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fetal hemoglobin newborns

Postnatal Changes in the Chemical Heterogeneity of Human Fetal Hemoglobin

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Extract

The fetal hemoglobin (Hb-F) of blood samples from 11 newborn babies (two normal infants, two sickle cell trait carriers, two Hb-C heterozygotes, two infants with Hb-SC disease, one infant with Hb-Richmond heterozygosity, one β-thalassemia heterozygote, and one infant with a heterozygosity for the hereditary persistence of fetal hemoglobin) and from 16 adults (eight normals, two Hb-S heterozygotes, one Hb-C heterozygote, and five SC patients) has been examined to determine the ratio of the two structurally different γ chains, namely the $^{G}\gamma$ and $^{A}\gamma$ chains. This ratio is about 2:3 in the Hb-F of the adults and, therefore, significantly different from the 3:1 ratio in the Hb-F of the newborn. This newborn ratio undergoes a considerable change between the 3rd and 4th months of life, at which time it approaches that of the Hb-F of adults.

Speculation

The mechanism by which the gradual change from γ chain synthesis to β and δ chain synthesis is controlled remains unclear. However, the change in the ratio of production of structurally different γ chains as a function of postnatal age indicates a rather complex mechanism which probably involves an unequal repression of the γ chain structural genes. Any explanation of the mechanism must take into account the fact that the production of two genes, the $^{G}\gamma$ and $^{A}\gamma$, is greatly decreased, whereas that of two other genes, the β and δ, is started. Perhaps a closely related or even identical mechanism controls not only the ratio of production of the $^{G}\gamma$ and $^{A}\gamma$ genes but also that of the β and δ genes.

Introduction

Fetal hemoglobin (Hb-F or $\alpha_2\gamma_2$) constitutes 50–90% of the total hemoglobin with an average value of about 75% in cord blood samples from full-term newborn infants [25]. The gradual replacement of Hb-F by adult hemoglobins A ($\alpha_2\beta_2$) and A_2 ($\alpha_2\delta_2$) is essentially complete 150 days after birth [12, 32], although levels of 1–3% are observed during the first 3 years of life [23].

In the hemoglobin of normal adults, the alkali-resistant residue is 0.4–1.0% [23]. Although this residue may not be Hb-F alone [10, 22], it is generally assumed to contain variable amounts of the same Hb-F that is present in cord blood [10, 13, 15, 26]. However, appre-

ciable amounts of Hb-F can be detected in the peripheral blood of individuals 6 months of age and older who have an acquired hematologic disorder or a genetic abnormality, such as β-thalassemia or a hemoglobinopathy [3, 4, 6, 7, 11, 31].

In a previous communication [29], we presented evidence that Hb-F of the newborn human infant is a chromatographically and electrophoretically inseparable mixture of two components that differ in the γ chains. In one type of γ chain (the $^{G}\gamma$ type [35]) a glycyl residue is present in position 136, whereas in the other (the $^{A}\gamma$ type) an alanyl residue is present in this position. The ratio of $^{G}\gamma$ to $^{A}\gamma$ chains in newborn Hb-F is about 3:1. Examination of Hb-F variants, abnormal in the γ chain, and of the Hb-F from individuals with hereditary persistence of fetal hemoglobin established that the $^{G}\gamma$ and $^{A}\gamma$ chains are the products of nonallelic structural genes for the γ chain [18–20, 29].

The question arises whether the 3:1 ratio of the $^{G}\gamma$ and $^{A}\gamma$ chains in the Hb-F of the newborn infant remains constant during early postnatal life and is the same in the minute amount of Hb-F found in adult blood. The pattern of this ratio was investigated by serial examination of Hb-F from infants in the 1st year of life and by analysis of Hb-F from normal adults and several patients with hemoglobinopathies. Some of these data have been described in a preliminary report [27].

Materials and Methods

Source of Blood Samples

Nine full-term Negro infants were studied at intervals during the 1st year of life. Two subjects had sickle cell trait, two were heterozygous for Hb-C, one was doubly heterozygous for hemoglobins S and C, one was heterozygous for Hb-Richmond [9], one was a heterozygous β-thalassemia carrier, one was heterozygous for the hereditary persistence of fetal hemoglobin, and one was normal. One normal infant and one with Hb-SC disease were studied only once. Blood samples (2–4 ml increasing to 5–15 ml at 5 months of age or older) were collected in ethylenediaminetetraacetate (EDTA).

Samples of blood, 250–500 ml, from three normal adult females, from two adult sickle cell carriers, and from one adult Hb-C trait carrier were collected in acid-citrate-dextrose (ACD). A few milliliters of blood were also placed in EDTA for hematologic analyses. Smaller volumes (50–100 ml in EDTA) were obtained from other adults: two Caucasian individuals from one family with slightly elevated Hb-F percentage [24], three members of a Negro family with a comparable elevation of Hb-F [14], and five patients with Hb-SC disease [38].

Hematologic Examination and Hemoglobin Analysis

Hemoglobin concentration (in g/100 ml), packed cell volume (PCV in percent), red cell (in $10^6/mm^3$), and reticulocyte counts (in percent) were determined by standard techniques [33].

Red cell hemolysates were studied by starch gel electrophoresis in Tris-EDTA-boric acid buffer, pH 8.1 [16], and by DEAE-Sephadex chromatography [8, 17]. The latter procedure allows the quantitation of relative amounts of hemoglobins A_2, A (and/or S and C), and F in the hemolysates. Quantitation of Hb-F involved alkali denaturation [5] and a recently developed method [30]. Data obtained with these two techniques are presented as $\%F_{AD}$ and $\%F_{IIe}$, respectively [30]. Comparative studies have shown that the F_{IIe} data are probably more reliable [30].

Isolation of Hb-F for Chemical Examination

Column chromatography on DEAE-Sephadex was also used for the isolation of larger amounts of Hb-F. The Hb-A_1 + F fractions from several preparative columns (2.5 by 50 cm) were combined and rechromatographed in the same way to remove residual Hb-A_0. The relative amount of Hb-F in these purified A_1 + F fractions varied greatly; rechromatography on a cation exchange resin was made when the Hb-F was estimated to be less than 15%. For this purpose the A_1 + F fraction was applied to a column (3.0 by 35 cm) of CM-cellulose [36] which was equilibrated with 0.01 M sodium phosphate buffer, pH 6.8. The chromatogram was developed with a pH gradient (0.01 M sodium phosphate buffers of pH 6.8, 7.2, 7.4, 7.6, and 7.8 with 100 mg KCN/100 ml) as previously described [17]. The Hb-A_1 fraction was eluted from the column as a broad heterogeneous zone that was followed by a small, rather homogeneous, zone primarily of Hb-F. The appropriate hemoglobin fractions were converted into globin by the procedure of Anson and Mirsky [1].

Chemical Examination of Hb-F

Investigation of the globins of the isolated Hb-F components was made as previously described [29]; the globin was cleaved with cyanogen bromide and the smallest peptide, γCB-3, was isolated and analyzed. The minimal amount of fetal globin for a single analy-

sis was 15 mg. The results of these analyses are presented as the numbers of glycyl and alanyl residues in peptide γCB-3 which comes from the C-terminal end of the γ chain and contains 13 amino acid residues. Its amino acid sequence is:

135 140 145
Val-Thr-Gly(orAla)-Val-Ala-Ser-Ala-Leu-Ser-Ser-Arg-Tyr-His

(The numbers refer to the positions of the residues in the γ chain.) As mentioned, the $^{G}\gamma$ and $^{A}\gamma$ chains differ in position 136 which is occupied either by glycine or by alanine. If the peptide comes from a $^{G}\gamma$ chain alone, amino acid analysis will show the presence of 1 residue of glycine (136) and of 2 residues of alanine in positions 138 and 140. Similarly, analysis of the peptide from a $^{A}\gamma$ chain only will show no glycine and 3 residues of alanine. The value for glycine in the analysis is the simplest indicator of proportion of the $^{G}\gamma$ and $^{A}\gamma$ chain; thus, if glycine is 0.50 (and alanine theoretically 2.50), the γCB-3 is derived from Hb-F which is a mixture of 50% each of Hb-F with $^{G}\gamma$ chains ($\alpha_2{}^{G}\gamma_2$) and of Hb-F with $^{A}\gamma$ chains ($\alpha_2{}^{A}\gamma_2$), and the ratio of the two types of chain is 1:1. The reliability of the data has been tested repeatedly by duplicate analyses of fetal globin samples. The results indicate an order of precision and accuracy of ±0.05 residue [18, 27, 28, 30].

Results

Data obtained from analyses of blood samples of the 11 infants are summarized in Table I. The table lists common hematologic variables, levels of Hb-A_2 (except for samples with Hb-C because of the inability to separate these two hemoglobins), levels of Hb-F as $\%F_{AD}$ and $\%F_{IIe}$, and number of glycyl and alanyl residues in the γCB-3 peptides of the isolated Hbs-F. Similar data on blood samples from eight normal adults, two sickle cell carriers, one Hb-C trait carrier, and five patients with Hb-SC disease are summarized in Table II.

Discussion

Since the original study [29], which examined the γCB-3 peptides of the Hb-F from 12 newborn infants, 32 additional cases including six from the present report have been studied. The mean value for glycine of these 44 cases is 0.73 with a range of 0.65 to 0.88 and that for alanine is 2.31 with a range of 2.19 to 2.43. The average ratio of $^{G}\gamma$ and $^{A}\gamma$ chains in the newborn, therefore, is almost exactly 3:1. Values from two fetuses of about 20 weeks gestational age (glycine 0.72 and 0.81; alanine 2.33 and 2.24, respectively) fall within the range observed for newborn infants and suggest a comparable relative production of $^{G}\gamma$ and $^{A}\gamma$ chains in early and late gestation.

Technical difficulties in the isolation of 0.5–1.0% Hb-F from 99–99.5% adult hemoglobins have limited the investigation of Hb-F in the normal adult. Data are available, however, from three normal adult females (*AD, NB,* and *GD*) and from two adult members of a Swiss family (*IF* and *LL*) as well as from three adult members of a Negro family (*SR, ER,* and *RR*) in which slightly elevated levels of Hb-F have been observed [14, 24] (Table II). The mean value for glycyl residues is 0.38 (range 0.23–0.55) and for alanyl residues 2.71 (range 2.52–2.99). These values differ significantly from those of the newborns and give a ratio of about 2:3 for the $^{G}\gamma$ and $^{A}\gamma$ chains of the Hb-F in the adult. The ratio is similar in the Hb-F of patients with sickle cell trait, Hb-C trait, and Hb-SC disease, although the relatively high values from *patient JM* with sickle cell trait and *patient HO* with Hb-SC disease remain unexplained.

The validity of the difference between the ratio of $^{G}\gamma$ and $^{A}\gamma$ chains in the Hb-F of the newborn and that in the adult is supported by the results of the serial examination of Hb-F from the newborns in the 1st year of life. Most data of Table I are also illustrated in Figure 1. The $^{G}\gamma$ and $^{A}\gamma$ ratio in the two newborns with AS, two with AC, one with A-Richmond, and one with SC changed considerably and, at the age of 150–200 days (at which time the level of Hb-F is less than 10%), approximates that seen in normal samples from adults or adults with sickle cell trait, Hb-C trait, or Hb-SC disease. Although comparable data were obtained for all six infants, individual variation is unexplained at present. Only a few analyses were made on the Hb-F from two normal infants; the results agree with those from the other infants. There is, in fact, good general agreement of all data from older infants and adults regardless of whether the individual is normal or heterozygous for an abnormal hemoglobin.

Of special interest are the data derived from *baby RS,* who presented with a β-thalassemia trait, and *baby RB,* who has the hereditary persistence of fetal hemoglobin. When *baby RS* was first studied at 56 days of age, the $^{G}\gamma$ to $^{A}\gamma$ ratio was about 2:1. This ratio steadily decreased with time and by 146 days approximated the adult value of 2:3. The nature of Hb-F from subjects with β-thalassemia places these individu-

Table I. Hematologic data on blood samples from infants 1 day–14 months of age, and the results of chemical analyses of Hb-F isolated from these samples

Case	Race and sex[1]	Hb type[2]	Age, days	Hb, g/100 ml	PCV, %	RBC, 10^6/mm^3	Retic., %	Hb-A_2, %	Hb-F[3] AD %	Hb-F[3] Ile %	γCB-3 Gly	γCB-3 Ala
CG	N-F	AA	1	18.6	50	5.07	n.d.[4]	<0.1	n.d.	(88.9)	0.72	2.33
			200	9.5	32	3.03	0.3	2.1	3.4	4.8	0.57	2.50
KO	N-M	AA	270	n.d.	n.d.	4.05	n.d.	2.4	1.8	n.d.	0.42	2.69
ST	N-F	AS	1	17.1	51	5.48	n.d.	<0.1	n.d.	(84.1)	0.71	2.34
			30	10.2	33	3.00	1.5	0.4	n.d.	61.8	0.68	2.37
			112	9.4	31	3.69	0.6	2.6	11.7	14.3	0.47	2.52
			156	10.0	30	4.15	0.7	3.4	5.9	6.3	0.40	2.62
			197	11.0	33	4.19	2.1	3.6	3.8	4.5	0.39	2.67
			253	10.4	31	4.09	0.5	3.1	3.4	2.8	0.44	2.65
			318	10.6	31	3.98	0.7	2.8	3.1	2.0	0.33	2.73
			373	11.2	34	4.32	0.9	2.8	2.2	1.5	0.33	2.85
MW	N-M	AS	1	n.d.	n.d.	n.d.	n.d.	<0.1	n.d.	(86.4)	0.72	2.34
			35	13.4	40	3.51	1.1	<0.1	n.d.	71.5	0.71	2.42
			65	9.1	30	2.99	n.d.	0.6	48.3	62.9	0.71	2.32
			183	11.0	35	3.42	n.d.	2.8	5.6	8.4	0.52	2.49
			336	9.4	30	4.18	3.2	2.8	3.4	2.7	0.39	2.66
AJ	N-M	AC	60	10.2	30	3.75	6.4	—[5]	20.3	23.5	0.57	2.45
			101	12.5	34	4.46	1.9	—	n.d.	9.1	0.48	2.53
CB	N-F	AC	14	n.d.	n.d.	n.d.	n.d.	—	n.d.	(86.9)	0.69	2.35
			28	11.2	38	3.37	0.3	—	n.d.	(79.2)	0.66	2.38
			56	10.7	33	4.01	n.d.	—	n.d.	(50.1)	0.66	2.42
			84	n.d.	n.d.	n.d.	n.d.	—	n.d.	19.3	0.55	2.49
			120	11.3	30	4.41	n.d.	—	n.d.	9.2	0.49	2.60
			150	11.1	28	3.95	n.d.	—	4.4	3.8	0.39	2.61
			210	8.8	29	4.30	n.d.	—	n.d.	3.4	0.38	2.70
			255	9.3	28	4.03	1.1	—	n.d.	3.8	0.41	2.68
			300	8.5	26	3.94	n.d.	—	1.6	2.5	0.30	2.78
KJ	N-M	SC	1	18.6	52	5.43	n.d.	—	n.d.	(89.8)	0.71	2.35
			51	9.6	30	3.28	n.d.	—	n.d.	(58.7)	0.64	2.34
			114	8.6	27	3.10	6.2	—	n.d.	14.4	0.56	2.48
			184	8.8	28	3.33	n.d.	—	7.4	7.0	0.48	2.63
			262	10.4	33	3.53	n.d.	—	4.1	4.5	0.45	2.59
			317	9.5	30	3.49	3.6	—	3.5	2.9	0.58	2.54
			409	9.7	28	4.30	1.2	—	2.8	n.d.	0.50	2.57
TH	N-M	SC	650	10.5	31	4.73	1.1	—	14.5	19.4	0.57	2.51
AS	N-F	A-Rich.	1	13.2	44	3.20	3.6	<0.1	42.9	70.5	0.72	2.30
			53	10.4	32	3.19	2.2	1.2	38.5	43.0	0.68	2.35
			127	10.8	32	3.60	0.6	4.4	6.1	6.7	0.48	2.57
RS	N-F	A-βTh.	56	10.6	31	4.24	n.d.	2.9	n.d.	29.9	0.56	2.54
			117	11.4	37	3.67	n.d.	4.5	4.1	4.8	0.45	2.62
			146	11.4	36	3.98	n.d.	3.9	n.d.	2.5	0.42	2.70
			439	9.7	32	5.30	1.8	4.4	1.6	1.6	0.30	2.72
RB	N-F	A-HPFH	1	n.d.	n.d.	n.d.	n.d.	0	59.3	87.2	0.67	2.29
			63	11.8	34	4.00	1.2	0.5	51.0	66.3	0.66	2.40
			91	13.2	39	4.57	1.2	1.5	n.d.	43.7	0.62	2.38
			127	11.2	36	3.92	1.2	1.2	34.4	38.8	0.58	2.38
			157	—	—	—	—	1.3	30.9	35.4	0.58	2.49
			190	12.8	38	5.25	0.5	1.6	30.7	36.7	0.55	2.45
			203	11.8	37	4.23	0.7	1.3	31.4	31.8	0.58	2.43

[1] N: Negro; F: female; M: male

[2] See *Materials and Methods* for definition of hemoglobin types.

[3] Percentages of Hb-F in parentheses were determined by DEAE-Sephadex chromatography only [8, 17]; such values usually agree with $\%F_{Ile}$ values if the percent Hb-F is large.

[4] n.d., not determined.

[5] Hb-A_2 was not determined because the hemoglobins C and A_2 are eluted together from columns of DEAE-Sephadex [8, 17].

Table II. Hematologic data on blood samples from adult subjects, and the results of chemical analyses of Hb-F isolated from these samples

Case	Race and sex[1]	Hb type[2]	Age, yr	Hb, g/100 ml	PCV, %	RBC, 10^6/mm^3	Retic., %	Hb-A_2, %	Hb-F AD %	Hb-F Ile %	γCB-3 Gly	γCB-3 Ala
AD	C-F	AA, 2nd sample	38	13.3	40	4.36	0.6	2.8	0.9	n.d.[3]	0.51 0.44[4]	2.59 2.67[4]
NB	C-F	AA	25	11.7	40	4.10	n.d.	2.7	1.0	n.d.	0.23	2.99
GD	N-F	AA	30	n.d.	n.d.	n.d.	n.d.	2.4	0.9	n.d.	0.29	2.80
IF	C-F	AA	30	15.1	49	4.47	0.9	2.5	1.2	n.d.	0.55	2.52
LL	C-M	AA	27	16.0	54	4.84	0.4	2.7	1.4	n.d.	0.55	2.52
SR	N-M	AA	43	14.2	50	4.98	0.8	2.8	1.4	0.8	0.37	2.67
ER	N-F	AA	12	11.3	39	3.90	2.0	3.2	1.9	1.3	0.31	2.73
RR	N-M	AA	10	11.6	38	4.19	1.4	3.4	1.8	1.0	0.25	2.82
JM	N-M	AS	26	n.d.	n.d.	n.d.	n.d.	3.4	0.8	0.9	0.65	2.33
MT	N-F	AS	30	11.4	34	3.87	0.9	3.3	0.9	0.6	0.36	2.72
AB	N-F	AC	28	13.6	39	5.48	1.1	—[5]	1.5	1.3	0.47	2.57
WC	N-M	SC	24	14.6	45	4.93	4.2	—	1.4	n.d.	0.48	2.64
JJ	N-M	SC	14	11.3	34	4.37	n.d.	—	5.4	4.7	0.45	2.58
HO	N-M	SC	31	11.9	37	3.84	1.1	—	2.4	2.4	0.66	2.42
MR	N-F	SC	60	9.3	29	3.00	5.4	—	2.6	2.1	0.36	2.59
AR	N-F	SC	23	8.2	28	2.50	8.3	—	7.3	—	0.38	2.65

[1] C: Caucasian; N: Negro; F: female; M: male.
[2] See *Materials and Methods* for definition of hemoglobin types.
[3] n.d., not determined.
[4] This analysis was made on globin prepared from the alkali-resistant hemoglobin fraction. Red cell hemolysate from about 250 ml blood was treated with alkali [31]. The soluble fraction was dialyzed overnight against running tap water and concentrated on a 8 by 20 cm column of CM-Sephadex in 0.05 M Tris-maleic acid, pH 6.5 [37], from which it was eluted with 0.1 M Tris-HCl, pH 8.0.
[5] Hb-A_2 was not determined because the hemoglobins C and A_2 are eluted together from columns of DEAE-Sephadex [8, 17].

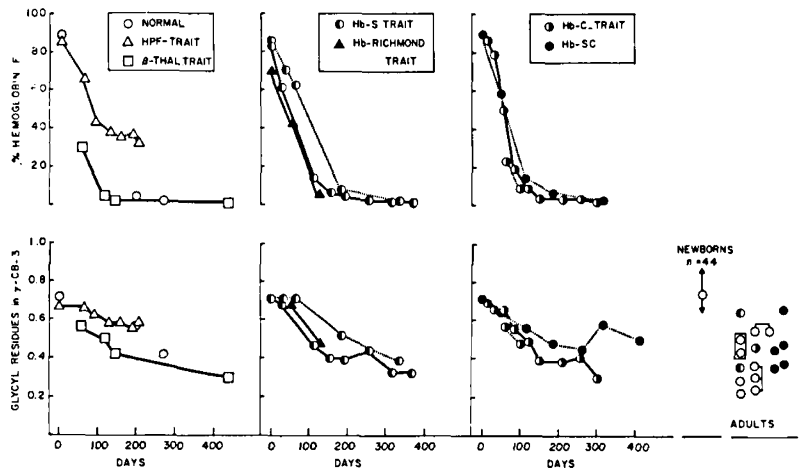

Fig. 1. Postnatal changes in the % Hb-F and the number of glycyl residues of the γCB-3 peptide of Hb-F from normal newborns and newborns with different hemoglobin abnormalities. See text and Tables I and II for further details.

als in one of two groups: in *group I*, the ratio of $^{G}\gamma$ to $^{A}\gamma$ chains is about 2:3 as in the adult, whereas in *group II* it is about 3:1 as in the newborn [28]. *Baby RS* obviously belongs in *group I*. There is convincing evidence that this ratio is an inheritable characteristic in β-thalassemia. This could not be tested in this family because attempts to isolate a sufficient quantity of Hb-F from the blood of the mother of *baby RS* who is also heterozygous for β-thalassemia were unsuccessful.

Baby RB is the child of *RE* (*family E* in Table IA [19]) who is an HPFH heterozygote with about 25% F_{Ile}. The $^{G}\gamma$ to $^{A}\gamma$ ratio in the Hb-F from the mother and three members of this family was about 3:2 and probably was different from the approxi-

mately 2:3 ratio in the Hb-F of most HPFH heterozygotes [19]. The Hb-F of *infant RB* descreased in about 150 days to the rather constant level of 35%, and a $^{G}\gamma$ to $^{A}\gamma$ ratio of 3:2 was reached at about the same time. It is also of interest that the glycine value in the Hb-F of the cord blood sample (0.67 residue) is in the lower part of the newborn range. This observation can be explained by assuming that at birth the γ chain loci *in cis* to the HPFH determinant produce $^{G}\gamma$ and $^{A}\gamma$ chains in a ratio of about 3:2 and those *in trans* produce these chains in the newborn ratio of about 3:1.

The orderly appearance and disappearance of fetal and adult forms of hemoglobin are probably the most obvious and best known examples of the regulation and control of protein synthesis and provide an excellent opportunity to study the genetic mechanisms involved. Although hypothetical explanations of the switch from Hb-F to Hb-A, or more precisely from γ to β and δ chains, have been advanced [2, 21, 34], insight into this mechanism is lacking. Our data add little to the understanding of this mechanism except to show a complexity greater than previously realized, because the evidence clearly suggests an unequal repression of the $^{G}\gamma$ and $^{A}\gamma$ structural genes as the switchover occurs. The determination of the ratio of $^{G}\gamma$ to $^{A}\gamma$, however, does allow an examination of the functioning of the switchover mechanism. Studies of Hb-F in β-thalassemia, for instance, suggest [28] that in one type it is partially inoperative whereas in another, as in the case of *baby RS,* it is fully functional. This is indicated by the fact that Hb-F in β-thalassemia heterozygotes is not elevated over normal in both groups that have been detected [28]. Because all hypothetical explanations of the switchover mechanism were published prior to the detection of the two types of γ chain, they could not take into account the presence of the nonallelic genes as any future explanation must do. One may well consider whether or not the eventual explanation for the control of the ratio of $^{G}\gamma$ to $^{A}\gamma$ chains and the change from 3:1 in the newborn infant to 2:3 in the adult may not also give the answer to the oft-asked question "Why are the β and δ chains normally in the ratio of 40 to 1?" We must appreciate that, in switching from the production of γ to β and δ chains, we actually are switching from the production of two genes, $^{G}\gamma$ and $^{A}\gamma$, to that of two other genes, β and δ. Is the factor that gives a ratio of 3:1 $^{G}\gamma$ to $^{A}\gamma$ chains in the prenatal state the same as or related to that which controls the ratio of 40:1 β to δ chains in the postnatal state? The present data do not really answer this question, but it may be possible to draw pertinent conclusions as more is learned about the ratio of the $^{G}\gamma$ to $^{A}\gamma$ chains in those instances in which Hb-F is elevated in adult life.

Summary

The fetal hemoglobin (Hb-F) of blood samples from 11 newborn babies and from 16 adults was examined to determine the ratio of the two structurally different γ chains, namely the $^{G}\gamma$ and $^{A}\gamma$ chains. This ratio is about 2:3 in the Hb-F of adults and, therefore, significantly different from the 3:1 ratio in the Hb-F found in newborns. The newborn ratio, however, undergoes a considerable change between the 3rd and 4th months of life, at which time it approaches that of the Hb-F of adults.

The mechanism by which the gradual change from γ chain synthesis to β and δ chain synthesis is controlled remains unclear and probably involves an unequal repression of the γ chain structural genes. Any explanation of the mechanism must take into account the fact that the production of two genes, the $^{G}\gamma$ and $^{A}\gamma$, is greatly decreased, whereas that of two other genes, the β and δ, is started. Perhaps a closely related or even identical mechanism controls not only the ratio of production of the $^{G}\gamma$ and $^{A}\gamma$ genes but also that of the β and δ genes.

References and Notes

1. ANSON, M. S., AND MIRSKY, A. E.: Protein coagulation and its reversal. The preparation of insoluble globin and heme. J. Gen. Physiol., *13:* 468 (1930).
2. BAGLIONI, C.: Correlations between genetics and chemistry of human hemoglobins. In: J. H. Taylor: Molecular Genetics, Part 1 (Academic Press, New York, 1963).
3. BEAVEN, G. H., ELLIS, M. J., AND WHITE, J. C.: Studies on human foetal haemoglobin. II. Foetal haemoglobin levels in healthly children and adults and in certain haematological disorders. Brit. J. Haemat., *6:* 201 (1960).
4. BEAVEN, G. H., ELLIS, M. J., AND WHITE, J. C.: Studies on human foetal haemoglobin. III. The hereditary haemoglobinopathies and thalassaemias. Brit. J. Haemat., *7:* 169 (1961).
5. BETKE, K., MARTI, H., AND SCHLICHT, I.: Estimation of small percentages of foetal haemoglobin. Nature, *184:* 1877 (1959).
6. BERTLES, J. E.: The occurrence and significance of foetal hemoglobins. In: A. S. Gordon: Regulation of Hematopoiesis (Appleton-Century-Crofts, New York, 1969).
7. BICKERS, J. N.: Alkali-resistant hemoglobin in sickle-cell disease. Ann. Intern. Med., *64:* 1628 (1966).
8. DOZY, A. M., KLEIHAUER, E. F., AND HUISMAN, T. H. J.: Studies on the heterogeneity of hemoglobin. XIII. Chromatography of various human and animal hemoglobin types on DEAE-Sephadex. J. Chromatogr., *32:* 723 (1968).
9. EFREMOV, G. D., HUISMAN, T. H. J., SMITH, L. L., WILSON, J. B., KITCHENS, J. L., WRIGHTSTONE, R. N., AND ADAMS, H. R.:

Hemoglobin Richmond, a human hemoglobin which forms asymmetric hybrids with other hemoglobins. J. Biol. Chem., *244:* 6105 (1969).
10. Falbe-Hansen, I.: The composition of the alkali-resistant haemoglobin fraction in blood from normal human adults. Brit. J. Haemat., *7:* 187 (1961).
11. Farrar, J. F., and Blomfield, F.: Alkali-resistant haemoglobin content of blood in congenital heart disease. Brit. J. Haemat., *9:* 278 (1963).
12. Garby, L., Sjölin, S., and Vuille, J. C.: Studies on erythrokinetics in infancy. II. The relative rate of synthesis of haemoglobin F and haemoglobin A during the first months of life. Acta Paediat., *51:* 245 (1962).
13. Hall, J. G., and Motulsky, A. G.: Production of foetal haemoglobin in marrow cultures of human adults. Nature, *217:* 569 (1968).
14. Horton, B. F., Hahn, D. A., and Huisman, T. H. J.: Slight increase of fetal hemoglobin in apparently healthy Negroes. Acta Haemat., *33:* 312 (1965).
15. Huisman, T. H. J., Jonxis, J. H. P., and Dozy, A. M.: Is foetal haemoglobin present in the blood of normal human adults? Biochim. Biophys. Acta, *18:* 576 (1955).
16. Huisman, T. H. J.: Normal and abnormal human hemoglobins. Advan. Clin. Chem., *6:* 231 (1963).
17. Huisman, T. H. J., and Dozy, A. M.: Studies on the heterogeneity of hemoglobin. IX. The use of tris(hydroxymethyl) aminomethane-HCl buffers in the anion exchange chromatography of hemoglobins. J. Chromatogr., *19:* 160 (1965).
18. Huisman, T. H. J., Schroeder, W. A., Adams, H. R., Shelton, J. R., Shelton, J. B., and Apell, G.: A possible subclass of the hereditary persistence of fetal hemoglobin. Blood, *36:* 1 (1970).
19. Huisman, T. H. J., Schroeder, W. A., Dozy, A. M., Shelton, J, R., Shelton, J. B., Boyd, E. M., and Apell, G.: Evidence for multiple structural genes for the γ chain of human fetal hemoglobin in hereditary persistence of fetal hemoglobin. Ann. N. Y. Acad. Sci., *165:* 320 (1969).
20. Huisman, T. H. J., Schroeder, W. A., Stamatoyannopoulos, G., Bouver, N., Shelton, J. R., Shelton, J. B., and Apell, G.: Nature of fetal hemoglobin in the Greek type of hereditary persistence of fetal hemoglobin with and without concurrent β-thalassemia. J. Clin. Invest., *49:* 1035 (1970).
21. Ingram, V. M.: A molecular model for thalassemia. Ann. N. Y. Acad. Sci., *119:* 485 (1963).
22. Jonxis, J. H. P., and Huisman, T. H. J.: The detection and estimation of fetal hemoglobin by means of the alkali denaturation test. Blood, *11:* 1009 (1956).
23. Kleihauer, E. F.: Fetales Hämoglobin und fetale Erythrozyten (Ferdinand Enke Verlag, Stuttgart, 1966).
24. Marti, H. R., and Bütler, R.: Hämoglobin F und hämoglobin A_2-Vermehrung bei der schweizer bevölkerung. Acta Haemat., *26:* 65 (1961).
25. Oski, F. A., and Naiman, J. L.: Hematologic Problems in the Newborn (W. B. Saunders Co., Philadelphia, 1966).
26. Rucknagel, D. L., and Chernoff, A. I.: Immunologic studies of hemoglobins. III. Fetal hemoglobin changes in the circulation of pregnant women. Blood, *10:* 1092 (1955).
27. Schroeder, W. A., and Huisman, T. H. J.: Investigations of molecular variation in human fetal hemoglobin in the infant and in certain hematological conditions in the adult. In: H. Peeters: Procedings of the 17th Colloquium on Protides of the Biological Fluids, p. 249 (Bruges, 1970).
28. Schroeder, W. A., Huisman, T. H. J., Shelton, J. R., Shelton, J. B., Apell, G., and Bouver, N.: Heterogeneity of fetal hemoglobin in β-thalassemia of the Negro. Amer. J. Hum. Genet., *22:* 505 (1970).
29. Schroeder, W. A., Huisman, T. H. J., Shelton, J. R., Shelton, J. B., Kleihauer, E. F., Dozy, A. M., and Robberson, B.: Evidence for multiple structural genes for the γ chain of human fetal hemoglobin. Proc. Nat. Acad. Sci. U. S. A., *60:* 537 (1968).
30. Schroeder, W. A., Huisman, T. H. J., Shelton, J. R., and Wilson, J. B.: An improved method for the quantitative determination of human fetal hemoglobin. Anal. Biochem., *35:* 235 (1970).
31. Singer, K., Chernoff, A. I., and Singer, L.: Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematological disorders by means of alkali denaturation. Blood, *6:* 413 (1951).
32. Wilson, M. G., Schroeder, W. A., Graves, D. A., and Kach, V. D.: Hemoglobin variation in D-trisomy syndrome. New Engl. J. Med., *277:* 953 (1968).
33. Wintrobe, M. M.: Clinical Hematology, ed. 5 (Lea and Febiger, Philadelphia, 1962.)
34. Zuckerkandl, E.: Controller-gene diseases: the operon model as applied to β-thalassemia, familial fetal hemoglobinemia, and the normal switch from the production of the fetal hemoglobin to that of adult hemoglobins. J. Mol. Biol., *8:* 128 (1964).
35. The terminology of these chains which formerly [29] were designated the $\gamma^{136G1\gamma}$ or γ^{G} chains and the γ^{136A1a} or γ^{A} chains has recently been modified [20].
36. Whatman microgranular CM-52, preswollen, ion capacity 1.0 mEq/g dry weight, Reeve Angel Co., New York, N. Y.
37. CM-Sephadex, C-50, capacity 4.5 ± 0.5 mEq/g, particle size 40–120 μ, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.
38. Informed consent was obtained for all subjects in this study.
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41. Requests for reprints should be addressed to: W. A. Schroeder, Division of Chemistry and Chemical Engineering, California Institute of Technology, 1201 East California Boulevard, Pasadena, Calif. 91109 (USA).
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