Structure and Properties of Hemoglobin CHarlem, a Human Hemoglobin Variant with Amino Acid Substitutions in 2 Residues of the β-Polypeptide Chain*

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ROBERT R. BOOKCHIN,† RONALD L. NAGEL, AND HELEN M. RANNEY§

From the Department of Medicine and Heredity Unit, Albert Einstein College of Medicine, Bronx, New York

SUMMARY

A new sickling hemoglobin variant designated hemoglobin CHarlem (Hb CH) migrated slightly anodally to the position of hemoglobin C on electrophoresis at pH 8.6, and has been shown to have the structure α2β2Glu → Val, 73 Asp → Asn. Hemoglobin CH comprised 40% of the hemoglobin in individuals of two generations of an American Negro family and is the first instance in which two amino acid substitutions in a single polypeptide chain have been defined in a human hemoglobin variant.

Hemoglobin CH appeared not to differ significantly from Hb A in its oxygen equilibria, ultraviolet spectra of the oxy and deoxy forms, and titratable sulfhydryl groups. Probably by virtue of its β2 Val substitution, Hb CH shared with Hb S (β2 Val → Val) the properties of erythrocyte sickling and relative insolubility and gelation of the deoxyhemoglobin. However, gelation experiments suggested that the β73 Asn substitution resulted in a decrease in the intermolecular interactions of deoxyhemoglobin CH as compared with deoxyhemoglobin S, and implied conformational differences between the two deoxyhemoglobins.

More than 40 variants of human hemoglobin have been described which differ in primary structure from their normal counterparts by a single amino acid substitution in each of the like polypeptide chains (1). The present report is concerned with a new hemoglobin variant, designated hemoglobin CHarlem (Hb CH), which is associated with erythrocyte sickling and which contains two amino acid substitutions in each β-polypeptide chain: its structure has been shown to be α2β2Glu → Val, 73 Asp → Asn. This report includes a description of the studies (some of which were the subject of a preliminary account (2)) leading to this structural formulation and a comparison of some properties of Hb CH and Hb S, particularly with reference to sickling.

EXPERIMENTAL PROCEDURE

Source of Hemoglobin CHarlem—The propositus was a 45-year-old Negro man referred by Miss Mary McKenna, senior chemist at Harlem Hospital, who observed erythrocyte sickling associated with a paper electrophoretic pattern of hemoglobins C and A and decreased solubility of the deoxyhemoglobin. Hemoglobin electrophoresis on starch gel at pH 8.6 (3) showed Hb A and a component migrating slightly anodal to the position of Hb C (Fig. 1). The hemolysate prepared by the method of Drabkin (4) contained 40% Hb CH, 2.3% fetal Hb (5), and the remainder Hb A. The normal minor basic component, Hb A2, could not be distinguished in the presence of Hb CH.

Hemoglobin CHarlem was found in similar proportions (40%) in six of the nine children of the propositus whose red cells also sickled in 2% sodium metabisulfite. These heterozygotes were clinically well. Further clinical studies will be described separately.

Studies of Primary Structure—Acid dissociation and recombination experiments were carried out by the method of Itano and Singer (6) with minor modifications. Hemoglobin CH was isolated by DEAE-cellulose chromatography by the method of Huisman and Dozy (7) or by starch granule electrophoresis (8) in Veronal buffer, pH 8.6. Neither method resulted in separation of Hb C from Hb A. The proportion of Hb A2 would be expected to double or triple (the usual proportion is 2 to 3%) during the isolation procedure. For comparative studies, Hb S was isolated by similar methods to remove most of the Hb F, which amounted to less than 2% following DEAE-cellulose chromatography. The β chains of Hb CH were isolated by a modification of the method of Bucci and Fronticelli (9) in which HMB was added to the hemoglobin which had been dialyzed for 4 hours against 0.05 m phosphate buffer, pH 5.8, containing 0.05 mole of NaCl, and the α and β chains were subsequently separated by electrophoresis on starch granules in Veronal buffer at pH 8.6. Since Hb A2 does not readily dissociate into α and β...
chains under these conditions, a appreciable contamination of Hb Cn with Hb A did not occur during isolation of $\beta^{\text{HM}}$ chains of Hb Cn. Globin was prepared from $\beta^{\text{HM}}$ chains of Hb Cn and Hb A by acid-acetone precipitation (10), washed with acetone, and lyophilized. Tryptic digestion was carried out for 90 min at pH 8.0 as described by Ingram (11) except that lyophilized globin rather than heat-denatured hemoglobin was digested in air rather than a nitrogen atmosphere. Peptide maps (combined paper electrophoresis and chromatography) were prepared by Baglioni's modification (12) of the method of Ingram (11) from the soluble tryptic digest of the $\beta^{\text{HM}}$-globin. Tryptic digests were also made of aminoethylated $\beta$-globin by the method of Clegg, Naughton, and Weatherall (13) to examine the otherwise insoluble "core" peptides. The peptide maps were stained for arginine, histidine, tryptophan, and sulfur (14).

Peptides which required further studies were stained with a dilute ninhydrin stain (0.01% ninhydrin in acetone). For acid hydrolysis, the peptides were eluted with 6 N HCl from the paper directly into capillary pipettes (15) which were then sealed and hydrolyzed at 105° for 22 hours. Contents of the capillary tubes after hydrolysis, the peptides were eluted with 6 N HCl from the paper directly into capillary pipettes (15) which were then sealed and hydrolyzed at 105° for 22 hours. Contents of the capillary tubes were analyzed directly (13) in a Beckman model 120B automatic amino acid analyzer.

Subtractive Edman degradation followed the method of Hill, Swenson, and Schwartz (16). Papain digestion was performed on $\beta^{\text{HM}}$ Tp I eluted from peptide maps with 10% acetic acid. Peptide, 2 to 3 μmoles (obtained from 10 to 12 peptide maps), was digested at 40° for 15 hours in 0.015% papain at pH 5.5 and at pH 4.5 by the methods of Hill, Swenson, and Schwartz (16).

Other Studies—Visible and ultraviolet spectra of oxy- and deoxyhemoglobin Cn were recorded on a Cary model 14R recording spectrophotometer. For preliminary observations of oxygen equilibria at 10°, the spectrophotometric technique of Allen, Guthe, and Wyman (19) with minor modifications was employed. Reactive sulfhydryl groups were titrated with HMB according to the method of Itano (21) with these modifications: phosphate buffers of varying ionic strength prepared from a single stock solution of 2.8 M potassium phosphate at pH 6.8 were used; the total solution for each determination was 5 ml; precipitates were separated by both centrifugation and filtration through Whatman No. 3 filter paper; and hemoglobin concentrations were determined after conversion to cyanmethemoglobin with a buffered solution of KCN and KFe(CN)$_6$ ($\varepsilon_{\text{max}} = 44.2$ with 64,500 as the molecular weight of hemoglobin). Duplicate determinations of solubility were carried out four times for deoxyhemoglobin Cn, three times for deoxyhemoglobin S, and once for oxyhemoglobin Cn and S.

Gelation—Solutions of hemoglobins Cn and S, isolated as described above, were concentrated by ultrafiltration and were dialyzed against 0.15 M potassium phosphate at pH 7.35 before gelation experiments. Gelling studies at 25° were carried out by the method of Singer and Singer (22) with the following modifications: a flow of nitrogen gas, rather than CO$_2$, was used for deoxygenation; gelation experiments with the isolated hemoglobins were performed on 0.5-m1 aliquots in 10-m1 Erlenmeyer flasks; and hemoglobin concentrations were determined as cyanmethemoglobin in a Beckman DU spectrophotometer. Fresh hemoglobin samples were diluted with distilled water to determine solubility.
mine minimum gelling concentrations. Sometimes the concentration of a nongelling solution was increased very slowly by evaporation in the testing apparatus; under the flow of water equilibrated nitrogen, the small increase (up to 2 to 3 g/100 ml after 2 hours) observed in the concentration of the hemoglobin solution probably accounted for eventual gelation of samples which were very viscous after 60 to 90 min of deoxygenation. The proportions obtained by mixing solutions of isolated hemoglobins were verified by quantitative elution after starch block electrophoresis and found to vary from the intended proportions within ±4% of the total hemoglobin. No difference in results of minimum gelling concentrations were observed in hemolysates prepared by freeze-thaw of erythrocytes followed by exposure to toluene and centrifugation.

**RESULTS**

**Structure of Hemoglobin Cn**—At pH 4.7, a single “new” hemoglobin species was observed in recombination experiments with the use of Hb Cn and Hb I, which contains abnormal α chains, and no new species were noted in experiments with Hb C and Hb Cn, which contains abnormal β chains (Fig. 2). These results were consistent with the observations of Itano and Robinson (23) on the hybridization of hemoglobins C and I in which the resulting new species (Hb A and the doubly abnormal hemoglobin CYPz) had the same electrophoretic mobility and were observed as a single electrophoretic component. Thus the results of recombination studies indicated that the β chains of Hb Cn were abnormal.

Preliminary peptide maps of the tryptic digest of electrophoretically isolated Hb Cn were initially thought (24) to show findings closely resembling those of Hb C Georgetown (25). However, when tryptic digests of isolated PCn chains were studied, the peptide maps contained β chain peptides differing from those reported for Hb C Georgetown. Some of the initial difficulties may have been related to contamination of Hb Cn with Hb A; the isolated β chains were essentially free of contamination with Hb A or with δ chains.

Comparison of peptide maps of the soluble tryptic peptides of isolated βn to chains with those prepared from isolated normal β chains (Fig. 3) showed the following differences: (a) βTp I (numbering system of Gerald and Ingram (26)) was missing, and a new peptide with a greater Rp moved further toward the cathode.
in the position of βTp I of Hb S; (6) βTp IX and βTp VIII-IX were replaced by two new peptides in a more cathodal position.

**Studies of βTp IX**—Amino acid analyses of the first variant peptide noted above were consistent with a βTp I in which 1 glutamyl residue was replaced by 1 valyl residue (Table I). The sequence of β*Tp I is as shown in Scheme 1 (27). To determine whether the substitution occurred at β7 or β7', the abnormal βTp I peptide was digested with papain (Fig. 4). Digestion at pH 5.5 yielded predominantly Peptides pap 1 and pap 2 and free lysine. At pH 4.5, Peptide pap 3 and free leucine were also prominent. Although free glutamic acid was found, a peptide containing only threonine, proline, and valine, as found by Hill, Swenson, and Schwartz in the papain digest of β*Tp I (16), was not recovered. The neutral zone on the peptide map of the papain digest contained contaminating peptides (not present in the original peptide and thought to be products of papain autodigestion), which could have masked a neutral Thr-Pro-Val peptide if it were present.

The peptide consisting of (Thr, Pro, Val, Glu) was eluted from the peptide map of a subsequent papain digest of β*Tp IX and Edman degradation was performed in three stages yielding the following molar ratios (glutamic acid = 1). The yields, from each step, based on the aliquots taken for amino acid analyses, are shown in parentheses. The products of the third stage were not acid hydrolyzed; this probably contributed to the low yield of glutamic acid, and accounted for the absence of traces of other amino acids. Stage 1 (99%): Thr, trace; Pro, 1.1; Val, 0.9; Glu, 1.0. Stage 2 (96%): Thr, trace; Pro, trace; Val, 0.7; Glu, 1.0. Stage 3 (27%): Thr, 0.0; Pro, 0.0; Val, 0.0; Glu, 1.0. These results established the substitution of valyl for glutamyl at the β6 position of Hb CTn.

**Studies of βTp IX and βTp VIII-IX**—βTp VIII is free lysine from position 66 which is variably cleaved during tryptic digestion from the β*6 valyl residue of βTp IX, resulting in the two soluble tryptic Peptides βTp IX and βTp VIII-IX (26) of the sequences shown in Scheme 2 (27). The amino acid composition of the acid hydrolysates of the other two different peptides was the same as the composition of their normal counterparts, β*Tp IX and β*Tp VIII-IX (Table II), but the electrophoretic mobilities (Fig. 3) indicated that each β* chain had a greater positive charge than the corresponding peptide from hemoglobin A. Such findings could result from the replacement of 1 of the aspartyl residues by asparaginyl since acid hydrolysis readily cleaves the amide group of asparagine (28) and aspartic acid is found on subsequent amino acid analysis. Findings similar to these were encountered by Lehmann, Beale, and Boi-Doku (29) in Hb G Accra (Q2 82 *W * Aan); the position on the peptide map of β*Tp IX appears the same as the position of β*~CtTp IX.

To define the abnormality in β*CTp IX, this peptide and β*Tp IX were digested with papain. Peptide maps of the resulting digests (Fig. 5) revealed that an anodally migrating peptide from β*Tp IX, which on analysis after acid hydrolysis (pep 2A, Fig. 6) corresponded to residues 71 to 75 of the β chain, was missing on the peptide map of β*CTp IX. The latter contained a new peptide which migrated in the neutral zone, and which represented residues 71 to 75 (71 to 74 of some digests) of the β* chain (pep 2C and 2C1 of Fig. 7), thus suggesting that the aspartyl residue at β7 was replaced by asparaginyl in Hb CTn.

To confirm this substitution, the peptic Peptide 2C from β*CTp IX was hydrolyzed enzymatically with urocin aminopeptidase for 4 hours and for 24 hours; amino acid analysis of the 24-hour digestion mixture showed equimolar quantities of

### Table II

Molar ratios of amino acid residues after acid hydrolysis of β-tryptic Peptides IX and VIII-IX from hemoglobin A and from hemoglobin CTn.

<table>
<thead>
<tr>
<th>Residues</th>
<th>β*Tp IX</th>
<th>β*CTp IX</th>
<th>β*Tp VIII-IX</th>
<th>β*CTp VIII-IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>1.0</td>
<td>1.0</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>His</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Asp</td>
<td>2.9</td>
<td>3.3</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Ser</td>
<td>1.2</td>
<td>1.0</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Gly</td>
<td>2.2</td>
<td>2.2</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Ala</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Val</td>
<td>1.1</td>
<td>0.9</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Leu</td>
<td>3.7</td>
<td>4.0</td>
<td>4.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Phe</td>
<td>0.9</td>
<td>0.9</td>
<td>1.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

### Scheme 1

NH₂-terminal-Val-His-Leu-Thr-Pro-Glu-Glu-Lys-

### Scheme 2

NH₂-terminal-Val-LeuCTly-Ala-Phe-Ser-Asp-Gly-Leu-Ala-His-Leu-Asp-Asn-Leu-Lys-

**Fig. 5.** Diagram of peptide maps of the products of pepsin digestion of β-tryptic Peptide IX from hemoglobin A (A) and hemoglobin CTn (B). The anodally migrating Peptide 2A from β*Tp IX is replaced in the peptic digest of β*CTp IX by the neutral Peptide 2C.
serine, asparagine, glycine, leucine, and phenylalanine. Addition of 0.02 μmole of aspartic acid to an aliquot of the mixture prior to amino acid analysis resulted in a separate peak at the appropriate elution time, as determined from a standard amino acid mixture. Analysis of the 4-hour leucine aminopeptidase digestion mixture also showed serine, asparagine, glycine, leucine, and phenylalanine, but the yield of amino acids varied, with phenylalanine present in largest amount and leucine in smallest yield. Hydrolysis of peptides by leucine aminopeptidase proceeds from the NH$_2$-terminal end (30). The yields suggest that at 4 hours there was incomplete digestion of the peptide, the sequence of which, from the NH$_2$-terminal, was presumed to be that shown in Scheme 3. Thus asparagine replaced aspartic acid as the β$_2$ residue of Hb C$_m$. Specific stains of all peptide maps made from tryptic digests of Hb A, Hb C$_m$, and their β chains showed no differences in occurrence of arginine, histidine, tryptophan, and sulfur. The β$^{1p}$ I and β$^{1p}$ IX peptides from both β$^A$ and β$^Ch$ stained positively for histidine.

Comparison of peptide maps made from tryptic digests of amino ethylated β$^{Ch}$ and β$^A$ chains showed no abnormalities in the position of the core peptides of β$^{Ch}$. Peptide maps of tryptic digests of whole globin from hemoglobins C$_m$ and A showed no differences in the α-tryptic peptides of the two hemoglobins.

Other Studies—The sedimentation coefficient ($s_{20},w$) of hemoglobin C$_m$ was 4.2 S as compared with 4.1 S for hemoglobin A, consistent with a tetrameric structure. Preliminary observations on the oxygen equilibria of electrophoretically isolated Hb C$_m$ showed no significant differences from isolated Hb A in the n value (hem-heme interaction) from Hill's equation or in the Bohr effect. A slight increase in oxygen affinity of hemoglobin C$_m$ as compared with Hb A has been noted (e.g. $p_50$ at 10$^o$ in 0.1 M phosphate buffer, pH 7.2, for Hb C$_m$ = 1.8 mm Hg, for Hb A = 2.6 mm Hg): further studies are necessary to establish the significance of the differences in oxygen affinity. Hemoglobin C$_m$ did not differ from Hb A in its visible and ultraviolet spectra in the oxy and deoxy states and in titration with HMB which showed two reactive sulfhydryl groups per tetramer (calculated values were 2.5 for Hb C$_m$ and 2.4 for Hb A). With these techniques, no evidence of gross conformational difference between Hb C$_m$, Hb S, and Hb A was found.

Observations of Sickling Properties—In addition to erythrocyte sickling in routine preparations (cells in 2% sodium metabisulfite, or a film of 0.9% NaCl-suspended cells sealed under a cover slip), almost 100% sickling was observed within 20 min when 1 drop of the red cells of the propositus, suspended in phosphate buffer at pH 7.2, was deoxygenated in a chamber with nitrogen; the erythrocytes reverted to normal shape on exposure to oxygen. Most of the sickled cells had the frayed ends (holly leaf forms) (Fig. 8) commonly encountered in sickled red cells from individuals with sickle trait, but a few filamentous forms were seen. Cooling the chamber in an ice bath prevented sickling during exposure to
FIG. 8. Sickling of the erythrocytes of the propositus. Erythrocytes were suspended in isotonic 0.9% NaCl under a sealed coverslip for 24 hours.

FIG. 9. Salting out curves of isolated hemoglobins $S$ and $C_{Harlem}$. Solubilities were determined at 25° in phosphate buffer at pH 6.8. The ionic strength of the Na$_2$S$_2$O$_4$ is included in the total ionic strength for the deoxyhemoglobins.

<table>
<thead>
<tr>
<th>Proportion of Hb $S$ to Hb $A$</th>
<th>Minimum gelling point of $S + A$ mixtures</th>
<th>Proportion of Hb $C_H$ to Hb $A$</th>
<th>Minimum gelling point of $C_H + A$ mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>g/100 ml</td>
<td>%</td>
<td>g/100 ml</td>
</tr>
<tr>
<td>100:0</td>
<td>24.5 24.5</td>
<td>100:0</td>
<td>36.2 36.2</td>
</tr>
<tr>
<td>75:25</td>
<td>20.8 27.7</td>
<td>75:25</td>
<td>22.9 30.5</td>
</tr>
<tr>
<td>50:50</td>
<td>15.1 30.1</td>
<td>50:50</td>
<td>19.0 29.2</td>
</tr>
<tr>
<td>40:60</td>
<td>12.8 32.1</td>
<td>40:60</td>
<td>16.1 32.1</td>
</tr>
<tr>
<td>25:75</td>
<td>8.4 33.5</td>
<td>25:75</td>
<td>7.9 31.4</td>
</tr>
<tr>
<td>15:85</td>
<td>6.0 39.8</td>
<td>15:85</td>
<td>5.6 37.2</td>
</tr>
<tr>
<td>7.5:92.5</td>
<td>No gelation</td>
<td>7.5:92.5</td>
<td>No gelation</td>
</tr>
</tbody>
</table>

Solubility Determination—Comparison of the solubilities of isolated hemoglobins $C_H$ and $S$ in the oxy and deoxy states (Fig. 9) revealed that the deoxy form of each was far less soluble than the oxy form, although deoxy-Hb $C_H$ appeared slightly more soluble than deoxy-Hb $S$. It is possible that Hb $A_S$, which is nitrogen for 2 hours; subsequent warming to room temperature resulted in almost complete sickling within 10 min. Under the polarizing microscope, the sickled cells of the propositus, as those containing Hb $S$ (31), showed a negative birefringence in the direction of the long axis of the sickle cell, independent of the membrane birefringence. The oxygenated, unsickled cells were not birefringent.
present in an undetermined amount (possibly as much as 7%) in the isolated Hb Ch could account for this difference.

Gelation Studies—The minimum hemoglobin concentrations required for gelation upon deoxygenation were determined for hemolysates from erythrocytes of the propositus, from persons with sickle cell anemia and sickle trait, and for prepared mixtures of the isolated hemoglobins. Comparison of the minimum gelling concentrations of mixtures of Hb S + Hb A and Hb Cn + Hb A (Table III; Fig. 10) revealed that, with mixtures containing over 50% Hb A (including hemolysates obtained from erythrocytes of patients with 40% Hb S or Cm and 60% Hb A), hemoglobins S and Cn behave the same: the greater the proportion of Hb A, the smaller the concentration of hemoglobins S or Cm required for gelation, while the total concentration required increases. These data resembled those obtained for Hb S by

![Fig. 10. Comparison of minimum gelling points of mixtures of hemoglobins S + A and hemoglobins Charn + A.](image)

**TABLE IV**
Comparison of minimum gelling points of Hb Ch + Hb S, Hb Cn + Hb A, and Hb S + Hb A

<table>
<thead>
<tr>
<th>Proportion of total hemoglobin</th>
<th>Minimum gelling point</th>
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</thead>
<tbody>
<tr>
<td><strong>Hb Cn</strong></td>
<td><strong>Hb S</strong></td>
</tr>
<tr>
<td>%</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>24.5</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
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<td>25</td>
</tr>
<tr>
<td>100</td>
<td>36.2</td>
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</tbody>
</table>

**DISCUSSION**

The new hemoglobin variant described contains two amino acid substitutions in each β-polypeptide chain: valyl for glutamyl at β6 as in Hb S and asparaginyl for aspartyl at β9. Two substitutions in a single polypeptide chain have not been previously defined for any hemoglobin variant; they could have originated either by a second mutation in a β chain gene which already carried a single mutation at the site for β6 or β9, or by homologous crossing over within the β chain locus in an individual doubly heterozygous for Hb S (β6' β9' Val) and for a hypothetical hemoglobin with the structure α2β6' 7 Asn. Each substitution in Hb Cn is consistent with a one-step mutation (a single base substitution) in the triplet code of messenger RNA, as has been observed with each of the human hemoglobin variants with a single amino acid substitution (1). It may be of

7 Indirect evidence recently presented by Gerald and Rath (33) suggests that Hb Cemstevan, originally believed to be α2β6' 7 Asn (25), is another example of sickle hemoglobin with a second β chain substitution. The location of the second substitution was not yet established.
evolutionary interest that the position in the α chain which is homologous to β^3, α^6 (34) is occupied by asparagine (17).

Like hemoglobin S, hemoglobin Cn was associated with erythrocyte sickling and relative insolubility of the deoxygenated form, but significant differences were observed in the gelation properties of the two deoxyhemoglobins. Since the initial observations of Harris and Bunting (35), that concentrated solutions of hemoglobin S will undergo gelation on deoxygenation, gelling techniques have been utilized for studies of hemoglobin variants (22, 32, 36) and minimum hemoglobin concentrations required for gelation have been taken as a measure of interactions between molecules of deoxyhemoglobin. The significantly higher concentration required for gelation of isolated Hb Cn as compared with Hb S suggested that the β^8 Asn substitution in Hb Cn resulted in decreased intermolecular interactions in solutions of this deoxyhemoglobin, and implied conformational differences between the deoxyhemoglobins Cn and S.

In contrast to the differences observed in gelation of isolated Hb S and Hb Cn, little difference in minimum gelling points was observed between mixtures of hemoglobins Cn and A and mixtures containing similar proportions of hemoglobins S and A. Thus, when the proportion of Hb A amounted to more than one-half of the mixture (as would be observed in the erythrocytes of heterozygotes), it made little difference whether the remainder of the hemoglobin was Hb S or Hb Cn. However, whereas Hb S had a lower minimum gelling point in the absence of Hb A, mixtures of deoxyhemoglobins Cn and A appeared to exhibit more or greater intermolecular interaction than did deoxyhemoglobin Cn alone. Current lack of knowledge of the structural basis of gelation of hemoglobin limits interpretation of these findings. It might, however, be suggested that the β^8 substitution disturbed a complementary binding site but did not interfere with the primary or β^8 Val determined site. In this case, if only Hb Cn were available for reaction, intermolecular interactions resulting in gelation would be smaller than they would be if Hb A (with a more favorable complementary binding site) were also available. Observations that a single amino acid substitution in the β chain may increase the interaction of another hemoglobin with Hb S, e.g., Hb Dmashab (36) and Hb C (22, 32) lend support to the idea that such a complementary binding site might involve the β chains. Hemoglobin F, which has γ chains and no β chains, appears not to interact with Hb S (22, 32).

The precise relationships between erythrocyte sickling and gelation of deoxyhemoglobin solutions are not clear, and inferences from gelation data about the mechanism of sickling must remain tentative. Nevertheless, it is of interest that a second substitution in the β^8 chain appeared to alter the intermolecular interactions of the deoxyhemoglobin.

Acknowledgments—The authors are grateful to Miss Mary H. McKenna for referring the propositus to us, to Dr. Janos Kurucz for his help in the use of the polarizing microscope, to Dr. Olga Blumenfeld for advice and discussions, to Dr. Robin W. Briel for advice on the ultracentrifugal studies, to Mr. Alan Jacobs for help and advice in the structural studies, and to Dr. Marlene Aldo and Miss Linda Donaldson for assistance.

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