

# The Postnatal Decline of Hemoglobin F Synthesis in Normal Full-Term Infants

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**ABSTRACT** Studies were carried out during the 1st yr of life in normal infants born at term to determine the proportions of fetal hemoglobin (Hb F) and adult hemoglobin (Hb A) being synthesized, in order to describe the complete switchover from Hb F to Hb A synthesis during postnatal life. 53 blood samples from 37 infants were incubated in an amino acid mixture containing [<sup>14</sup>C]leucine and chromatographed on DEAE-Sephadex for separation of Hb F and Hb A fractions. The completeness of the DEAE-Sephadex separation of Hb A and Hb F at an age when the major portion of synthesis was of the adult type of hemoglobin was confirmed by globin chain chromatography with the use of carboxymethyl cellulose. There was a rapid decline in Hb F synthesis postnatally until 16–20 wk of age when levels of 3.2% ±SD 2.1% were reached. By combining this data with that previously published, the complete switchover from Hb F to Hb A synthesis can be described in humans in relation to postconceptional age. It follows a sigmoid curve; the steep portion, which lies between the 30th and 52nd postconceptional week, is preceded and followed by plateaus averaging 95% and 7% Hb F synthesis, respectively.

## INTRODUCTION

The orderly appearance and disappearance of adult and fetal forms of hemoglobin constitute one of the best known examples of the regulation of protein synthesis. During intrauterine life the proportion of fetal hemoglobin (Hb F)<sup>1</sup> to adult hemoglobin (Hb A) synthesis decreases as gestation progresses (1) and the rate of the transition from Hb F to Hb A synthesis is not affected by a precocious exposure to extrauterine life (2).

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<sup>1</sup>Abbreviations used in this paper: CMC, carboxymethyl cellulose; Hb A, adult hemoglobin; Hb F, fetal hemoglobin.

The duration of postnatal synthesis of Hb F is an issue that is now assuming increasing importance as information is being sought to further the understanding of the mechanisms, genetic and nongenetic, that control both the orderly switchover from Hb F to Hb A synthesis and the reappearance of Hb F in certain disease states (3). Furthermore, elevated levels of Hb F synthesis may persist in various congenital diseases, the most frequent of which are the hemoglobinopathies and thalassemias (4).

Little is known of the postnatal synthesis of Hb F in individual normal infants where the differences introduced by prematurity and other pathological conditions are absent. Therefore, the purpose of this investigation was to study Hb F synthesis during the 1st yr of life in normal full-term infants.

## METHODS

The study was carried out with the goal of determining serially Hb F and Hb A synthesis postnatally in normal full-term infants. 37 healthy, normal full-term infants (born between 38 and 42 wk gestation) appropriate in weight for gestational age (5) were selected for the study. They were all chosen at birth on the basis of a close correlation between the gestational age determined by the menstrual history and the clinical assessment based on external and neurological examination. These infants had blood samples drawn during follow-up for their routine "well baby" care.

The synthesis of Hb F and Hb A was determined by measuring the incorporation of [<sup>14</sup>C]leucine into the hemoglobins formed during the *in vitro* incubation of reticulocytes by methods described previously (2). The radioactive hemoglobins were prepared from samples obtained for analysis from term infants immediately after birth and at 4–6 wk intervals. Many of the infants, although selected at birth for the study, were sampled for the first time during their subsequent visits to the clinic. Because of difficulties in obtaining 3–5 cm<sup>3</sup> of blood adequate for reticulocyte yields and radioactive incorporation, only nine of the infants had more than one sample drawn for sequential studies, three of which had a sample at birth. 37 infants were studied, and a total of 53 blood incubations were obtained.

The separation of small amounts of Hb F in adult blood by chromatographic methods can be subject to error due

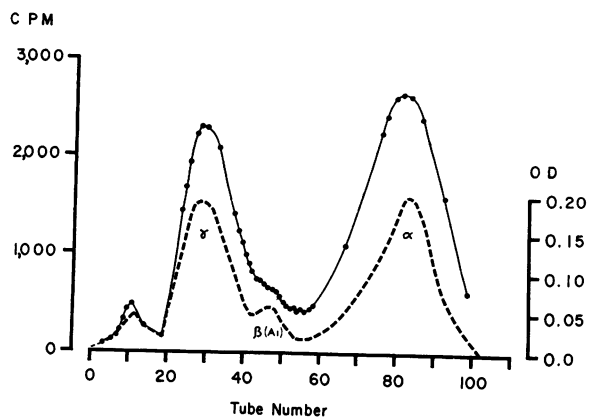


FIGURE 1 Gradient elution chromatography on CMC of globin from hemoglobins obtained after DEAE-Sephadex separation. Absorbance at 280 nm (---) and the counts (●—●—●) are superimposed from a mixture of non-radioactive HbA<sub>1</sub> and radioactive Hb F pooled from two infants of 18 and 20 wk of age.

to the minor component HbA<sub>1</sub> (also known as HbA<sub>1c</sub>) in the zone of Hb F (6). Because of this, further analyses were carried out on pooled radioactive Hb F obtained

from two infants 18 and 20 wk of age after the incubation and DEAE chromatographic separation described herein. This pooled radioactive Hb F was then mixed with an aliquot of nonradioactive hemoglobin corresponding to the A<sub>1</sub> fraction that was obtained after Sephadex-DEAE chromatographic separation of adult blood. Carboxymethyl cellulose (CMC) column chromatograph was carried on this mixture of radioactive Hb F and nonlabeled HbA<sub>1</sub>. The chromatograms were obtained using de-ionized 8 M urea buffers as described by Clegg, Naughton, and Weatherall (7). Finally, liquid scintillation counting was performed on 0.5-ml aliquots of the fractions of gamma, beta (HbA<sub>1</sub>), and alpha globin separated by the CMC column. There was no significant radioactivity detected in the beta (HbA<sub>1</sub>) globin peak. The optical density of the CMC chromatograph and the counts per minute are shown in Fig. 1.

These findings demonstrate that the minor HbA<sub>1</sub> component found in adult blood accounts for an insignificant amount of the radioactivity within the Hb F peaks obtained in this study.

## RESULTS

Fig. 2 demonstrates the chromatograms and superimposed incorporation of [<sup>14</sup>C]leucine into the Hb A and Hb F fractions from a full-term infant whose hemoglobin synthesis was studied at birth, and 8, 28, and

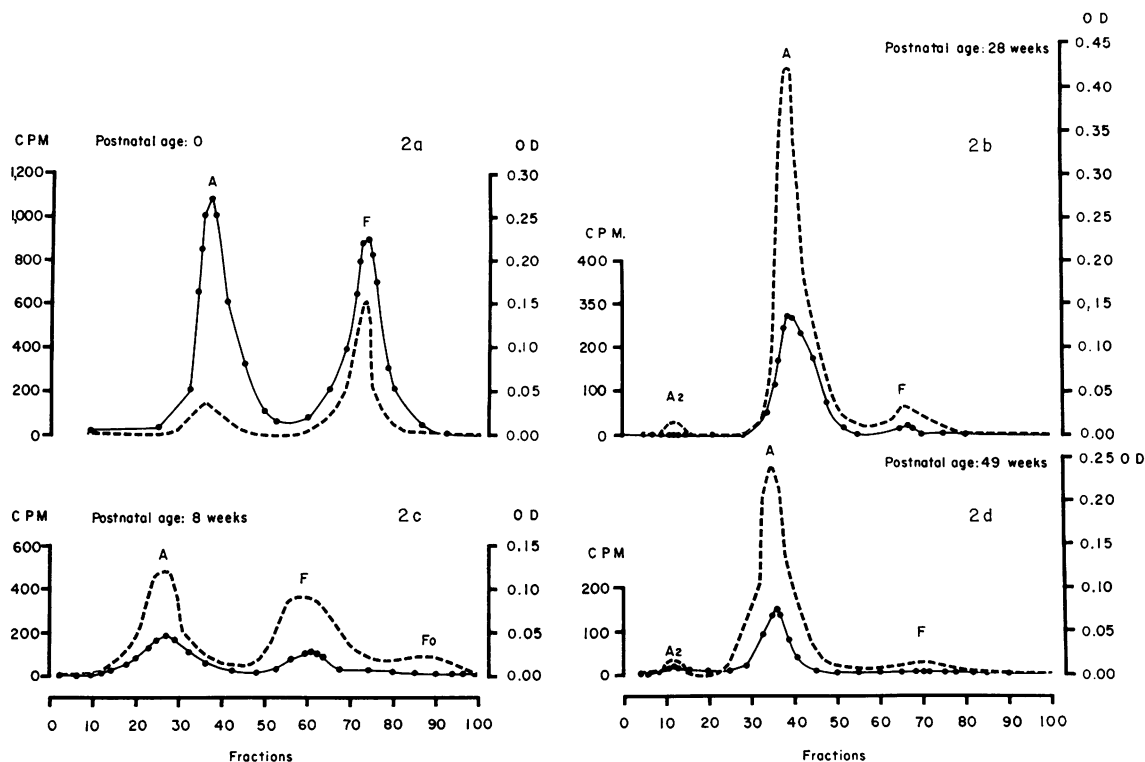


FIGURE 2 Postnatal Hb A and Hb F synthesis in an infant born at term determined at birth (a), 8 wk of age (b), 28 wk (c), and 49 wk of age (d). The superimposed tracings represent the Sephadex-DEAE chromatographic separation of Hb A and Hb F, absorbance 280 nm (---), and counts per minute of [<sup>14</sup>C]leucine-labeled hemoglobins (●—●—●). (a) Total Hb F is 77.5%, radioactive Hb F 46.9%. (b) Total Hb F is 63.4%, radioactive Hb F 30.1%. (c) Total Hb F is 9.9%, radioactive Hb F 3.6%. (d) Total Hb F is 4.0%, radioactive Hb F 0.8%.

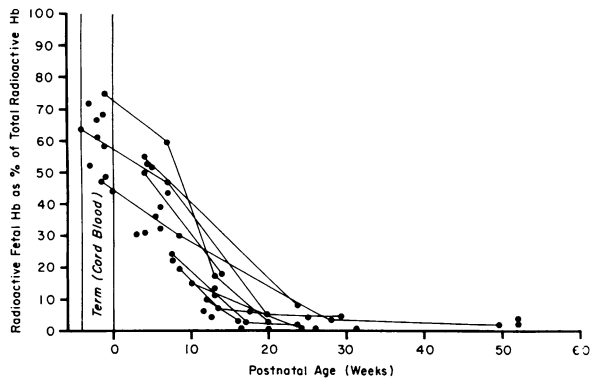


FIGURE 3 Radioactive Hb F as a percentage of the total radioactive hemoglobin in relation to postnatal age. All the infants were born at term. Lines uniting different points represent sampling of the same infant.

49 wk of postnatal age. The Hb A and Hb F peaks are symmetrical in shape, and the separations were complete. The proportion of Hb F to total hemoglobin being synthesized was 46.9% at birth, 30.1% at 8 wk postnatally, 3.6% at 28 wk of age, and only 0.8% at 49 wk after birth.

Fig. 3 illustrates the time-course of radioactive Hb F as a percent of total radioactive hemoglobin in the 37 infants studied who were born at term. There is a rapid decline in Hb F synthesis postnatally until about 12 wk of age, when levels averaging 7% are reached. From this age on, the decline in Hb F synthesis is more gradual.

A histogram of the postnatal proportion of Hb F to total Hb synthesis at 4 postnatal wk intervals is shown in Fig. 4. There is little change in Hb F synthesis beyond 16 postnatal wk. At the 48-52-wk age, interval Hb F synthesis was only  $1.7\% \pm \text{SD } 1.0\%$ .

To describe the complete switchover from Hb F to Hb A synthesis during pre- and postnatal life, the data from a previous study (1), where Hb F synthesis was determined using cord blood obtained from both preterm and term newborn infants, were combined with the results from the present study. The combination of these data in terms of postconceptional age is shown in Fig. 5. Clearly the switchover from Hb F to Hb A synthesis follows a sigmoid curve. The steep portion lies between 30 and 52 postconceptional wk preceded and followed by plateaus of approximately 95% and 7%, respectively.

## DISCUSSION

When studying adult blood, the separation of small amounts of Hb F can be subject to error because of the minor component HbA<sub>1</sub> which is found in the zone of the Hb F on chromatographic separation (6). In a normal adult, HbA<sub>1</sub> can amount to 5-8% of the total

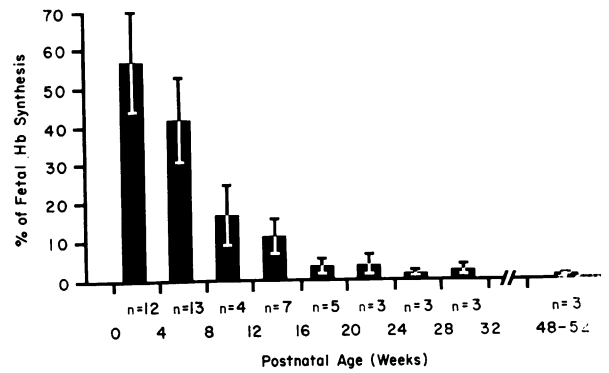


FIGURE 4 The percent of Hb F synthesis and a SD are shown at intervals of 4 postnatal wk.

hemoglobin. In a previous publication (2), which dealt with infants where Hb F made up a large proportion of the total amount of hemoglobin, the quantity of the minor component HbA<sub>1</sub> found after DEAE-Sephadex separation was of no particular consequence. A similar conclusion was reached when further analyses were carried out on the fetal peaks at 18 and 24 wk of age. Since at that postnatal age Hb F synthesis was found to be equal or less than 4% of the total, the amount of possible contamination was considered insignificant. HbA<sub>1</sub> may well be a product of the normal ageing of erythrocytes, as there appear to be insignificant amounts present in newly synthesized Hb F.

All of the infants included in this study were normal, born after a term gestation, and healthy during their postnatal follow up. This selection of subjects thus differed from those included in a similar investigation performed by Garby, Sjölin, and Vuille (8). They used two methods, one based on the relative rate of the appearance of i.v. injected radioactive iron in circulating Hb F and the other based on the semi-

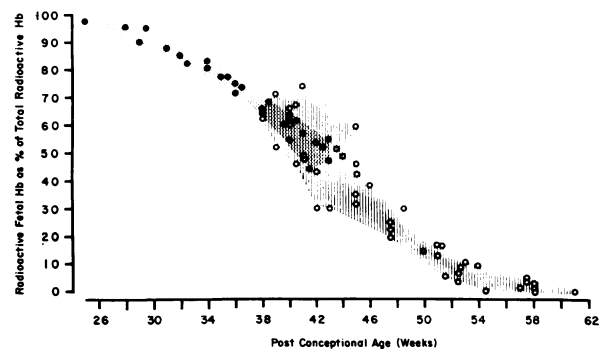


FIGURE 5 The decline of Hb F synthesis during the pre-natal period: ● represents cord blood data obtained from Bard, Makowski, Meschia, and Battaglia (1) from preterm newborn infants appropriate in weight for gestational age. ○ represents data on the postnatal blood samples in infants obtained from the present study.

quantitative evaluation of Hb F in reticulocytes. Contrary to our study, their infants varied in gestation and more than half the infants had some central nervous system pathology. Their data when compared to our study demonstrated a faster rate in the decline of Hb F synthesis soon after birth. The results from the two studies became similar after the 10th postnatal wk.

Human fetal studies have shown that Hb A synthesis is underway by the end of the first trimester. By cation exchange resin it has been demonstrated that Hb A synthesis is present in 55-day embryos (9). Others (10), by means of electrophoresis, have detected Hb A after 11 wk of gestation and have described a synthesis of 5% of adult hemoglobin by the end of the first and throughout the second trimester of gestation. Wood and Weatherall (11), using incubations of DEAE-Sephadex separations on blood samples from 8-, 13-, 15-, and 16-wk normal fetuses, also have confirmed the presence of Hb A as early as the 8th wk of gestation. The complete orderly switchover from Hb F to Hb A synthesis in humans can now be described by adding the data on very immature fetuses (11) and preterm infants previously published (1) to these infants of the present study.

It appears that Hb A synthesis is present at 8 wk of gestation and that its relative rate of production changes little until about 30 wk of gestation at which point the main switchover begins. The period of rapid switchover lasts until approximately 52 postconceptional wk when once again the relative rates of Hb A and Hb F synthesis change slowly.

The transition from Hb F and Hb A synthesis in humans can be correlated in terms of postconceptional age. Hb F synthesis is 95–90% of the total hemoglobin being synthesized from 8 to 30 wk of gestation, after which there is rapid switchover to Hb A synthesis. The period of rapid transition lasts about 22 wk, after which at 52 wk of postconceptional age the hemoglobin F synthesis is approximately 7%. There is then a gradual decline in Hb F synthesis over the next few months until the levels of 1–2% found in adults are attained.

In term newborn infants Hb F synthesis declines to levels of less than 10% by 16 wk of age. The finding of elevated levels of Hb F synthesis beyond this point can be considered abnormal in infants born at term.

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