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The $^{G}\gamma$ and $^{A}\gamma$ Hemoglobin Chains during Human Fetal Development

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In humans, nonallelic loci direct the synthesis of the γ chains of fetal hemoglobin (Hb F) [1–3]. The γ chains produced by these loci differ in the presence of glycine or alanine at position 136 and are thus designated the $^{G}\gamma$ and $^{A}\gamma$ chains, respectively. Combination of these chains with α chains produces tetrameric fetal hemoglobins ($\alpha_2{}^{G}\gamma_2$, $\alpha_2{}^{A}\gamma_2$, and perhaps $\alpha_2{}^{G}\gamma{}^{A}\gamma$) which are identical in electrophoretic and chromatographic behavior. However, detection and quantitation of the two chains may be accomplished by isolation of the C-terminal cyanogen bromide peptides ($^{G}\gamma$CB-3 and $^{A}\gamma$CB-3) from a solution of fetal hemoglobins containing both types of chains, followed by determination of the amino acid composition of the isolated material [1].

The structurally different γ chains are present in unequal proportions in neonates [1–3]. At birth, approximately 75% are $^{G}\gamma$ while the remaining 25% are $^{A}\gamma$, the ratio of $^{G}\gamma$:$^{A}\gamma$ being very close to 3:1. With growth of the infants, however, this ratio changes, and, near the sixth month of life, is almost reversed: approximately 40% of the chains are $^{G}\gamma$ while the remaining 60% are $^{A}\gamma$ ($^{G}\gamma$:$^{A}\gamma = 2{:}3$) [3]. Similar proportions of the two fetal chains are found in normal adults [3], in the Hb F isolated from adults with one variety of hereditary persistence of fetal hemoglobin (HPFH) [4, 5], as well as from persons with certain forms of β- and $\beta\delta$-thalassemia [6, 7].

Given these observations, the question arises as to whether the unequal proportions of the two γ chains at birth reflect asynchronous activation of the two types of loci during gestation, the $^{A}\gamma$ locus being activated at a later date than the $^{G}\gamma$ locus. This situation would require that in young fetuses the proportions of γ chains be different from those observed at birth, with significantly higher proportions of $^{G}\gamma$ chains. Schroeder and Huisman [3] found proportions of $^{A}\gamma$ and $^{G}\gamma$ chains in the red cells of two fetuses of approximately 20 weeks' gestational age which were essentially identical with those typical of neonates. While these observations suggest that the $3{}^{G}\gamma{:}1{}^{A}\gamma$ ratio is maintained throughout the final 4 months of intrauterine

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development, further measurements of $^{G}\gamma$:$^{A}\gamma$ ratios in several fetuses of different ages are required before the presence or absence of shifts in proportions of the chains can be established. To this end, we measured the proportions of the two types of chains in fetuses ranging in crown-rump length from 81 to 200 mm (that is, from the end of the first through the second trimester of gestation). In addition, similar determinations were carried out using material from hematologically normal neonates.

MATERIALS AND METHODS

Globins from 26 fetuses and nine neonates were prepared by acid-acetone precipitation [8]. Several fetal samples were pooled according to crown-rump length to provide sufficient material for subsequent analyses (table 1). Individual or pooled samples, ranging from 26 to 200 mg, were dissolved in 70% formic acid with equal weights of cyanogen bromide (CNBr) and placed in glass-stoppered vials. After 18 hr, the reaction was stopped by adding 20 vol of deionized water, and the reaction products were dried by lyophilization. In each case, the entire CNBr digest was then dissolved in 1% formic acid to a concentration of 40 mg/milliliter and applied to a 2 × 293 cm column of Sephadex G-50 (fine) equilibrated with 1% formic acid. The column was developed with 1% formic acid at a flow rate of 10 ml per fraction per 32 min (fig. 1).

The CNBr fragments comprising all of the peaks in the elution profile of the initial column run were dried by lyophilization, hydrolyzed under vacuum in 6N HCl for 24 and 48 hr at 108° C, and subjected to analysis on a Beckman model 120B amino acid analyzer. After this preliminary identification of the various cleavage products of fetal globin, only the γCB-3 peptides from subsequent samples were so analyzed. Amino acid compositions of γCB-3 peptides from normal neonates provided a basis for assessing the efficacy of the preparative techniques.

RESULTS

The $^{A}\gamma$CB-3 peptide contains three residues of alanine and none of glycine; the $^{G}\gamma$CB-3 peptide contains one glycyl and two alanyl residues [1]. Thus, the proportion of $^{G}\gamma$ chains in a sample of fetal globin is readily obtained from the number of residues of glycine found upon compositional analysis of the $^{G}\gamma$CB-3 and $^{A}\gamma$CB-3 peptides prepared from the sample, while the amount of alanine in excess of two residues serves as an approximation of the proportion of $^{A}\gamma$ chains.

Schroeder et al. [2] found mean values for glycine and alanine of 0.71 and 2.32 residues in the γCB-3 peptides prepared from 108 cord blood samples gathered from all over the world, indicating that close to 71% of the fetal chains analyzed were of the $^{G}\gamma$ type. The mean values for glycyl (0.74) and alanyl (2.30) residues in the γCB-3 peptides from the nine neonatal samples examined in this study (table 1) are in accord with the above findings.

The sum of glycyl and alanyl residues may be used as a standard of purity. If the sum falls between 3.0 and 3.1, the γCB-3 preparation is considered to be of sufficient purity to obviate further treatment [1, 3]. In this investigation, the numbers of glycyl residues per γCB-3 peptide prepared from neonatal samples range from 0.66 to 0.81; values for alanine range from 2.20 to 2.44. In only one instance was the sum of glycyl and alanyl residues in excess of 3.06 (Gly = 0.72, Ala = 2.44, sum = 3.16), and no sum fell below 3.00.

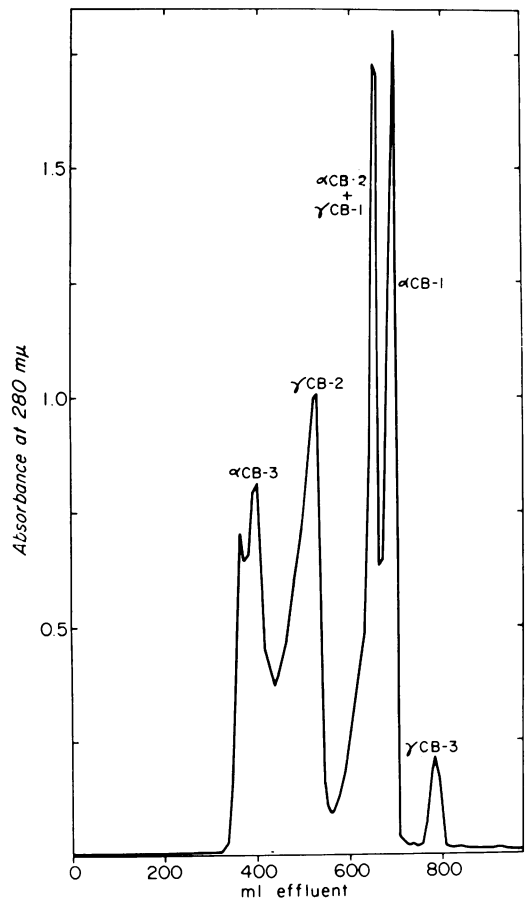

FIG. 1.—Elution profile produced by gel filtration of CNBr peptides prepared from 200 mg of human fetal globin. Peaks are labeled according to which of the three α-chain or three γ-chain CNBr fragments they contain.

Similar results were obtained for the γCB-3 peptides from fetal blood samples (table 1). Fetuses ranging in crown-rump length from 81 to 200 mm show little variation in numbers of glycyl (0.67 to 0.81) or alanyl (2.21 to 2.37) residues per γCB-3 peptide. Furthermore, in no instance was the sum of glycine and alanine below 3.00 or above 3.09. The values appearing in table 1 thus demonstrate that there was little contamination of our preparations from extraneous sources.

DISCUSSION

It is clear from these results that the proportions of the two human γ chains are maintained within narrow limits from the end of the first through the second trimester of gestation. Furthermore, there is no tendency for higher or lower values of either amino acid to be clustered at one end of the developmental sequence. In

TABLE 1

$^{G}\gamma$ AND $^{A}\gamma$ CHAINS FROM FETUSES AND NEONATES

FETUS CROWN-RUMP LENGTH (mm)	FERTILIZATION AGE (WEEKS)*	RESIDUES PER CB-3 PEPTIDE†			$^{G}\gamma$ CHAINS: TOTAL γ CHAINS (%)
		Gly	Ala	GLY + ALA	
81–100‡	12–13	0.80	2.21	3.01	80
101–110	14	0.76	2.25	3.01	76
111–120‡	14–15	0.80	2.25	3.05	80
131–140	16	0.67	2.37	3.04	67
141–150	17	0.79	2.22	3.01	79
151–160	17–18	0.76	2.33	3.09	76
161–170‡	18–19	0.81	2.25	3.06	81
171–180‡	19–20	0.72	2.30	3.02	72
181–190‡	20	0.71	2.30	3.01	71
191–200‡	21	0.72	2.28	3.00	72
		Neonates (Mean ± SD)§			
		0.74 ±0.05	2.30 ±0.07	3.04 ±0.05	

* Calculated according to Patten [9].

† Averages of two determinations each after 24 and 48 hr of hydrolysis calculated on the basis of the known sequence of γCB-3 [1, 10].

‡ Globins from two to five different fetal blood samples are included.

§ Based on analyses of globins from nine neonates.

addition, the identity of the ratios derived from fetal material with those obtained for normal neonates indicates that the proportions of $^{G}\gamma$ and $^{A}\gamma$ chains remain unchanged throughout the latter two-thirds of gestation, an interval during which the level of Hb F declines from approximately 95% to about 80%. Thus the only observed change in proportions of $^{G}\gamma$ and $^{A}\gamma$ chains coincides with the precipitous decline in levels of Hb F from about 80% to less than 5% during the first 6 months of postnatal development [3].

Kabat [11] has proposed a mechanism by which successive replacement of ϵ by γ and γ by β and δ chains might be achieved during human development. The proposal, in its simplest terms, states that a promotor locus (site of attachment of RNA polymerase to the chromosome) is the first in a series of closely linked loci. The promotor is followed in sequence by the ϵ-chain locus, the $^{G}\gamma$ locus, the $^{A}\gamma$ locus, and the β and δ loci. Moreover, because all but the β and δ loci are assumed to be separated by terminator sites, only the locus next to the promotor will be operative at any given time. Successive excision of promotor-proximal loci by intrachromosomal crossing over might then produce a series of stem cell populations, the earliest of which synthesizes ϵ chains, the next $^{G}\gamma$ chains, then $^{A}\gamma$ chains and, finally, β and δ chains. It is noteworthy that this theory predicts the elimination of $^{G}\gamma$ loci prior to that of $^{A}\gamma$ loci, a logical assumption based on the fact that the ratio of $^{G}\gamma$:$^{A}\gamma$ chains declines following parturition.

It is difficult to explain these data using Kabat's hypothesis of gene activation [11]. The hypothesis, in its native form, would require that the proportion of cells

synthesizing $^{G}\gamma$ chains keep pace with that of $^{A}\gamma$-synthesizing cells throughout the last 6 months of gestation, a period during which β-chain levels steadily increase from about 5% to 20% of the total non-α chains. This situation would demand that excision of three $^{G}\gamma$ loci be matched by the further excision of a single $^{A}\gamma$ locus over the last two-thirds of fetal development. Moreover, continuation of this process could not account for the shift in $^{G}\gamma$:$^{A}\gamma$ ratios following birth.

On the other hand, if excision were to occur at a later stage of red cell development, coincident with mRNA synthesis, then the quantities of various chains produced would vary according to the time of excision relative to the length of time transcription has been proceeding. Under these conditions, one might expect to find single erythrocytes containing more than two types of non-α chains (excluding δ chains). This modified form of Kabat's hypothesis introduces further difficulties, inasmuch as it requires that looping-out excision be either speeded up or initiated earlier relative to transcription as fetal and neonatal development continues. Moreover, the relatively rapid changes in proportions of $^{A}\gamma$, $^{G}\gamma$, and β chains following parturition necessitate that such changes in timing themselves occur at nonuniform rates.

The interpretive complexities required by Kabat's hypothesis in its native or modified forms lead us to reject asynchronous gene activation as an adequate explanation for the changes in the proportions of $^{G}\gamma$ and $^{A}\gamma$ chains during development. The fact that changes in the proportions of the two γ chains coincide with the precipitous decline in levels of Hb F from about 80% to less than 5% during the first 6 months of postnatal development indicates that the shifts in γ-chain proportions result from differential repression of the two types of γ loci.

The switch from synthesis of fetal to that of adult hemoglobin appears highly complicated, involving finely coordinated action upon four loci: the activities of two loci are stimulated (β and δ), while the activities of two loci ($^{A}\gamma$ and $^{G}\gamma$) are subjected to differential repression.

SUMMARY

The relative amounts of the $^{A}\gamma$ and $^{G}\gamma$ chains of human fetal hemoglobin are identical in neonates and in fetuses ranging in crown-rump length from 81 to 200 mm. This observation indicates that the unequal proportions of the two chains characteristic of neonates are not a reflection of asynchronous activation of the $^{G}\gamma$ and $^{A}\gamma$ loci. Furthermore, the postnatal changes in proportion of the two γ chains normally observed during the period of rapid decline in levels of Hb F are most likely consequences of differential repression of their respective loci.

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