HEMOGLOBIN I is an extremely rare variant of human hemoglobin with a considerably lower isoelectric point than hemoglobin A. Indeed, its unusually fast electrophoretic mobility at pH 8.6 is equalled only by hemoglobin H, which is composed exclusively of β chains.

Hemoglobin I originally was found in the United States by Page, Rucknagel, and Jensen1 in a Negro (G. H.) whose features suggested Indian ancestry, and in Algeria by Cabannes, Sendra, and Dalaut2 who designated it hemoglobin H. Subsequently, Rucknagel, Page, and Jensen3 identified the latter as hemoglobin I. In 1957, Schwartz, Atwater, Repplinger, and Tocantins4 reported a case of hemoglobin A/I in a woman of Negro and American Indian ancestry. Other cases of hemoglobin A/I recently have been found in a Negro family by Levin and Schneider (personal communication) in Galveston, and in an Algerian Mussulman family by Boulard et al.5

The nature of the biochemical substitution in the hemoglobin molecule resulting from the mutation at the I locus was determined by Murayama and Ingram.6 Comparisons of “fingerprints” of hemoglobins I and A revealed that one of the peptides, No. 23,7 had a tryptophan residue which was not present in peptide 23 of hemoglobin A. The new number for this peptide is αT-4, according to the recently recommended system of hemoglobin peptides by Gerald and Ingram.8 Conversely, peptide 15 of hemoglobin A, which has a tryptophan residue, is not present in hemoglobin I. The abnormal peptide in hemoglobin I was located in the α chain. The demonstration of “hybrid” molecules resulting from recombinations of the dissociated α and β chains of hemoglobins S and I added to the evidence of the chain being the site of the amino acid substitution of hemoglobin I.9 Murayama10 found that in hemoglobin I the amino acid substitution was an aspartyl residue in place of a lysyl residue. Later, Murayama11 found that from the N-terminus of the α chain the sixteenth amino acid residue, which is lysine in hemoglobin A, had been replaced by an aspartyl residue in hemoglobin I.

We recently have found a new case of hemoglobin A/I in a healthy, 19 year old Negro female (J. A.). The blood of the propositus was made available through the courtesy of Dr. B. E. Conner and was among some 300 specimens obtained during the physical examinations of the freshman class of a local college. Five relatives of the proband were found to have hemoglobin I.

METHODS

Hemoglobin solutions were prepared by the method of Drabkin.12 Each sample was analyzed by paper electrophoresis in 0.05 M veronal buffer, pH 8.6, using the hanging
strip procedure in a Durrum-type cell. In some instances, electrophoresis was performed on paper dipped in 0.05 per cent egg albumin and dried before use, in order to diminish adsorptive effects. Hemoglobin samples from some affected members of Family A were subjected to agar gel electrophoresis, citrate buffer, pH 6.2, on microscope slides. Separation of the minor component, I₂, from Hb A was accomplished with agar gel in the same manner with a potential gradient of 120 volts for 3 hours.

Hemoglobin I was separated from the accompanying hemoglobin A by starch block electrophoresis. Concentration of the hemoglobin on carboxymethylcellulose was carried out following the procedure of Ingram and Stretton. The hemoglobin was denatured by heating for 6 minutes at 90°C, and it was then cooled and digested with trypsin (1 mg of hemoglobin) in an automatic titrator for 1½ hours at 38°C. After the digested hemoglobin had been adjusted with 0.1 N HCl to pH 6.5, it was centrifuged and concentrated in vacuo.

Fingerprinting was performed according to the method of Ingram and the chromatography solvent was that used by Baglioni. Hemoglobins I and A were simultaneously "fingerprinted" between glass plates using the usual buffer (pH 6.4) consisting of pyridine:acetic acid:water (100:4:900 v/v). Ascending chromatography was carried out in the solvent consisting of pyridine:isooamyl alcohol:water (35:35:30). Peptides of hemoglobin digests of A and I were separated by ionophoresis using the same pH 6.4 buffer as mentioned above on strips of Whatman 3MM paper 100 cm. long. The ionophoresis was carried on for 1 hour at 5000 volts. The peptide bands on the ionogram, located in a guide strip dipped in 0.2 per cent ninhydrin in acetone, were cut out and sewed on a new sheet of Whatman 3MM paper. Corresponding peptide bands were then subjected to ascending chromatography, using the same solvent that was used for chromatography in the "fingerprinting" procedure. Peptides A-23 and I-23 were first located by staining guide strips with ninhydrin, and positive identification of A-23 was made with a reagent specific for tyrosine which was used to dip the same guide strip. Positive identification of I-23 was made in a similar manner using Erlich's reagent to identify tryptophan. The peptides were eluted with 1 M acetic acid, then dried in vacuo, and hydrolyzed with 6 N HCl in an evacuated tube for 16 hours at 110°C. The mixture of amino acids was dried in vacuo and analyzed according to the method of Moore, Spackman, and Stein.

Separation of the α and β chains was accomplished by employing a modification of the counter current distribution method of Hill and Craig and described recently by Bowman and Ingram.

RESULTS

Figure 1 shows the pedigree of Family A, according to the hemoglobin phenotypes when known. Those who have been tested and who have only normal hemoglobin are II-2, II-4, II-5, III-1, III-5, III-7, and IV-5. Those having hemoglobin I are II-1, II-7, III-3, III-9, III-11, and the proband, IV-4. The other members were not tested.

A photograph of the great-grandfather of the propositus (father of II-7) revealed features which could be described as more white or American Indian (i.e., high cheek bones, thin lips, almost straight hair, and extremely light complexion) than typically negroid. The family has resided in the Austin area for at least the past four generations.

Hemoglobin A/I is present along with hemoglobin A in the propositus, IV-4, and also exists in her father, III-9, a healthy 45 year old man. An uncle of the propositus, III-3, has hemoglobin A/I, and is a 56 year old man who is extremely healthy. The first cousin, once removed, III-11, of the propositus also has hemoglobin A/I. He is 49 years old and is in good health. The paternal grandmother, II-7, of the propositus is a healthy woman 71
A FAMILY WITH HEMOGLOBIN I

Fig. 1.—Pedigree of Family A showing incidence of Hb I trait.

Fig. 2.—Paper electrophoresis of hemoglobins I, N, A, S, and C at pH 8.6.

years old. She has five living children. She has a half-brother, II-1, who also has hemoglobin A/I. The amount of hemoglobin I in the affected members varies from 35 per cent in III-9 to 15 per cent in II-7. It accounts for 16 per cent of total hemoglobin in III-3 and in the propositus (IV-4).

The members of this family with hemoglobin I trait have no other obvious clinical abnormalities. There has been no history of chronic illnesses or malformations in this family as far as the investigators have been able to find out.

Electrophoresis of hemoglobin A/I (obtained from the father, III-9, of
Fig. 3.—Paper electrophoresis of hemoglobin from members of Family A and C. S. having hemoglobin A/I trait at pH 8.6.

the propositus) on citrate agar at pH 6.2 failed to separate A from I. Rucknagle et al. found hemoglobins A and I did not separate at pH 6.5 on paper electrophoresis, although hemoglobins A and H did separate under these conditions.

In zone electrophoresis in alkaline buffers, however, hemoglobins A and I are widely separated. In figure 2 is a pattern obtained by paper electrophoresis, veronal buffer, pH 8.6, of hemoglobin types I, N, A, S, and C. The 0.05 per cent of albumin present in the electrophoresis paper alters the mobility of all the hemoglobins; however, the relative mobilities remain the same. Hemoglobin I migrates toward the anode about as far from hemoglobin A as does hemoglobin C toward the cathode.

Figure 3 is a photograph of a paper electrophoretic separation, pH 8.6, of hemoglobins A/I from individuals III-3, III-9, IV-4, III-11, II-1, II-7, and C.S., respectively. Hemoglobin A/C at the extreme left serves as a contrast. C.S., who is not of that kinship, but who has hemoglobin A/I, is a Negro woman living in Galveston, studied by Levin and Schneider.

Hemoglobins A and I from an uncle of the proband (III-3) were separated by means of starch block electrophoresis (fig. 4). Hemoglobins I, A, and the minor component, A₂, were readily isolated and eluted. Hemoglobin I migrated far in front of hemoglobin A toward the anode. The minor component, hemoglobin A₂, migrated more slowly than hemoglobin A. Hemoglobin A₂ ranged from a concentration of 1.7 per cent to 2.5 per cent in the blood of the members having A/I hemoglobin. The reason for the high concentrations of A₂ in some of the individuals having hemoglobin I is unknown; there was no history or indication of thalassaemia in this family. In the others
the concentration of $A_2$ was very close to 2.5 per cent. No other minor component was separated on starch block. $I_2$, a new minor component, was found to migrate with hemoglobin A at pH 8.6 as well as pH 6.0, 6.5, 6.8, 7.0, and 8.0. Only by agar gel electrophoresis at pH 6.2 could this minor component, $I_2$, be separated from hemoglobin A. Figure 5 is a photograph of the migration of the minor component $I_2$ and the slower major component A. The source of the mixture of A and $I_2$ was the fraction migrating as hemoglobin A in the starch block electrophoresis analysis shown in figure 4.

Photographs and tracings of "fingerprints" of the tryptic digests of hemoglobins A and I are shown in figures 6 and 7. Examination of each of the "fingerprints" revealed a striking difference in the location of I-23 as compared to the corresponding peptide in hemoglobin A. Furthermore, Erlich's reagent demonstrated the presence of tryptophan in peptide I-23. In hemoglobin A, peptide 23 contains, among other amino acids, arginine, histidine,
and tyrosine, but not tryptophan. On the other hand, peptide 15 α, which contains tryptophan in hemoglobin A, was missing in hemoglobin I. The results from the “fingerprinting” of our hemoglobin I are essentially the same as those reported previously by Murayama and Ingram. It seemed unlikely
that the amino acid substitution would be different from that which Dr. Murayama found in hemoglobin I obtained from Dr. Tocantins; however, in order to confirm the fact that the two hemoglobins I are of the same molecular species, amino acid analyses of the peptides 23 from hemoglobin A and I were compared. First, the peptides 23 from the two hemoglobins were purified by ionophoresis, the appropriate bands were cut from the main portion of the paper and sewed on another sheet of filter paper and chromatographed. Peptide 23 was thus obtained free from any other peptides.

Peptide I-23 was difficult to analyze because of the presence of tryptophan. Not only was tryptophan destroyed, but other amino acids did not have consistent concentrations from one hydrolysis to the next because of the destructive action of tryptophan during hydrolysis. Evacuation of the test tube in which the hydrolysis was carried out was necessary before repeatable
Table 1.—Sequence of Amino Acids in Peptides 15 and 23 of Hemoglobins A and I

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Results were obtained. This was carried out with a vacuum pump after freezing the contents of the hydrolysis tube.

Amino acid analyses of corresponding peptides of hemoglobin A and I confirmed the identity of hemoglobin I from Family "A" with the hemoglobin I described by Murayama. Table 1 shows the results of amino acid analyses of peptides 23 and 15 from hemoglobin I and hemoglobin A. The amino acids have been arranged sequentially according to the findings of Braunitzer et al. A dotted line above indicates a tryptic cleavage in the peptide chain.

Since lysine has been changed to aspartic acid in hemoglobin I, there is no tryptic break at that position and the two peptides of the tryptic digests of hemoglobin A become a single, long peptide in hemoglobin I.

After separating the hemoglobin chains by countercurrent distribution, the isolated α and β chains of hemoglobins A and I were digested with trypsin. Fingerprints of digests of α chains of hemoglobin A and hemoglobin I revealed that the tryptophan-containing peptide 23 was present in the α chain of hemoglobin I and the peptide 15 α was in the α chain of hemoglobin A.

The abnormal hemoglobin I described in this investigation is of identical molecular species to the one studied by Murayama and Ingram. Lysine has been substituted by aspartic acid in the sixteenth amino acid position from the N-terminus of the α chain. According to pedigree studies, this hemoglobin variant appears to be transmitted by a co-dominant gene.

Hemoglobin I has furnished information concerning the genetic control of the structure of the α chains in fetal hemoglobin (α^A_2β^A_2) and in the minor hemoglobin component A_2 (α^A_2β^A_2). Boulard et al. isolated the abnormal
minor component, $I_2 (\alpha_1^1 \delta^1 \delta^2)$ by elution from a carboxymethyl cellulose column. The peptide pattern of this component revealed the same altered peptide (23) as was found in hemoglobin I; this confirmed the presence of the same $\alpha$ chain in adult hemoglobin as in minor hemoglobin component. Furthermore, the finding of an abnormal fetal hemoglobin whose abnormality is most probably attributed to the $\alpha$ chains found in the abnormal adult hemoglobin helps confirm the fact that there is only a single set of $\alpha$ chain genes in the fetal cell supplying chains to hemoglobin A and hemoglobin F.

**SUMMARY**

Hemoglobin I was recently found in a Negro family. The amino acid substitution was shown to occur in the sixteenth residue of the $\alpha$ chain (lys $\rightarrow$ asp) and to be identical with hemoglobin I described by Murayama. The minor component, $I_2$, was demonstrated by agar gel electrophoresis.

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A Family with Hemoglobin I

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