Perinatal Metabolism of Hemopexin and Heme[1] (35210)

JEAN D. ROSS, H. H. LIEM, AND U. MULLER-EBERHARD[2]
(Introduced by William O. Weigle)
Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, California 92037

It has been suggested that heme may predispose the newborn to kernicterus (1). High concentrations of heme pigments in amniotic fluid or in the serum of erythroblastotic infants are known indicators of severe hemolytic diseases (1–3). Moreover, heme, like bilirubin, depresses the uptake of oxygen by rat brain *in vitro* and is lethal to newborn rats when injected intraperitoneally (4). Several investigators have demonstrated that heme also represses or inhibits the function of various serum and tissue-bound proteins (5–9).

Two heme-binding proteins, hemopexin[3] and albumin, protect the organism from the toxic effects of heme. Both accept heme simultaneously, but the β-globulin Hx has the greater affinity for this pigment (10) and is responsible for its transfer to the liver (11). There it is converted enzymatically to bilirubin (12). Although Hx is synthesized by the fetal liver (13), its serum levels are low in fetuses (13) and newborns (14). Hx is also present in human amniotic fluid, where its concentration decreases in the presence of fetal hemolytic disease (15), indicating that an intrauterine disposal mechanism exists for the heme–Hx complex.

[1] Supported by research grants HE 08660 from the National Heart Institute, HD 04445 from the Institute of Child Health and Human Development, San Diego County Heart Association #83, and the National Institute of Health Special Fellowship 4 FO3 HD-06213-12A1.

[2] Recipient of Career Development Award 5-K3-AM-19, 923 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

[3] Abbreviations: Hx = hemopexin; RHx = rabbit hemopexin; HHx = human hemopexin; sp act = specific activity.

It is not known whether heme or Hx crosses the materno–fetal barrier. The present study concerns materno–fetal transport of these two molecules. Also, normal values in pregnant, postpartum, and neonatal rabbits are given. The rabbit was utilized in this investigation, since the function and metabolism of Hx and heme have been studied extensively in this species (11, 16, 17).

Materials and Methods. Perinatal Hx values. Serum Hx concentrations were determined by radial immunodiffusion (14) in 11 female New Zealand white rabbits at various times before and after delivery of their litters and in 38 of their progeny. Blood was obtained from the neonates by intracardiac puncture, serially when possible. Serum Hx levels were also measured in pooled sera obtained from two litters upon hysterotomy 3 and 1 days prior to estimated date of delivery.

Transplacental transfer of heme and Hx. ^{59}Fe- and ^{14}C-hemin were prepared from duck erythrocytes as described elsewhere (16). Rabbit and human β-fractions and RHx were purified according to a modification of the method previously described for HHx (18). The RHx was labeled with ^{125}I through the courtesy of Dr. F. J. Dixon, Jr. (19). Hysterotomies were performed either following terminal exsanguination of the doe (Expt. 1) or after intravenous sodium pentothal anesthesia (Expts. 2 and 3). Blood samples were obtained by intracardiac puncture, and those from fetuses were pooled. Tissues and whole fetuses (Expt. 2) were homogenized in 3 to 8 vol of sucrose–PO_4 solution, final pH 7.8, and the supernatants were separated from crude cartilaginous, tendonous, and bony debris by centrifugation at 1°. Radioactivity of the cloudy supernatants was measured in a well scintillation counter

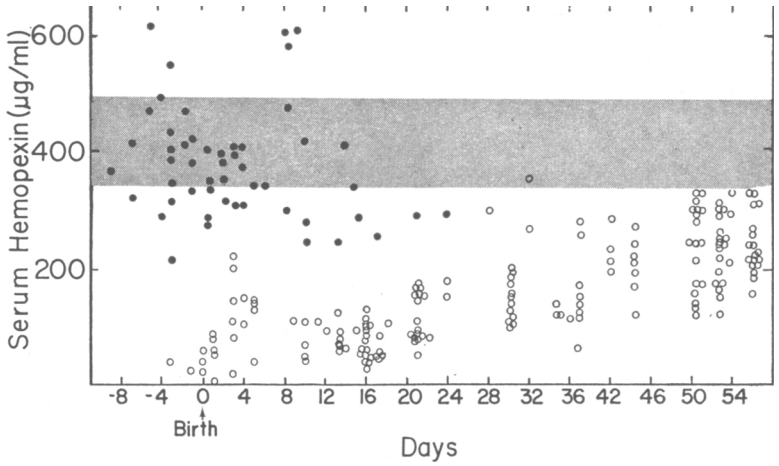

FIG. 1. Serum hemopexin values for mother rabbits (●); and their offspring (○) at various times before and after delivery. The shaded area represents normal values for adult male rabbits within 2 SD of the mean.

and results are expressed as percentage of injected radioactivity per 10 g (for tissues), per 10 ml (fluids) or per total tissue, when possible. RHx and HHx were assayed as above (14) using species-specific antibodies. The following experiments were performed:

Expt. 1: A 4.4-kg pregnant rabbit (27th day of gestation) was given 116 µg of ^{125}I-RHx (sp act = 23 µCi/mg) intravenously. Two hr later, following a hysterotomy, radioactivity was determined in maternal plasma, liver, lung, kidney, spleen, and bone marrow, as well as in fetal serum, placentas, and livers.

Expt. 2: ^{59}Fe-heme (sp act = 5.4 µCi/µmole), previously incubated with human β-fraction containing 80 mg of HHx, was administered intravenously to a rabbit 21 days pregnant. Grouped fetuses (2 to 4 at a time), amniotic fluid, and the respective placentae basales and fetal placentas were removed every 20 to 30 min over a 2-hr period. Fetal and maternal sera were assayed for HHx and radioactivity. The ^{59}Fe content of amniotic fluid and maternal serum was measured, as well as that of supernatants from fetuses, fetal placentas, and placentae basales.

Expt. 3: Two pregnant rabbits (27th day of gestation) were injected intravenously with 0.15 or 0.16 mg of ^{14}C-hemin (sp act = 2.5 µCi/µmole), previously incubated with 2 ml of plasma for 30 min at 37°. Fetal and maternal serum radioactivity was measured between 30 and 150 min postinjection. Results, in this instance, were expressed as percentage of injected radioactivity per milliliter of serum.

Observations and Discussion. Fetal, newborn, and juvenile rabbits, like humans (13, 14), had much lower serum Hx levels than their mothers (Fig. 1). Values gradually increased and usually approached those of normal adults by age 30 to 60 days (Figs. 1 and 2). There was no difference between Hx levels of males and females (Fig. 2). Maternal values before delivery were generally in the normal range but several were increased or diminished; most decreased during lactation (Fig. 1). During the first 48 hr after birth the mean maternal serum Hx concentration was 7.8 times that of the neonates. These results suggest that either fetal synthesis of Hx, its transport across fetal membranes, or both are inefficient in immature animals; alternatively, consumption of the protein is increased in the perinatal period.

In Exp. 1 (Table I) over 72% of the injected radioactivity was recovered from the total maternal and fetal tissues, with the exception of crude debris and of amniotic fluid, the total volume of which was not measurable. The greatest portion of organ-deposited ^{125}I was found in the doe liver. Since in maternal liver the total radioactivity was ¼ of that seen in estimated total plas-

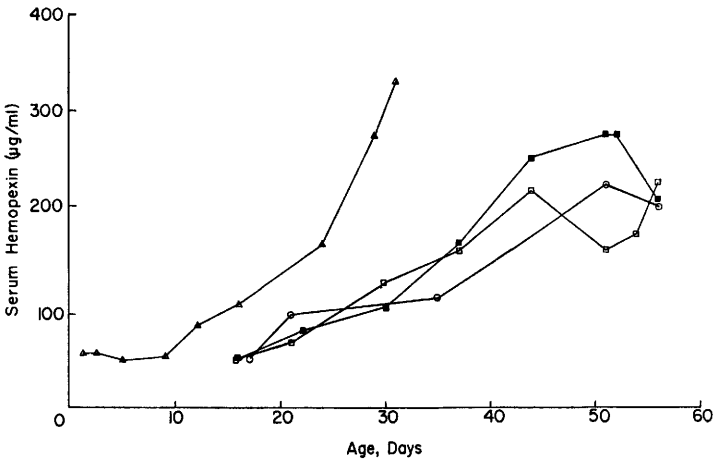

FIG. 2. Representative sequential serum hemopexin values for four newborn or juvenile rabbits: (○) female; (□, ■) males; (△) sex unknown.

ma, contamination by intrahepatic blood cannot be ruled out. However, studies currently in progress indicate that blood contamination is minor and previous observations have indicated that the liver is a major site of removal of Hx (11)

Expt. 1 also demonstrates that in rabbits small amounts of homologous Hx (RHx) pass from the mother to amniotic fluid and to fetal placenta, serum, and liver. ^{125}I-RHx was detected in fetal plasma, although in much smaller amounts than in that of the mother (maternal:fetal ratio of radioactivity 12.0:1.0). Substantial amounts of ^{125}I-RHx were found in homogenates of fetal placenta, while lesser quantities were seen in fetal liver and plasma, as well as in amniotic fluid. Fetal liver contained a total radioactivity 15 times that estimated as circulating in fetal plasma. This finding suggests that there is greater intrahepatic deposition of Hx in fetal than in maternal liver and, additionally, that plasma contamination was obviously an insignificant factor in fetal hepatic radioactivity. Placental radioactivity exceeded that of maternal lung, kidney, spleen, and bone marrow.

On the other hand, the heterologous protein HHx does not pass the rabbit placental barrier. In Expt. 2, HHx was found neither

TABLE I. Expt. 1. Distribution of Radioactivity Following Injection of ^{125}I-Rabbit Hemopexin into a Pregnant Doe.[a]

	Maternal		Fetal	
Tissue	Per 10 g or 10 ml of tissue	In total tissue	Per 10 g or 10 ml of tissue	In total tissue
Plasma	3.40	50.70[b]	0.28	0.12[b]
Liver	1.20	12.70	0.075	1.79
Lung	0.51	0.73	—	—
Kidney	0.85	1.77	—	—
Spleen	0.28	0.48	—	—
Bone marrow	0.39	—	—	—
Amniotic fluid	—	—	0.32	—
Placenta	—	—	0.70	5.04

[a] Percentage of injected radioactivity 2 hr after injection.

[b] Results for maternal and fetal plasma are approximated, using an estimated plasma volume of 34 ml/kg.

in fetal sera nor in amniotic fluid, although it was present in significant amounts (above 96 μg/ml) in maternal plasma throughout the observation period. Thus, transmission of RHx to amniotic fluid is apparently favored over that of the heterologous protein. In rabbits, no distinction has previously been noted between the entry of homologous versus heterologous proteins into amniotic fluid, although preferential transport of homologous proteins to fetal sera is known to occur (20).

Definitive knowledge of the transport of serum proteins from mother to fetus is meager and principally concerns immunoglobulins. Gitlin *et al.* (21), in a study of the transplacental passage of radioiodinated proteins in human pregnancies, demonstrated that only small amounts of transferrin, 19 S globulin and fibrinogen are transported, while variable amounts of albumin and large quantities of 7 S globulin pass to the fetus. Similar observations have been made in monkeys (20).

In rabbits, whose placental and fetal membranes differ from those of man and monkey, significant transmission of immunoglobulins to fetal blood occurs via the yolk sac splanchnopleure rather than directly across the placenta (20). Recently, it has been shown that small amounts of transferrin, a β-globulin with a molecular weight similar to that of Hx (22), pass to the fetus (23), and that the fetal placenta binds maternal transferrin avidly (23). Similarly, in Expt. 1, maternal ^{125}I-RHx appeared in significant amounts in the placenta (Table I). The minimal amounts of radioactivity found in maternal kidney suggest that only a little ^{125}I dissociated from RHx and was excreted during the experimental period.

Small quantities of heme were also transported across the rabbit materno–fetal barrier. Six percent of the total ^{14}C-heme injected into the maternal circulation was detected 30 to 60 min later in sera of fetuses removed at that time (Expt. 3: Table II, litter 1). After 2.5 hr, only 0.8% of the injected radioactivity was found in fetal plasma while 7% remained in maternal plasma. The more rapid elimination of ^{14}C-heme from fetal serum suggests an accessory mechanism for clearance

TABLE II. Expt. 3. Passage of ^{14}C-Heme across the Rabbit Materno–Fetal Barrier.

Rabbit and litter no.	After injection (min)	% of injected radioactivity per ml of plasma
1 a. Mother	30	15
b. Litter (8 fetuses)	30–60	6
2 a. Mother	120	7
b. Litter (6 fetuses)	130–150	0.8

of the molecule, as could occur with bidirectional passage across the placenta. Similarly, in Expt. 2 the ^{59}Fe content of maternal plasma decreased only slightly between 30 and 120 min (Fig. 3A), while radioactivity was detected only in the first fetal supernate, obtained at 28 min (Fig. 3B). In contrast, ^{59}Fe was found in every specimen of placenta basalis and fetal placenta (Fig. 3C) but never in amniotic fluid. Significantly, radioactivity decreased in both placental supernates between 30 and 90 min postinjection, and rose at least to the earlier levels by 110 min. Although transport of uncoupled ^{59}Fe cannot be excluded, these results suggest that heme passes from mother to fetus directly across the placenta rather than by way of the yolk sac splanchnopleure, or amniotic fluid. Moreover, bidirectional passage of heme may occur, similar to that seen with several molecules, including bilirubin (24) and albumin (20).

Low levels of Hx may render the neonate more susceptible to the toxic effects of heme and related compounds. While transfer of heme to the amniotic fluid is excluded, its transport from the fetus back to the mother, as suggested by our results, may protect the fetus *in utero* from injury by heme in a manner similar to that seen with bilirubin (24). In addition, inadequate materno–fetal transport of the protein appears to be one factor contributing to low fetal and neonatal serum Hx concentations.

Summary. Sera from fetal, newborn, and juvenile rabbits were found to have lower Hx levels than those of their mothers but approached the normal adult range by 30 to 60 days of age. Inadequate materno–fetal transport of Hx contributed to low fetal and ne-

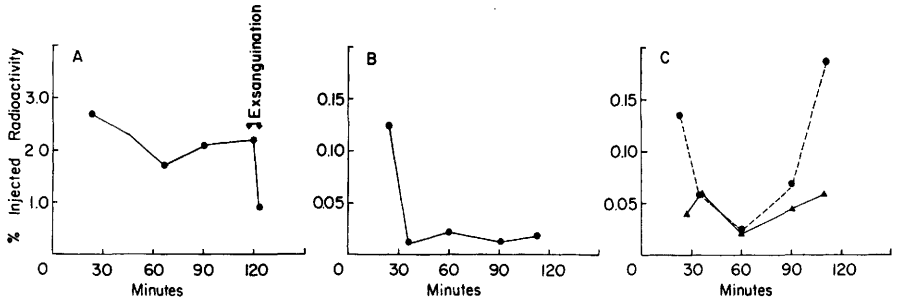

FIG. 3. Percentage of administered ^{59}Fe at various intervals during Expt. 2: (A) maternal serum; (B) fetal supernatant; (C) supernatants from fetal (▲); and maternal (●) placentae.

onatal serum levels, since only small amounts of homologous ^{125}I-Hx passed from the maternal circulation to fetal serum and tissues. However, substantial amounts were found in fetal and maternal placentae. Heterologous HHx did not pass. Small amounts of ^{14}C- and ^{59}Fe-heme also crossed the materno–fetal barrier of rabbits, probably directly across the placenta. Furthermore, our findings suggested bidirectional transplacental passage of heme.

1. Tuttle, A. H., Amer. J. Dis. Child. **89**, 544 (1955).

2. Abelson, N. M., and Boggs, T. R., Pediatrics **17**, 452 (1956).

3. Zetterström, R., Stempfel, R., and Escardo, F. E., Acta Paediat. **45**, 241 (1956).

4. Day, R., Pediatrics **17**, 925 (1956).

5. Kappas, A., and Granick, S., J. Biol. Chem. **243**, 346 (1968).

6. Davis, R. B., Biggs, R., and Denson, K. W. E., Thromb. Diath. Haemorrh. **17**, 299 (1967).

7. Hayashi, N., Yoda, B., and Kikuchi, G., J. Biochem. **63**, 446 (1968).

8. Scholnick, P. L., Hammaker, L. E., and Marver, H. S., Proc. Nat. Acad. Sci. U.S.A. **63**, 65 (1969).

9. Bottomley, S. S., and Smithee, G. A., Proc. Annu. Meet. Amer. Soc. Hematol., 12th, Cleveland, 1969, p. 67 (Abstr.)

10. Liem, H. H., and Muller-Eberhard, U., J. Lab. Clin. Med., 1970, in press.

11. Muller-Eberhard, U., Bosman, C., and Liem, H. H., J. Lab. Clin. Med. **76**, 426 (1970).

12. Tenhunen, R., Marver, H. S., and Schmid, R., J. Biol. Chem. **244**, 6388 (1969).

13. Gitlin, D., and Biasucci, A., J. Clin. Invest. **48**, 1433 (1969).

14. Hanstein, A., and Muller-Eberhard, U., J. Lab. Clin. Med. **71**, 232 (1968).

15. Muller-Eberhard, U., and Bashore, R., N. Engl. J. Med. **282**, 1163 (1970).

16. Muller-Eberhard, U., Liem, H. H., Hanstein, A., and Saarinen, P. A., J. Lab. Clin. Med. **73**, 210 (1969).

17. Ross, J. D., and Muller-Eberhard, U., J. Lab. Clin. Med. **75**, 694 (1970).

18. Muller-Eberhard, U., and English, E. C., J. Lab. Clin. Med. **70**, 619 (1967).

19. McConahey, P. J., and Dixon, F. J., Int. Arch. Allergy **29**, 185 (1966).

20. Wild, A. E., Sci. Progr. (London) **54**, 351 (1966).

21. Gitlin, D., Kumate, J., Urrusti, J., and Morales, C., J. Clin. Invest. **43**, 1938 (1964).

22. Hrkal, Z., and Muller-Eberhard, U., Pac. Slope Conf., 1969, p. 16 (Abstr.).

23. Baker, E., and Morgan, E. H., Quart. J. Exp. Physiol. Cog. Med. Sci. **54**, 173 (1969).

24. Bashore, R. A., Smith F., and Schenker, S., Amer. J. Obstet. Gynecol. **103**, 950 (1969).

Received May 20, 1970. P.S.E.B.M., 1971, Vol. 136.