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The Proportions of $^{G}\gamma$- and $^{A}\gamma$-Globins in the Fetal Hemoglobin Synthesized in Preterm and Term Infants[1]

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ABSTRACT

The change in $^{G}\gamma$ to $^{A}\gamma$ ratio in relation to the switchover of fetal HB (HbF) to adult Hb ($\alpha_2\beta_2$) synthesis has not been well defined. The γ-globins of HbF ($\alpha_2\gamma_2$) have either glycine ($^{G}\gamma$) or alanine ($^{A}\gamma$) at position 136. During fetal life the $^{G}\gamma$ makes up 70% of the γ-globins, although they are 40% of the small amounts of HbF in the adult. To further the understanding of this switchover, globin chain synthesis was determined sequentially in eight preterm and 20 full-term infants postnatally. To complete the study, single analysis was also carried out in eight term infants at birth and six preterm infants at the postconceptional age equivalent to term. Blood samples were incubated in an amino acid mixture containing ^{3}H-leucine and subjected to reversed-phase liquid chromatography. The results demonstrated that the fetal proportions of $^{G}\gamma$ to $^{A}\gamma$ are rigidly controlled according to postconceptional age and not affected by postnatal age after preterm birth. During the early postconceptional age switchover from HbF to adult Hb synthesis, an equal repression of the $^{G}\gamma$ and $^{A}\gamma$ chains was found. However, based on the values obtained from the term infants, after a postconceptional age of 44 wk, the levels of $^{G}\gamma$ to total γ synthesis begin to decrease and become more variable. (***Pediatr Res* 37: 361–364, 1995**)

Abbreviations

HbF, fetal Hb
PCA, postconceptional age

The switchover from fetal ($\alpha_2\gamma_2$) to adult ($\alpha_2\beta_2$) Hb synthesis during the perinatal period has been a subject of major interest to both clinicians and molecular biologists. To the former it has therapeutic implications, whereas to the latter it serves as an example of sequential gene activation proceeding in a biologically controlled manner (1).

Three types of γ chains normally compose fetal Hb. They are coded by two closely linked γ genes, $^{G}\gamma$ and $^{A}\gamma$, present in the β gene cluster on chromosome 11. At position 136, the $^{G}\gamma$ chain has glycine, whereas the $^{A}\gamma$ has alanine. In addition, the $^{A}\gamma$ chain is polymorphic, the residue 75 being occupied either by isoleucine ($^{A}\gamma^{I}$ chain) or threonine ($^{A}\gamma^{T}$ chain) (2).

It has been noted that the relative amount of the $^{G}\gamma$ to total γ chains ($^{G}\gamma/^{A}\gamma+^{G}\gamma$) produced during the second and third trimester of fetal life is 70%, whereas the small amount of HbF synthesized during adult life is made up of 40% $^{G}\gamma$ (3–5). However, the timing of the $^{G}\gamma$ to $^{A}\gamma$ switch is not the same as the γ to β switch. Whereas in the full-term infant there appears to be no postnatal decrease in $^{G}\gamma$ chain until total γ chain synthesis has dropped below β-chain production, it has not been distinguished whether the change in $^{G}\gamma$ ratio is related to postnatal or postconceptional age. A study was therefore designed to determine in preterm and full-term infants whether the change in the ratio of $^{G}\gamma$ to total γ synthesis is triggered by the birth process or controlled by a developmental clock similar to that controlling the switchover from γ to β globin synthesis.

METHODS

Three groups of newborn infants were included in this study. Studies in progress at the University of Iowa Hospital and Clinics provided an opportunity for postnatal sequential blood samples to be obtained from preterm and term newborn infants. There were eight preterm newborn infants and 20 normal full-term infants whose gestational age at birth was 28 ± 1 and 40 ± 1 wk, respectively, cared for at the University of Iowa Hospitals and Clinics. Samples were obtained weekly for 7 wk beginning at 3 wk of life in the preterm group and monthly in the term infants starting from 28 d of age. Not all of the infants were examined at each time point. A group of eight normal

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full-term newborns (gestational age 40.3 ± 0.7 wk) delivered at St. Justine's Hospital, Montreal, as well as six prematurely born infants (gestational age 28.6 ± 2 wk) at a postconceptional age corresponding to the full-term infants (gestational age 39.8 ± 0.8 wk) were also included to complete the developmental age range of interest.

The preterm and term infants included in this study had their gestational age based on the ultrasound examination performed at 18–20 wk of pregnancy, the mother's menstrual history, or the confirmation of the infant's physical examination on the day of birth. All infants included were normal white infants without known hemoglobinopathies. The only infants who received transfusions were in the preterm group.

The red blood cells obtained from the infants by heel stick were incubated for 6 h in an amino acid mixture containing [^{3}H]-leucine as previously described (6). The cells were then washed and lysed and the hemolysate was subjected to globin chain separation and quantitation by reverse phase HPLC equipped with an integrator using the method described by Shelton *et al.* (7). This method uses a gradient between aqueous trifluoroacetic acid and trifluoroacetic acid in acetonitrile and gives an excellent resolution of human globin chains. The equipment consisted of a Waters automated gradient controller, model 680; a Waters data system, model QA-1; two Waters pumps, model 510; and a Waters absorbance detector, model 441 (Millipore Corporation, Waters Chromatography Division, Milford, MA). The chromatographic column was a Vydac large-pore (300 Å) C4 column (4.6 × 250 mm) (The Separations Group, Hesperia, CA). The absorbance was read at 214 mm. The procedure of Shelton *et al.* (7) was followed with minor modifications. Mixture A contained 20% of acetonitrile in water, and mixture B contained 70% acetonitrile. The two solutions contained 0.1% trifluoroacetic acid. The elution profile of newborn human globin chains was obtained with a gradient of 40 to 50% solvent B in 70 min at a flow rate of 1 mL/min. This method provided a separation of the α and β chains, as well as the three types of γ chains ($^A\gamma^T$, $^G\gamma$, and $^A\gamma^I$). The γ-globin chains were quantified by adding $^G\gamma$ and $^A\gamma$ (which included $^A\gamma^T$ when present). Liquid scintillation counting was carried out on the separated globin fractions. The relative amounts of the separated fractions were obtained using a computer program (Inplot 4.02, Graphpad Software, Inc., San Diego, CA) that provided an integrated profile of the incorporation of [^{3}H]-leucine into the globin chains as well as their proportions. The results concurred with those obtained by the cut-out and weighing procedure. The percentage of HbF was calculated by the ratio of $\gamma/\gamma+\beta \times 100$. The synthesis of the different γ-globins was too low and too close to detectability for accuracy after an age of 60 wk postconception. This study was reviewed and approved by both the University of Iowa Committee on Research Involving Human Subjects and St. Justine's Hospital. Written parental informed consent was obtained.

RESULTS

An example of an HPLC separation of the globin chains of a preterm infant born at 29 wk of gestation, sampled at a PCA of 34 wk, is illustrated in Figure 1. The total HbF was 89.5%, and HbF synthesis was 84.0%. The total $^G\gamma$ and $^G\gamma$ synthesis was 69.7 and 67.5%, respectively. The sequential changes of $^G\gamma$ production as well as HbF synthesis in relation to PCA are shown in Figure 2. The results show that in preterm infants, despite a gradual decrease in HbF synthesis, there is a rigid control of γ synthesis. This control is still tight at birth (40 wk PCA) but not at 44 wk PCA, at which point a wider range is observed. The postnatal changes in both total HbF and HbF synthesis obtained by HPLC in relation to PCA in the full-term infants are shown in Figure 3. The mean HbF decreases from 70.7% at 1 mo to 14.1% at 5 mo of age, whereas HbF synthesis changed from 42.6 to 11.1% during the same period. There was no relationship between the level of HbF synthesis and $^G\gamma$ synthesis at the 4-wk intervals of the study beyond 44-wk PCA.

Table 1 lists the mean ± SD of the percentage of HbF synthesis to total HbF synthesis concomitantly with the per-

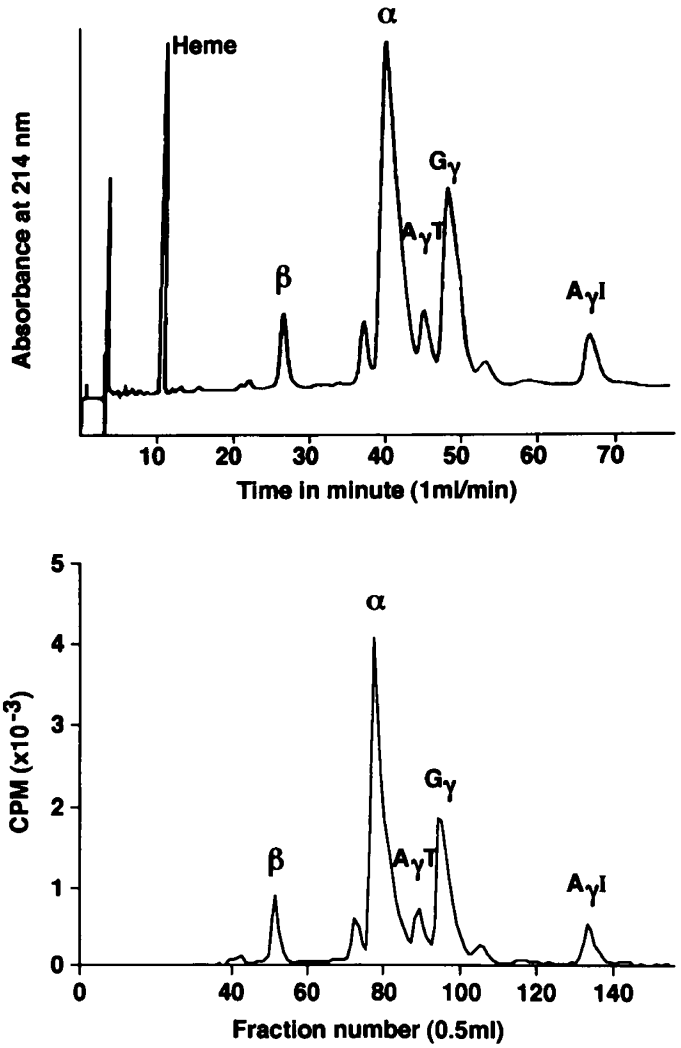

Figure 1. Chromatography of [^{3}H]-labeled globins separated on HPLC from a preterm infant born at 29 wk of gestation sampled at a postconceptional age of 34 wk. *Top*, The absorbance at 214 nm. *Bottom*, The counts per minute in each 0.5 mL fraction. The percentage of total globins that were γ-globins (HbF) was 89.5%, and the percentage of total globin synthesis that was γ-globin (HbF) synthesis was 84.0%. The total $^G\gamma$ to total γ was 69.7%, and the $^G\gamma$ synthesis to total γ synthesis was 67.5%.

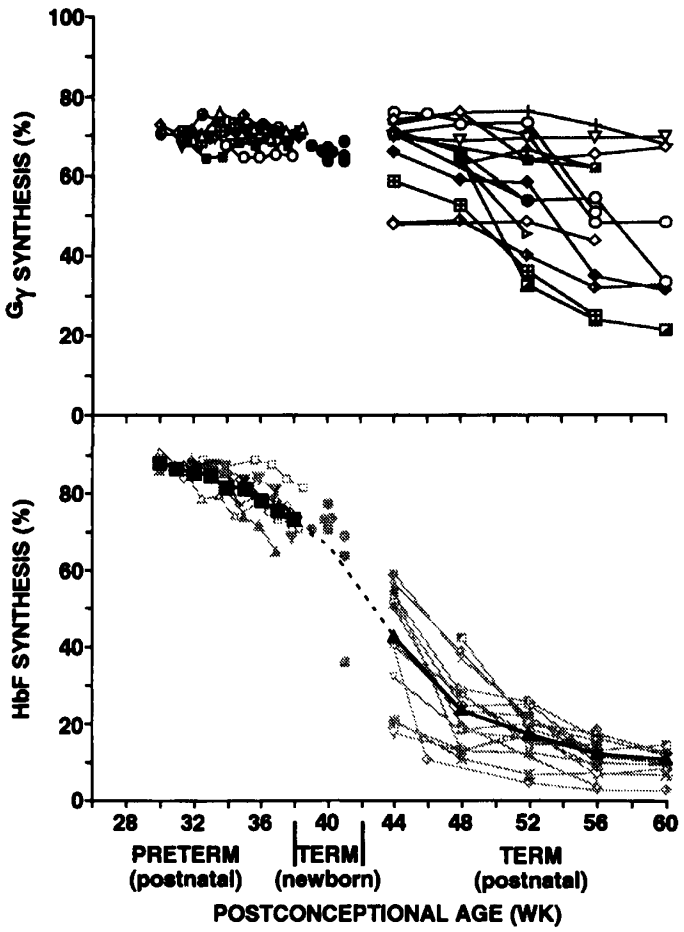

Figure 2. The sequential changes in $^{G}\gamma$ synthesis (*top*) and HbF synthesis (*bottom*) in relation to PCA. ■-■-■-, the postnatal mean values of the preterm infants; ▲-▲-▲, postnatal mean values of the term infants; - - -, joins the postnatal preterm and term infants through the mean level at 40 wk PCA of the term newborns. The lines and symbols unite values obtained from the same infants.

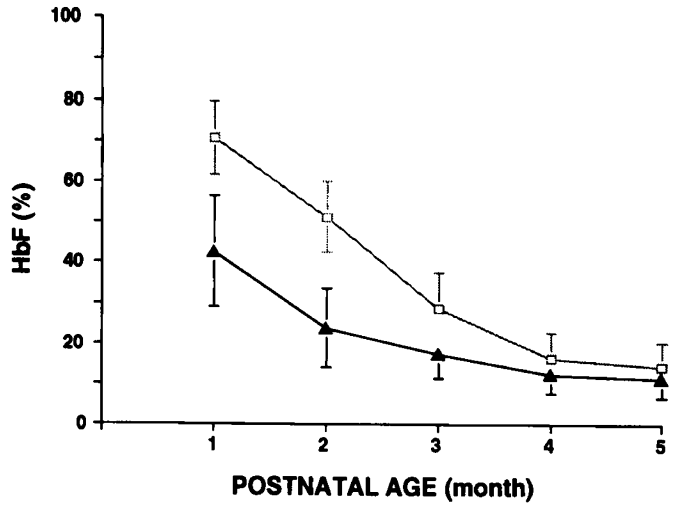

Figure 3. The postnatal changes in total HbF and HbF synthesis in relation to age in months in normal term infants. □-□-□, mean ± SD for HbF; ▲-▲-▲, mean ± SD for HbF synthesis.

centage range of $^{G}\gamma$ to total γ synthesis at 3- to 4-wk intervals from 30 wk to 60 wk PCA. Also shown in Table 1 are the data obtained from cord blood at birth of eight full-term infants as well as six preterm infants born at 26–30 wk of gestation,

Developmental changes in HbF and $^{G}\gamma$-globin chain synthesis

	n	PCA (wk)	Ratio of HbF to total Hb synthesis (%)	Range of $^{G}\gamma$ to total γ synthesis (%)
Preterm (postnatal)	7	31	86.6 ± 2	67.0–71.2
	7	34	80.9 ± 5	65.1–72.9
	8	37	73.1 ± 8	63.4–72.2
Term*	8	39–41	66.6 ± 13	63.4–68.4
Preterm†	6	39–41	68.6 ± 9	64.9–70.4
Term (postnatal)	14	44	42.6 ± 14	47.7–76.1
	14	48	23.6 ± 10	48.9–78.2
	17	52	17.1 ± 6	32.4–76.3
	15	56	12.1 ± 5	24.0–72.7
	10	60	11.1 ± 4	21.5–69.4

Values are mean ± SD.
* Term infants sampled at birth.
† Preterm infants born at 26–30 wk sampled at term PCA.

sampled at a postconceptional age corresponding to term. These data show that the levels of $^{G}\gamma$-globin synthesis at a postconceptional age corresponding to term in these different groups of infants are within the same range.

DISCUSSION

Because of ethical considerations, a study of this nature could only be carried out with different groups of infants. By determining globin chain synthesis longitudinally in preterm as well as term infants, this study was able to evaluate the control of $^{G}\gamma$ gene expression during the perinatal and early postnatal period. Some of the preterm infants received adult blood as volume replacement as part of their neonatal care; by determining the levels of the newly synthesized globins, there was no contamination of the data by any transfused adult red blood cells. Reversed-phase HPLC provided a rapid means of quantifying globins synthesized during the perinatal period. The values of the ratio of $^{G}\gamma$ to total γ obtained agree with those documented in cross-sectional studies reported previously (3–5).

By measuring the different types of γ-globin synthesis sequentially in both preterm and term infants, it was possible to determine the point during human development when the pattern of the γ-globin chains produced in the fetal period changes. There was little variation in $^{G}\gamma$ expression postnatally in preterm infants during their development until a postconceptional age considered to be term. However, there were gradual changes during the first month after birth in the term infants. At that stage of development, the pattern of $^{G}\gamma$ gene expression changed. There was a progressive dispersion of the levels of $^{G}\gamma$ production, with a shift toward lower $^{G}\gamma$ synthesis. At 44 wk PCA, 71% of the term infants were synthesizing more than 65% of $^{G}\gamma$. This number decreased to 57 and 33% at 48 and 52 wk, respectively. These observations suggest that the change from fetal to adult γ-globin ratios is closely related to the postconceptional age, supporting the likelihood that it is controlled intrinsically.

Single nucleotide substitution in the promoter regions of $^{A}\gamma$ and $^{G}\gamma$-globin genes have been shown to be associated with increased fetal Hb production in patients with sickle cell anemia (8), β thalassemia (9), and cases of $^{G}\gamma$-hereditary persistence of fetal Hb (10). These hemoglobinopathies with increased HbF are also associated with increased $^{G}\gamma$-globin

production; this has led to the theory that these promoter regions are involved in the modulation of HbF production and that mutations within the promoter regions could be responsible for high levels of $^{G}\gamma$ in adults.

Elevated $^{G}\gamma$ production related to DNA sequence variation of C *versus* T at 158 bp of the 5′ of $^{G}\gamma$ gene Cap sites has been shown to occur in certain hemoglobinopathies (9). In these hemoglobinopathies the levels of $^{G}\gamma$ production in relation to 158 C-T polymorphism were evaluated by analyzing DNA using restriction endonuclease *Xmn*I, which recognizes the sequence from -157 to -166 only if T is at the position -158. To examine this possibility, a DNA fragment of $^{G}\gamma$ promoter from 1500 to 2121 nucleotides (11) was amplified by polymerase chain reaction with specific primers (5′-primer: nucleotides 1500–1518; and 3′-primer: nucleotides 2101–2121) from the cellular DNA isolated from the hemolysates of the samples. The resulting 622-bp DNA fragment was digested with *Xmn*I. The presence of *Xmn*I site in DNA did not correlate with high levels of $^{G}\gamma$ production in the term infants followed between 44 and 60 wk.

Economou *et al.* (12) have published data that indicate that nucleotide substitutions in the regions of the γ promoters are not responsible for the variations in HbF found in normal adults. In a more recent study carried out in a normal Japanese population, Enoki *et al.* (13) have shown that a wide range of $^{G}\gamma$-globins exists in normal populations; based on their data, the conventionally accepted $^{A}\gamma/^{G}\gamma$ ratios can be interpreted as not representative of normal subjects. The results from the present report on the sequential analysis of $^{G}\gamma$ chain synthesis are consistent with the findings of the cross-sectional study carried out in a normal Japanese population (13).

The present report illustrates that during the switchover from HbF to adult Hb synthesis, when γ-globin synthesis has decreased to less than 20%, the narrow range of $^{G}\gamma$ levels that existed during early development becomes widespread. At that point the decline in $^{G}\gamma$ is not in synchrony with the decline in HbF synthesis. This information could provide a new understanding of the normal regulatory mechanism of $^{G}\gamma$ and $^{A}\gamma$ gene expression during development.

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