Hemoglobin Sealy ($\alpha_2^{47\,His}\,\beta_2$): A New Variant in a Jewish Family

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Hemoglobin (Hb) variants have been found in nearly every ethnic group which has been well investigated. One variant, Hb Beilinson ($\alpha_2^{47\,Gly}\,\beta_2$), has been reported (De Vries *et al.*, 1963) in an Ashkenazi family. A variant with this same structure was also found in Italians, designated Hb L Ferrara (Baglioni, cited by Huehns and Shooter, 1965). We now report the occurrence in an Ashkenazi family of a new hemoglobin variant in which there is also a substitution of the α 47 aspartyl residue but in which the replacement consists of a histidyl rather than a glycyl residue.

MATERIAL AND METHODS

Hemolyzates were prepared by addition of water and toluene to erythrocytes which had been washed three times with 0.85% NaCl. Electrophoresis was performed on starch gel and cellulose acetate, tris-ethylenediamine tetraacetic acid–borate (TEB) buffer, *p*H 8.5 (Smithies, 1965), and on citrate agar, *p*H 6.2 (Robinson *et al.*, 1957). Chromatography was performed on diethylaminoethyl cellulose (DEAE) (Huisman and Dozy, 1965) and on amberlite resin, IRC 50, citrate buffer *p*H 6.0 (Huisman and Prins, 1955). Fetal hemoglobin was measured by the method of alkali denaturation (Betke *et al.*, 1959). Hemoglobin A_2 values were obtained by spectrophotometric measurement of the hemoglobin components, which were eluted from cellulose acetate after electrophoresis. Hemoglobin A_2 values were also obtained by DEAE chromatography. Structural analyses were performed according to procedures previously described for the preparation of amino ethylated α chains from globin, column chromatography of the tryptic hydrolyzates, and amino acid analyses of the peptides (Jones, 1964; Jones *et al.*, 1966).

RESULTS

The new variant, called Hb Sealy after the hospital where it was found, was first detected in the cord blood sample of a newborn infant during the course of a survey. It was also found, together with Hb A, in the hemolyzates of the infant's mother, maternal uncle, and maternal grandmother. The latter's family came from the prov-

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ince of Galicia in the former Austro-Hungarian Empire. Like other α chain variants, Hb Sealy was detectable in three forms, involving the α chains of each of the three normally occurring hemoglobins—adult (A), $\alpha_2\beta_2$; fetal (F), $\alpha_2\gamma_2$; and A_2, $\alpha_2\delta_2$. Although most hemoglobin variants in the trait condition comprise 25–45% of the total hemoglobin, Hb Sealy accounts for only 14–18% of the hemoglobin of its carriers (Table 1). No distinctive clinical or hematologic abnormalities were found in these individuals. The hemoglobin of the infant's father was of type A, but a slight increase of fetal hemoglobin (about 2%) was consistently present in his hemolyzates and is detectable in Figure 1. This was unaccompanied by any other hematologic abnormalities.

In zone electrophoresis in alkaline buffers (Fig. 1), the adult form of Hb Sealy moves like Hb S, while the fetal variant is slower than the adult by about as much as

TABLE 1

QUANTITATIVE DISTRIBUTION OF HEMOGLOBINS IN SUBJECTS WITH HB SEALY (EXPRESSED AS PERCENTAGE)

Subject	Sealy	F[a]	Normal A_2	Variant A_2	A
Proposita (age 3 weeks)....	8.2[b] (18.8, fetal form)[b]	62.5	.....	.....	10.5
Mother.....	17.0	1.0	1.1	0.7	80.2
Uncle.....	14.2	0.9	1.9	0.8	82.2
Grandmother.....	17.7	1.0	1.7	0.6	79.0

[a] Determined by the method of alkali denaturation. Other values obtained by DEAE chromatography, except for Hb A, which was obtained by subtraction.

[b] Determined by chromatography on amberlite resin IRC 50, citrate buffer, *p*H 6.0. The value for the fetal form includes some Hb A, from which it was incompletely separated.

Hb F is slower than Hb A. In agar electrophoresis, citrate buffer, *p*H 6.2 (Fig. 2), the adult variant moves between Hb S and Hb C, but the fetal variant does not separate from Hb A and Hb F. In chromatography on amberlite resin IRC 50, the adult variant moves between Hb S and Hb C, while the mobility of the fetal variant is similar to that of Hb S. Quantitative evaluation of the hemoglobin components, given in Table 1, suggests that the synthesis of the α chains of Hb Sealy is considerably less than that of normal α chains, since each type of variant hemoglobin (adult, fetal, and A_2) is present in a much smaller percentage than the corresponding normal form.

Hb Sealy from the uncle of the proposita was isolated by DEAE chromatography and subjected to structural analysis. The chromatogram in Figure 3 illustrates the peptide pattern of 28 mg of aminoethylated α chain of Hb Sealy separated on a 0.9×16 cm column of Spinco 15A resin, using a linear gradient of pyridine–acetic acid beginning at *p*H 3.1, 0.2M and ending at *p*H 5.0, 2.0M. One-tenth of the effluent was reacted with ninhydrin at 570 mμ to obtain the pattern, and nine-tenths was collected for structural studies. Each zone was rechromatographed in order to obtain pure peptides, generally using the same gradient and a 0.9×60 cm column of Dowex 50W-X2. However, the abnormal peptide in Zone XIV was rechromatographed on Spinco PA-35 resin, and the two peptides in Zone XI were resolved on

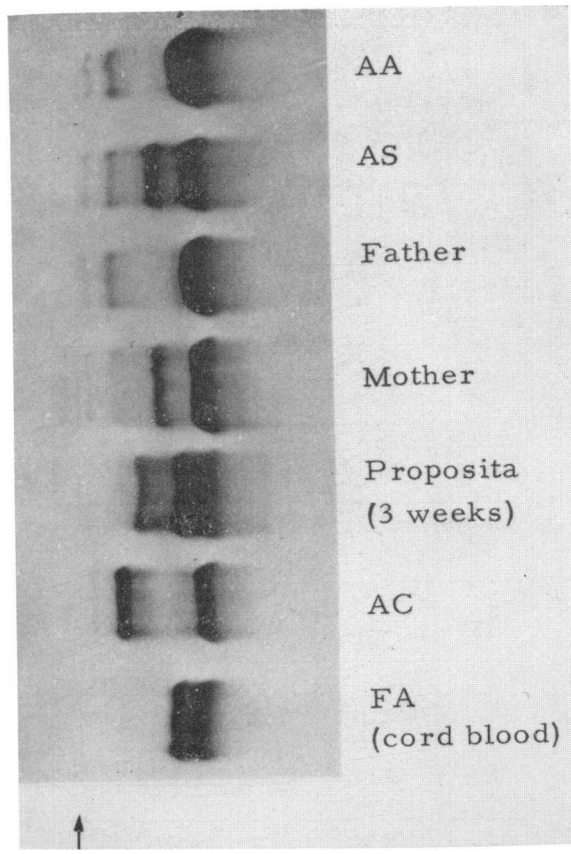

FIG. 1.—Electrophoresis of hemolyzates on cellulose acetate, *p*H 8.5. Anode to the right. Arrow indicates point of origin. In the sample from the mother, a faint band, representing the variant form of Hb A_2, was detectable slightly cathodal to the point of origin.

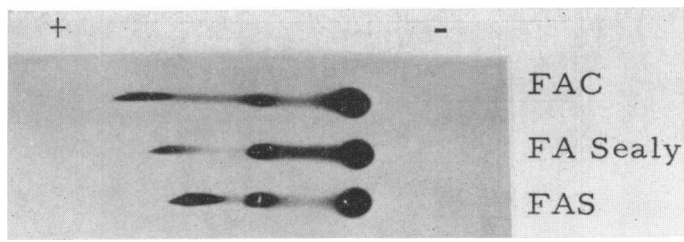

FIG. 2.—Citrate agar electrophoresis, *p*H 6.2, of cord blood hemolyzate of infant with Hb Sealy, compared to cord bloods containing Hb S or Hb C.

TABLE 2

AMINO ACID COMPOSITION OF TRYPTIC PEPTIDES OF AMINOETHYLATED ALPHA CHAIN OF HB SEALY[a]

Amino Acid	αT-1 (IV)[b]	αT-2 (VII)	αT-3 (XI)	αT-4 (VI)	αT-5 (IV)	αT-6 (XIV)	αT-7 (XIII)	αT-8 (VIII)	αT-9 (III)	αT-10 (XII)	αT-11 (IV)	αT-12a (XI)	αT-12b (III)	αT-13 (II)	αT-14 (XIV)
Lysine	1.00	0.83	1.02	.....	1.02	0.97	1.01	1.00	1.07	.....	1.06	.....	1.04	1.03	.....
S-aminoethylcysteine	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.83	.....	.....	.....
Histidine	.....	.....	.....	1.05	.....	**3.06**	1.01	.....	2.97	.....	.....	1.03	1.92	.....	.....
Arginine	.....	.....	.....	0.97	.....	.....	.....	.....	.....	1.01	.....	.....	.....	.....	1.01
Tryptophan	.....	.....	0.23	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Aspartic acid	1.08	1.13	.....	.....	0.22	**0.00**	.....	.....	5.87	.....	2.04	.....	1.04	2.03	.....
Threonine	.....	1.11	.....	.....	1.99	1.01	.....	.....	1.13	.....	.....	.....	2.00	2.85	.....
Serine	0.97	.....	.....	.....	1.04	1.92	.....	.....	1.99	.....	.....	0.99	1.00	.....	.....
Glutamic acid	.....	.....	.....	3.01	.....	0.99	.....	.....	0.18	.....	.....	.....	1.01	.....	.....
Proline	0.96	.....	.....	.....	0.93	0.94	.....	.....	1.17	.....	0.99	.....	1.99	.....	.....
Glycine	.....	.....	1.10	2.99	0.26	1.08	1.98	.....	0.34	.....	.....	.....	.....	1.05	.....
Alanine	1.06	.....	1.88	3.96	0.14	1.08	.....	.....	6.65	.....	.....	.....	5.05	2.00	.....
Valine	0.94	0.93	.....	0.99	.....	0.94	.....	.....	3.09	.....	1.98	.....	1.76	.....	.....
Methionine	.....	.....	.....	.....	0.81	.....	.....	.....	0.60	.....	.....	.....	.....	2.03	.....
Leucine	0.98	.....	.....	1.05	1.20	1.09	.....	.....	4.04	0.99	.....	1.99	4.94	.....	0.99
Tyrosine	.....	.....	.....	0.98	.....	0.98	.....	.....	.....	.....	.....	.....	.....	0.99	.....
Phenylalanine	.....	.....	.....	.....	2.01	1.95	.....	.....	.....	.....	0.94	.....	0.99	.....	.....

[a] Ratios of amino acids recovered following hydrolysis *in vacuo* with 6 N HCl for 22 hr at 110 C.

[b] Roman numerals designate zone from chromatogram in Fig. 3. Each zone was rechromatographed.

Sephadex G-10. The normal αT-6 peptide was missing from its usual position (Zone XII, Fig. 3, in Jones *et al.*, 1966), but another peptide was eluted in Zone XIV along with the normal αT-14. The amino acid composition of the new peptide corresponds to that of αT-6 except for an extra histidine and no aspartic acid (see Table 2). The same abnormal αT-6 peptide was also isolated from the abnormal hemoglobin of the proposita's mother.

The amino acid analyses of the other tryptic peptides of the α chain of Hb Sealy are also given in Table 2. The values are similar to those for the corresponding α chain peptides from Hb A and represent less than 0.12 residue of any contaminating amino acids, except for αT-5 and αT-9, which still contain appreciable amounts. However, the analyses of these two peptides yield data similar to those for Hb A.

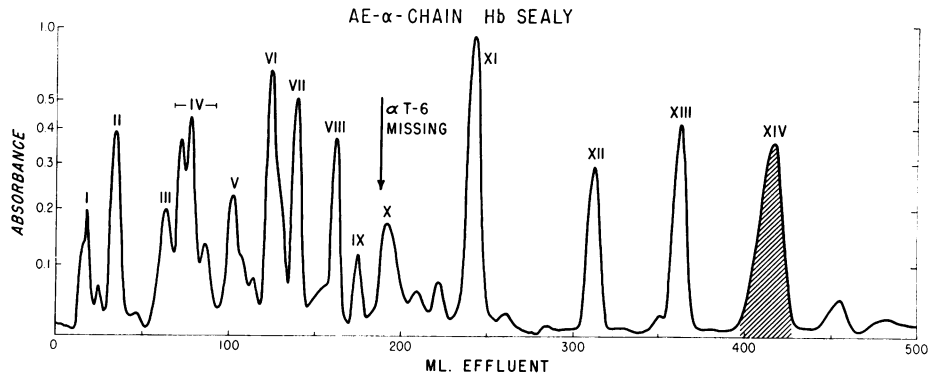

FIG. 3.—Peptide pattern of tryptic hydrolyzate of the aminoethylated α chains from Hb Sealy. The absorbance of the reaction products of ninhydrin and peptides was measured continuously at 570 mμ.

Because the normal αT-6 contains only one aspartyl residue which is situated in position 47, it may be concluded that Hb Sealy differs from Hb A by the substitution of histidine for aspartic acid in position 47 of the α chain, and its structure, therefore, is $\alpha_2{}^{47\ His}\beta_2$.

SUMMARY

A new variant, Hb Sealy, $\alpha_2{}^{47\ His}\beta_2$, has been found in heterozygous combination with Hb A in three generations of an Ashkenazi family. It comprises only 14–18% of the total hemoglobin of the adult carriers and is not associated with any distinctive clinical or hematologic abnormalities.

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