HEMOGLOBIN C_{HARLEM}: A SICKLING VARIANT CONTAINING AMINO ACID SUBSTITUTIONS IN TWO RESIDUES OF THE β-POLYPEPTIDE CHAIN

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Many of the variants of human hemoglobin have been shown to result from a single amino acid substitution in each of the pair of like polypeptide chains in the tetrameric hemoglobin. This report describes an abnormal hemoglobin, designated hemoglobin C_{Harlem}, which has been found to contain two amino acid substitutions in the β-polypeptide chains; one substitution is probably that of hemoglobin S, $\beta^{6\ val}$, but $\beta^{7\ val}$ has not been ruled out. The second is a substitution in peptide βTp IX, in which asparagine replaces aspartic acid at β73. Such an abnormal hemoglobin could have its origin in a second mutation in a β chain which already carried a single mutation in either the 6 or 73 residue, or could result from crossing over in an individual who was doubly heterozygous for hemoglobin S and, for example, for a hemoglobin with the structure $\alpha_2^A\ \beta_2^{73\ asn}$.

Hemoglobin C_{Harlem} was observed in 7 members of an American Negro family referred to us by Miss Mary H. McKenna of the laboratories of Harlem Hospital, who observed the association of what appeared to be hemoglobin C trait with erythrocyte sickling. The hemoglobin migrated slightly anodal to hemoglobin C at pH 8.6, and amounted to 40 per cent of the hemoglobin in each of the affected members of the family. Isolated hemoglobin C_{Harlem} exhibited an

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oxygen equilibrium like that of hemoglobin A, and in high concentration, like hemoglobin S, gelled on deoxygenation with subsequent liquefaction on cooling. Both deoxyhemoglobins C_{Harlem} and S are relatively insoluble as compared with hemoglobin A: by the method of Itano (1953), the solubility of deoxyhemoglobin C_{Harlem} was 0.17 grams per liter and that of deoxyhemoglobin 0.12 grams per liter in 2.24M phosphate buffer at pH 6.8, while deoxyhemoglobin A and the oxy-form of each show no precipitate under these conditions.

Recombination experiments carried out at pH 4.7 (Itano and Singer, 1958) disclosed new species on recombination of hemoglobin C_{Harlem} with hemoglobin I (an α chain abnormality) but none on recombination with hemoglobin C, a β chain abnormality. The recovery of new species, corresponding to $\alpha_2^A \beta_2^A$ and $\alpha^I \beta^{C_{Harlem}}$ indicated that the abnormality was in the β chain of hemoglobin C_{Harlem}.

Hemoglobin C_{Harlem} was purified on DEAE-cellulose (Huisman and Dozy, 1962) and the β chains were isolated by a modification of the method of Bucci and Fronticelli (1965), in which PMB* was added to the hemoglobin at pH 5.8 and the α and β chains subsequently separated by starch block electrophoresis in veronal at pH 8.6. Peptide maps of the tryptic digests of the isolated β chains were made by Baglioni's modification (1961) of the method of Ingram (1958). Comparison of these peptide maps with those prepared from isolated normal β chains (Fig. 1) showed the following differences: (1) βTp I (designated according to numbering system of Gerald and Ingram, 1961) was missing and a new peptide with a greater Rf moved further toward the cathode. (2) βTp IX and βTp VIII-IX were missing and two other new peptides were cathodal to the βTp IX and βTp VIII-IX positions. The first abnormal peptide was in the position of βTp I of hemoglobin S. Following acid hydrolysis of this abnormal peptide amino acid analysis (Beckman model 120B amino acid analyzer) demonstrated one glutamic acid and two valine residues compared

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* PMB = parahydroxymercuribenzoate

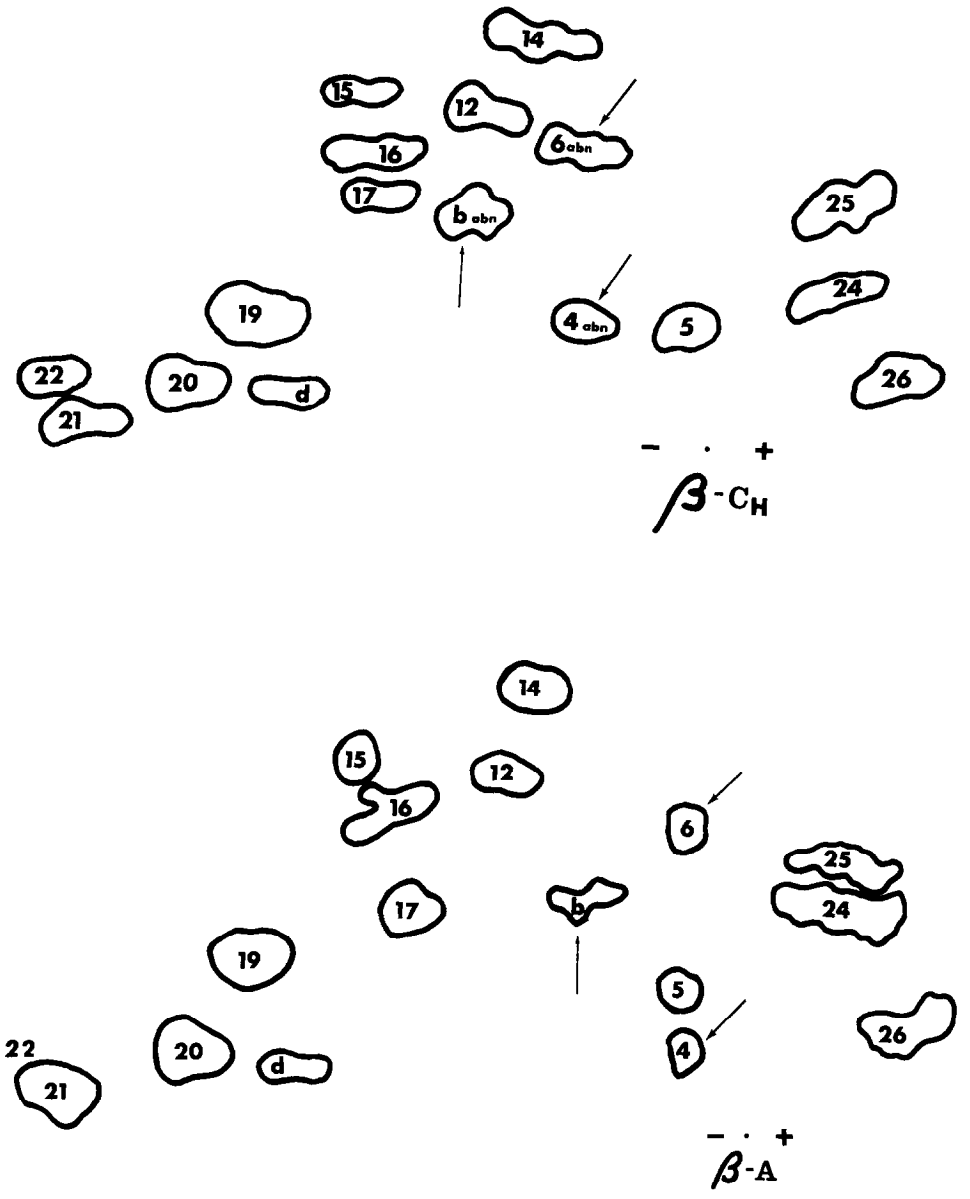

Figure 1. Tracings of the tryptic peptide maps of $\beta^{C_{Harlem}}$ globin and β^{A} globin. Peptides are numbered according to Ingram (1958). Note that peptide 4 (βTp I) of hemoglobin C_{Harlem} migrates cathodal to the normal position of βTp I with a greater Rf, in the position of β^{S}Tp I, and that peptides 6 (βTp IX) and b (βTp VIII-IX) of hemoglobin C_{Harlem} are cathodal to the position of these peptides of hemoglobin A. In the fingerprint of β^{A} globin peptide 22 did not separate from peptide 21.

with two glutamic and one valine residue in hemoglobin A (Table 1). The sequence of β^{A}Tp I is as follows (Goldstein et al., 1963):

val-his-leu-thr-pro-glu-glu-lys-
1 2 3 4 5 6 7 8

Thus a substitution of valine for glutamic acid apparently occurred in position 6 or 7 of the β chain.

Table 1

Molar ratios of amino-acid residues after acid hydrolysis of β tryptic peptides I from hemoglobin A and hemoglobin C_{Harlem}.

Residues	β^{A}Tp I	β^{C}HTp I
Lys	1.0	1.0
His	0.9	0.7
Thr	1.0	0.9
Glu	1.9	1.2
Pro	1.2	1.1
Val	0.9	1.8
Leu	1.3	1.0

βTp VIII is free lysine from position 66 which is variably split during tryptic digestion from the 67 valine residue of βTp IX, resulting in the two peptides, βTp IX and βTp VIII-IX:

-lys-	-lys-	val-leu-gly-ala-phe-ser-asp-gly-leu-ala-his-leu-asp-asn-leu-lys-
65	66	67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82
	βTp VIII	βTp IX

Similar analysis of the other two abnormal peptides (Table 2) demonstrated no difference from their normal counterparts, β^{A}Tp IX and β^{A}Tp VIII-IX, but their electrophoretic mobilities indicated that each abnormal peptide had a greater positive charge than the corresponding peptide from hemoglobin A. These findings could be explained only by the replacement of one of the two aspartic acid residues by an asparaginyl, which would be converted by acid hydrolysis into aspartic acid. This situation was encountered previously with hemoglobin G_{Accra} (Lehmann et al., 1964) in which the aspartic acid residue at β79 was replaced by asparagine. The published fingerprint of the tryptic digest of hemoglobin G_{Accra} shows βTp IX having moved apparently to the same position as βTp IX of hemoglobin C_{Harlem}. Either of the two

aspartic acid residues in βTp IX, at positions 73 or 79, could be replaced by asparagine in hemoglobin C_{Harlem}. To distinguish between these two

Table 2

Molar ratios of amino-acid residues after acid hydrolysis of β tryptic peptides IX and VIII-IX from hemoglobin A and hemoglobin C_{Harlem}.

Residues	β^{A}Tp IX	β^{C_H}Tp IX	β^{A}Tp VIII-IX	β^{C_H}Tp VIII-IX
Lys	1.0	1.0	2.2	1.8
His	0.9	0.9	1.0	0.8
Asp	2.9	3.3	3.0	3.0
Ser	1.2	1.0	1.4	1.3
Gly	2.2	2.2	2.1	2.2
Ala	2.1	2.1	2.1	2.2
Val	1.1	0.9	0.7	0.7
Leu	3.7	4.0	4.2	4.0
Phe	0.9	0.9	1.3	1.0

possibilities the normal and abnormal peptides βTp IX were eluted with 10 per cent pyridine, lyophilized, and digested with 0.01 per cent pepsin at 25°C for 20 hours as described by Goldstein et al. (1963). Fingerprints of the resulting digests revealed an anodally migrating peptide from β^{A}Tp IX which was absent in the digest of $\beta^{C_{Harlem}}$Tp IX; the latter contained a new peptide migrating in the neutral zone. Amino acid analyses of these two peptides yielded equimolar quantities of phe, ser, asp, gly and leu from β^{A}Tp IX and phe, ser, asp and gly from $\beta^{C_{Harlem}}$Tp IX, apparantly representing positions 71-75 and 71-74 of the respective βTp IX peptides. The difference in leucine between these two peptides is consistent with the products of its pepsin digestion (Goldstein et al., 1963) and does not account for their different electrophoretic mobilities. These preliminary results suggest that the aspartic acid residue at β73 is replaced by asparagine.

The occurrence of two substitutions in a single polypeptide chain of hemoglobin has not been previously observed; however the high frequency of hemoglobins S and C in persons of African descent increases the probability of encountering a second amino-acid substitution in these mutant β-polypeptide chains. The substitutions in hemoglobin C_{Harlem} (valine for glutamic, asparagine for aspartic) conform to the expected results of substitution of a

single base in the triplet code of messenger RNA as do all the known substitutions in hemoglobin variants (Beale and Lehmann, 1965).

Hemoglobin C_{Harlem} could have originated when a second mutation occurred in an individual with hemoglobin S (such individuals may constitute 40 per cent of the population in certain areas of East Africa), or by homologous crossing over within the β chain locus between determinants for β6 and β73. The greater distance between these positions on the β chain mightfavor crossing over between the two, as compared with crossing over between sites for hemoglobin S and certain other β abnormalities, e.g. β7 (hemoglobin $G_{San\ Jose}$), or β16 (hemoglobin $J_{Baltimore}$).

In tertiary structure, residue β73 is spatially closer to β6 than the residue numbers would imply. From the recent model of Perutz (1965), β73 occupies the 17th position of the E helix which is near the N-terminal portion of the β chain. While hemoglobin C_{Harlem} retains many properties of hemoglobin S, viz. sickling, gelation of the hemolysate on deoxygenation with liquefaction on cooling, and relative insolubility of the deoxy form, a detailed comparison of these properties reveals differences which must result from the second substitution in hemoglobin C_{Harlem}. An account of these studies and of the clinical findings associated with hemoglobin C_{Harlem} will be part of a separate report.

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References

Baglioni, C. Biochim. Biophys. Acta 48,392 (1961).
Beale, D. and Lehmann, H. Nature 207,259 (1965).
Bucci, E. and Fronticelli, C. J. Biol. Chem. 240,PC 551 (1965).
Clegg, J.B., Naughton, M.A. and Weatherall, D.J. Nature 207,945 (1965).
Gerald, P.S. and Ingram, V.M. J. Biol. Chem. 236,2155 (1961).
Goldstein, J., Konigsberg, W. and Hill, R.J. J. Biol. Chem. 238,2016 (1963).
Huisman, T.H.J. and Dozy, A.M. J. Chromat. 7,180 (1962).
Ingram, V.M. Biochim. Biophys. Acta 28,539 (1958).
Itano, H.A. Arch. Biochem. 47,148 (1953).
Itano, H.A. and Singer, S.J. Proc. Natl. Acad. Sci. U.S., 44,522 (1958).
Lehmann, H., Beale, D. and Boi-Doku, F.S. Nature 203,363 (1964).