	7.34	7.33	—	48	—	30	165	115	55/35	46/27
D-3	7.46	7.41	28	35	22	20	180	175	56/33	56/35
D-4	7.40	7.41	34	32	23	26	220	150	46/28	43/24
D-5	7.05	6.95	54	56	11	13	210	225	—	—
D-6	7.24	7.23	42	44	24	23	180	150	—	—
D-7	—	—	—	—	—	—	180	200	—	—
D-8	7.30	7.27	34	30	24	15	180	140	46/38	39/32
D-9										
M-1	—	7.20	—	—	—	—	165	150	—	—
M-2	7.28	7.25	41	43	15	17	170	165	45/36	43/34
M-3	7.30	7.21	45	48	15	13	180	165	45/36	42/30

* Blood samples obtained from carotid artery.

Malloy and Evelyn (12). Crystalline bilirubin was prepared from bile samples by the method of Ostrow, Hammaker, and Schmid (13). Fetal bile samples were reacted with diazotized 2,4-dichloroanaline (14). The resultant diazo derivatives of fetal and maternal bile pigment and of standards prepared from crystalline unconjugated bilirubin in rat bile were extracted into ethyl acetate and resolved on silica gel G by thin-layer chromatography in the following solvent systems: system I, methyl ethyl ketone: propionic acid: water (75: 25: 35) (15); system II, isoamyl acetate: propionic acid: n-propanol: water (4:3:2:1) (16).⁸ Chloroform extracts of acidified bile, urine, and homogenized intestine were examined in a Beckman DB spectrophotometer. These extracts were then chromatographed in systems I and II described above. Fetal and adult dog liver homogenates were assayed for o-aminophenol glucuronyl transferase activity by the method of Levvy and Storey (17).

Radioactivity in bile, serum, urine, allantoic fluid, and amniotic fluid was assayed in a Packard Tri-Carb liquid scintillation spectrometer as described previously (18). Solid tissues including liver, intestine, spleen, gall bladder, kidney, lung, heart, and brain were homogenized, lyophylized, combusted in a Thomas-Ogg oxygen flash ignition chamber, and counted by established methods (19). The specific activity of bilirubin crystals was determined (18). Thin-layer plates used for the characterization of diazo derivatives of bilirubin, and bile and tissue pigments were sectioned, scraped into counting vials, and counted by techniques described previously (20).

RESULTS

Effects of experimental manipulation on fetal physiologic processes. Nearly all of the fetal preparations remained in excellent condition during the course of study (Table II). Fetal arterial blood pressure remained stable. Fetal heart rate showed a moderate tendency to slow

under the influence of anesthesia, but rates almost invariably in excess of 140 were recorded throughout the experiments. Determinations of fetal arterial blood pH, Po₂, and Pco₂ were obtained in eight fetal preparations. As can be seen in Table II, these values remain constant in five of six detailed studies in dogs and in two detailed studies in monkeys. In animal D-5 (see Table II) apparent metabolic acidosis developed, coincident with the onset of the early stages of labor. Fetal electrocardiographic tracings remained essentially unaltered throughout all studies.

Fetal bilirubin metabolism in dogs after administration of a "low dose" quantity of bilirubin-³H. The highest fetal plasma concentrations of radioactivity and the lowest percentage of placental transfer were obtained in the preparation which received ether anesthesia (Figs. 2 and 3, Table III D-1). In all other studies the fetal plasma concentration of radioactivity rose to a plateau at approximately 3-4 hr during the infusion of bilirubin-³H, and diminished after the infusion was terminated (Fig. 2). At the end of study, plasma concentrations of radioactivity equalled 25-50% of peak values. It could be estimated from the concentration of radioactivity in the plasma and the average plasma volume (9% body weight) that no more than 12% of the administered radioactivity infused over 6 hr remained in the plasma at the end of infusion. Maternal biliary excretion of radioactivity is shown in Fig. 3. Tritium was detectable in the bile usually within 1-2 hr of the start of the fetal infusion and continued throughout the period of observation. (On the average, 1 hr of the initial lag phase was due to maternal biliary catheter dead space.) From 3 to 20% of the bilirubin administered to the fetus ap-
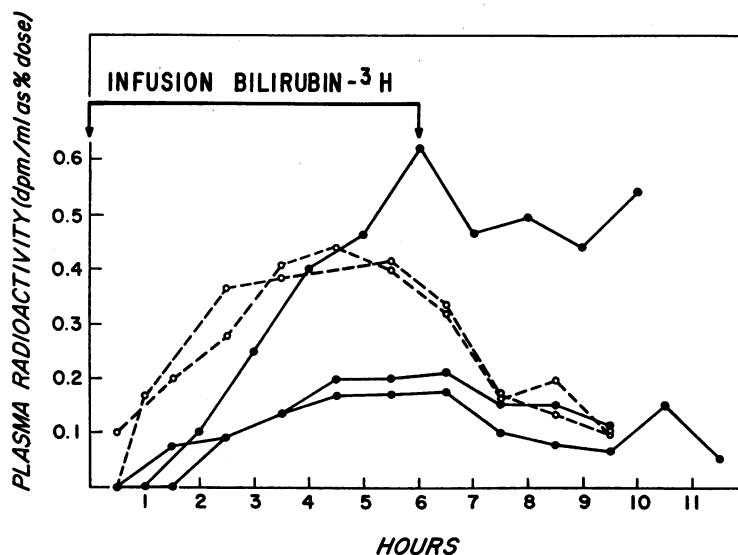
FIGURE 2 Plasma levels of tritium in the dog fetus during and after 6-hr intravenous infusions of bilirubin-³H. Studies in which "low dose" amounts of bilirubin-³H (——) and others in which "overload" amounts (———) were administered are included. The fetus with the highest percentage of the dose persisting in the plasma at the end of study received ether anesthesia (see text). For convenience in comparing experiments, the ordinate is expressed as the per cent of the total dose infused in 6 hr contained in a milliliter of plasma.
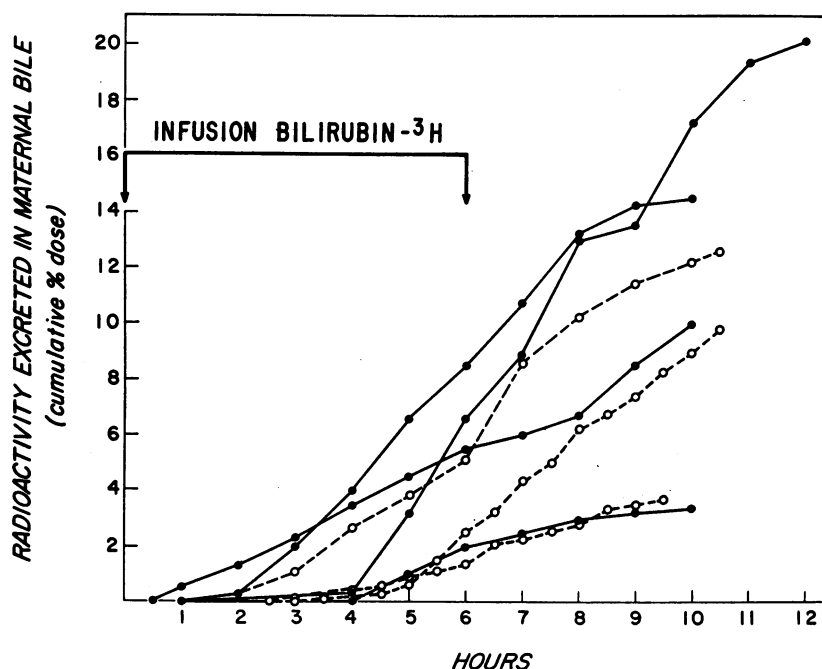

FIGURE 3 Placental transfer of tritium in the dog fetus. "Low dose" (——) and "overload" (———) studies are included. The fetus with the lowest placental transfer received ether anesthesia (see text). Placental transfer is measured in terms of maternal biliary excretion, since virtually all bilirubin transferred into maternal serum is eliminated in maternal bile. The ordinate is expressed as the cumulative per cent of the administered dose appearing in maternal bile.

TABLE III
Recovery of Radiolabel at Termination of Studies

Experiment	Dose	Estimated fetal plasma*	Fetal liver	Fetal bile and intestine	Placental transfer	Total recovery
		% dose	% dose	% dose	% dose	% dose
D-1	"Low dose"§	12	3	35	3	53
D-2	"Low dose"	3	5	46	10	64
D-3	"Low dose"	1	<1	51	15	67
D-4	"Low dose"	—	<1	42	20	62
D-5	"Low dose"	5	2	35	9	51
D-6	"Overload"§	3	27	21	13	64
D-7	"Overload"	3	25	26	10	64
D-8	"Overload"	2	25	24	4	55
D-9‡	"Low dose"	—	1	33	—	33
M-1	"Low dose"	14	2	6	24	46
M-2	"Low dose"	15	1	6	49	70
M-3	"Low dose"	12	6	3	46	67

* For estimation of volume of fetal plasma see text.
‡ The bile duct was catheterized in this fetus and bile flow was external.
§ The use of the term "low dose" and "overload" is defined in text. The amounts of bilirubin-³H infused are included in Table I.

peared as labeled material in maternal bile (Table III). More than three quarters of that portion of the administered material which was transferred across the placenta and excreted by the maternal liver could be identified as bilirubin-³H by hydrolysis and crystallization of the *maternal* bile pigment (13).

At the conclusion of study, relatively small amounts of radioactivity remained in fetal plasma (1–12% of the dose) or in the fetal liver (1–5% of the dose) (Table III). 35–51% of the administered radioactivity was recovered from the combined fetal gall bladder bile and

intestinal contents. 82–100% of radioactive label in *fetal* bile was recovered as bilirubin-³H by hydrolysis and crystallization (13). Thin-layer chromatography of the diazo derivatives of fetal bile in solvent systems I and II established that 65–70% of counts on the chromatograms coincided with the conjugate zone, indicating that the fetus probably excreted bilirubin glucuronide-³H. "Direct-reacting" bilirubin (12) in the fetal dog gall bladder equalled 1.2–3.4 mg. In no study was the amount of radioactivity in organs other than the intestine and liver significantly greater than that which would be an-
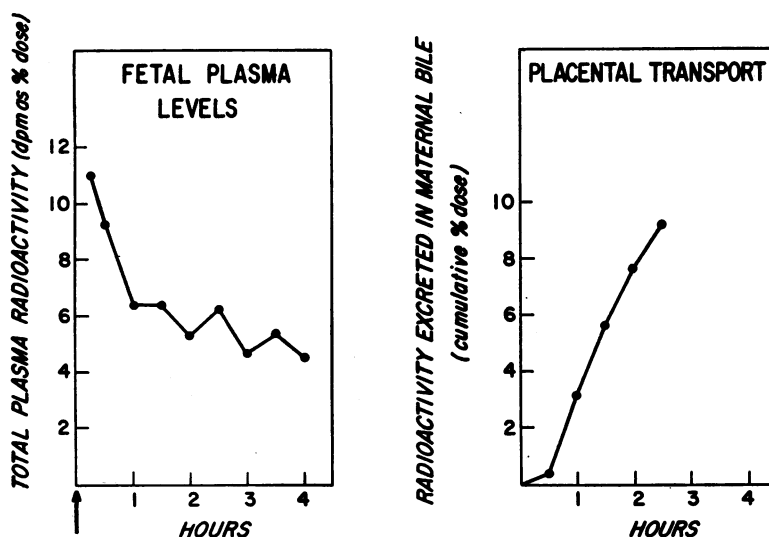

FIGURE 4 Plasma levels of tritium and placental transfer in one study with rapid intravenous injection of bilirubin-³H into a dog fetus.

ticipated on the basis of plasma content. Only minute amounts of radioactivity were present in amniotic and allantoic fluid and in fetal and maternal urine.

The results of administering a rapid intravenous injection of bilirubin-³H to a fetus are depicted in Fig. 4. Total plasma radioactivity fell from 11 to 4% of the dose from the initial observation to the final observation at 4 hr, while placental transfer equalled 9% of the dose at the end of study. 35% of the administered material was excreted in fetal bile (Table III, D-5).

Fetal liver showed approximately three-quarters the glucuronyl transferase activity present in adult liver: mean fetal hepatic activity = 0.220 μmoles of o-aminophenol glucuronide formed per hr per g wet weight liver (range three determinations = 0.182–0.250); mean adult hepatic activity = 0.286 μmoles per hr per g (range, five determinations = 0.258–0.328).

Fetal bilirubin metabolism in dogs after administration of an "overload" of bilirubin-³H. As can be seen in Figs. 2 and 3, results similar to those described above were obtained when 2.8–4.3 mg of bilirubin-³H were infused intravenously into a fetus. Plasma concentrations of radioactivity were somewhat higher than those observed in "low dose" studies when expressed as a percentage of the administered dose (Fig. 2). Essentially all of the pigment in the plasma was in the form of "unconjugated" (i.e., "indirect-reacting") bilirubin. The percentages of administered label which crossed the placenta and were excreted in maternal bile were comparable to those in the "low dose" studies (Fig. 3).

The major difference between these three experiments and the "low dose" studies can be seen in Fig. 5 and Table III. Whereas in the "low dose" studies the proportion of label excreted in fetal bile was great and that accumulated and retained in the fetal liver was small, in the studies in which an "overload" of biliru-

FIGURE 6 Rate of excretion of tritium in fetal dog bile. Biliary excretion of tritium after intravenous injection into a pregnant adult dog of a comparable dose (weight for weight) is included for comparison of fetal and adult rates of disposition. For the study of fetal biliary excretion, the fetal bile duct was catheterized and bile flow was diverted exterior to the uterus.

bin-³H was administered, the converse was true. From 21 to 36% of the administered dose was excreted in fetal bile, but an additional 25–27% was retained in the fetal liver at the end of study. The major proportion of tritium present in fetal and maternal bile was shown by crystallization and chromatography of diazo derivatives to be in the form of bilirubin glucuronide-³H.

Rate of fetal dog biliary excretion of bilirubin-³H. The rate of fetal bile flow and bilirubin excretion during the period 48–57 hr after institution of external biliary drainage equalled 0.06 and 70 μg/hr respectively (fetal weight equalled 323 g). This rate of bilirubin excretion per unit body weight in fetuses equals approximately one-half the rate observed in adults. The rate of fetal biliary excretion of radioactive label is shown in Fig. 6. A representative curve of biliary excretion after intravenous administration of bilirubin-¹⁴C (13) to an adult pregnant dog is included for comparison. It can be seen that fetal excretion of radioactivity is less rapid; that is, the per cent of the administered dose excreted is less than one-third that excreted by the pregnant adult animal. As noted above, tritium appeared in fetal bile in the form of bilirubin glucuronide-³H. Small amounts of tritium transferred directly from the plasma into the intestine.
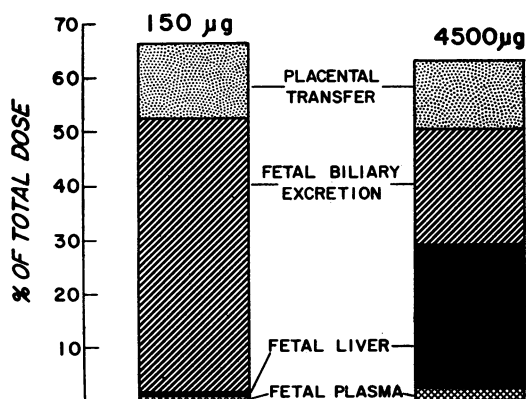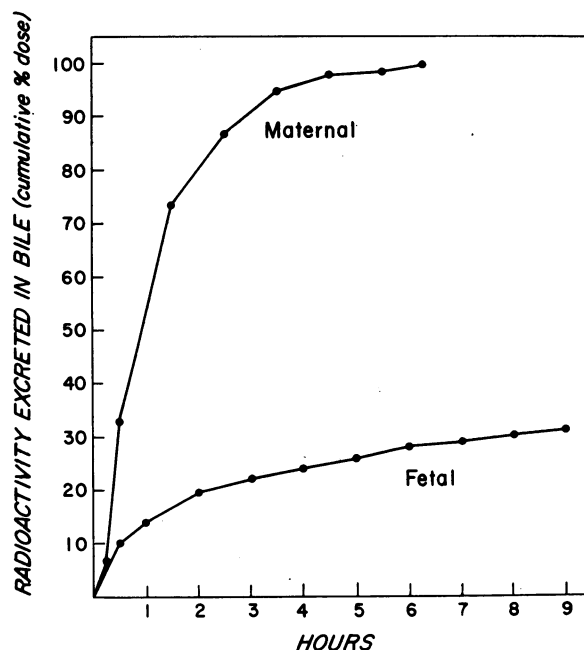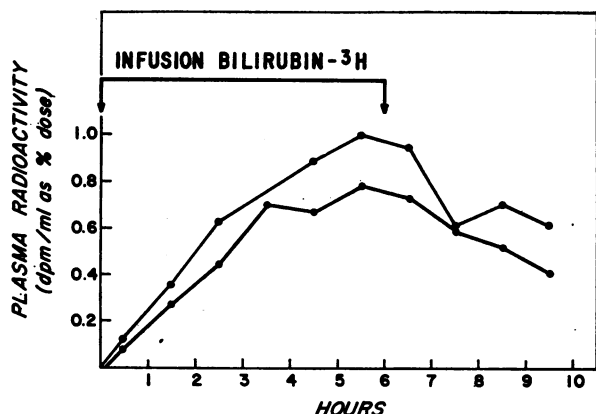

FIGURE 5 Final disposition of tritium after representative "low dose" and "overload" studies in the dog fetus. The amounts of label in fetal liver are greatly increased in the "overload" study.

FIGURE 7 Plasma levels of tritium in two monkey fetuses during and after 6-hr fetal intravenous infusions of bilirubin-³H.

Fetal bilirubin metabolism in monkeys. The results of infusing bilirubin-³H intravenously in three fetal monkey preparations are depicted in Figs. 7 and 8 and Table III. An analysis of samples obtained from two of the animals showed that the concentration of plasma radioactivity rose to peak values at 6 hr of infusion of bilirubin-³H, and tapered after completion of infusion. At the end of study, plasma concentrations of radioactivity equalled 50–60% of peak values. The change with time of the plasma tritium concentration as a per cent of the total infused dose is similar for both animals (Fig. 7). If an allowance is made for the 10–15% difference in fetal weight and presumed plasma volume, the curves become nearly identical. At the end of infusion, approximately 20% of the bilirubin-³H administered over the 6 hr period was present within the intravascular compartment (calculation based on an assumed fetal

monkey plasma volume of 10% of body weight [21]). When bilirubin-³H was infused over a 2 hr period and collections were continued for a total of 6½ hr (monkey fetus M-1, Table III), 24 % of the administered dose was transferred across the placenta and appeared in maternal bile. In two studies with 6 hr infusions and a total of 11 hr of collection (M-2, M-3), 46–49% of the infused tritium crossed the placenta and was excreted by the maternal liver. 58–76% of the label appearing in maternal bile was recovered as bilirubin-³H by hydrolysis and crystallization (13). Only 3–6% of the administered label was present in fetal bile, and of this small quantity less than one-fifth was bilirubin-³H. Fetal liver also contained little radioactivity and there was none measurable in the fetal intestinal contents and intestinal wall. Bilirubin in the fetal gall bladder ranged from 0 to 100 μg (12).

Chromatography of fetal tissue extracts. Fetal intestinal wall was homogenized and extracted with chloroform. A diazo-negative yellow pigment with peak absorption at 410 mμ was observed in the chloroform extracts. When this material was chromatographed on silica gel G thin-layer plates in systems I and II (described above), the yellow pigment migrated in a discrete band with R_f values 0.9 and 0.8 respectively, and with clear separation from the solvent front. Approximately two-thirds of the tritium present in the intestinal wall (radioactivity in the intestinal wall at the conclusion of study equalled from 10 to 20% of the administered dose) coincided on chromatography in both solvent systems with the diazo-negative yellow pigment. A pigment with similar spectral and chromatographic properties was extracted from fetal dog and monkey urine and fetal monkey bile.
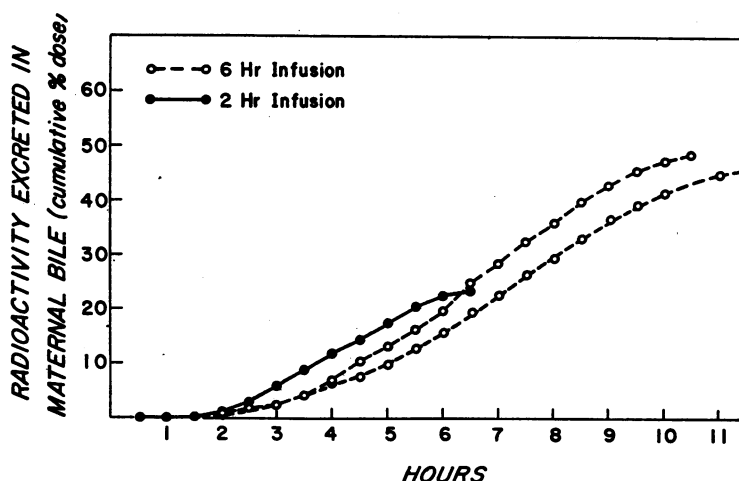

FIGURE 8 Placental transfer of tritium in the monkey fetus during and after 2-hr and 6-hr infusions of bilirubin-³H.

DISCUSSION

Hepatic uptake, conjugation, and biliary secretion of bilirubin are extremely rapid processes in the adult. Half of a moderate load of bilirubin is taken up by the liver within minutes of injection, and within 1–3 hr, 70–90% of the administered material is excreted. Conjugation plays a large role in the adult excretory process, and the major proportion of pigment present in the bile is in the form of bilirubin glucuronide (22, 23).

When one compares the results in the fetus described above with the known characteristics of adult bilirubin metabolism, the methodologic limitations of the present investigation should be recognized. Although all studies were performed at least 24 hr after fetal surgery, and although the fetus was maintained undisturbed during study, it was necessary to administer low levels of halothane anesthesia while investigations of bilirubin metabolism were in progress. With one exception, the fetal heart rate, arterial blood pressure, arterial blood gases, and pH were maintained at physiologic levels during the course of study. The ether anesthesia used in one study did appear to depress placental transfer of bilirubin-^{3}H, and it is conceivable that fetal placental and (or) hepatic function were affected by halothane anesthesia. The term "low dose" is used in a relative sense. While 33–189 µg of bilirubin (Table I) is a small quantity relative to the rate of adult production during a 6 hr period, the rate of fetal bile pigment production is unknown. The amounts of pigment excreted per unit body weight by the fetal dog equalled roughly half the amounts excreted by an adult. When this amount is combined with the amounts transferred across the placenta, or metabolized by alternate pathways, it seems probable that fetal and adult excretion, and therefore production, are of the same order of magnitude. One suggestive study, however, does not establish the point, and it is possible that in so-called "low dose" studies, the amounts of administered bilirubin were proportionally greater than recognized. Endogenous fetal plasma bilirubin concentrations were below the limits of accuracy of the Malloy-Evelyn method, and plasma bilirubin concentrations remained below measurable levels in the majority of experiments. In those preparations infused with larger concentrations of bilirubin-^{3}H, the relative proportions of plasma unconjugated and conjugated bilirubin were assessed only approximately by means of the "indirect" and "direct" diazo reaction.

Within these limitations, the present investigations establish that fetal and adult bilirubin metabolism are grossly different. The rapid and efficient pattern of hepatic excretion characteristic of virtually all normal adult mammalian species clearly differs from that described above in the fetus (as seen, for example, in Fig. 6). Moreover, and more surprisingly, major fetal species differences exist. Placental transfer of bilirubin probably plays a limited excretory role in the dog fetus. Only an average of 10% of 33–189 µg of bilirubin-^{3}H infused into the fetus was transferred across the placenta during the 10–12 hr period of study. On the other hand, the fetal liver in the dog is relatively "mature." Homogenates of fetal dog liver form o-aminophenol glucuronide at rates which average three-quarters of adult values, and although less effective than the adult's, the fetal dog liver was capable of forming and excreting appreciable quantities of bilirubin glucuronide. When given the maximal load studied (4.3 mg infused intravenously over 6 hr), fetal biliary excretion equalled approximately 1.1 mg of bilirubin glucuronide-^{3}H. In this experiment and in others in which an "overload" of bilirubin-^{3}H was administered (D 6-8, Table III), the amount of tritium retained in the liver increased from < 1–5% to 25–27% of the dose. Presumably, in these circumstances the capacity of the fetal liver to conjugate and(or) secrete bilirubin was exceeded.

The over-all capacity for bilirubin excretion in the monkey fetus was equally limited. In contrast to the findings in dogs, however, the placenta in monkeys is *a* major, or *the* major, site of bilirubin excretion. When 91 or 239 µg of bilirubin-^{3}H was infused into a monkey fetus over 6 hr, roughly half the administered labeled pigment was transferred intact across the placenta into the maternal circulation. Moreover, the primate liver is relatively "immature." *In vitro* assays of glucuronyl transferase activity yield values one-third as great as those of the adult (24). Less than 10% of the modest amounts of bilirubin-^{3}H administered to the fetus appeared in fetal bile, and most of this small fraction was in the form of diazo-negative ^{3}H-labeled bilirubin derivatives. The present studies are thus in close agreement with previous investigations of fetal primate bilirubin metabolism performed with short-term infusions using diverse surgical techniques (4, 6). In sum, the fetal dog liver is relatively "mature," and little bilirubin is excreted via the placenta. It is, therefore, not surprising that during the neonatal period, dogs show no evidence of "physiologic jaundice." [3] In the monkey, the placenta appears to be a major fetal excretory organ, and since the fetal liver is functionally immature it is not surprising that newborn monkeys regularly develop "physiologic jaundice."

Several other features of fetal bilirubin metabolism in the two species deserve comment. Maximal plasma bilirubin-^{3}H concentrations (as per cent dose) tended to be higher in the monkey fetus than in the dog fetus. These, in part, may reflect the greater affinity of monkey albumin for bilirubin. Primate (human) albumin binds

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[3] Bernstein, R. B. Unpublished observations.

bilirubin with greater avidity than that of certain other species (25), and this in turn results in a smaller volume of pigment distribution (26). If the binding of bilirubin to fetal monkey albumin were greater than to fetal dog albumin, it would be anticipated that comparable infusions to both animals would lead to higher plasma concentrations in the fetal monkey.

In both species, but especially in the dog, the approximate volume of distribution of the label greatly exceeded the plasma volume. Part of the bilirubin-^{3}H entered the liver, biliary tree, and intestine, but a wider tissue distribution would appear probable judging, for example, from the maximal value of 11% of the radioactive dose contained within the fetal plasma after rapid intravenous injection of bilirubin-^{3}H (see Fig. 4). Moreover, by comparing the rate of plasma decay with the rate of placental transfer, it can be seen that plasma levels probably represent a dynamic equilibrium between intravascular entry and exit of radiolabel. For example, during the period from 1 to 4 hr when the amounts of radioactivity in fetal plasma decreased by less than 2% of the dose, the amount of placental transfer and maternal biliary secretion equalled 6% (Fig. 4). It, therefore, appears probable that reentry into plasma of labeled pigment from fetal liver and(or) other extravascular sources occurred.

A diazo-negative yellow pigment with spectral and chromatographic characteristics distinct from those of bilirubin was extracted from the small intestinal wall of fetal dogs. Tritium extracted from the small bowel wall representing 10–20% of the administered dose migrated in two solvent systems at the same rate as the yellow pigment. Small quantities of similar radiolabeled pigmented materials also were present in fetal dog and monkey urine and in fetal monkey bile. It is probable, therefore, that this diazo-negative pigment is a degradation product of bilirubin, perhaps related to the unidentified pigments found in the amniotic fluid of fetuses with severe hemolysis (27), or to the bilirubin derivatives in the bile of Gunn rats (26).

The significance of fetal bilirubin glucuronide formation and excretion may be considered in this light. A part of the excreted conjugate may be sequestered in the intestine. Bilirubin glucuronide is not absorbed from the intestine of adults, but whether or not intact conjugate is absorbed from fetal intestine is unknown. Similarly, it is not known whether the quantity of mammalian β-glucuronidase and the physical conditions present in the fetal intestine are suitable for the hydrolysis of bilirubin glucuronide. The site and magnitude of formation of the labeled diazo-negative bilirubin derivative(s) are unknown. Whether this material represents a significant alternate path of bilirubin degradation must remain speculative.

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