The Interaction of Hemoglobin E With β Thalassemia: A Study of Hemoglobin Synthesis in a Family of Mixed Burmese and Iranian Origin

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A 5-yr-old girl with hemoglobin E-β thalassemia was discovered in a family of mixed origin. The father is Iranian (β-thalassemia trait) and the mother is Burmese (hemoglobin-E trait). Hemoglobin synthesis was studied in vitro in the blood of the proposita and family members. In the subjects with hemoglobin E trait the ratio of the quantity of hemoglobin A to hemoglobin E was 3:1. However, the β^A/β^E synthesis ratio in reticulocytes was in the range of 1.5-2.18, and the specific activity of β^E was 31%-49% greater than β^A, suggesting instability of hemoglobin E with preferential destruction of abnormal hemoglobin. The blood of the proposita exhibited only hemoglobin F and hemoglobin E and reticulocytes and bone marrow showed no β^A synthesis. This Iranian β-thalassemia gene is therefore of the β^0 type. The β^E/α synthesis ratio (approximately 0.74) in blood of the proposita was similar to the β^A/α ratio in mildly affected relatives with thalassemia trait. These results suggest that the severity of the hemoglobin E-β thalassemia syndrome is attributable to both instability and defective synthesis of hemoglobin E in association with absent β^A synthesis due to a β^0 thalassemia gene.

HEMOGLOBIN E (β^{22} lys) is found with high frequency in Southeast Asia, especially in Thailand.[1,2] This abnormal hemoglobin has a rather limited geographical distribution, although it has also been described in low frequency in the neighboring Chinese and Indians.[3] In the Far East, hemoglobin E often occurs in association with β thalassemia, and the interaction of the two genes produces a moderately severe thalassemia syndrome characterized most often by the complete absence of hemoglobin A and the failure to synthesize β^A globin chains.[4,5,6] The basis for the severity of the hemoglobin E-β thalassemia syndrome has not been completely defined. The present paper reports studies of hemoglobin synthesis in a family of mixed Burmese and Iranian origin. This interracial marriage provided the unique opportunity of investigating the effects on hemoglobin production of the interaction of hemoglobin E with β thalassemia derived from a gene pool from Western Asia.

MATERIALS AND METHODS

Routine hematological studies were performed by standard methods.[7] The starch block, starch gel, and hemoglobin "fingerprinting" techniques have been described previously.[8] Hemoglobin E and hemoglobin A_2 were measured by starch block electrophoresis. Hemoglobin F was measured by the alkali denaturation technique.[9] Peripheral blood and bone marrow samples for in

I

	1	2	3
β/a		0.60	1.21
HbF(%)		1.5	1.0
HbE(%)		—	24.5
HbA₂(%)		7.5	—

II

	1	2	3	4	5
β/a	0.58	1.10	0.46	0.74	1.33
HbF(%)	1.2	1.4	2.5	31.5	
HbE(%)	—	25.6	—	68.5	
HbA₂(%)	8.1	—	4.4	—	

III

	1
β/a	1.24
HbF(%)	42-46
HbE	18-25

PROPOSITA

NORMAL

HbE TRAIT
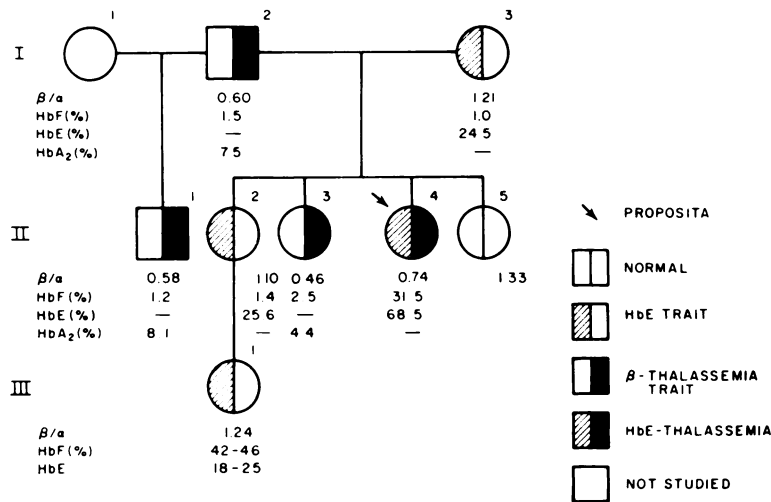
β-THALASSEMIA TRAIT

HbE-THALASSEMIA

NOT STUDIED

Fig. 1. Family pedigree. The β/α synthesis ratio in vitro and the percentage of each hemoglobin found in peripheral blood is indicated for each member of the family.

vitro study of hemoglobin synthesis were drawn into heparinized tubes and immediately placed in ice. The cells were washed and incubated in the medium of Lingrel and Borsook[10] in the presence of 100 μc of ^{3}H-leucine.[8] After incubation, globin was prepared from the entire hemolysate including membranes. The globin polypeptide chains were separated by column chromatography on carboxymethylcellulose in 8 M urea-containing buffers.[11] Radioactivity was measured by liquid scintillation counting after adding 1 ml of sample from each fraction collector tube to 10 ml of Aquasol (New England Nuclear Corp.). Graphs of radioactivity versus sample tube were drawn, and the total radioactivity in each polypeptide fraction was determined by measuring the area under the curves. This technique has been found to give results accurate to within 1°ₒ 2°ₒ of the results obtained by counting aliquots of pooled eluates. Specific activities of the separated globin chains were determined after dialysis to remove urea. Protein was measured by the biuret method.

RESULTS

Clinical Studies

The family pedigree is shown in Fig. 1. The proposita (II-4) was 5 yr old when first admitted to Metropolitan Hospital. She had a history of anemia since the age of 18 mo and had received a total of three blood transfusions during her life; the last transfusion was given 7 mo prior to admission. The patient exhibited pallor, mild scleral icterus, and an enlarged liver and spleen. The peripheral blood revealed a hemoglobin concentration of 6.4 g/100 ml, a hematocrit value of 20.3°ₒ, and a reticulocyte count of 4.5°ₒ (Table 1). The blood film showed extreme anisopoikilocytosis, severe hypochromia, many normoblasts, target cells, and basophilic stippling. The Cr^{51} red cell half-life was 12 days. Starch gel electrophoresis revealed absence of hemoglobin A with hemoglobin bands only in the positions of hemoglobin F and hemoglobin E, suggesting a diagnosis of hemoglobin E thalassemia (Fig. 2). The amount of hemoglobin F was estimated to be 31.5°ₒ by alkali denaturation (Fig. 1) and 37°ₒ by chromatography of globin on carboxymethylcellulose in 8 M urea.

The father (I-2) is of Iranian origin. His blood revealed microcytosis, hypochromia, and an elevated percentage of hemoglobin A₂ (7.5°ₒ), suggesting a

Table 1. Hematological Data on Family Members

Subject	Age (yr)	Hb (g/100 ml)	RBC (x 10⁶/μl)	Hct (%)	MCH (pg)	MCV (μ³)	MCHC (%)	Serum Fe (μg/100 ml)	TIBC (μg/100 ml)
I-2	42	12.5	6.22	37.9	20.2	62	33.2	104	324
I-3	34	10.8	4.55	33.6	23.7	73	32.4	29.8	301
II-1	13	11.0	6.23	34.6	17.6	55	32.2	110	295
II-2	18	11.8	5.11	36.8	22.8	72	32.1	68	325
II-3	7	10.6	5.60	32	18.9	57.1	33.1	71.6	316
II-4	5	6.4	3.45	20.3	18.6	58	31.8	176	240
II-5	4	11.7	4.61	36.1	25.3	78	32.1	119	327
III-1	$\frac{1}{12}$	12.8	4.30	33	29.7	76.7	38.7		

diagnosis of thalassemia minor (Table 1, Fig. 1). Similar hematological findings were present in two siblings of the proposita (subjects II-1 and II-3).

The mother (I-3) is Burmese and has always been in good health. She had a mild hypochromic anemia with a low serum iron (Table 1). Starch gel electrophoresis revealed major hemoglobin bands in the positions of hemoglobin A and hemoglobin E suggesting a diagnosis of hemoglobin E trait (Fig. 2). Similar results were found in another sister of the proposita (subject II-2).

Fingerprint Analysis

Peptide maps of the β chain of the abnormal hemoglobin agreed with those previously reported for β^E.[12] Tryptic peptide III was absent and two new peptides, a neutral and an arginine-containing basic peptide were present.

Studies of Hemoglobin Synthesis

The relative synthesis of the various globin chains was measured in vitro in the peripheral blood of all the members of the family. The incubations were carried out for 60 min in the presence of ³H-leucine. Figure 1 shows the total
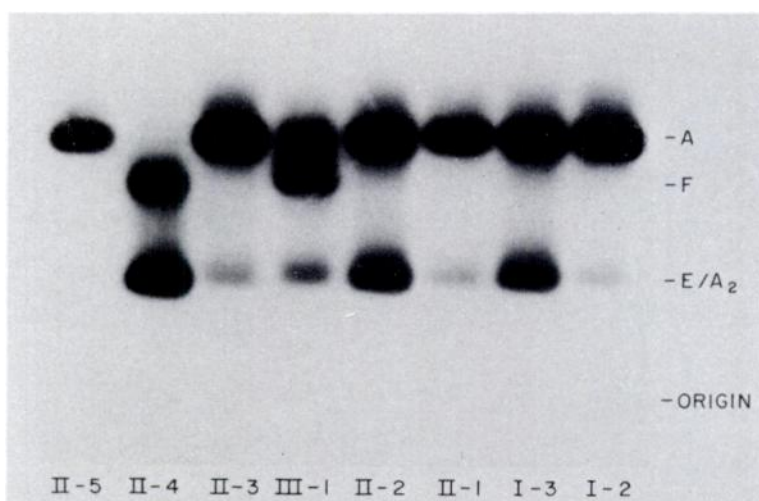
Fig. 2. Starch gel electrophoresis at pH 8.6 of hemolysates from family members. The anode is towards the top of the figure. Hemoglobin A is absent in the sample from the proposita, II-4. Benzidine stain.

Table 2. Biosynthetic Data on Family Members

| Patient	Synthesis Ratio			Specific Activity			
	γ/α	β^A/α	β^E/α	γ	β^A	β^E (CPM/mg)	α
I-2		0.6			384		594
I-3		0.73	0.48		171	254	203
II-1		0.58			154		298
II-2		0.67	0.43		328	429	351
II-3		0.46			181		390
II-4	0.18		0.74	12,509		33,990	33,117
II-5		1.33			257		193
III-1	0.36	0.75	0.49	416	1,294	1,123	612

β/α synthesis ratios and Table 2 lists the various non-α/α synthesis ratios and the specific activities of the γ, β^A, β^E, and α chains.

β Thalassemia Minor

On the basis of clinical and electrophoretic evidence (Table 1, Fig. 1) subjects I-2, II-1, and II-3 were considered to have β thalassemia minor. The results of the studies of hemoglobin biosynthesis confirmed the clinical impression. These three subjects had β/α synthesis ratios of 0.6, 0.58, and 0.46, all of which are markedly below the value of approximately 1.0 expected for the balanced synthesis found in normal individuals.[13,14] Figure 3 shows the pattern of radioactivity obtained by column chromatography of the globin chains of subject II-3. The radioactivity peak in the β-chain region is smaller than that in the α-chain region, illustrating the deficit in β-chain synthesis. The specific activities of the α chains in the three experiments were also 1.5–2.2 times greater than the specific activities of the β^A chains (Table 2). Since these subjects had not been transfused this increase in α-chain specific activity probably reflects the prior accelerated destruction of the excess nonradioactive α chains produced during the development of the red cells.[6,15,16]

Normal Subject

Subject II-5 demonstrated no evidence of hemoglobinopathy by clinical or electrophoretic criteria. The study of hemoglobin biosynthesis revealed a β/α
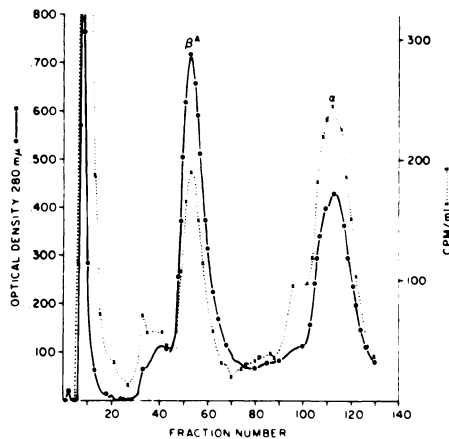
Fig. 3. Synthesis in vitro of α and β chains in β thalassemia minor. Column chromatography on carboxymethylcellulose in 8 M urea of globin prepared from the blood of subject II-3. The β/α ratio of incorporated radioactivity is 0.46.

synthesis ratio of 1.33, which is within the expected range for normal individuals.[17,18]

Hemoglobin E Trait

Subjects I-3 and II-2 were found to have hemoglobin E trait by starch gel electrophoresis. Incubation of peripheral blood samples with radioactive amino acid indicated balanced total β/α synthesis ratios of 1.21 and 1.1 (Fig. 1 and Table 2). Figure 4 shows the chromatographic pattern of radioactivity incorporated into the globin chains of subject II-2. When the total radioactivity in the individual chains was estimated from the chromatography graphs, the ratio of synthesis of β^A/β^E was 1.5 in subject I-3 and 1.6 in subject II-2 (Table 2). When calculated from the specific activities of the chains, the β^A/β^E synthesis ratios were 2.18 and 2.05 for the same subjects. These values are all less than the 3:1 ratio of hemoglobin A to hemoglobin E found in peripheral blood. In addition, the specific activity of β^E was $31\%-49\%$ greater than that of β^A in both experiments (Table 2). The results suggest that hemoglobin E is synthesized in relatively greater amounts than it is found in peripheral blood. These findings are compatible with relative instability of hemoglobin E resulting in preferential destruction of the abnormal hemoglobin during the maturation of the erythrocyte.

Subject III-1 was a 1-mo-old infant at the time of study. Starch gel electrophoresis revealed the major hemoglobin component to be hemoglobin F (Fig. 2). Prominent hemoglobin bands were also present in the positions of hemoglobin A and hemoglobin E suggesting a diagnosis of hemoglobin E trait. On the basis of urea-CMC chromatography of globin, the γ chains were estimated to comprise $42\%-46\%$ of the non-α chains in the blood (Fig. 1); $33\%-36\%$ was β^A, and $18\%-25\%$ was β^E. Thus the β^A/β^E ratio (1.3-2) was lower than that found in the peripheral blood of the adult members of the family with hemoglobin E trait (3:1). When globin synthesis was measured the total non-α/α radioactivity was 1.6 (Table 2). This result is greater than the 1.2 and 1.1 values
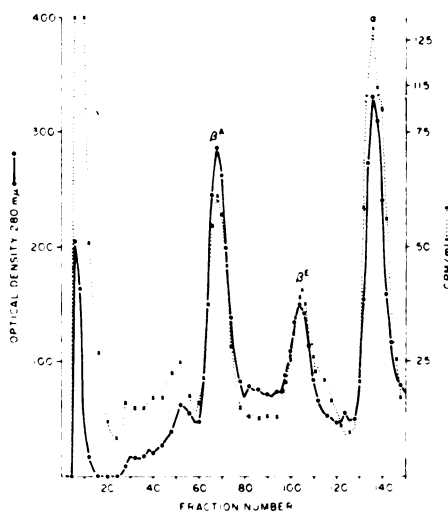

Fig. 4. Synthesis in vitro of globin chains in hemoglobin E trait. Column chromatography in 8 M urea of globin prepared from the blood of subject II-2. The specific activity of β^E is greater than that of β^A, although the β^A/β^E synthesis ratio is 1.5.

found in the adult members with hemoglobin E trait and may reflect some imbalance in globin chain synthesis in early infancy. The β^A/β^E synthesis ratio was 1.53, the same as obtained with cells from the adult subjects with hemoglobin E trait. However, unlike the experiments with the blood of adult family members, the specific activity of β^E was not greater than β^A in the blood of subject III-1 (Table 2).

Hemoglobin E Thalassemia

Hemoglobin synthesis was studied in vitro in both the peripheral blood and bone marrow of subject II-4. Figure 5 shows the chromatographic pattern of radioactivity incorporated into globin by the peripheral blood. Radioactive peaks were present in the region of γ, β^E, and α chains. There was no evidence of any synthesis of β^A. Total non-α/α synthesis was almost balanced; a ratio of 0.92 was found when the sum of the radioactivity under the optical density peaks of γ and β^E was compared to the radioactivity in α globin. The β^E/α synthesis ratio was 0.76. This value is slightly higher than the β^A/α synthesis ratios found in the members of the family with thalassemia minor. However, as seen in Fig. 5, the peak of radioactivity in the β^E region was not symmetrical and suggested the presence of a second component migrating in the "pre-α" region. This could falsely raise both total non-α/α and β^E/α ratios. The specific activities of β^E and α were approximately equal (Table 2). The specific activity of γ globin was one-third that of the other two chains suggesting a longer life span of those cells containing larger amounts of hemoglobin F, or a greater stability of hemoglobin F than hemoglobin E, or the excess free α chains. In an experiment utilizing a bone marrow specimen from patient II-4, no radioactive peak was found in the β^A region, again indicating that this thalassemia gene is characterized by complete suppression of β^A globin synthesis. However, a significant peak of radioactivity was found migrating between the β^E and α regions and because of the obvious contamination of the β^E peak by the "pre-α" radioactivity, direct accurate measurement of the β^E/α synthesis ratio was not possible in the bone marrow sample.
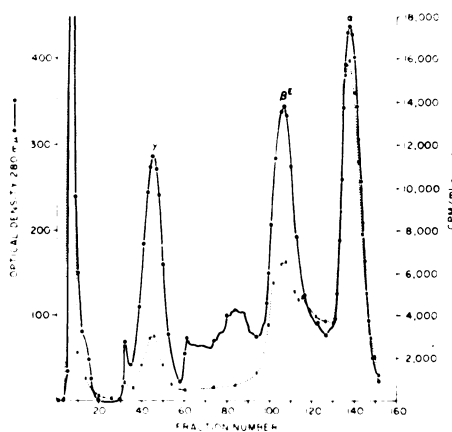

Fig. 5. Hemoglobin synthesis by peripheral blood in hemoglobin E-β thalassemia. The assymetry of the peak of radioactivity in the β^E region suggests the presence of another component between β^E and α.

DISCUSSION

It is now apparent that there are at least two broad categories of β-thalassemia genes: One form, termed $\beta+$, results in variably decreased production of β^A globin; the other, termed β^o, causes complete suppression of β^A globin with total absence of hemoglobin A in homozygotes.[19] Certain geographic areas exhibit a preponderance of one or the other of the two types of β-thalassemia genes.[19]

Because patients with hemoglobin E-β thalassemia almost invariably have a total absence of hemoglobin A,[4,5] the predominant β thalassemia gene occurring in Southeast Asia has been considered to be the β^o type.[20] It was therefore of interest to investigate the clinical and hemoglobin biosynthetic characteristics of hemoglobin E-β thalassemia found in a racially mixed family whose β thalassemia gene was of Western Asian origin.

The clinical features of the proposita in the present study resembled those described previously in cases from Thailand. The thalassemia syndrome was severe and no hemoglobin A was detected. Studies of hemoglobin synthesis in the bone marrow and peripheral blood demonstrated absence of β^A synthesis. This result is similar to the findings reported by Weatherall and his associates in studies of hemoglobin synthesis in peripheral blood of three Thai patients.[6] Thus the β thalassemia gene of Iranian origin in the present family is also of the β^o type.

Despite the presence of only one functional gene for the production of β chains, the subjects with thalassemia minor had only a mild clinical disorder resembling the form of heterozygous β thalassemia caused by a $\beta+$ gene and commonly seen in individuals of Mediterranean origin. The β/α synthesis ratio in members of the present family varied from 0.46 to 0.6, similar to previously reported values for subjects with thalassemia minor from an Italian family with a $\beta+$ gene.[21]

The results of the studies on the three patients (I-3, II-2, III-1) with hemoglobin E trait indicated that β^E is produced in amounts one-third to one-half less than β^A (Table 2). Prior reports of markedly decreased erythrocyte mean corpuscular hemoglobin and volume in hemoglobin E disease and trait were suggestive of defective hemoglobin production.[4,22,23] However, on the basis of the total incorporated radioactivity, and the specific activities of β^E and β^A, the present study suggests that hemoglobin E is synthesized in slightly greater relative amounts than it is found in the peripheral blood. This finding implies preferential destruction of the abnormal hemoglobin and is analogous to results obtained in studies of several unstable hemoglobins.[8,24,25] Instability of hemoglobin E has previously been predicted from structural considerations.[26] Incubation of the blood of the infant III-1 resulted in equal specific activities for β^A and β^E, although the β^A/β^E synthesis ratio was equal to that found in the adult subjects. This may reflect a lessened destruction of performed hemoglobin E at this early period of life.

The β^E/α synthesis ratio in the patient with hemoglobin E thalassemia was estimated to be approximately 0.74 in the experiment utilizing peripheral blood. This may be a slight overestimation due to admixture of the β^E with "pre-α" material. Weatherall and associates reported more severe imbalance in the

β^E/α ratios of their subjects with hemoglobin E thalassemia.[6] Nevertheless, despite the severity of the disease, the balance in α- and β-chain synthesis in the present study did not appear to be much more deranged in the proposita than in her mildly affected relations with thalassemia minor. Thus, in addition to its reduced synthetic rate, the nature of the hemoglobin E molecule must also be a major factor in the difference in the morbidity of the two conditions. It is possible that production of both α and β chains is depressed in hemoglobin E thalassemia, but absolute rates of globin synthesis could not be compared with that in hemoglobin E trait or thalassemia minor. Decreased stability of hemoglobin E may contribute to the severity of the hemoglobin E-β thalassemia syndrome, along with the instability and tendency towards precipitation of the excess α chains,[27,28] and the dual reduction in hemoglobinization of the erythrocyte due to defective synthesis of β^E and absence of β^A.

ACKNOWLEDGMENT

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