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Characterization of $\alpha^{23GluNH_2}$ in Hemoglobin Memphis. Hemoglobin Memphis/S, a New Variant of Molecular Disease*

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ABSTRACT: A previously unreported mutant α chain, $\alpha^{23GluNH_2}$, was found associated with β^A as hemoglobin Memphis, with β^{6Val} as Hb Memphis/S, or with β^{6Lys} as Hb Memphis/C. The two hemoglobins, Hb Memphis/S and Hb S, found together cause a new, clinically mild variant of sickle cell anemia. The hemoglobins were characterized by electrophoresis, hybridization, separation of the α and β chains by countercurrent distribution, tryptic and chymotryptic digestion, peptide maps, amino acid analysis, whole blood viscosity, and minimal hemoglobin gelling concentration. The mutation is a substitution of glutamine for glutamic acid at residue 23 in the corner between the A- and B-helical regions of the α chain. The loss of a negative charge in this region may alter the configuration of the α chain.

The molecular behavior of the mutant α-chain combination with β^S differs from that of α^A with β^S. The viscosity of the blood, gelling of the hemoglobin, and the clinical history of the individuals with Hb Memphis/S and Hb S deviate from the findings in a sickle cell anemia (Hb S) individual without the α-chain variant. Thus, the sickling phenomenon may be related to polypeptide chain interaction.

The characterization of a mutation in an abnormal human hemoglobin provides additional confirmation of the genetic code (Beale and Lehmann, 1965) and a means of correlating a change in primary structure of a protein with its tertiary configuration and any resulting change in the molecular behavior. Herein is reported a previously undescribed mutation in the α chain of hemoglobin where glutamine is substituted for glutamic acid at residue 23 ($\alpha^{23GluNH_2} = \alpha^{Mem}$). This abnormal α chain is found associated with another abnormal chain, β^{6Val} (β^S). In this individual no normal β chains are present; therefore, the molecular criterion for identification of sickle cell anemia is met. However, the alleviation of the classical symptoms of sickle cell anemia and the behavior of cells and hemoglobin under low-oxygen tension indicate that the hemoglobin $\alpha_2^{Mem}\beta_2^S$ does not follow the same molecular behavior as $\alpha_2^A\beta_2^S$. This then may be an indication that interaction between α and β chains

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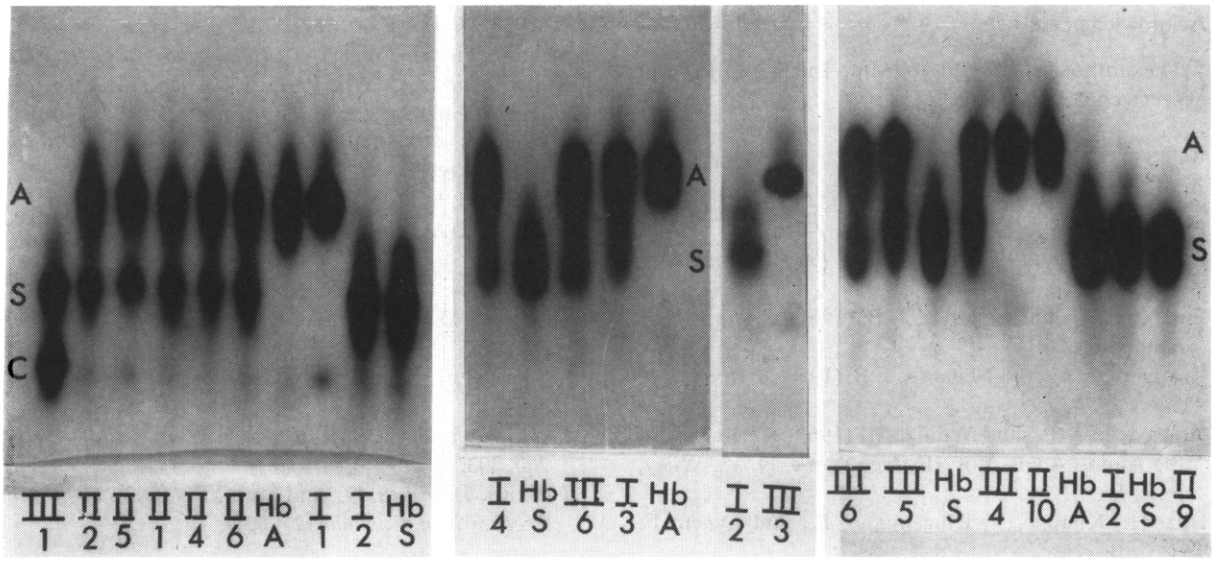

FIGURE 1: Vertical starch gel electrophoresis, Tris–EDTA–boric acid buffer, pH 8.6, 15ma, 16 hr, benzidine stain. The position of hemoglobins A, S, and C are noted. When $\alpha^{23\text{GluNH}}$ was present, the resultant hemoglobins, Hb Memphis, Hb Memphis/S, or Hb Memphis/C were found in the same position as Hb A, Hb S, or Hb C depending upon the β chain with which α^{Memphis} associated. The source of the hemoglobin is designated according to Figure 5.

is an essential part of the sickling phenomenon. The loss of a negative charge at position 23 near the corner between the A- and B-helical regions of the α chain may alter the configuration of the mutant α chain and this in turn may alter the relationship of α^{Mem} chains to the β^{S} chains. The presence and reactions of this new molecule alter the classical picture of sickle cell anemia. Chemical and clinical characterization of the new mutant α chain and the new hemoglobins resulting from its association with different β chains found in the pedigree, including three generations, is also reported.

Materials and Methods

Venous blood was collected using 0.1 ml of 10% disodium EDTA as anticoagulant and hemolysates were prepared (Drabkin, 1946). The hemoglobin concentration of the hemolysate was determined by the cyanmethemoglobin method (Crosby *et al.*, 1954) after dialysis against 10^{-4} M disodium EDTA solution for 24 hr at 4° and the hemoglobin was stored in the carboxyhemoglobin form.

Electrophoresis and Chromatography. Agar gel electrophoresis (Shibata and Iuchi, 1961) was performed using a 2 g % cyanmethemoglobin solution at pH 6.2, 7.2, and 8.6. The protein was stained with bromophenol blue in order to see the separated bands more clearly.

Starch gel electrophoresis using Tris–EDTA–boric acid buffer (Boyer *et al.*, 1963) at pH 8.6, 15 ma for 16 hr, was performed by the method of Smithies (1959). After electrophoresis the starch gel was cut into two slices and stained either by benzidine or by amido-black 10 B. The hemoglobins were isolated by column chromatography using Amberlite IRC 50 (Bio-Rex 70) and developer 2 (Allen *et al.*, 1958).

Hybridization. The abnormal hemoglobin was hybridized with Hb C for the detection of a chain anomaly (Huehns and Shooter, 1962). Since the isolation of this abnormal hemoglobin from Hb S was difficult, the hemolysate of the propositus (I-2) and the Hb C solution were mixed in the proportion of 1:1, then dissociated and recombined (Itano and Robinson, 1959). The recombinant mixture was subjected to starch gel electrophoresis and the hemoglobin bands on the starch gel were examined.

Peptide Mapping. Tryptic digestion and peptide mapping of the cell hemolysates and of the isolated polypeptide chains were performed by the method of Ingram (1958). When cell hemolysates were used, the heme was removed first (Anson and Mirsky, 1930) and then the globin was digested with trypsin. When polypeptide chains were fingerprinted, both the preparation of globin and the isolation of the chains by countercurrent distribution were done according to the method of Hill *et al.* (1962). The tryptic digest was electrophoresed at 2000 v. Ingram's solvent system was used for chromatography. Peptides were located by the ninhydrin reaction and the amino acids histidine, tyrosine, and arginine were identified by special staining methods (Block *et al.*, 1958).

Chymotryptic Peptides. In order to investigate the amino acid substitution, abnormal peptides which appeared on the fingerprint were collected and further digested with chymotrypsin. The abnormal peptides were isolated using one-dimensional, high-voltage paper electrophoresis (2000 v/45 cm) at pH 6.5, pyri-

dine–acetic acid buffer. The tryptic digest was applied to Whatman 3MM paper (55 × 14 cm) in a line at a distance 24 cm from the anode end. Four narrow guide strips were cut lengthwise, two from each side of the air-dried paper. The outer strips were stained with ninhydrin and the other strips were stained for histidine. The abnormal peptide was located on the unstained paper with the aid of the stained guide strips and then eluted with 0.5% NH_4HCO_3 solution (Clegg *et al.*, 1965). The abnormal peptide was collected from this solution in pure form by repeated lyophilization. The peptide thus obtained was dissolved in 0.5% NH_4HCO_3 at a concentration of 0.2 g %. The peptide was digested by incubating with chymotrypsin (1:50, w/w) at 37° for 13 hr (Ingram, 1959). The chymotryptic hydrolysate was lyophilized and fractionated using one-dimensional, high-voltage paper electrophoresis. The peptides were located by ninhydrin reaction. All enzymes used were two times recrystallized as supplied by Worthington Biochemical Corp.

Amino Acid Analysis. Amino acid analysis of the tryptic and chymotryptic peptides was determined using a Technicon amino acid analyzer. The peptide was eluted from the paper with 2 ml of constant-boiling HCl and then hydrolyzed at 110° under nitrogen for 24 hr. The acid was evaporated from the hydrolysate immediately before amino acid analysis.

Quantitation of Peptides. The abnormal peptide and the normal peptide corresponding to it were treated by quantitative phenylthiocarbamylation (Sjöquist and Sjöquist, 1963). The phenylthiocarbamyl peptides were eluted individually with 50% ethanol, the optical density was measured at 243 mμ, and the percentage of the two peptides was calculated. The relative amounts of the hemoglobins present were extrapolated from these values.

Estimation of Degree of Sickling. Viscosity of deoxygenated whole blood was determined (Charache and Conley, 1964). All viscosity measurements were made after exposure of the blood to an atmosphere of 95% N and 5% CO_2 for 30 min. Minimal gelling concentrations of hemoglobin were obtained in an atmosphere of 95% N and 5% CO_2 (Singer and Singer, 1953). Results were expressed in concentration of Hb S as calculated from the amount of fetal hemoglobin present.

Family Study. After discovering the abnormal hemoglobin in the propositus, blood from other members of the family was examined in a similar manner.

Results

Electrophoresis and Chromatography. Agar gel electrophoresis of the hemolysate of the propositus (I-2) at pH 6.2 and 7.2 revealed clear-cut separation of Hb F, Hb A, and Hb S, starting at the cathodic end in the order mentioned. The Hb S appeared to be divided into two fragments. The slower moving one was assumed to be another type of abnormal hemoglobin after comparison with the mobility of known Hb S.

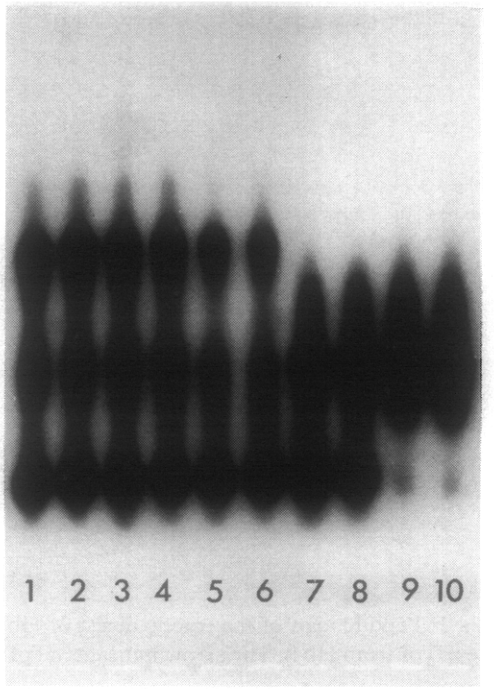

FIGURE 2: Vertical starch gel electrophoresis, pH 8.6, of hemoglobin dissociated at pH 4.7 and recombined (hybridized) with Hb C. 1 and 2: hemoglobin from II-4; 3 and 4: hemoglobin from II-6; 5 and 6: hemoglobin from II-2; 7 and 8: hemoglobin from I-2; 9 and 10: hemoglobin from I-2 without Hb C (pH control). 1, 3, 5, 7, and 9: hemoglobins dissociated at pH 4.7 and recombined; 2, 4, 6, 8, and 10: control mixtures not dissociated. Note absence of band in posititon of Hb A in 7, indicating absence of β^A chains in this individual.

It was located to the cathodic side of Hb S and so close to it that its isolation was thought to be impossible. The fact that the divided Hb S band did not separate with a portion migrating to the position of Hb A on agar, but remained in the position of Hb S, clearly indicated that it was not identical with Hb D (Marder and Conley, 1959).

Agar or starch gel electrophoresis at pH 8.6 of the hemolysate of the blood of the propositus did not show a separation of the abnormal hemoglobin from Hb S. It was located in close proximity to Hb S on the cathodic side. The total hemoglobin fraction was heavier than that of the control Hb S at the cathode end (Figure 1). Amberlite IRC 50 (Bio-Rex 70) chromatography, which gave satisfactory separation of Hb F, Hb A, and Hb S, failed to reveal the abnormal hemoglobin.

Hybridization. Hybridization of hemoglobin of the propositus (I-2) with Hb C did not result in formation of Hb A ($\alpha_2^A\beta_2^A$) or Hb Memphis ($\alpha_2^{Mem}\beta_2^A$), demonstrating the absence of β^A chains in this individual (Figure 2).

Peptide Composition and Identification of Amino

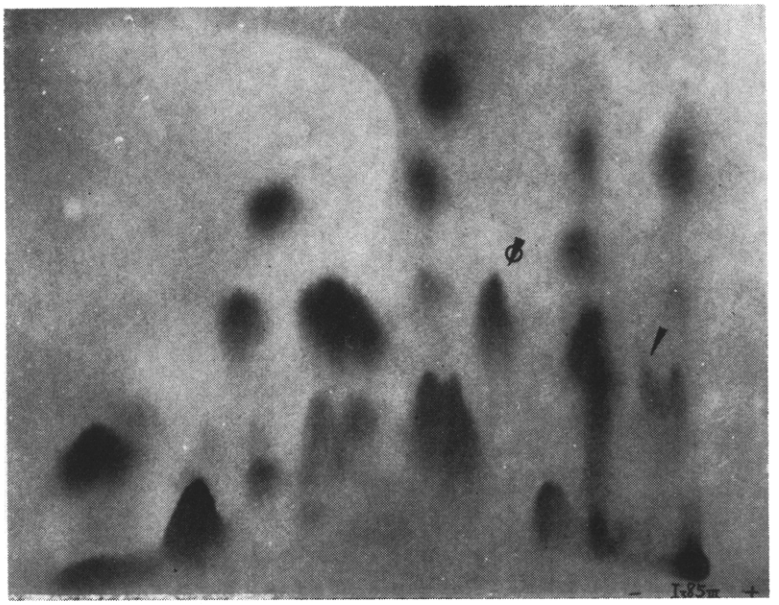

FIGURE 3: Peptide map of the tryptic digest of Hb Memphis/S and Hb S from individual II-9. The circled arrow designates βTpI from Hb S. The arrow indicates αTpIV-x.

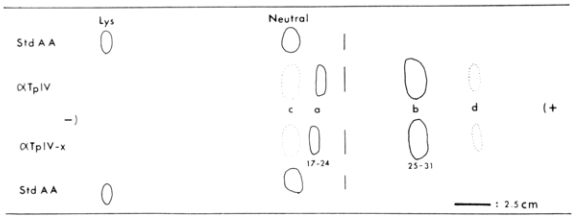

FIGURE 4: Electrophoresis of the chymotryptic digests of αTpIV and αTpIV-x (2000 v/45 cm, 68 ma/14 cm, 90 min, pyridine–acetate buffer, pH 6.5). a: Amino acids 17–24; note difference in mobility. b: Amino acids 25–31; mobility identical. c and d: Ninhydrin-positive bands with trace amounts of amino acids. Std AA indicates standard amino acid mixture. The figure is a tracing of the original electrophoretic separation.

TABLE I: Amino Acid Composition of an Abnormal Peptide Appearing at the Characteristic Position of βTpl from Hb S.

Amino Acid	Amino Acid Residues per Molecule of Peptide	
	βTpI[a]	Theoretical βTpI of Hb S
Threonine	1.05	1.00
Glutamic acid	1.12	1.00
Proline	0.92	1.00
Valine[b]	1.61	2.00
Leucine	0.92	1.00
Lysine	0.91	1.00
Histidine	1.09	1.00

[a] The values of the amino acid residues are the values obtained after correction for the presence of the contaminant, βTpVIII,IX. [b] One of the two valines is an N-terminal amino acid and is partially destroyed by ninhydrin staining.

Acid Substitution. Countercurrent distribution of globin separates only α chains from β chains but does not isolate different α chains or different β chains from each other. Polypeptide chains of the propositus (I-2) were examined. Only one aberrant peptide, βTpI of Hb S, and no normal counterpart was found in peptide maps of the β-chain tryptic digest, thus indicating the presence of a single type of β chain. However, what appeared to be two αTpIV peptides were found in the peptide maps of the α-chain fraction, thus indicating the presence of two different α chains. In confirmation of this, the fingerprint of the tryptic digest of the globin of the propositus revealed the appearance of two abnormal peptides and the lack of a normal βTpI peptide (Figure 3). One of the abnormal peptides which appeared on the fingerprint was identified as βTpI of Hb S by location and by amino acid analysis (Table I). The other abnormal peptide was located just to the cathodic side of its neighboring αTpIV and it showed positive histidine, tyrosine, and arginine staining reactions as did the normal αTpIV. This peptide, because of the specific amino acid staining reactions and the slow-moving phenomenon of the abnormal hemoglobin, may be considered to be an aberrant αTpIV and will be referred to as αTpIV-x. Amino acid analysis (Table II) confirmed this. Since no peptides were evident in the chromatographic direc-

TABLE II: Amino Acid Composition of αTpIV and αTpIV-x.

Amino Acid	Amino Acid Residues per Molecule of Peptide		
	αTpIV-x	αTpIV	Theoretical αTpIV
Aspartic acid	0.24	0.23	0.00
Threonine	0.11	0.09	0.00
Serine	0.29	0.27	0.00
Glutamic acid	2.95	3.23	3.00
Glycine	3.06	3.26	3.00
Alanine	3.74	3.91	4.00
Valine	0.89	0.93	1.00
Leucine	1.03	1.09	1.00
Tyrosine	0.45	0.72	1.00
Lysine	0.16	0.13	0.00
Histidine	0.96	1.03	1.00
Arginine	1.02	1.09	1.00

TABLE III: Amino Acid Composition of the Acid Hydrolysate Obtained after Electrophoresis of the Chymotryptic Digest from Peptides a and b of αTpIV-x and αTpIV.

Amino Acid	Amino Acid Residues Per Molecule of Peptide		
	αTpIV-x	αTpIV	Theoretical αTpIV
	Peptide a		
Glutamic acid	0.97	1.03	1.00
Glycine	2.04	2.02	2.00
Alanine	1.96	1.98	2.00
Valine[a]	0.57	0.83	1.00
Tyrosine	0.63	0.54	1.00
Histidine	1.00	1.00	1.00
	Peptide b		
Glutamic acid	2.09	2.04	2.00
Glycine[a]	0.46	0.52	1.00
Alanine	2.00	2.00	2.00
Leucine	0.99	0.98	1.00
Arginine	1.00	1.00	1.00

[a] Valine and glycine are N-terminal amino acids and are partially destroyed by ninhydrin staining.

tion on the fingerprints, the peptides αTpIV and αTp-IV-x were isolated by one-dimensional, high-voltage paper electrophoresis.

One-dimensional, high-voltage paper electrophoresis of the chymotryptic digest of both αTpIV-x and αTpIV at pH 6.5 revealed four ninhydrin-positive bands illustrated as a–d in Figure 4. Of the four bands, the a band of αTpIV-x was clearly demonstrated to be slower moving toward the anode when compared with the corresponding band of the normal αTpIV. The other three bands of the αTpIV-x were identical in electrophoretic mobility with those of αTpIV. Amino acid analysis of the hydrolysate of the a band of the αTpIV-x showed the presence of two glycine, two alanine, one glutamic acid, one tyrosine, and 0.6 valine residues, indicating that this peptide arose from residues 17 to 24 of the α chain, the first eight amino acids of α TpIV-x (Table III). The amino acid analysis of the a band of αTpIV was the same as the a band of αTpIV-x (Table III) even though αTpIV-x, upon electrophoresis, had a less negative charge. This would indicate that, before acid hydrolysis of the peptide, the glutamic acid residue in a αTpIV-x was, in fact, glutamine. It is known that acid hydrolysis converts glutamine to glutamic acid.

The analysis of the b bands of αTpIV-x and αTpIV gave identical results, namely, two glutamic acid, two alanine, one leucine, one arginine, and one-half glycine residues (Table III). These results were consistent with the assumption that the peptide constituents arose from residues 25 to 31 of the α chain.

The c and d bands of both the αTpIV-x and αTpIV were markedly different from the a and b bands because the amounts of amino acids present were too small to be analyzed. From all the results, it was concluded that the 23rd residue in the α chain, normally glutamic acid, had been replaced by glutamine in the mutant α chain.

Hemoglobin, Genotype, and Phenotype. The amount of the abnormal hemoglobin present was extrapolated from the determination of the phenylthiocarbamyl αTpIV-x and αTpIV peptides. By this method, the propositus had equal amounts of Hb Memphis/S and Hb S. The name of Hb Memphis/S was given to this abnormal hemoglobin ($\alpha_2{}^{23GluNH_2}\beta_2{}^{6Val}$). The abnormal α chain and its structural gene were designated as $\alpha^{Memphis}$. When $\alpha^{Memphis}$ was found in combination with normal β chains, the resulting hemoglobin was called Hb Memphis. The respective distribution of the structural genes in the members of the family was established by checking the presence of the various polypeptide chains of hemoglobin using fingerprints and starch gel electrophoresis. The results are shown in Figure 5. The hemoglobin, phenotype, and genotype of each member of the family are described in Table IV. In the expression of major adult hemoglobins, if three different structural genes are present, two hemoglobins are found; when four different structural genes are present, four hemoglobins are found; and two different structural genes result in one hemoglobin (Figure 5 and Table IV).

Estimation of Degree of Sickling. Viscosity of deoxygenated whole blood was measured (Charache and Conley, 1964). In blood from nine controls (Hb A) the steel ball traversed 10 cm in 60.5–72.8 sec, as compared to 30.2 sec in water. The same distance of

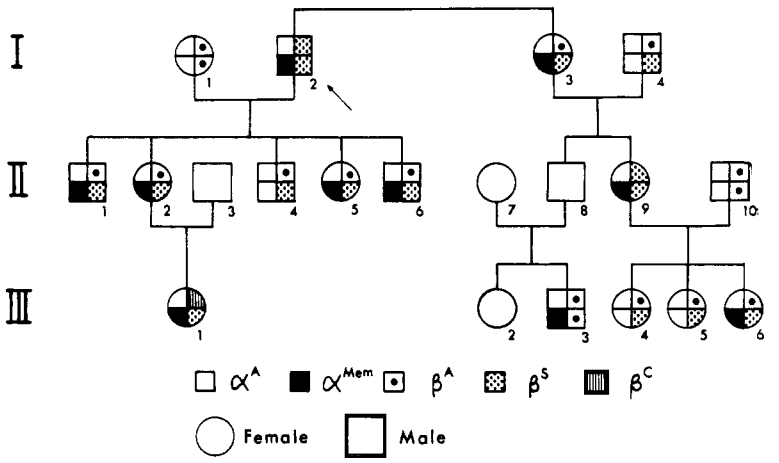

FIGURE 5: Family tree showing the respective distribution of structural genes as expressed by the polypeptide chains found. The arrow indicates the propositus (index case), the first individual studied in this family. Blank symbols indicate patient not available for examination.

fall was observed to require 125.2–198.2 sec in blood from nine sickle cell anemia patients (Hb S). In marked contrast, results obtained in blood from the propositus (I-2) and his niece (II-9) were 77.2 and 88.4 sec, respectively. The same steel ball and glass tube were used in all determinations.

Minimal concentrations of hemoglobin solutions required for gelling upon deoxygenation were expressed in g/100 ml of Hb S because the level of fetal hemoglobin in the propositus and his niece were not equal (10.1 and 2.9%, respectively). Hemolysates of six sickle cell anemia patients (Hb S) required minimal hemoglobin concentrations of from 19 to 24 g/100 ml for gelling, a range in agreement with that reported by Singer and Singer (1953). The lowest Hb S concentration sufficient for gel formation of a hemolysate of the propositus was 26.8 g/100 ml and his niece 26.0 g/100 ml.

The individual III-3 with Hb Memphis and Hb A did not show any clinical or hematologic abnormalities. The six family members with four hemoglobins (Table IV) were clinically indistinguishable from sickle cell trait patients. The propositus (I-2), age 72, and his niece (II-9), age 40, had experienced occasional painful swelling of the joints of the extremities during their teens, but had not suffered any of the severe painful crises usually seen in sickle cell anemia patients. Both had a normochromic, hemolytic anemia with hematocrit readings of 21.5 and 27%, respectively, and positive sickle cell preparations.

Discussion

According to the mild clinical symptoms, hematologic tests, and electrophoretic analysis of his blood, the propositus (I-2), a 72-year-old male, was diagnosed as having atypical sickle cell anemia. A single abnormal hemoglobin, Hb S, was seen on routine paper electrophoresis at pH 8.6. Only upon careful observation was any other abnormality detected by agar gel electrophoresis at pH 6.2 and 7.2 and in starch gel electrophoresis at pH 8.6. The aberrant hemoglobin migrates in the hemoglobin S region at the cathodic side and is almost indistinguishable from the usual Hb S. Accordingly, attempts to separate and isolate the pure abnormal hemoglobin from Hb S by the use of agar, starch gel, and Amberlite IRC 50 chromatography were unsuccessful. Indisputable evidence for the presence of an abnormal hemoglobin, except for Hb S, was not obtainable until an abnormal peptide was detected on a fingerprint. This abnormal peptide (αTpIV-x) was located to the cathodic side of the neighboring normal αTpIV peptide. The slower anodic migration of the αTpIV-x on the fingerprint and of the a band of the chymotryptic digest of the αTpIV-x in one-dimensional paper electrophoresis and the results of the amino acid analysis of these peptides compared to the normal αTpIV peptides indicated that in this abnormal hemoglobin glutamic acid is replaced by glutamine at residue 23 of the α chain. The amino acid replacement, glutamine for glutamic acid, as a result of a single base change in the structural gene, is possible and consistent with the current knowledge of the genetic code.

The names Hb Memphis, Hb Memphis/S, and Hb Memphis/C were given to the abnormal hemoglobins expressed as $\alpha_2{}^{23\mathrm{GluNH_2}}\beta_2{}^{A}$, $\alpha_2{}^{23\mathrm{GluNH_2}}\beta_2{}^{6\mathrm{Val}}$, and $\alpha_2{}^{23\mathrm{GluNH_2}}\beta_2{}^{6\mathrm{Lys}}$, respectively. In these abnormal hemoglobins, there is only a barely discernible difference in electrophoretic mobility in contrast with other mutant hemoglobins which have been first noted because of their different electrophoretic mobility. In the normal α chain, the 23rd residue, glutamic acid, does not seem to provide the hemoglobin molecule with an increase in net negative charge because of its proximity to histidine at position 20 with the possibility of interaction between the two molecules (H. Lehmann, personal communication). The substitution of the neutral amino acid glutamine for the glutamic acid at this

TABLE IV: Representation of Structural Genes and Resulting Hemoglobins Observed in This Family.

Individuals	Genotype	Phenotype	Hemoglobins
I-1, II-10	$\alpha^A\alpha^A/\beta^A\beta^A$	$\alpha_2^A\beta_2^A$	A
I-4, II-4, III-4,5	$\alpha^A\alpha^A/\beta^A\beta^S$	$\alpha_2^A\beta_2^A$, $\alpha_2^A\beta_2^S$	A S
III-3	$\alpha^A\alpha^{Memphis}/\beta^A\beta^A$	$\alpha_2^A\beta_2^A$, $\alpha_2^{Memphis}\beta_2^A$	A Memphis
I-3, II-1,2,5,6, III-6	$\alpha^A\alpha^{Memphis}/\beta^A\beta^S$	$\alpha_2^A\beta_2^A$, $\alpha_2^{Memphis}\beta_2^A$ $\alpha_2^S\beta_2^A$, $\alpha_2^{Memphis}\beta_2^S$	A Memphis S Memphis/S
I-2, II-9	$\alpha^A\alpha^{Memphis}/\beta^S\beta^S$	$\alpha_2^A\beta_2^S$, $\alpha_2^{Memphis}\beta_2^S$	S Memphis/S
III-1	$\alpha^A\alpha^{Memphis}/\beta^S\beta^C$	$\alpha_2^A\beta_2^S$, $\alpha_2^{Memphis}\beta_2^S$ $\alpha_2^A\beta_2^C$, $\alpha_2^{Memphis}\beta_2^C$	S Memphis/S C Memphis/C

position does not appear to cause any appreciable charge difference. Thus, any hemoglobin containing α^{Mem} will be difficult to distinguish from a hemoglobin containing the normal α chain. This effect made it impossible to detect the chain anomaly by the hybridization test because the two hybrids, $\alpha_2^{Mem}\beta_2^C$ and $\alpha_2^{Mem}\beta_2^S$, have the same electrophoretic mobility as Hb C and Hb S which are present at all times in the mixture of the recombinants. This loss of a negative charge at position 23 near the corner of the A- and B-helical regions of the α chain may alter the configuration of the mutant α chain. This may in turn alter the relationship of the α^{Mem} chains to the β^S chains, since the behavior of the cells and hemoglobin under low-oxygen tension indicate that the hemoglobin $\alpha_2^{Mem}\beta_2^S$ does not follow the same molecular behavior as $\alpha_2^A\beta_2^S$. This then may be an indication that the interaction between α and β chains is an essential part of the sickling phenomenon.

Only two instances of sickle cell anemia patients who also have an α-chain variant have been found. One is heterozygous for α^A and $\alpha^{Stanleyville\ II}$ (Hall-Craggs *et al.*, 1964) and the other has α^{68Lys} (the α chain of Hb $G^{Philadelphia}$) (Pugh *et al.*, 1964). Both patients have typical sickle cell anemia, but the α-chain variants differ from the one described in this report and do not appear to alter the sickling phenomenon.

The propositus and his niece, who have Hb Memphis/S, have little history of suffering from the typical painful crisis of the usual sickle cell anemia. The rigid erythrocytes, due to tactoid formation of deoxygenated Hb S, are thought to result in partial obstruction of microcirculation, tissue anoxia, and therefore painful crisis. Since this did not appear to occur in the presence of α^{Mem}, it was determined that the presence of this chain does change deoxygenated blood viscosity indicating a decrease in erythrocyte deformation and thus a decrease in sickling. Likewise, the gelling phenomenon indicates a difference in the interaction of the α^{Mem} with β^S as contrasted with α^A and β^S. According to Murayama (1966) the sickling phenomenon requires an interaction of the abnormal β chain of one molecule of hemoglobin with the α chain of another molecule. It may be that this interaction occurs in the region changed by this α-chain mutation, thereby interfering with the interaction necessary for the sickling phenomenon. This in turn would lead to a milder form of sickle cell disease as found in the individuals in this study.

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CORRECTIONS

In the paper "Initial Attempts at a Theoretical Calculation of the Conformation of Gramicidin-S," by Garret Vanderkooi, S. J. Leach, George Némethy, Roy A. Scott, and Harold A. Scheraga, Volume 5, September 1966, in footnote 3 on p 2996 the correct ADI document number is 9016.

In the paper by Herbert M. Kagan and Alton Meister, Volume 5, July 1966, p 2423, the title should be corrected to read "Activity of Glutamine Synthetase toward *threo*-γ-Methyl-L-glutamic Acid and the Isomers of γ-Hydroxyglutamic Acid."

In the paper "Studies on Verdohemochrome," by Ephraim Y. Levin, Volume 5, September 1966, p 2845, all ϵ values should be reported as $\epsilon \times 10^{-3}$.