Hemoglobin NYU, a Delta Chain Variant, $\alpha_2\delta_2^{12Lys}$

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ABSTRACT A minor hemoglobin (Hb) component with the electrophoretic properties of the δ-chain variant Hb A_2' was encountered in two unrelated families of Russian-Jewish ancestry. This minor component, designated Hb NYU, was shown to result from the substitution of lysine for asparagine at δ^{12}. We have confirmed studies of others that hemoglobin A_2' isolated from the hemoglobin of some African subjects, results from the replacement of the normal glycine at δ^{16} by arginine. Thus for interpretations of the incidence of δ-chain variants in different populations, electrophoretic data are not sufficient.

In members of one of the families in the present study, the visual estimations of normal Hb A_2 and of Hb NYU on starch-gel electrophoretic patterns suggested the presence of δ-thalassemia. In hemolysates of one of the heterozygotes for Hb NYU, hemoglobin A_2 was not demonstrable with starch-gel electrophoretic methods but was readily recovered by column chromatography in approximately the amounts expected for δ-chain heterozygotes.

INTRODUCTION

2–3% of normal human hemoglobin (Hb) prepared from the erythrocytes of healthy adults is the minor basic component, Hb A_2, described by Kunkel, Ceppellini, Müller-Eberhard, and Wolf in 1955 (1). Hb A_2 has the same α-polypeptide chains as the major component, Hb A ($\alpha_2^A\beta_2^A$), but the non α-chains, designated δ-chains, differ from the β-chains in 10 amino acid residues (2). The structural locus for δ-chain synthesis is closely linked to the β-locus (3–5) and probably arose from the β-locus by the process of gene duplication (6). Several variants of the δ-chain resulting from single amino acid substitutions have been described. The present report is concerned with another δ-chain variant, designated Hb NYU, which was found in two families of Russian-Jewish extraction, one residing in New York and the other in Israel. The observations on the first family were the subject of a preliminary report.

METHODS

Hemolysates were prepared by the lysis of washed erythrocytes with water and toluene. Vertical starch-gel electrophoresis was carried out by the method of Smithies (7) with minor modifications: "Electrostarch"[1] with a Tris-ethylenediamine tetraacetate (EDTA)-borate buffer, pH 8.6, was used (8). The proportions of Hb components were determined by the method of Kunkel and coworkers (1). The minor basic components of Hb were isolated by chromatography on demountable columns (20 or 25 mm × 400 mm) of microgranular diethylaminoethyl (DEAE)-cellulose (Whatman DE-52) developed with tris (hydroxymethyl) aminomethane–HCl buffers, 0.04 mole/liter, pH 8.2. About 1 g of Hb was applied to the column. Free α-chains in trace amounts frequently constituted the first component to be eluted. The second component, Hb NYU, was eluted with the same buffer and was clearly separated from both α-chains and normal Hb A_2. Hb A_2 could be eluted on prolonged chromatography without change in buffer, or more rapidly by using a linear pH gradient (to pH 8.0), or alternatively by removing the top half of the column when the Hb A_2 was the sole Hb in the lower half and eluting the Hb A_2 with 0.2 M sodium phosphate buffer, pH 6.4. The purity of the isolated hemoglobins was checked by starch-gel electrophoresis. The α- and δ-polypeptide chains of globin were separated by the method of Clegg, Naughton, and Weatherall (9). Trypsin digestions were carried out on globins prepared from Hb NYU and from normal Hb A_2, on isolated δ-chains from Hb NYU, and from Hb A_2, and on the S-(β-aminoethyl) derivatives of the isolated δ-chains. Peptide maps were prepared by the method of Clegg and coworkers (9), except that ascending chromatography was used. Peptide maps of globin of Hb A_2 and of Hb NYU and of the corresponding aminoethylated δ-chains were used for preliminary comparisons. Peptide maps of nonaminoethylated δ-chain preparations, stained with 0.02% ninhydrin, were used for elution of peptides by the method of Sanger and Tuppy (10). Amino acid analyses of the peptides, hydrolyzed for 18 hr in 6 N HCl, were carried out on a Beckman-Spinco amino acid analyser, model 120B, equipped with a long path flow cell.

[1] Obtained from O. Hiller, Madison, Wis.

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RESULTS

Family I (AECOM kindred 225) The propositus referred to the Heredity Clinic at the Albert Einstein College of Medicine in New York at age 17 because of a history of red urine on exercise and the finding of an abnormal Hb. The exercise-induced pigmenturia was probably myoglobinuria associated with muscle phosphofructokinase deficiency; studies dealing with this rare disorder in the propositus have been published elsewhere (11). The mother of the propositus was not anemic (Hb 13.1 g/100 ml, reticulocytes 1.5%). In the propositus the Hb concentration varied between 14.1 and 16.7 g/100 ml, and reticulocytes ranged from 2.7 to 4.6%. Decreased erythrocyte phosphofructokinase deficiency was observed in mother, father, and propositus (11), by Layzer and coworkers.

Fig. 1 is a photograph of an electrophoresis on starch gel of Hb samples prepared from members of this family, and Fig. 2 shows the variations in hemoglobins containing δ-chains in all members of the family who were studied. The unusual aspects of Hb A_2 in this family were apparent: (*a*) the mother and propositus had a minor Hb component more basic than Hb A_2, designated Hb NYU. This basic Hb variant was electrophoretically indistinguishable from Hb A_2' described by Ceppellini (3); (*b*) the mother's hemolysate showed Hb NYU and Hb A but Hb A_2 was not seen. Furthermore, the proportions of Hb A_2 in the siblings and in the maternal grandmother of the propositus were lower than those usually encountered (Table I). (The minor abnormal component migrating in the position of Hb A_2' was designated Hb NYU by subject II-5 of this family who was a member of the faculty of New York University.)

In Fig. 1, a component migrating between Hb A_2 and Hb A may be seen in the hemolysates of the mother,

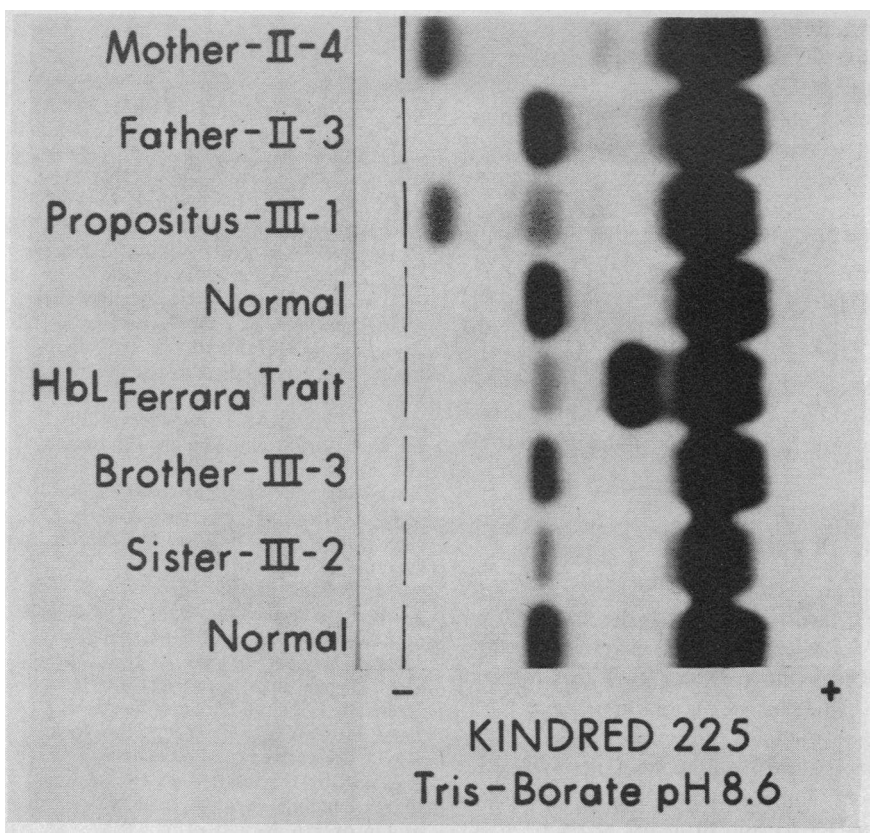

FIGURE 1 Starch-gel electrophoresis of hemolysates of kindred 225 (family I). Hemoglobin concentrations, 3 g/100 ml. Presence of Hb NYU (nearest cathode) in propositus (III-1) and in his mother as well as absence of Hb A_2 in mother and decreased proportions of Hb A_2 in brother and sister of propositus are evident. The significance of the component migrating between Hb A_2 and Hb A in II-4, II-3, and III-1 is not clear. It was not regularly seen in other studies of these subjects and was not encountered in hemolysates from subject II-5 whose hemoglobin was used for structural studies. Benzidine stain. Numbers refer to designation of individuals in Fig. 2.

father, and propositus; on the original gel the presence of such a component in III-3 (brother) was suggested but not clearly visualized. This trace component (from the appearance of gels, one might estimate that it would amount to about 0.1–0.2% of the total Hb) was not seen on starch-gel electrophoresis of hemolysates (from subject II-5) which were used for structural studies of Hb NYU (Fig. 4). No studies of the structure of this trace component were undertaken. It might be pointed out that in subjects II-4, II-3, and III-1, the presence of this component was associated with an approximate 50% decrease in erythrocyte phosphofructokinase activity (11), but the relationship, if any, between the presence of the trace component and the enzyme deficiency is not known.

Structural studies of hemoglobin NYU. Family I: peptide maps of tryptic digests of globin prepared from Hb NYU and from normal Hb A_2 are shown in Fig. 3. In these maps, as well as in peptide maps of aminoethylated δ-chains of the two Hb, the only difference observed was in δ-T-2. Peptide maps of the δ-chains of Hb NYU lacked the normal peptide δ-T-2; two "new" peptides not seen in the maps of δ-chains of normal Hb A_2 were observed. The amino acid composition of the new peptides indicated, as shown in Table II, that lysine had replaced the asparagine at position 12 of the δ-chain (helical residue A9 in the notation of Perutz [12]). Since δ-T-2 is a soluble peptide, aminoethylation was omitted for the preparative maps in order to reduce the number of peptides. Table II contains the results of amino acid analyses of δ-T-2 from normal Hb A_2 and from the two "new" peptides of δ-NYU, and the sequence of normal δ-T-2.

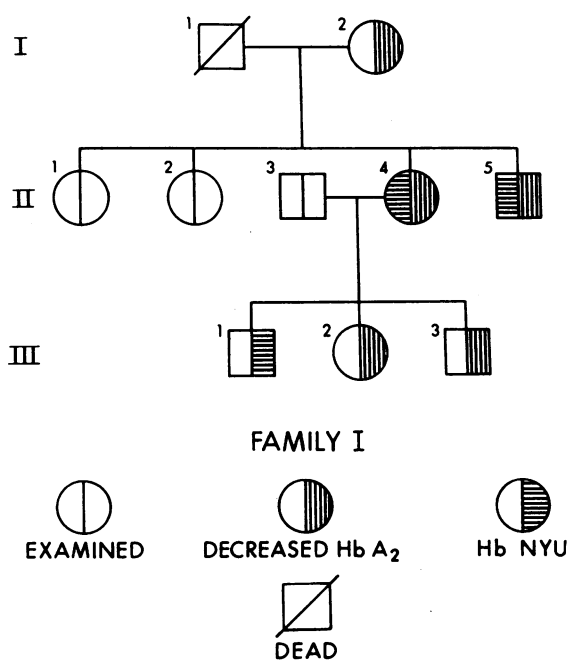

FIGURE 2 Occurrence of Hb NYU and of decreased Hb A_2 in family I (kindred 225).

TABLE I

Proportions of Hemoglobins NYU, A_2, and A in Members of Kindred 225, as Determined by Starch-Block Electrophoresis (1)

Relationship to propositus	Number, from Fig. 2	Hemoglobin analysis, starch block		
		Hb NYU	Hb A_2	Hb A
		%	%	%
(Propositus)	III-1	1.3	1.3	97.4
Mother	II-4	1.4	—	98.6
Father	II-3	—	2.4	97.6
Sister	III-2	—	1.4	98.6
Brother	III-3	—	1.8	98.2
Maternal uncle	II-5	0.9	—	99.1
Maternal aunt	II-1	—	2.5	97.5
Maternal aunt	II-2	—	2.7	97.3
Maternal grandmother	I-2	—	1.5	98.5

Family II: after structural studies of Hb NYU had been completed, the second example of a minor Hb component with the electrophoretic mobility of Hb A_2' was encountered at Tel Hashomer Hospital in Israel in a Russian-Jewish man with myelofibrosis. The propositus in this family as well as two of his three children (who had no evidence of hematologic disease) had the unusual minor basic Hb component which migrated in the same position as Hb NYU (or Hb A_2') (Fig. 4) on starch-gel electrophoresis at pH 8.6. Hb NYU and Hb A_2 were present in approximately equal (1%) proportions in the

TABLE II

Amino Acid Composition of δ-T-2 of Hb A_2 and of δ-T-2a and of δ-T-2b of Hb NYU

	δ-T-2 normal, Hb A_2		Family I, Hb NYU		Family II, Hb NYU	
	Expected	Found	δ-T-2a	δ-T-2b	δ-T-2a	δ-T-2b
Lys	1.0	1.0	1.0	1.0	1.0	1.0
Asp*	1.0	1.2	0	0	0	0
Thr	1.0	1.0	0.9	0	1.0	0
Gly	1.0	1.4	0	1.2	0	1.2
Ala	2.0	2.3	1.2	1.2	1.2	0.9
Val	1.0	1.2	1.0	—	0.9	—
Leu	1.0	1.2	0	1.2	0	1.0
Try	1.0	‡	0	‡	0	‡

Sequence of normal δ-T-2

Position:	9	10	11	12	13	14	15	16	17
Residue:	Tyr	Ala	Val	Asn	Ala	Leu	Try	Gly	Lys

Vertical line indicates site of cleavage by trypsin into δ-T-2a and δ-T-2b in Hb NYU in which Lys replaces Asn at position 12. Molar ratios are calculated on the basis of Lys = 1.

* Asn which occurs in position 12 would be recovered after acid hydolysis as Asp.

‡ Indicates positive staining reaction for tryptophan (Ehrlich's reagent).

hemolysates of each of the three affected individuals in this family. Hb NYU was isolated and the composition of δ-T-2 studied as described above. The peptide maps and the amino acid composition of the two "new" peptides closely resembled the findings in family I (Table II).

Each of the two families was of Russian-Jewish ancestry but the two families were not known to be related.

Studies of Hb A₂ in subject II-5 of family I. When preparative chromatography of 1–2 g of hemolysate from subject II-5 (in whose hemolysate Hb A₂ was not visualized on starch-gel electrophoresis, Fig. 4) was carried out on DE-52, a second minor component was eluted after Hb NYU. The proportion of this component was approximately equal to the proportion of Hb NYU in the total hemolysate, i.e., each was about 1% of the total Hb applied to the column. After concentration by ultrafiltration, this component migrated in starch gels in the position of Hb A₂. Peptide maps of tryptic digests of the globin and of its separated δ-chains closely resembled similar preparations of Hb A₂ prepared from normal subjects, and the amino acid composition of δ-T-2 was that found in normal δ-chains. A hemoglobin solution of 30 g/100 ml was prepared from washed eryth-

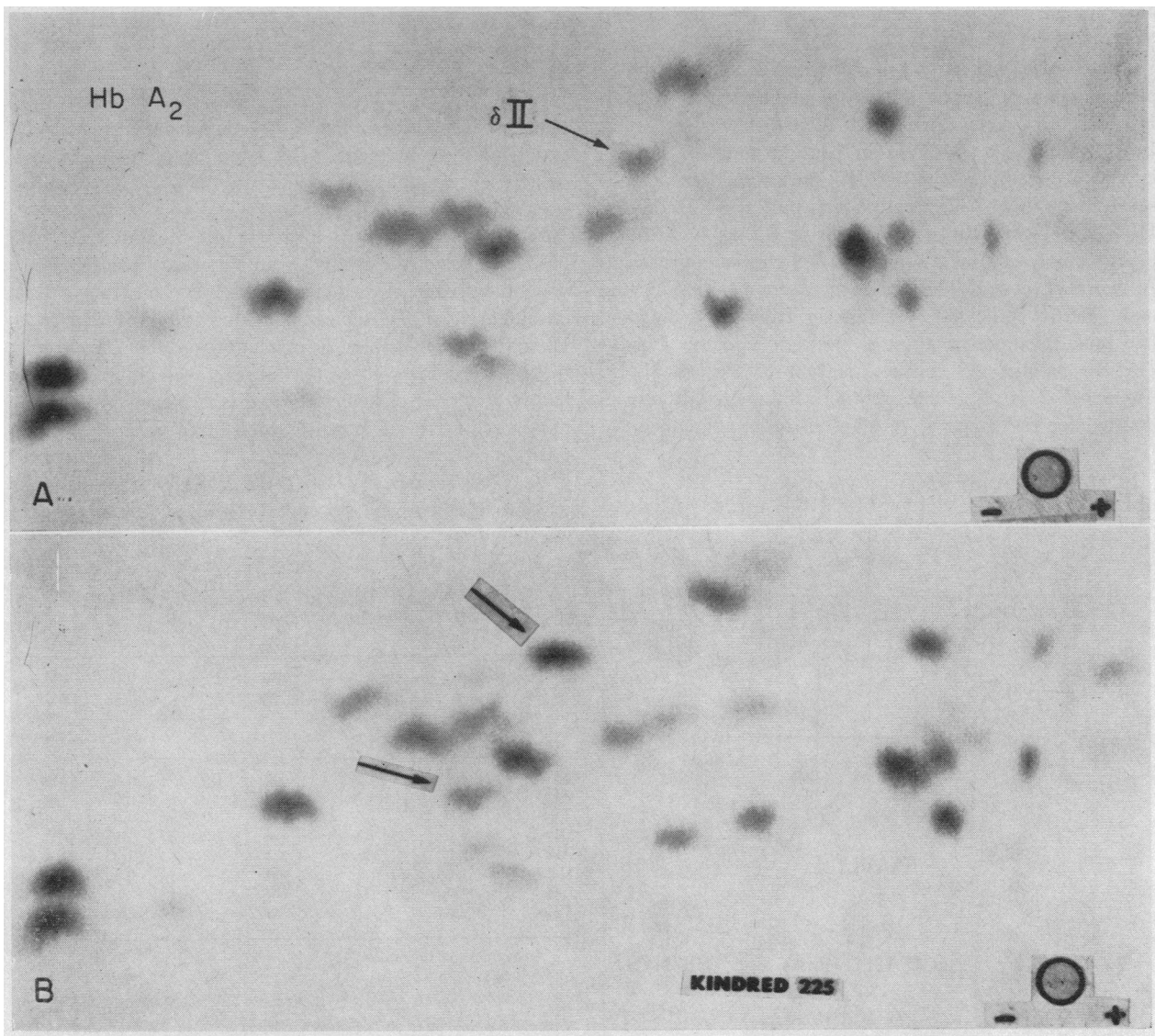

FIGURE 3 A and B Peptide maps of tryptic digests of globin. (A) Hb A₂ and (B) Hb NYU. Peptides which differ are indicated by arrows. The peptide with the greater R_f in Hb NYU is δ-T-2b; the lower, δ-T-2a. The amino acid composition of peptides was determined on eluates of maps prepared from separated δ-chains (10).

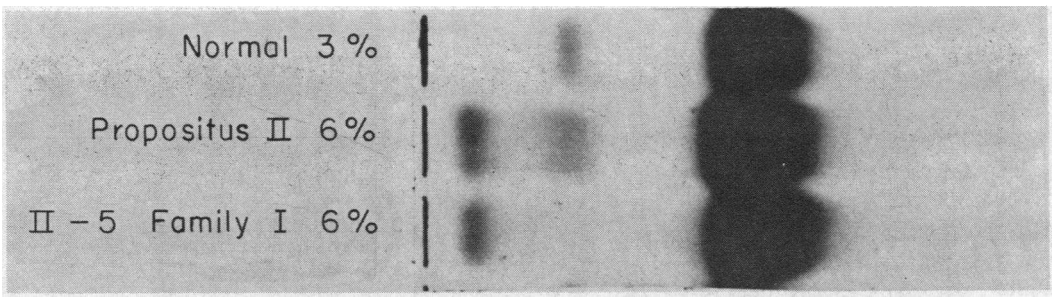

FIGURE 4 Starch-gel electrophoresis of hemolysates containing Hb NYU from propositus of family II and uncle (II-5) of propositus in family I. Numbers at left are concentration of hemoglobin (grams per 100 ml) in hemolysates used for gels. Higher concentrations of hemolysates containing Hb NYU were employed in order to demonstrate minor components. Tris-ethylenediamine tetraacetate (EDTA)-borate buffer, pH 8.6. Benzidine stain.

rocytes of subject II-5 by freezing and thawing. Even in this concentrated solution Hb A_2 was not seen on starch-gel electrophoresis. When Hb A_2 isolated from the hemolysate of a normal subject by electrophoresis on starch blocks was added to a 3% solution of unfractioned Hb of subject II-5, Hb A_2 was apparent in the expected proportion by visual estimation after starch-gel electrophoresis. Prior dialysis of the hemolysate of subject II-5 for 3 days against Tris-HCl buffer, 0.04 mole/liter, pH 8.2, or against 0.04 M Tris did not result in the appearance of Hb A_2. Hb A_2 was not seen on electrophoresis on starch blocks (1), but when the segment of starch between Hb NYU and Hb A was eluted and concentrated by ultrafiltration, a band in the position of Hb A_2 was seen on starch-gel electrophoresis.

DISCUSSION

Ceppellini (3) described the first δ-chain variant of human hemoglobin, Hb A_2', in American Negroes. Hemoglobin A_2' has since been shown to result from the substitution of arginine for glycine at δ^{16} (Table III) (13, 14). Hemoglobin NYU ($\delta^{12\ Lys}$) is indistinguishable from Hb A_2' by electrophoretic techniques, and the methods utilized in reports of the occurrence of Hb A_2' in non-African subjects would not have distinguished Hb NYU from Hb A_2'. Since in both of the families in the present report the electrophoretically slow moving minor component proved to be Hb NYU, it is clear that electrophoretic identification is insufficient for interpretation of the genetic significance of these slow components in different populations. Hemoglobin NYU is the fifth δ-chain variant resulting from a single amino acid substitution to be described (Table III) (15, 16). We have found the structure of an electrophoretically similar component from a Negro subject to be $\alpha_2^A\ \delta_2^{16\ Arg}$, as reported by others for Hb A_2' (13, 14), and the structure of an example of the δ-variant, Hb Flatbush, obtained from members of the first family with this variant to be recognized (4), to be δ^{22} glutamic acid for alanine as first described by Jones, Brimhall, and Huisman (14).

TABLE III

Variants in the Delta Chain of Human Hemoglobin A_2

Designation	δ-Residue, No.	Substitution		References	Electrophoretic mobility, pH 8.6
		Normal	Variant		
A_2'	δ 16	Gly	Arg	(13,14)	Slower than A_2
Flatbush	δ 22	Ala	Glu	(14)	Faster than A_2
Sphakiá	δ 2	Arg	His	(15)	Between A_2' and A_2
Babinga	δ 136	Gly	Asp	(16)	Slightly faster than A_2
NYU	δ 12	Asn	Lys		Same as A_2'

An additional factor affecting the δ-chain phenotype appeared to be present in family I: starch-gel electrophoresis yielded Hb NYU with no evidence of normal Hb A_2 in hemolysates prepared from both II-4 and II-5 even when concentrations of Hb in the hemolysate as high as 30 g/100 ml were used for gels. Yet these two individuals could not be homozygous for Hb NYU, since (1) their mother I-2 did not have the δ-chain variant and (2) two children, III-2 and III-3 of II-4, did not have Hb NYU. From Table II, the values for Hb A_2 in the maternal grandmother and siblings of the propositus are distinctly low. The findings were reminiscent of δ-thalassemia (17) in which a genetically determined decrease in δ-chain synthesis is postulated. However, when 1 or more g of Hb from subject II-5 was chromatographed on DE-52, Hb A_2 in proportions approximately equal to those of Hb NYU were regularly recovered. Since the component appeared to be Hb A_2 electrophoretically and on peptide maps of its δ-chains, its absence on gel electrophoresis of hemolysates suggested the possibility that this Hb A_2 was modified in the hemolysate.

Prolonged dialysis of the hemolysate against the buffer used for the chromatographic separations, or against Tris, did not lead to detectable alterations of the starch gel electrophoretic patterns which continued to show only Hb A and Hb NYU. This "masking" of Hb A_2 was

not present in the propositus of family I (Fig. 3) or in members of family II heterozygous for Hb NYU; in these individuals the expected nearly equal proportions of Hb NYU and A_2 were encountered. Thus, while the findings in family I superficially resembled δ-thalassemia, more detailed studies indicated that the deficient synthesis of normal δ-chains was an unlikely explanation for the findings in this family. Further experiments concerned with the detection of Hb A_2 in subjects II-4 and II-5 of family I are being carried out.

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