The Presence of Hemoglobin S and C Harlem in an Individual in the United States

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The first reported case of hemoglobin S and C Harlem in an individual is described. The patient, a 35-yr-old female, had numerous crises during adolescence and early adulthood, but these occurred more infrequently as she grew older. Chemical evidence is presented for the characterization of both variant hemoglobins. The clinical course of this individual with Hb S in combination with Hb C Harlem appears to be similar to that for persons with sickle cell anemia.

Both the sickle cell gene and the gene producing the mutant β-chain in hemoglobin C Harlem (Georgetown) are present in the black population in the United States. The sickle cell gene produces an abnormal β-chain, resulting in hemoglobin S, αβ6Glu→Val, in which glutamic acid in position 6 in the primary sequence of the β-chain is replaced by valine. In the case of hemoglobin C Harlem, αβ6Glu→Val,73Asp→Asn, there are two substitutions in each β-chain: the first is identical to that found in Hb S, and the second is a substitution of aspartic acid at position 73 by asparagine. The latter substitution has also been found as a single alteration in Hb Korle Bu.

Hemoglobin S can be characterized by its low solubility in concentrated phosphate buffers when in the reduced form, as well as by its electrophoretic mobility on cellulose acetate and citrate agar. Hb C Harlem, which migrates in the position of Hb C at alkaline pH, also has a low solubility, and on citrate agar electrophoresis at pH 6.2 it migrates like Hb S. In this paper, we report the simultaneous occurrence of two sickling hemoglobins, Hb S and Hb C Harlem, in the same individual.

CASE REPORT

A 35-yr-old black female was apparently in good health until the age of 13 when she began experiencing "weak spells," joint pains especially in her legs, and sometimes abdominal and back pains. At first, the attacks occurred frequently, then gradually diminished in number as she grew older. The last attack occurred approximately 3 yr ago. These attacks usually lasted from 3 to 7 days; however, some lasted as long as 3 wk. None of the episodes occurred during any of the patient's pregnancies. Physical examination revealed no evidence of jaundice or leg ulcers, although she had surgery for bilateral aseptic necrosis of the femoral heads 6 yr ago.

Her hemoglobin was 9.3 g/100 ml, with a hematocrit of 28.5%; the white cell count was 9400/cu mm, with 64% neutrophils, 13% lymphocytes, and 15% monocytes; the platelet count was 108,000/cu mm. Her blood smear showed the presence of macrocytic target cells as well as typical sickled cells. There was also slight polychromasia with basophilic stippling.
The patient's mother and father could not be located, and she had no siblings. She was married twice and had a 13-yr-old son and two daughters, one 7 yr old and the other 15 yr old. One child died in childbirth. The son and the 7-yr-old daughter have Hb A and C Harlem, while the other daughter has Hb A and S. The children appear normal and have had no history of crises or attacks. The patient's two previous husbands could not be located.

MATERIALS AND METHODS

Hemoglobin S and C Harlem were separated by DEAE chromatography to determine the amount of each variant hemoglobin present. For structural analysis, abnormal $\beta$-chains were prepared directly from a hemolysate by the method of Clegg et al. without prior separation of the abnormal hemoglobins. The separated chains were reduced, aminoethylated, and digested with trypsin. Peptide maps were prepared on Whatman 3MM paper and then stained with ninhydrin. Cyanogen bromide was used to cleave the $\beta$-chain at the methionine residue, and the peptides were separated on a G-50 Fine Sephadex column (manuscript in preparation). Automated Edman degradation was carried out on a Beckman Model 890C Sequencer, and the DMAA fast peptide program was used. The PTH-amino acids were identified by gas and thin-layer chromatography. Amino acid analyses were done on a Beckman 121 Amino Acid Analyzer according to the method of Spackman et al., and routine hematologic studies were performed by standard methods.

RESULTS

Blood obtained from the patient and from her two older children was tested by electrophoresis on both cellulose acetate at pH 8.4 and citrate agar at pH 6.2. The results are shown in Fig. 1. All these samples gave a positive solubility test identical to that of sickle hemoglobin which forms an insoluble precipitate in the presence of concentrated phosphate buffer and a reducing agent. At alkaline pH, Hb S and Hb C were demonstrable in the sample from the patient, but on citrate agar, there was only one band in the position of Hb S. Quantitation of Hb S and C Harlem gave 49.9% Hb S and 43.6% Hb C Harlem and Hb A2. Alkali denaturation by the method of Singer et al. gave 7.2% alkali-resistant hemoglobin.

The separation of the $\beta^S$-chain and $\beta^{C\text{Harlem}}$-chain was carried out directly on a hemolysate rather than on purified hemoglobins because of the charge differences between the $\beta^S$- and $\beta^{C\text{Harlem}}$-chains.

![Fig. 1. Electrophoretic patterns of hemoglobins obtained by cellulose acetate (upper) and citrate agar (lower) electrophoresis (Schmidt and Brosious, 1974).]
The initial sample of each cyanogen bromide peptide was 500 nmoles.

From the peptide maps of the tryptic digests of the $\beta^S$ and $\beta^C$ Harlem chains, the $\beta^S$ peptide is readily identifiable, but the abnormal $\beta^C$ is not obvious. The compositions of these peptides were determined by elution and subsequent amino acid analysis.

The $\beta^C$ Harlem substitutions were confirmed by sequencing the cyanogen bro-

*Identified by spot test.
†Estimate.
‡Identified as trimethylsilyl derivatives and confirmed by thin-layer chromatography.
mide peptides from the \( \beta^C_{\text{Harlem}} \)-chain (Fig. 2). These results are shown in Table 1. Sequencing methodology and identification procedures provided direct evidence for the substitution of glutamic acid by valine at position 6 and the replacement of aspartic acid by asparagine at position 73.

**DISCUSSION**

The detection of the double heterozygote for Hb S and Hb C Harlem emphasizes the importance of doing electrophoresis at both pH 8.4 and on citrate agar at pH 6.2. Electrophoretic procedures at pH 8.4 that are used in detecting abnormal hemoglobins do not distinguish between Hb C, O Arab, and C Harlem. Nevertheless, these hemoglobins can be separated on citrate agar at pH 6.2. On this medium, Hb C moves anodal to Hb A, while Hb C Harlem appears as a band in the same position as Hb S (Fig. 1). Hemoglobin O Arab can be observed as a sharp band very close to Hb A and between Hb A and Hb S. Citrate agar electrophoresis at pH 6.2 is therefore essential in order to differentiate between these hemoglobins, particularly in cases where electrophoresis at pH 8.4 indicates Hb S/C, S/O Arab, S/E and S/C Harlem patterns.

The reports on Hb C Harlem by Bookchin et al. indicate that, although this hemoglobin variant is associated with erythrocyte sickling and relative insolubility in the deoxygenated form, there are significant differences between Hb C Harlem and Hb S. However, they also showed that equimolar mixtures of Hb S and Hb C Harlem have gelling properties almost identical to those of 100% Hb S, and on this basis it was suggested that the double heterozygote for Hb S and Hb C Harlem might resemble sickle cell anemia. From the data that we have been able to accumulate, it appears that these predictions are to a large extent valid.

In addition, studies have been reported on the interaction of Hb S and Hb C Harlem with Hb Korle Bu, in which the single amino acid substitution \( \beta^{79} \text{Asp} \rightarrow \text{Asn} \) is identical to one of the substitutions in the \( \beta^C_{\text{Harlem}} \)-chain. Individuals with Hb Korle Bu and Hb S resemble those with the sickle cell trait condition. The patient we have described has had numerous crises similar to those observed in persons with sickle cell anemia, but these have become less frequent as the patient has grown older. Our results indicate that the double heterozygous condition for Hb S and Hb C Harlem shows characteristics similar to those of patients homozygous for Hb S, and the clinical course for an individual with this combination of variant hemoglobins closely resembles that for an individual with sickle cell anemia.

**ACKNOWLEDGMENT**

We would like to thank Dr. P. F. Milner for his interest and comments during this investigation.

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