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Hemoglobin Kenya, the Product of a γ-β Fusion Gene: Studies of the Family

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Recently, we reported the discovery of a new hemoglobin variant, Hb Kenya, in a 26-year-old Kenyan of the Nilotic Luo tribe [1]. Chemical characterization of the non-α chain of this hemoglobin showed that the amino terminal segment has the sequence of the γ chain of fetal hemoglobin (Hb F) and the carboxy terminal segment that of the β chain of Hb A. It was concluded that the non-α chain of Hb Kenya is the product of a hybrid gene that has been formed by nonhomologous crossing over within gene loci.

On the basis primarily of electrophoretic and hematological observations (A. G. Kendall, unpublished), the propositus was originally diagnosed as a heterozygote for Hb S and the hereditary persistence of Hb F (HPFH). Because of the discovery of Hb Kenya in the propositus, a reevaluation of his condition and that of members of his immediate family was highly desirable. This report describes the results of these studies as well as additional chemical evidence to support the γ-β structure of the non-α chain of Hb Kenya.

MATERIALS AND METHODS

Source of Blood Samples

Blood samples were obtained from the propositus (P. O.) and his relatives on several occasions. Hematological investigations were made on fresh material in Kenya by one of us (A. G. K.) as well as on samples that had been in transit to Georgia for less than 7 days. After collection in Vacutainers with EDTA as anticoagulant, the samples were airmailed from Kenya to Augusta, Georgia, without refrigeration.

Hematological Examination

Hemoglobin concentration (in grams per 100 ml), packed cell volume (percentage), red cell counts (in 10^6 per cubic millimeter), white cell counts (per cubic millimeter),

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and red cell indices were determined by standard techniques [2]. Peripheral blood smears were inspected after staining with Jenner-Giemsa stain. Distribution of Hb F in the cells was studied by the acid elution technique [3].

Hemoglobin Analyses

Starch gel electrophoresis of hemoglobin in hemolysates was made as described before [4]. The amount of alkali-resistant hemoglobin (presented as percentage F_{AD}) was determined by the method of Betke et al. [5]. The quantities of the several hemoglobin variants were determined by chromatography on columns (0.9 × 55 cm) of DEAE-Sephadex [6, 7] and occasionally on columns (1.8 × 35 cm) of carboxymethyl cellulose [8]. The purity of the hemoglobins so isolated was evaluated by starch gel electrophoresis. The amount of Hb F (presented as percentage F_{Ile}) was also determined by a procedure that is based on the determination of the ratios of isoleucine to leucine and to phenylalanine in chromatographically isolated zones of Hb F [9].

DEAE-Sephadex chromatography with larger columns was used for the isolation of preparative amounts of Hb Kenya and Hb F from hemolysates of subjects with the Hb S–Hb Kenya combination and of Hb A_2' and Hb A_2 from the hemolysate of subject Ro. O. When Hb A and not Hb S is present, as in subject A. O., the isolation of Hb F and Hb Kenya required two chromatograms. The first, on a column of CM cellulose (2.5 × 40 cm), separated a mixture of Hb A and Hb F from a mixture of Hb Kenya and Hb A_2. By rechromatographing these two zones on columns of DEAE-Sephadex (2.5 × 40 cm), Hb F was separated from Hb A, and Hb Kenya from Hb A_2.

Chemical Analyses

The α and non-α chains of Hb Kenya were separated by CM-cellulose chromatography in urea [10]. The S-β-aminoethyl (AE) derivative was prepared with ethylenimine [11]. A 135-mg sample of the abnormal AE chain was hydrolyzed with trypsin (Worthington Biochemical Corp., Freehold, N.J.; crystallized three times, salt-free) in a Radiometer *p*H stat at *p*H 9 for 20 hr at room temperature. Trypsin equal to 0.5% of the weight of the chain was added at zero time and again 2 hr later. Upon completion of the hydrolysis, the *p*H was lowered to 2.5 with 1N HCl, and the solvent was removed by flash evaporation. The peptides were separated by chromatography on a 0.9 × 60-cm column of Chromobead resin type P (Technicon Instruments) at 37°. The gradient for the development of the chromatogram has been described [1]. Several zones required rechromatography on columns (0.6 × 60 cm) of Dowex 1-X2 [12].

After a 40-mg portion of unmodified abnormal chain had been cleaved with cyanogen bromide as described in [13], the fragments were separated on two 1 × 160-cm columns of Sephadex G-50 in tandem with 25% acetic acid as the solvent.

Amino acid analyses were made with a Spinco model 124 automated amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) equipped with high-sensitivity cuvettes and an Infotronics model CRS-110 A integrator (Infotronics Co., River Forest, Ill.). Samples were hydrolyzed for 24 and occasionally 72 hr at 110° C under reduced pressure with 6N HCl. Tryptophan could be identified by amino acid analysis because a portion survived the hydrolysis with hydrochloric acid.

In order to determine the type of γ chains in the Hb F, globins from the chromatographically isolated Hb F were examined as previously described [13].

RESULTS

Initial Examination of Family O

The propositus, P. O., is a healthy 26-year-old male. He was first studied by one of us (A. G. K.) in the hematology laboratory of the University of Nairobi Medical

School in Kenya. On the basis of hematological examination, cellulose acetate electrophoresis, alkali denaturation determination, and cellular distribution of Hb F, the tentative diagnosis was a Hb S–HPFH condition. Two of the eight siblings and a paternal aunt appeared also to have the Hb S–HPFH condition; two were diagnosed simply as HPFH heterozygotes; the mother, a second aunt, and two siblings were found to have Hb S trait; and two siblings appeared to be normal. The father of the propositus died in 1968 of a liver ailment. The low level of Hb F, which was between 7% and 10%F_{AD} in the HPFH heterozygotes and between 11% and 15% in the subjects with the Hb S–HPFH condition, suggested a need for a more detailed investigation. The pedigree of family O is given in figure 1.

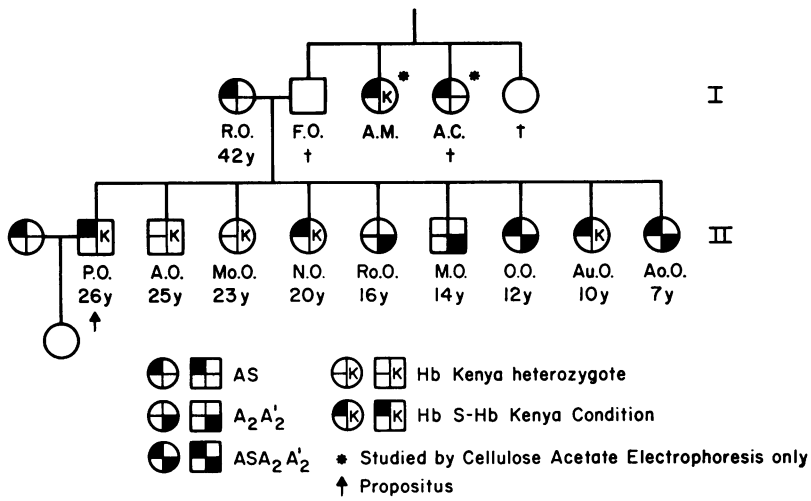

FIG. 1.—Pedigree of family O

Electrophoretic and Chromatographic Analyses

Figure 2 illustrates starch gel electrophoretic patterns of the hemoglobins from the propositus (P. O.), his mother (R. O.), and seven of his eight siblings. The pattern in the propositus, which confirms a previous observation [1], shows the presence of four hemoglobins (A_2, Kenya, S, and F in order of increasing mobility) and the absence of Hb A. The same pattern is present in siblings N. O. and Au. O. A brother of the propositus, A. O., has small amounts of Hb Kenya and Hb F in addition to Hb A and Hb A_2. The mother, R. O., is simply a Hb S heterozygote. Subjects Ro. O. and M. O. are heterozygous for the δ chain variant, Hb B_2 or Hb A_2' [14, 15], whereas subjects O. O. and Ao. O. have this variant as well as Hb S. The father, F. O., then must have had the Hb Kenya and Hb A_2' genes in *trans*.

Figure 3 depicts DEAE-Sephadex chromatograms of the hemoglobin components of subject A. O., who is heterozygous for Hb Kenya, and of subject N. O., who has the Hb S–Kenya combination. Good separation of the various zones allowed quantitation of the different hemoglobins. The Hb Kenya that was isolated from both

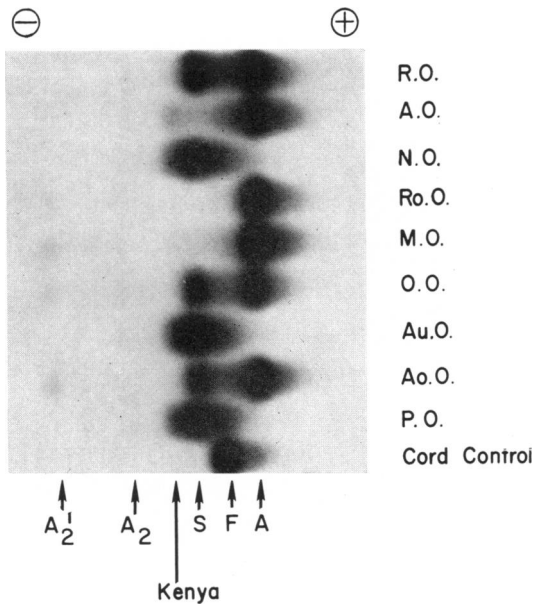

FIG. 2.—Starch gel electrophoresis of hemoglobin from nine members of family O in Tris-EDTA-borate buffer at *p*H 9.0 Stain is o-Dianisidine.

chromatograms behaved as a single band in starch gel electrophoresis. Zone X contains an electrophoretically more rapidly moving derivative of Hb Kenya and the minor Hb S_1 fraction; both fractions could easily be distinguished from Hb A.

Figure 4 illustrates the CM-cellulose chromatograms of the hemoglobins from subject A. O., who is heterozygous for Hb Kenya, and from subject M. O., who has Hb A_2'. Although Hb Kenya could not be separated from Hb A_2 by this technique, the separation of Hb A_2' from the other hemoglobins was complete.

Quantitative Hemoglobin Data

The results of quantitative analyses for the various hemoglobins are listed in table 1. Although the data derive mainly from DEAE-Sephadex chromatography, other methods, when advantageous, were also used. Hb Kenya, which is about 6% in subject A. O., the Kenya heterozygote, is increased to 17%–19% in the three siblings with the Hb S–Hb Kenya condition. The amount of F_{IIe} is 5% in the Hb Kenya heterozygote and 7%–11% in the three subjects with the Hb S–Hb Kenya combination. The levels of Hb A_2 are variable and should be compared with values of 2.2%–3.0% in normal controls, 2.8%–3.4% in Hb S heterozygotes, and 1.1%–4.8% in patients with sickle cell anemia [16, 17]. The level in subject A. O. with the Hb Kenya heterozygosity is low normal and that in the subjects with the Hb S–Hb Kenya condition appears to be decreased. (Subject Au. O. is an exception.)

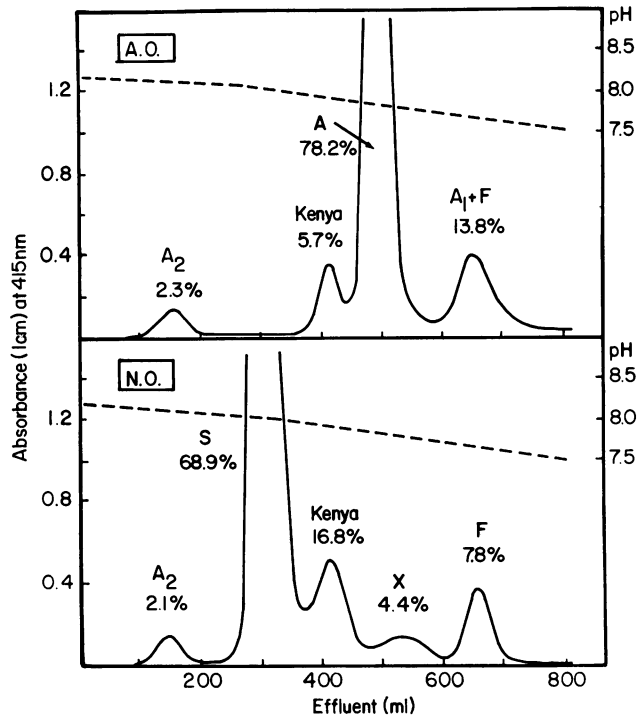

FIG. 3.—Chromatographic separation of the components from approximately 50 mg of hemoglobin from subject A. O., who is heterozygous for Hb Kenya, and subject N. O., who has the Hb S–Hb Kenya condition, on DEAE-Sephadex (0.9 × 55 cm).

Chemical Analyses of Hb Kenya

On the basis of data previously published [1], the non-α chain of Hb Kenya is a hybrid chain which consists partly of γ chain (the amino terminal portion) and partly of β chain (the carboxy terminal portion). This conclusion stems from amino acid analysis of the total chain and of tryptic peptides identical with γT-2, γT-3, γT-9a,* βT-13, and βT-14. (Although peptides T-4, T-6, T-7, T-8, and T-15 were also isolated, they are identical in the β and γ chains.) Now peptides γT-1, γT-9b,* βT-10a,* and βT-11, of which the amino acid composition is given in table 2, have also been isolated from the digest of the AE-γ-β chain of Hb Kenya. Peptide γT-9b* is crucial for delineating more exactly the point of crossing over. The sequence His-Leu-Asp-Asp(or Asn)-Leu-Lys of the six residues in this peptide is identical in both β and γ chains except for the fourth residue of the peptide (residue 80 of the

* Nomenclature for the tryptic peptides of the γ-β chain is difficult because the β chain has 15 tryptic peptides and the γ chain has 16. Peptides T-1 thru T-8 are homologous. However, γT-9 plus γT-10 [18] are equivalent to βT-9. In order that the numbering of the peptides from the β portion be normal, γT-9 and γT-10 are designated γT-9a and γT-9b, respectively, in this discussion. Peptide βT-10a is a fragment of βT-10 that results from tryptic cleavage at the aminoethylated cysteinyl residue in position 93.

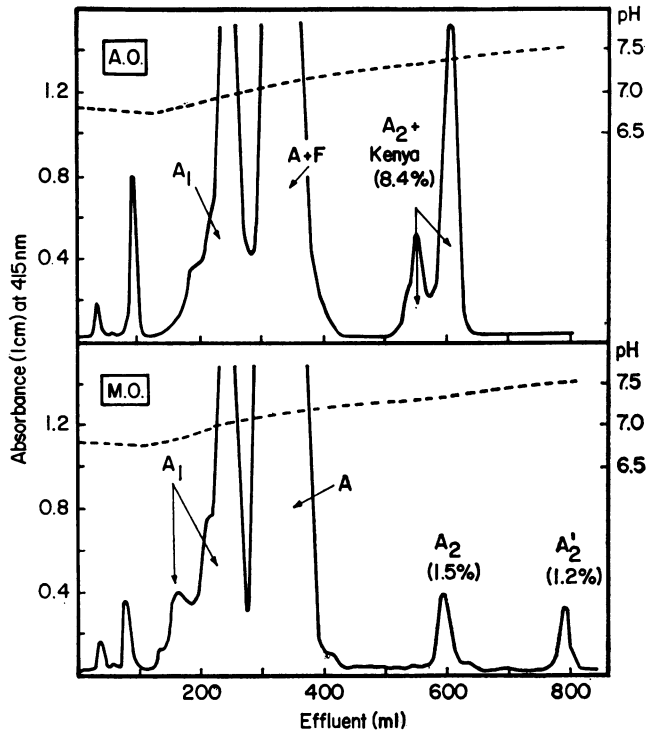

FIG. 4.—Chromatographic separation of the components from approximately 50 mg of hemoglobin from subject A. O., who is heterozygous for Hb Kenya, and from subject M. O., who is heterozygous for Hb A_2', on CM-cellulose (1.8 × 35 cm).

TABLE 1

HEMOGLOBIN DATA BY DEAE-SEPHADEX CHROMATOGRAPHY [6, 7]

SUBJECT	Hb A_2' (%)	Hb A_2 (%)	Hb S (%)	Hb A (%)	Hb Kenya (%)	Hb A_1+F (%)	Hb F (%) F_{AD}*	Hb F (%) F_{IIe}†	OTHERS‡	γCB-3§ Gly	γCB-3§ Ala
R. O.	0	4.4	35.4	60.2	0	0	0.8	0.8	...	...	...
P. O.	0	1.7	61.7	0	17.6	12.9	8.2	11.0	6.1	1.02	2.01
A. O.	0	2.3‖	0	78.2	5.7‖	13.8	5.6	5.6	...	1.05	2.02
N. O.	0	2.1	68.9	0	16.8	7.8	...	6.6	4.4	1.08	1.95
Ro. O.	1.6	0.7	0	88.7	0	9.0	0.7	1.6	...	...	...
M. O.	1.7	1.3	0	91.6	0	5.4	0.6	1.3	...	...	...
O. O.	2.1	2.8	36.5	56.3	0	2.3	0.6	0.9	...	...	...
Au. O.	0	3.3	64.5	0	19.0	10.8	...	8.9	2.4	1.01	1.96
Ao. O.	2.0	1.8	25.8	66.0	0	4.4	0.7	0.6	...	...	...

* By an alkali denaturation procedure [5].

† By a chemical procedure [9].

‡ A minor zone mainly consisting of S_2 and $Kenya_1$, which are electrophoretically more rapidly moving zones.

§ Isolated from Hb F by a procedure described previously [13].

‖ CM-cellulose chromatography separates Hb Kenya from Hb A but not from Hb A_2; the combined amount of Hb Kenya plus Hb A_2 by this method was 8.4%.

TABLE 2

ADDITIONAL AMINO ACID ANALYSES IN RESIDUES PER MOLECULE

Amino Acid	γ-β Chain of Hb Kenya			Tryptic Peptides of γ-β Chain§				γ-βCB-1		γ-βCB-2		Isolated Hemoglobins‖		
	Original*	Oxidized†	Theory‡	γT-1	γT-9b	βT-10a	βT-11	Found	Theory	Found	Theory	A_2	A_2'	Theory αδ
Trp	0.9	...	2	...	...	...	...	0.2	2	0	0	...	...	3
Lys	12.1	11.7	12	0.96	0.96	...	...	1.8	2	9.5	10	22.3	22.3	22
AE-Cys	...	...	...	...	...	0.74	...	...	...	...	...	...	...	...
His	9.2	9.2	9	0.89	0.84	0.91	0.86	0.8	1	8.0	8	16.2	16.6	17
NH_3	10.0	12.2	9	...	0.36	...	...	...	...	...	...	...	...	...
Arg	2.7	2.7	3	...	...	...	0.94	1.8	2	0.9	1	7.1	8.0	7
$CySO_3H$	...	2.0	...	...	...	...	...	...	...	...	...	...	...	...
Asp	13.8	14.0	14	1.07	2.07	...	2.03	4.9	5	8.1	9	27.8	28.3	27
$MetSO_2$	...	0.8	...	...	...	...	...	...	...	...	...	...	...	...
Thr	8.2	8.5	9	1.05	...	1.81	...	4.7	5	3.9	4	12.1	12.3	14
Ser	6.0	6.3	7	...	...	1.10	...	4.3	5	2.2	2	14.3	14.2	17
Glu	10.0	10.4	10	2.13	...	0.91	1.05	5.4	5	5.2	5	17.4	16.6	17
Pro	6.1	5.6	5	...	...	...	1.01	1.2	1	4.0	4	12.9	12.8	13
Gly	12.6	12.9	13	0.97	...	0.91	...	5.9	6	7.2	7	19.2	18.2	20
Ala	14.0	14.5	14	...	...	1.01	...	4.1	4	10.0	10	37.6	36.8	36
Cys/2	0.6	...	2	...	...	...	...	...	...	...	2	...	...	...
Val	13.2	13.9	14	...	...	...	1.03	4.2	4	8.8	10	31.0	29.7	30
Met	1.1	0.1	1	...	...	...	...	...	...	0	0	...	...	4
Ile	2.2	2.2	3	...	...	...	...	1.7	2	0.8	1	0	0	0
Leu	17.3	16.6	17	...	2.13	2.06	1.02	5.4	5	11.8	12	33.6	34.8	36
Tyr	2.9	3.0	3	...	...	...	...	1.1	1	2.0	2	5.6	5.6	6
Phe	7.8	8.2	8	...	...	1.00	1.05	3.9	4	4.3	4	15.8	15.3	15
Hser	...	...	...	...	...	...	...	0.5	1	0	0	...	...	...

* Analyses made prior to oxidation.

† According to the procedure of Hirs [19].

‡ Assuming that the crossover occurred between residues 81 and 86.

§ Isolated from the digest of the AE-γ-β chain. Residues present in less than 0.1 residue molecules are omitted.

‖ From chromatogram shown in fig. 4; the data are given in residues/αδ subunit.

whole chain), which is aspartyl (Asp) in the γ chain and asparginyl (Asn) in the β chain. The low recovery of NH_3 (0.36 residues per molecule) in the amino acid analysis of γT-9b indicates that the fourth residue is aspartyl and that a section of the γ chain is the source; application of the Edman procedure substantiated this conclusion. Thus, the crossover is limited to positions 81–86 inclusive because position 80 is occupied by an aspartyl residue as in the γ chain whereas position 87 is occupied by a threonyl residue as in the β chain (fig. 5).

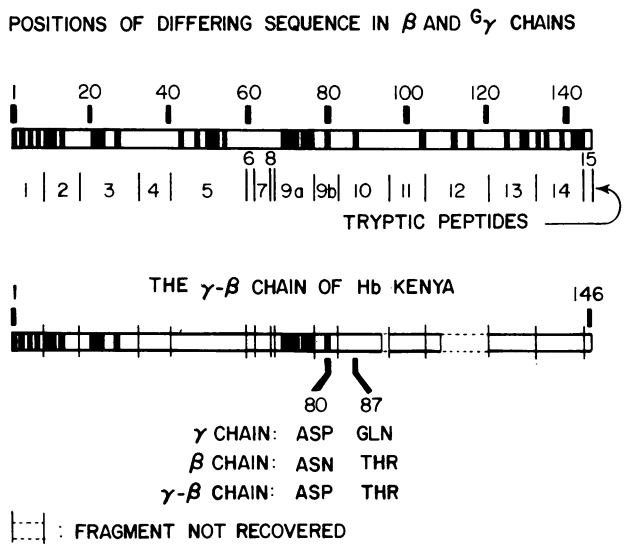

FIG. 5.—Summary of data from structural studies of the non-α chain of Hb Kenya. Bars denote differing residues in β and γ chains.

Table 2 also lists the amino acid composition of the γ-β chain of Hb Kenya before and after oxidation. Determined and calculated data are in good agreement, with the exception of the values for proline and isoleucine. Two of the three expected residues of isoleucine were detected in peptides γT-2 and γT-9a*. Peptide γT-5, which has the third isoleucyl residue, was not isolated as such. However, peptide γ-βCB-1 from cyanogen bromide cleavage of the γ-β chain contains most of γT-5 in its 55 residues from the amino terminal portion of the chain. The amino acid composition of both cyanogen bromide fragments of the γ-β chain agrees well with theory (table 2).

Chemical Analyses of the Electrophoretically Slowly Moving Minor Variant

The amino acid compositions of this hemoglobin and of Hb A_2 are given in table 2. The data are in general agreement with the calculated composition of the $\alpha\delta$ dimer. Because the slow component contains one more residue of arginine and perhaps one less residue of glycine, these results together with the electrophoretic and chromatographic observations are evidence that this variant is indeed the δ-

chain variant Hb A_2' which is characterized by a Gly → Arg replacement at position 16 [14, 15]. The limited data, however, do not exclude other replacements.

Chemical Analyses of Hb F

Globin F from blood of subject A. O. with the Hb Kenya heterozygosity and of the three siblings with the Hb S–Hb Kenya condition was examined to determine the amino acid composition of the γCB-3 peptide (residues 134–146 inclusive of the γ-chain), which is formed by reaction with cyanogen bromide. The data in table 1 are stated as the numbers of glycyl and alanyl residues in this peptide. In all four cases the integral value for glycyl residues equal to 1.0 means that a single type of γ chain is present; this is the $^{G}\gamma$ chain with a glycyl residue in position 136 [13]

Hematological Observations

Samples from the nine members of family O were examined immediately on arrival in Augusta but after several days in transit (table 3). Despite these sub-optimal conditions, the data are rather normal for subjects who live in the malarial belt near Lake Victoria and often suffer from a low-grade chronic parasitemia (*P. falciparum*) as well as iron deficiency due to hookworm infestation. The values are also very similar to those obtained in Kenya by one of us in 1970 and 1971. At that time on several occasions, stigmata of β thalassemia were absent in fresh films of blood from subjects A. O. and N. O., as well as relatively fresh films from seven other siblings (fig. 6). Subjects with Hb S–Hb Kenya had somewhat more target cells than the Hb Kenya heterozygote or the AS-trait individual.

The acid elution technique was applied to 8-day-old samples from subjects R. O., A. O., and P. O. Results are shown in figure 7. Aged blood samples of a normal infant, a patient with Fanconi anemia, and a patient with sickle cell anemia served as controls. The distribution of Hb F in the red cells of subjects A. O. and P. O. is fairly uniform and distinctly different from that in the control samples. A similar uniformity of distribution has been observed with fresh blood films and is comparable to that in red cells of HPFH heterozygotes.

DISCUSSION

Sequence of the γ-β Chain

The positions of the 39 differences in sequences of the γ and β chains [18] are depicted in figure 5. The differing peptides γT-1, γT-2, γT-3, γT-9a, γT-9b, βT-10a, βT-11, βT-13, and βT-14 as well as the identical peptides T-4, T-6, T-7, T-8, and T-15 have been isolated from tryptic digests of the non-α chain of Hb Kenya. Peptides γT-5, βT-10b, and βT-12b have not been recovered. There can be little doubt that the amino terminal part of the chain has the sequence of the γ chain and the carboxy terminal part has that of the β chain. That the carboxy terminal part is identical with the β rather than with the similar δ chain is established by peptides βT-10a and βT-13, which have the compositions of the β peptides and not those of the δ peptides. Thus, the non-α chain of Hb Kenya results from a fusion of parts of the γ and β chains. This γ-β chain presumably is the product of a

TABLE 3

HEMATOLOGICAL DATA COLLECTED ON BLOOD SAMPLES 7 DAYS OLD

Analyses	R. O.	P. O.	A. O.	N. O.	Ro. O.	M. O.	O. O.	Au. O.	Ao. O.
WBC (per mm^3)	3,400	3,500	4,000	4,900	3,000	5,500	5,200	4,400	3,700
RBC ($10^6/mm^3$)	3.89	5.16	6.61	4.96	4.39	4.87	4.25	4.94	5.40
Hb (g/100 ml)	10.9	13.9	16.4	11.8	10.6	12.7	11.4	12.5	12.0
PCV (%)	36	42.5	55.8	37.1	35.5	40.7	37.2	39.1	39.2
MCV (μ^3)	91	82	84	74	80	83	87	78	72
MCH ($\mu\mu g$)	27.9	26.8	24.5	23.6	24.0	25.9	26.7	25.2	22.1
MCHC (%)	30.5	32.9	29.3	31.9	30.1	31.4	30.8	32.2	30.8
Age (yr) and sex	42, F	26, M	25, M	20, F	16, F	14, M	12, F	10, F	7, F
Condition	AS	S Kenya	A Kenya	S Kenya	A_2A_2'	A_2A_2'	AS A_2A_2'	S Kenya	AS A_2A_2'

NOTE.—Fresh films of blood from all nine members of family O were evaluated on several occasions in 1970 and 1971 by one of the authors (A. G. K.) with these assessments: R. O., occasional target cells, normochromic and normocytic; P. O. occasional target cells, otherwise normal; A. O., normal; N. O., mild hypochromia due to iron deficiency, became normochromic after iron therapy, occasional target cells; Ro. O., slightly hypochromic; M. O., slight polychromasia, low-grade *P. falciparum* parasitemia; O. O., occasional target cells; Au. O., occasional target cells, slight polychromasia, low-grade *P. falciparum* parasitemia; Ao. O., normal. Reticulocyte counts were less than 3% in all cases.

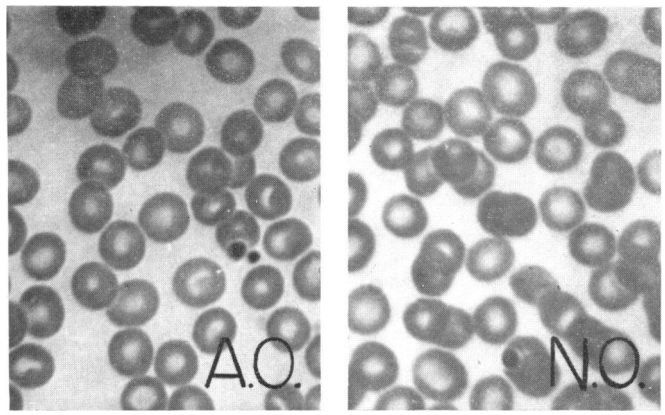

FIG. 6.—Peripheral blood smears of subject A. O., who is a heterozygote for Hb Kenya (left), and subject N. O., who has the Hb S–Hb Kenya condition (right).

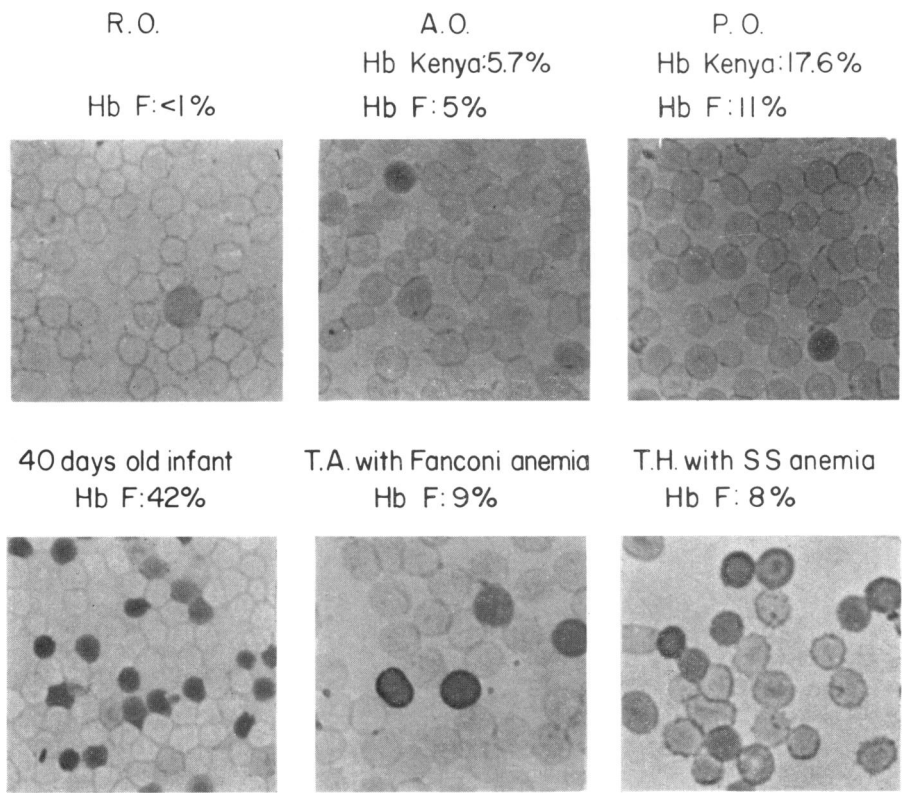

FIG. 7.—Red cell smears after application of the acid elution technique. The blood samples from R. O., A. O., and P. O. were 8 days old; that of the infant 25 days old; that of the patient with Fanconi anemia 10 days old; and that of the sickle cell anemia patient 30 days old.

hybrid gene that is formed by nonhomologous crossing over of the γ and β genes with deletion. The point of crossing over can be narrowed to the section that codes for residues 81–86 inclusive (fig. 5).

Genetic Aspects

Similar hybrid chains, but between δ and β chains, are found in the Lepore hemoglobins [20–22]. Their existence has been taken as additional evidence for linkage of the Hb_δ and Hb_β loci: our results similarly suggest a close linkage between a Hb_γ chain locus and the Hb_β locus. Consequently, an arrangement of genes of the type $Hb_\gamma.Hb_\delta.Hb_\beta$ is required to explain the occurrence of hybrid genes that produce the δ-β chains of the Lepore hemoglobins and the γ-β chain of Hb Kenya.

The γ chain of human Hb F is known to be a mixture of very similar chains. These differ at a minimum in position 136, which can be occupied by either a glycyl residue or an alanyl residue [13]. The ratio between these two types of chains, termed ${}^{G}\gamma$ and ${}^{A}\gamma$, respectively, is about 70:30 in the Hb F of the newborn and about 40:60 in the small amount of Hb F of the normal adult. It has been concluded that nonallelic Hb_γ structural genes are responsible for this chemical heterogeneity of the γ chain [13, 23, 24]. Accordingly, if close linkage exists as noted above, the arrangement of genes must be either $Hb_{G_\gamma}.Hb_{A_\gamma}.Hb_\delta.Hb_\beta$ or $Hb_{A_\gamma}.Hb_{G_\gamma}.Hb_\delta.Hb_\beta$.

The Hb F from subjects with the Hb Kenya condition has ${}^{G}\gamma$ chains only. Consequently, an intact ${}^{G}\gamma$ locus must be present. A deletion chromosome that contains such an intact locus will result if the arrangement of scheme I is present:

$$\underset{|\leftarrow\text{deletion}\rightarrow|}{Hb_{G_\gamma}.Hb_{A_\gamma}.Hb_\delta.Hb_\beta} \rightarrow Hb_{G_\gamma}.Hb_{A_\gamma-\beta}, \qquad \text{(scheme I)}$$

and if the Hb_{A_γ} and Hb_β loci participated in crossover with the deletion of parts of both and of the entire Hb_δ locus. An alternative arrangement (scheme II) cannot be excluded:

$$\underset{|\leftarrow\text{deletion}\rightarrow|}{Hb_{A_\gamma}.Hb_\delta.Hb_\beta.Hb_{G_\gamma}} \rightarrow Hb_{A_\gamma-\beta}.Hb_{G_\gamma}. \qquad \text{(scheme II)}$$

Recent evidence suggests that there may be four Hb_γ loci that have been designated

$$Hb_{\underset{m}{G_\gamma}},\ Hb_{\underset{l}{G_\gamma}},\ Hb_{\underset{m}{A_\gamma}},\ \text{and}\ \ Hb_{\underset{l}{A_\gamma}},$$

respectively. The G and A superscripts denote the type of γ chain and the m (more) and l (less) subscripts the relative amounts of γ chain that are synthesized by these separate genes [25]. This model with four Hb_γ loci will also explain adequately the genetic basis of Hb Kenya. If the chromosomal arrangement of the various structural loci is assumed to be

$$Hb_{\underset{m}{G_\gamma}}.Hb_{\underset{l}{G_\gamma}}.H_{\underset{m}{A_\gamma}}.Hb_{\underset{l}{A_\gamma}}.Hb_\delta.Hb_\beta,$$

either of two specific deletions (schemes III and IV) yields a

$$\underset{m}{Hb_{G_\gamma}}.\underset{l}{Hb_{G_\gamma}}.\underset{m}{Hb_{A_\gamma}}.\underset{l}{Hb_{A_\gamma}}.Hb_\delta.Hb_\beta \rightarrow \underset{m}{Hb_{G_\gamma}}.\underset{l}{Hb_{G_\gamma}}.\underset{m}{Hb_{A_\gamma-\beta}} \quad \text{(scheme III)}$$

$$\underset{m}{Hb_{G_\gamma}}.\underset{l}{Hb_{G_\gamma}}.\underset{m}{Hb_{A_\gamma}}.\underset{l}{Hb_{A_\gamma}}.Hb_\delta.Hb_\beta \rightarrow \underset{m}{Hb_{G_\gamma}}.\underset{l}{Hb_{G_\gamma-\beta}} \quad \text{(scheme IV)}$$

chromosome that provides a genetic basis for the observations on Hb Kenya. Scheme II with four Hb_γ loci would provide other possibilities. The observations on Hb Kenya, however, are of no value in deciding the actual number of γ genes.

Phenotypic Expression of the Hb Kenya Condition

Because both the Lepore and Kenya hemoglobins presumably are the products of hybrid genes that result from the deletion of genetic material, the phenotypic expression might be expected to be similar. However, a comparison of hematological and hemoglobin data from subjects with these abnormalities shows that such is not the case (table 4). Although the Hb Lepore and Hb Kenya heterozygotes have compa-

TABLE 4

COMPARISON OF HEMATOLOGICAL AND HEMOGLOBIN DATA ON SUBJECTS WITH Hb $Lepore_{Washington}$* AND WITH Hb KENYA

	Hb Lepore Trait	Hb Lepore–Hb S–(Hb C) Condition	Hb Kenya Trait	Hb Kenya–Hb S Condition
Red cell morphology	Thalassemic	Thalassemic	Normal	Normal
Abnormal Hb (%)	7.3–10.4	10.3–10.6†	5.7	16.8–19.0
Hb A_2 (%)	2.1–2.8	2.6‡	2.3	1.7–3.3
Hb F (% F_{AD})	1.5–9.3	4.4–10.4	5.6	...
Hb F (% F_{IIe})	...	...	5.0	6.6–11.0
Hb A	...	Absent	...	Absent
Distribution of Hb F in RBC	Unequal	...	Equal	Equal
No. glycyl residues in γCB-3	0.27–0.51§	...	1.05	1.04

* Data are mainly from family A described in [26]; the methodology in that study is similar to that used in the analyses described here.

† Two patients with Hb Lepore–Hb C condition.

‡ One patient with Hb Lepore–Hb S condition.

§ From [17].

rable amounts of the hemoglobin variant, of Hb F, and of Hb A_2, the Hb Lepore heterozygote has the erythrocyte morphology of β thalassemia whereas the Hb Kenya heterozygote lacks these morphologic changes. Furthermore, the distribution of Hb F which is distinctly unequal in red cells of Hb Lepore heterozygotes is nearly equal in the cells of the Hb Kenya heterozygote. The same situations obtain when a $Hb_{\beta S}$ (or $Hb_{\beta C}$) locus is in *trans* to the Hb Lepore or Hb Kenya determinant. In addition, in these double heterozygotes Hb A is absent, and the level of

Hb A_2 is lower than that in subjects with Hb S trait or sickle cell anemia [16, 17]. The Hb_{β^S} gene in *trans* to the Hb Lepore determinant does not significantly influence the level of the Hb Lepore: in striking contrast, the Hb_{β^S} gene in *trans* to the Hb Kenya determinant leads to about a threefold increase in the level of Hb Kenya. When the Hb S–Hb Kenya combination is present, the level of Hb F is approximately double that in the Hb Kenya heterozygote. The clinical course, the normal red cell morphology, the proportions of the hemoglobin components, and the equal distribution of Hb F within the erythrocytes exclude the possibility that the Hb Kenya anomaly is related to a β thalassemia condition. On the contrary, the Hb Kenya heterozygote must be considered a special type of HPFH.

The difference in phenotypic expression of the Lepore and Kenya anomalies is one of the more fascinating aspects of the present study. It is not easy to determine why the presence of a δ-β gene produces a β-thalassemia-like condition and the presence of a γ-β gene produces an HPFH-like state. The answer possibly lies in controlling factors responsible to greater or lesser degree for switching from the production of γ chains to that of β and δ chains. One can assume a factor Y and place it between the Hb_{A_γ} and Hb_δ loci; thus, $Hb_{G_\gamma}.Hb_{A_\gamma}.Y.Hb_\delta.\ Hb_\beta$ (for simplicity, two instead of four γ loci are indicated). The Lepore deletion, to produce the $Hb_{\delta\text{-}\beta}$ locus, leaves factor Y untouched and active. The Kenya deletion, to produce the $Hb_{\gamma\text{-}\beta}$ locus, deletes factor Y and causes inactivity. It should be realized that factor Y is active or inactive only in *cis*. This conclusion derives from the fact that Hb F, not only in the heterozygote but also in the Hb S–Hb Kenya state where its quantity is doubled, has $^G\gamma$ chains alone. The deletion (and hence, inactivity) of this factor permits production from the Hb_{G_γ} gene in *cis* to the deletion. If its inactivity were effective also in *trans* where the chromosome is normal (or has a Hb_{β^S} gene), both $^G\gamma$ and $^A\gamma$ chains would be apparent in the Hb F because of production from Hb_{G_γ} and Hb_{A_γ} genes in *trans*.* The activity of factor Y in *cis* also follows from information from the HPFH condition [23, 27]. In Lepore heterozygotes, the situation is different; the Hb F of these individuals has $^G\gamma$ and $^A\gamma$ chains in a ratio that is found in Hb F of the normal adult and of many β thalassemia heterozygotes [28]. Whether the Hb F in these individuals derives from *cis, trans,* or both, to the determinant cannot be decided, because both types of γ chains are produced. The level of Hb F in heterozygotes for Hb Lepore or β thalassemia often is normal or only moderately elevated.

Genetic Basis for HPFH

Chemical analysis of Hb F in HPFH has led to grouping into three basic types of HPFH: the Hb_{G_γ}, the Hb_{A_γ}, and the $Hb_{A_\gamma}Hb_{G_\gamma}$ classes, in which, respectively, $^G\gamma$ chains, $^A\gamma$ chains, or a mixture of both are produced [23, 27]. As a direct consequence of these data, it has been concluded that the genetic basis for HPFH may reasonably be deleted genes and that various specific deletions of $^G\gamma$, $^A\gamma$, δ, and

* Traces of $^A\gamma$ chain from Hb_{A_γ} genes in *trans* would be expected but undetectable within the precision of the method. Obviously, production in *trans* from this gene is not increased.

β genes account for the classes of HPFH. The observations that we have been discussing support this hypothesis, because the nature of Hb Kenya requires the deletion of parts of Hb_{γ} and Hb_{β} loci, of a Hb_{δ} locus, and perhaps of one or more additional Hb_{γ} loci. In other types of HPFH, the deletion of entire loci for a specific hemoglobin chain makes far less obvious the recognition of the genetic defect.

SUMMARY

The non-α chain of Hb Kenya is considered to be the product of a hybrid gene from nonhomologous crossing over of γ and β loci with deletion. The N-terminal part derives from the γ gene and the C-terminal part from the β gene. Further chemical investigation has localized this crossing over between residues 81 and 86.

Examination of immediate relatives of the propositus has shown the presence of not only Hb Kenya but also Hb S and Hb A_2' in addition to Hb A, Hb A_2, and Hb F in a variety of combinations.

The data permit the several genes to be arranged in the order $Hb_{G_{\gamma}}$.$Hb_{A_{\gamma}}$.Hb_{δ}.Hb_{β}.

Although the deletion of genetic material that results in the hemoglobins Lepore with their δ-β chain produces symptoms of β-thalassemia trait, the deletion that produces Hb Kenya leads to the characteristics of the hereditary persistence of fetal hemoglobin. The genetic significance of this observation is discussed.

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