The Synthesis of Fetal and Adult Hemoglobin in Sheep During the Perinatal Period[1] (36317)

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The transition from fetal to adult hemoglobin synthesis has been studied in several mammalian species including sheep. Previous studies do not agree on the time of the transition in sheep. Karvonen (1) found that adult hemoglobin represented approximately 50% of the total hemoglobin at birth. Breathnach (2), on the other hand, identified adult hemoglobin only after birth. In order to describe the transition from fetal to adult hemoglobin in the sheep fetus more precisely, we have studied the incorporation of ^{14}C-leucine into adult and fetal hemoglobins by reticulocytes obtained from peripheral blood samples of unanesthetized, unstressed fetal, and newborn sheep.

Materials and Methods. Pregnant Dorset and mixed breed (western) sheep, at approximately 100 days gestation, were used in this study. The hemoglobin type of the adult pregnant sheep was determined by electrophoresis on cellulose acetate. All sheep were either BB or AB hemoglobin types. The surgical preparation was as described by Meschia *et al.* (3), but instead of using umbilical vessels, fetal carotid and jugular vessels were catheterized. The surgery was well tolerated, and the ewe was usually standing and feeding within 6 hr of the surgical closure.

Blood samples were obtained for study from the fetal vessels at intervals from 3 to 5 days. Each sample was dated and the corresponding fetal age of each sample was calculated from the breeding date, as well as retrospectively from the weight and vertebral column length of the lamb at birth (4). A total of 36 incubations were carried out *in vitro* under sterile conditions using fresh sam-

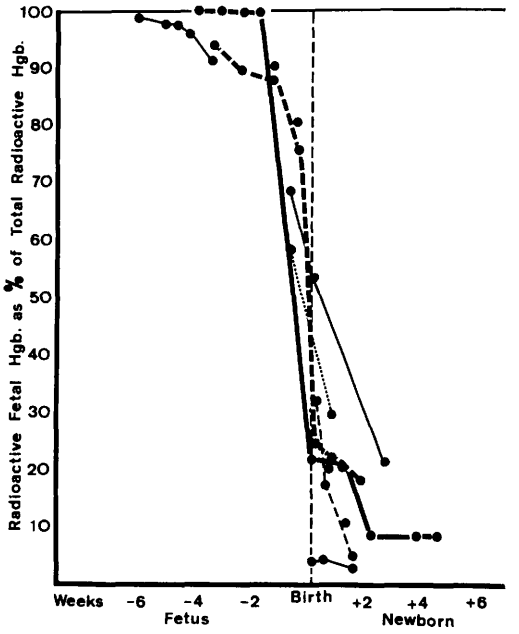

FIG. 1. Radioactive fetal hemoglobin as a percentage of total radioactive hemoglobin in relation to gestational age, measured antenatally and postnatally from the day of birth: The lines uniting the different points represent repeated sampling of the same fetus and newborn. Elution of fetal and adult hemoglobin from DEAE Sephadex A-50 was performed with a decreasing pH gradient (7.9 to 7.1) using Tris–HCl buffers. The absorbance of each protein fraction at 280 mμ was determined. Liquid scintillation counting of the hemoglobin fractions was then carried out. The percentage of adult and fetal hemoglobin in duplicate blood samples after incubation and separation agreed within ±2%.

[1] This study was supported in part by U.S. Public Health Service Grants HD 00781 and HD 02348.

[2] Holder of a Fellowship from the Queen Elizabeth II Canadian Research Fund.

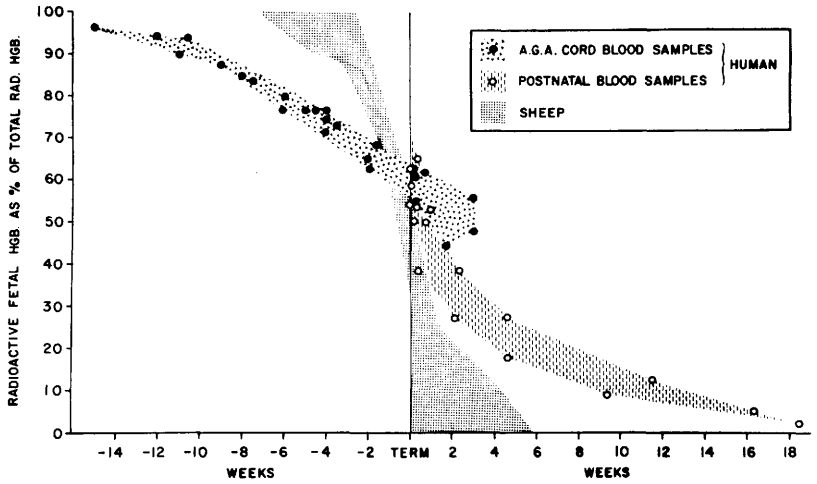

FIG. 2. The synthesis of fetal and adult hemoglobin during the perinatal period: Age was calculated from birth antenatally and postnatally, using term as 40 weeks gestation for the human data and 148 days for the sheep data. Data on cord blood samples in man (●) were obtained from Bard *et al.* (7) from pre-term newborn infants appropriate in weight for gestational age (AGA). Data on postnatal blood samples in man (○) were obtained from Table 2 of Garby *et al.* (10). Data on lamb blood is from the present study.

ples obtained from 11 different animals. The reagent mixture was prepared as by Lingrel and Borsook (5). To 2.64 ml of reagent mixture, 1 cm^3 of packed RBC, 0.15 ml of ferrous ammonium sulfate (10.5 mg in 10 ml of 0.9% NaCl) and 0.1 ml of leucine-^{14}C (DL-leucine-1-^{14}C obtained from Amersham Searle; sp act 55.2 mCi/mmole) were added. The incubation was carried out with agitation under air at 37°. After a 6-hr incubation, the cells were washed three times in 20 vol of isotonic saline and then lysed by freezing and thawing. The hemolysate was then desalted and purified by passage through a G-25 Sephadex column.

Separation of adult and fetal hemoglobins. The purified hemoglobin solution containing 60–80 mg of hemoglobin was subjected to column chromatography on a DEAE Sephadex A-50 medium (Pharmacia Fine Chemicals) which provided a separation of adult and fetal hemoglobin fractions in the manner described previously (6). Finally liquid scintillation counting on the adult and fetal fractions was carried out.

Results. Figure 1 presents the data on the concentration of radioactive fetal hemoglobin as a percentage of the total radioactive hemoglobin of 36 fetal and newborn samples incubated with leucine-1-^{14}C. The lines connect the different points which represent repeated studies on the same fetus and/or newborn lamb. In all instances, a sharp decrease in the relative proportion of fetal hemoglobin being synthesized occurred within the 2 weeks prior to birth.

Discussion. The transition from fetal to adult hemoglobin synthesis in sheep occurs over a comparatively short time span of approximately 4 weeks prior to delivery. The rapid transition from fetal to adult hemoglobin synthesis near term means that relatively minor differences in the state of maturation of fetal lambs, growth rate and hemoglobin turnover will result in large differences in the proportion of fetal and adult hemoglobins of mature fetal lambs. Such variation in the quantities of adult and fetal hemoglobin of sheep fetuses near term may be a factor in explaining the differences of umbilical venous O_2 saturations observed in term fetal lambs (3).

In the human fetus, the onset of adult hemoglobin synthesis has been detected as early as 9 weeks (8). *In vitro* synthesis of adult hemoglobin has been demonstrated in

cell suspensions of fetal hematopoietic tissues at 17 weeks gestation.

In a previous study, data of the relative rates of fetal and adult hemoglobin synthesis was obtained using cord blood reticulocytes from newborn infants appropriate in weight for gestational age (AGA) where the length of gestation ranged from 25 to 43 full weeks (7). Garby *et al.* (10) studied postnatal hemoglobin synthesis by reticulocytes from the time of birth up to 20 weeks of age. Figure 2 combines the data in man from these two studies on the synthesis of hemoglobin by reticulocytes and compares them with the data on sheep obtained in the present study. Clearly, in both cases the transition describes a sigmoid curve; and there is a marked difference in the rate of transition for these two species.

This maturational difference is comparable to other differences in biological maturation of these species (*e.g.*, the length of gestation, 148 days in sheep *vs* 280 days in human; sexual maturation, 2 years in sheep *vs* 12–13 years in human; and life span, 6–7 years in sheep *vs* 65 years in human).

Summary. The presence of adult hemoglobin has been detected in fetal sheep blood by column chromatography as early as 4 weeks prior to birth. The transition from fetal to adult hemoglobin synthesis in sheep is essentially completed within a 2-month period including one prenatal month and one postnatal month. This rate of transition is much faster than in man.

1. Karvonen, M. J., *in* "Haemoglobin" (F. J. W. Roughton and J. C. Kendrew, eds.), p. 279. Butterworths, London (1949).

2. Breathnach, C. S., Quart. J. Exp. Physiol. **49**, 277 (1964).

3. Meschia, G., Makowski, E. L., and Battaglia, F., Yale J. Biol. Med. **24**, 154 (1969).

4. Cloete, J. H. L., and Onderstepoort, J., Vet. Sci. Anim. Ind. **13**, 417 (1939).

5. Lingrel, J. B., and Borsook, H., Biochemistry **2**, 309 (1963).

6. Huisman, T., and Dozy, A. M., J. Chromatogr. **19**, 160 (1965).

7. Bard, H., Makowski, E. L., Meschia, G., and Battaglia, F. C., Pediatrics **45**, 766 (1970).

8. Zilliacus, H., and Ottelin, A. M., Biol. Neonatorum **11**, 389 (1967).

9. Thomas, E. D., Lochte, H. L., Greenough, W. B., and Wales, M., Nature (London) **185**, 396 (1960).

10. Garby, L., Sjolin, S., and Vuille, J., Acta Paediat. Scand. **51**, 245 (1962).

Received July 22, 1971. P.S.E.B.M., 1972, Vol. 139.