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Research Article

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β-Thalassemia in the American Negro

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ABSTRACT In Italian patients with high hemoglobin A_2 β-thalassemia trait, the synthesis of β-chains of adult hemoglobin in the peripheral blood is approximately one-half that of α-chains. In this study the relative rates of β- and α-chain synthesis were determined in 26 Negro heterozygotes and five homozygotes for β-thalassemia in six families. The β/α ratio of globin synthesis was decreased in only 15 heterozygotes, whereas in the other 11, β/α globin synthesis was in the normal range or was slightly increased. These unusual findings did not appear to be due to the presence of α-thalassemia or a hyperactive "normal" β-allele. This study demonstrates that the β/α ratio of globin synthesis in the peripheral blood is normal in some patients with β-thalassemia trait. In five Negro homozygotes with relatively mild clinical disease the β/α ratios were similar to those of Caucasians with Cooley's anemia. Further studies are needed to explore the relationship between normal synthesis ratios in many Negro heterozygotes and milder clinical disease in homozygotes in the same families.

INTRODUCTION

β-Thalassemia trait is a common disorder in the Negro, occurring with an incidence of approximately 0.8% in the United States (1) and Nigeria (2). Hematological findings including red cell morphology and levels of hemoglobin A_2 (Hb A_2) and hemoglobin F (Hb F) are similar in Negro and non-Negro patients with β-thalassemia trait (2–4). Homozygous β-thalassemia in the Negro has been reported infrequently with approximately 20 cases appearing in the American literature (5). In general these patients have had a milder clinical disorder than patients with homozygous β-thalassemia in other racial groups. The reasons for the generally higher hemoglobin levels and decreased needs for transfusion in Negro homozygotes are not known.

We have studied globin synthesis in the heterozygotes and homozygotes of six Negro families with β-thalassemia in order to compare their biochemical findings with those seen in other racial groups.

METHODS

Patients. Six Negro families that included five patients with homozygous β-thalassemia and 26 members with heterozygous β-thalassemia have been studied. The hematologic data on these patients are summarized in Table I.

The "S" family included two sisters aged 51 and 50 yr with homozygous β-thalassemia. The older unmarried sister has been anemic all of her life. She has been transfused regularly with 1 U of packed red blood cells every 2 mo since 26 yr of age. She is short in stature and has marked hepatosplenomegaly and cardiomegaly with compensated congestive heart failure. She sustained two fractures of the right humerus and a compression fracture of the body of a lumbar vertebra in the past 10 yr, presumably due to a weakening of the bones by excessive hematopoiesis. The younger sister was married and had one child. She was first seen in Jefferson Hospital at age 26 for marked cardiomegaly and hepatosplenomegaly with a hemoglobin concentration of 2.5 g/100 ml. Since then she had been transfused on a regular basis with 1½ U of blood per mo. In 1957 she underwent splenectomy for severe splenic pain and hypersplenism. After surgery she did not require blood transfusions for 2 yr. In 1970 she began to suffer from headaches that were presumably due to pressure from hematopoiesis in the frontal bones of her skull. The headaches stopped when transfusions were administered more frequently. She died recently in cardiac failure due to severe hemosiderosis. Ferrokinetic studies in both sisters have been previously reported (6). The pedigree of this family is shown in Fig. 1.

The propositus in the "M" family was a 33-yr old woman with thalassemia major. At age 20 she was found to have hepatosplenomegaly and a hemoglobin concentration of 4.5 g/100 ml. From that time on she received 1 U of blood each month. She had multiple hospitalizations for pericarditis, thrombophlebitis, cholecystitis, and spontaneous abortion. Her operations included removal of an ectopic pregnancy, cholecystectomy, splenectomy, and oophorectomy. In 1967, a chest X-ray examination revealed soft tissue masses compatible with extramedullary hematopoiesis on both sides

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TABLE I

Hematologic and Subunit Synthesis Data

Subject	Age	Hemoglobin	Hematocrit	Red blood cells	Reticulocytes	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular hemoglobin concn.	Hemoglobin A_2	Hemoglobin F	Globin synthesis ratio β/α
	yr	*g/100 ml*	%	*10^{-6}/mm³*	%	*μm³*	*pg*	*g/100 ml*	%	%	
Family S											
I-2	75	10.5	38.0	4.41	3.4	86.0	24.0	28.0	5.1*	1.2	1.38
I-3	70	13.1	42.0	5.51	2.7	76.0	24.0	31.0	5.8*	1.1	0.63
I-4	63	12.2	39.0	4.85	3.0	81.0	25.0	31.0	4.5*	3.2	0.74
I-5	61	12.4	43.0	4.50	2.0	96.0	28.0	29.0	5.5*	1.4	1.15
I-6	59	12.8	41.0	5.43	4.0	76.0	24.0	31.0	5.5*	2.6	0.76
I-7	59	13.9	45.0	4.97	1.0	91.0	28.0	31.0	3.0	0.7	1.10
I-8	49	11.7	38.5	4.35	4.1	89.0	27.0	31.0	5.3	3.0	1.03
II-1	51	6.1‡	17.9	2.32	7.4	75.0	25.9	35.9	3.5	18.0	0.25
II-2	50	6.9‡	23.5	3.01	20.6	78.0	23.0	29.0	5.2	20.0	0.22
II-3	50	13.7	44.0	4.50	1.0	97.2	30.3	31.2	2.6	0.2	0.95
II-5	31	12.8	40.5	5.11	7.0	80.0	25.0	31.0	6.6	2.9	0.52
III-1	30	11.1	36.0	4.77	5.9	70.0	24.0	31.0	5.5	5.4	1.09
III-3	5	10.7	36.0	5.14	4.4	70.0	21.0	30.0	6.1	3.8	0.55
IV-1	11	10.7	37.5	4.89	1.4	76.0	22.0	29.0	5.7	3.9	1.02
Family M											
I-1	50	6.8	21.5	2.82	18.7	76.0	24.0	32.0	§	3.8	0.64
II-1	33	9.8‡	30.0	3.80	7.6	79.0	26.0	33.0	6.3	5.8	0.13
II-2	31	12.1	39.5	5.88	1.8	67.5	20.5	30.4	5.6	1.6	0.83
II-5	13	12.1	39.0	5.71	3.6	68.0	21.0	31.0	5.6	1.3	1.02
III-1	13	11.3	37.0	4.93	2.1	75.1	22.9	30.5	5.5	1.8	0.66
III-2	11	10.9	36.0	6.00	1.2	60.0	18.0	30.0	7.4*	0	0.53
III-3	4	11.9	36.0	4.56	1.1	78.9	26.1	33.1	6.0*	1.4	1.14
Family R											
I-2	32	13.9	44.0	5.19	2.6	85.5	27.0	31.7	4.5	0.3	0.93
II-1	9	11.8	38.0	5.62	2.2	67.0	21.0	31.0	4.4	1.6	0.69
II-3	6	12.0	38.5	5.18	1.0	75.0	23.2	31.5	4.5	1.3	0.83
II-5	10/12	9.6	30.0	4.14	5.6	72.8	23.2	32.2	§	12.2	0.71
Family H											
Mother	31	12.9	42.0	5.21	3.2	81.0	24.7	30.7	5.6	3.1	0.94
Daughter 1	11	10.0	34.0	5.85	8.4	58.1	17.1	29.4	7.5	45.7	0.24
Daughter 2	8	7.8	28.0	4.78	11.8	58.5	16.6	27.8	6.5	43.5	0.21
Son	6	11.3	37.0	4.98	2.0	74.7	22.5	30.2	6.0	5.5	0.86
Daughter 3	4	12.9	41.0	5.68	2.2	72.5	22.7	31.6	5.7	4.2	1.00
Family N											
Mother	24	11.5	36.5	4.78	2.0	77.0	24.0	31.5	5.5	2.7	0.83
Daughter	5	10.8	33.5	4.85	3.0	60.0	22.0	32.0	4.7	2.5	0.78
Family P											
Mother	22	10.3	35.0	5.40	1.7	64.8	19.1	29.5	4.7	0.5	0.69
Daughter	5	11.4	34.0	3.70	3.5	91.1	30.8	33.5	3.2	2.3	1.19

* Figure is total of Hb A_2 and Hb $A_{2'}$.

‡ Patient was on a regular blood transfusion program.

§ Not done. Patient has sickle β-thalassemia.

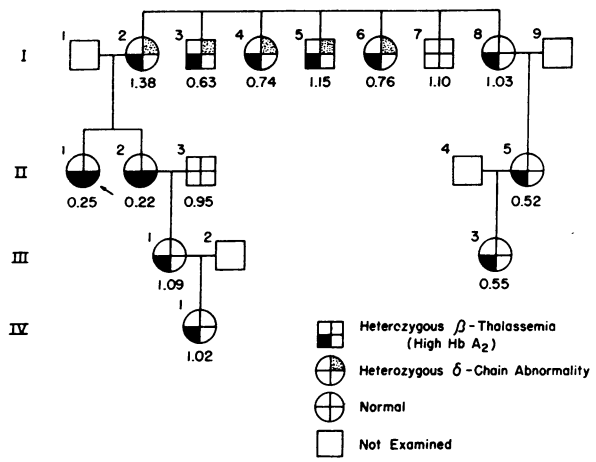

FIGURE 1 Pedigree of family S. The numbers below the symbols are the β/α ratios.

of the dorsal spine. This patient died recently. The cause of death was cardiac arrhythmia due to severe hemosiderosis and fibrosis of the myocardium. Her 52-yr old mother has sickle β-thalassemia. She has had intermittent severe low back pains and cardiomegaly with congestive heart failure controlled by digitoxin, diuretics, and an occasional blood transfusion. She has hepatomegaly but her spleen is not palpable. Her sclerae are mildly icteric. The pedigree of this family is shown in Fig. 2.

The "R" family has four members with β-thalassemia trait or sickle β-thalassemia (Fig. 3). The "H" family has been previously described (7). This family contains two girls ages 11 and 8 with homozygous β-thalassemia. The members of the "N" and "P" families with high Hb A_2 β-thalassemia trait are listed in Table I.

21 control subjects without evidence of thalassemia, 7 homozygotes, and 11 heterozygotes for β-thalassemia of Italian, Greek, and English ancestry were also studied.

Laboratory studies. Hematologic studies were done by standard methods (8). Hemoglobin A_2 levels were determined by starch granule electrophoresis (9). The normal range by this method in our laboratory is 1.8–3.3% (mean ±2 SD). Hb F levels were determined by alkali denaturation (10). Normal subjects have less than 2% Hb F.

Globin synthesis studies. Globin synthesis in the peripheral blood was studied by methods previously described (11–13). Peripheral blood was incubated with [^{14}C]leucine for 2 h, the α- and β-chains were separated, and radioactivities and specific activities were determined for each chain. In the heterozygotes, where less radioactivity was incorporated and α- and β-chain pools were approximately equal, β/α ratios of specific activity were used to express relative synthesis of globin chains. In the homozygotes, where large amounts of radioactivity were incorporated during the incubation and where an excess of nonradioactive α-chain might be present, β/α ratios of radioactivity were used to express relative globin synthesis. In the one study of globin synthesis in the bone marrow, 4 ml of aspirated marrow was studied in a manner identical with that of peripheral blood.

RESULTS

The hematologic and globin synthesis data on the members of the Negro families are summarized in Table I and Figs. 1–3. Serum iron levels were determined and were found to be normal in all the heterozygotes in family S; in patients I-2, II-2, and II-5 in family M; I-2 in family R; and I-1 in family P.

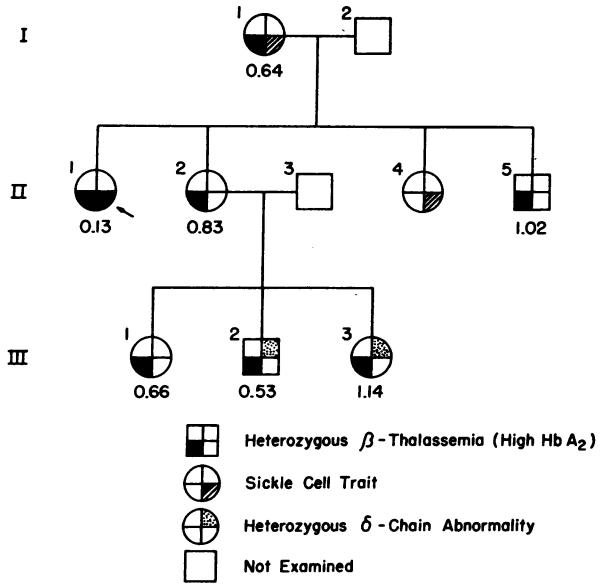

FIGURE 2 Pedigree of family M. The numbers below the symbols are the β/α ratios.

The 24 patients with β-thalassemia trait were identified by elevated levels of Hb A_2, abnormal red cell morphology, and genetic studies. In each of these patients the Hb A_2 value was elevated, the mean for the group being 5.47±0.69 (1 SD). 12 of these 24 heterozygotes had elevated levels of Hb F, the highest being 5.5%. The mean percentage of Hb F for the entire group was 2.35±1.40 (1 SD). These findings are similar to those of Caucasians (14) and Negroes (3) with β-thalassemia trait previously reported. Two family members with sickle β-thalassemia were also included to make a total group of 26 heterozygotes. They were

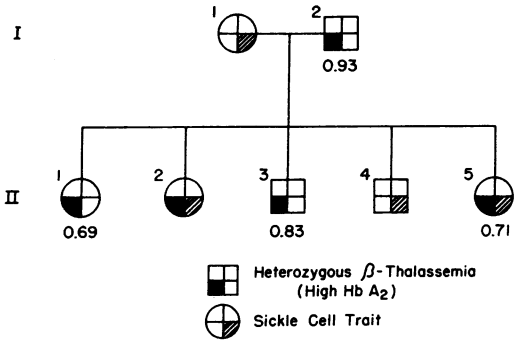

FIGURE 3 Pedigree of family R. The numbers below the symbols are the β/α ratios.

identified by genetic studies, abnormal red cell morphology, a preponderance of Hb S and a small amount (family R, II-5) or an absence of Hb A (family M, I-1) on electrophoresis. The Negro homozygotes included three adult patients who needed transfusions and two children who maintained adequate hemoglobin levels without therapy. Each of these patients had elevated levels of Hb F and abnormalities of red cell morphology which were similar to those in non-Negro patients.

The mean β/α ratio in the control group was 0.99 $\pm$0.05 (1 SD). The Caucasian patients with β-thalassemia trait and high levels of Hb A_2 had a mean β/α ratio of 0.57$\pm$0.08 (1 SD). Ratios reported for Caucasian heterozygotes from other laboratories are similar to those in our group (15–17). The seven Caucasian homozygotes had a mean β/α ratio of 0.17, with a range from 0.02 to 0.25. Ratios in homozygotes reported by other laboratories ranged from 0 to 0.24 (15–19).

The values of the β/α ratios in the Caucasian group with β-thalassemia trait did not overlap those of the control group. In contrast, the β/α ratios of the Negro group with heterozygous β-thalassemia ranged from 0.52 to 1.38, overlapping the Caucasian heterozygotes and the control group (Fig. 4). 11 of the 26 Negro patients had β/α ratios within the normal range or above. The mean β/α ratio of the Negro group was 0.84$\pm$0.21 (1 SD).

The β/α ratios in the five patients with homozygous β-thalassemia ranged from 0.13 to 0.25, values similar to those found in Caucasians. Studies of globin synthesis in the bone marrow of two of these patients (family "H", daughters 1 and 2) have been previously reported (7) and were also found to be similar to those in Caucasian patients.

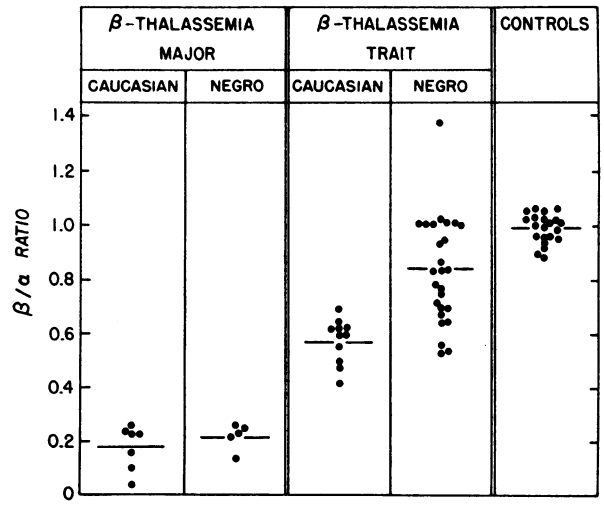

FIGURE 4 β/α Ratios in the five groups studied. The mean of each group is indicated by a horizontal line.

Peripheral blood and bone marrow studies were done on one Negro heterozygote (family "S", I-8). The β/α ratio in the peripheral blood was 1.03, and that in the bone marrow was 1.13. There was 22 times as much radioactivity incorporated in the bone marrow sample as in the same volume of peripheral blood. There were adequate iron stores in the marrow.

Studies of three Negro patients were repeated on a second occasion to test the constancy of the unusual findings in this group. The results using the same methods have been found to be highly reproducible in our laboratory and by others (17). The paired results in patients I-2 and I-8 from family "S" and the mother from family "P" were 1.38 and 1.36, 1.03 and 1.02, and 0.69 and 0.68, respectively. The second study in the first two patients was done 2 yr after the first study, while the interval for the third patient was 2 mo. These results indicate that the β/α ratios in these patients, whether high, normal, or low, did not vary with time. Two chromatographic studies are shown in Fig. 5.

Several patients in families S and M were found to have Hb A_2', a δ-chain variant found commonly in Negroes (20) (Figs. 1, 2). The Hb A_2 values given for these patients (Table I) are the sum of the Hb A_2 and Hb A_2' bands on starch block electrophoresis.

DISCUSSION

β-Thalassemia is an inherited disorder of hemoglobin synthesis found in many ethnic groups, including Italians, Greeks, Turks, Arabs, Indians, Southeast Asians, and Chinese. The most common heterozygous form of this disorder is associated with hypochromia, microcytosis, an elevated red cell count, and a high level of Hb A_2. In many of the patients with elevated Hb A_2, there is also a mild elevation of Hb F. Although the heterozygotes are usually asymptomatic, they may have mild anemia. The clinical features of β-thalassemia trait in the Negro do not seem to differ from those seen in other ethnic groups. The mean Hb A_2 level in the 24 Negro heterozygotes in this study was 5.5%, a value similar to those of Negro heterozygotes (5.1%) previously reported (3). The mean Hb F level (2.3%) and the range of Hb F are also similar to those previously reported (3).

Homozygotes for β-thalassemia in the non-Negro groups usually have severe clinical disease which starts in the 1st yr of life, requires frequent blood transfusions and results in death during or before the third decade of life. In contrast, most Negro homozygotes have a relatively mild disease, frequently live to the fourth decade and beyond and occasionally give birth with uneventful pregnancies. Although some of the patients

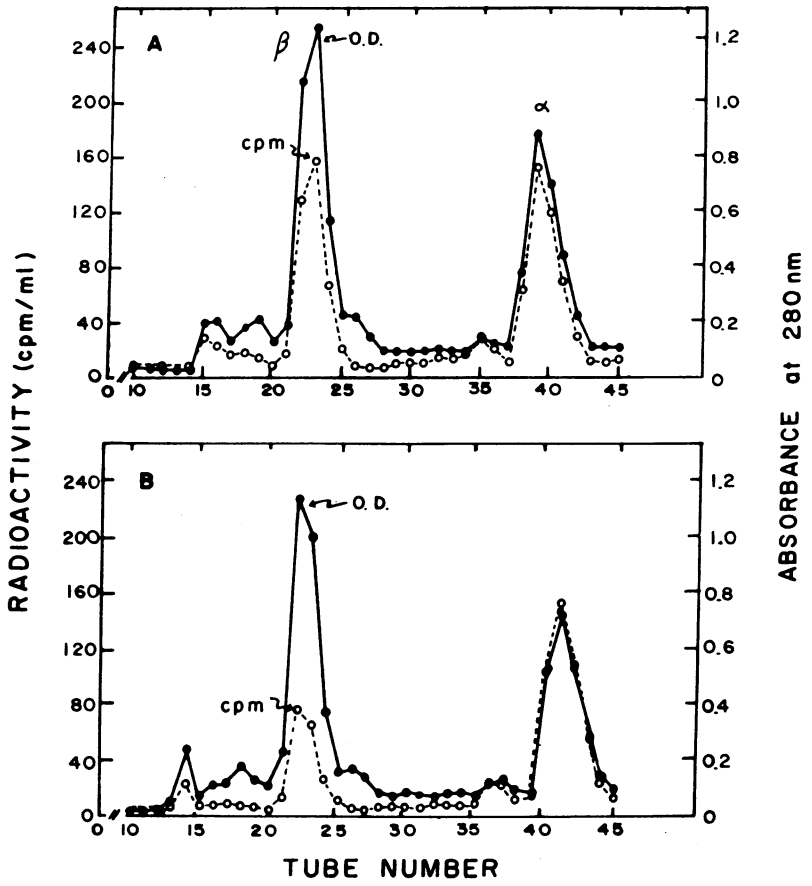

FIGURE 5 Chromatograms of globin from patients I-8 (A) and II-5 (B) in family S. The β/α specific activity ratios in these Negro patients with high Hb A_2 are 1.02 (A) and 0.52 (B). The first patient was studied on separate occasions 2 yr apart, with similar results each time.

do not need regular transfusions, others may develop symptoms of severe anemia in the third or fourth decade necessitating the initiation of a regular transfusion program (21). The three Negro adult patients reported here were well until they were approximately 20 yr old, when decreasing hemoglobin levels necessitated transfusion therapy on a regular basis. The prognosis of young Negro children with homozygous β-thalassemia is therefore guarded, despite relative well-being earlier in life. The reason for the generally milder clinical disease in Negro patients and the deterioration in some of them as they grow older is not known.

In normal persons there is equal synthesis of α- and β-chains in peripheral blood reticulocytes. In Caucasians with heterozygous β-thalassemia, the synthesis of β-chain is approximately one-half that of α-chain in the peripheral blood (15–17). The results of peripheral blood studies in the Negro heterozygotes reported here differ markedly from those in Caucasians. Reports of unexpected results in Negro heterozygotes have also recently appeared in abstracts (22, 23). Only 58% of the Negro patients in our studies had deficient β-chain synthesis, whereas the remainder were in the normal range (mean ± 2 SD) or above. These two groups of Negro heterozygotes could not be distinguished by differences in hemoglobin concentrations, red cell indices, Hb A_2 or Hb F levels. Repetition of the globin synthesis studies in three patients with high, normal, or low β/α ratios, as well as in other patients studied in our laboratory, indicates that the results are reproducible and are characteristic of globin synthesis in any patient.

An analysis of the pedigrees shows the distribution of different globin synthesis ratios within individual families. Both types of patients with β-thalassemia trait (low β/α ratio, normal β/α ratio) are found in each of three families (Figs. 1–3). In another family (H) all high Hb A_2 heterozygotes had globin synthesis ratios within the range of normal controls, although in a fifth family

(N) both heterozygotes had low β/α ratios (Table I). In family "S", patient 1–8 had a normal β/α ratio, whereas her daughter (II-5) and granddaughter (III-3) had ratios of approximately 0.5 (Fig. 1). These findings could possibly be due to the presence of both α-thalassemia and β-thalassemia in patient I-8, with a lack of transmission of α-thalassemia to her daughter. In both Italian and Chinese patients, doubly heterozygous for α- and β-thalassemia, the β/α ratios have ranged from 0.95–1.03 (17). Further analysis of family "S" indicates that unusual ratios were most probably not due to the modifying influence of α-thalassemia. Patient I-2 with β-thalassemia trait had a high ratio of 1.38, whereas her two daughters (II-1, II-2) with homozygous β-thalassemia had β/α ratios of 0.22 and 0.25, in the range of those found in Caucasians with Cooley's anemia (15–19) (Fig. 4). The daughter (III-1) and granddaughter (IV-1) of one of the homozygous patients (II-2) had balanced globin synthesis. In an Italian patient with homozygous β-thalassemia and heterozygous α-thalassemia and a relatively mild clinical disorder, the β/α ratio was 0.45, indicating the modifying effect of the α-thalassemia gene on the expression of the two β-thalassemic genes (17). The globin synthesis ratios in the two Negro homozygotes in family "S" show no evidence of a modifying effect of an α-thalassemia gene. The daughter (III-1) of one homozygote inherited a gene for β-thalassemia from her mother but did not inherit α-thalassemia from either her mother ($\beta/\alpha = 0.22$) or father ($\beta/\alpha = 0.95$). The normal globin synthesis ratios in patients III-1 and IV-1 thus are not due to the presence of α-thalassemia. Indeed, it would be highly unlikely to find α-thalassemia trait in 42% of the β-thalassemia heterozygotes in this study, when the incidence of α-thalassemia in the Negro is approximately 2% (24). No patients with simple α-thalassemia trait were found in any of the Negro families included in this study. The presence of an interacting gene for the silent carrier state of α-thalassemia in some of the patients could not be ruled out by these studies.

Itano (25) has postulated the existence of several normal globin chain alleles which differ in their synthetic capacities. The presence of a hyperactive β-chain allele in the trans position to the thalassemic gene in some heterozygous patients might explain the occurrence of balanced globin synthesis ratios in these patients. A single gene of this type does not seem to have been present in the two largest families studied (families S and M). In both of these families there is an abnormal δ-chain in some individuals which serves as a marker for the $\delta\beta$-loci. Patients I-2 and I-5 in family "S" (Fig. 1) had the abnormal δ-chain and unusually high β/α ratios for β-thalassemia, whereas siblings I-3, I-4, and I-6 had low β/α ratios and also possessed the abnormal δ-allele. In family "M" (Fig. 2) one sibling with an abnormal δ-chain (III-2) had a β/α ratio of 0.53, whereas a sister with the same δ-variant (III-3) had a ratio of 1.14. It is thus unlikely that a "super-gene" produced synthesis in some of the Negro patients, since the β- and δ-loci are closely linked (26), and the factor producing balanced synthesis is not linked to the δ-locus.

A recent study of α-thalassemia in the Negro concludes that there is a much wider range of α/β synthesis ratios than in Caucasian patients (27). The values in the Negroes overlap the normal range, and the average ratio indicates less imbalance of globin synthesis than in the Caucasian group. The results of the studies reported in this paper demonstrate a similar finding in β-thalassemia trait in the Negro. The mean β/α ratio is higher than in Caucasians, and many patients have ratios in the normal range.

Although Caucasian heterozygotes for β-thalassemia have a relative decrease in synthesis of β-chains in their peripheral blood, β- and α-chain synthesis are equal in the bone marrow (28). The presence of balanced globin chain synthesis in the bone marrow suggests that compensatory β-chain synthesis for the thalassemic defect is present in the nucleated red cells of some patients. The possibility that decreased β-chain synthesis was present in the bone marrow but not in the peripheral blood of the Negro heterozygotes with unusually high ratios was tested in one patient (family S, I-8). Unbalanced globin synthesis was not detected in either bone marrow or peripheral blood of this patient, the β/α ratios being 1.13 and 1.03, respectively. It has been suggested that the difference previously observed in globin synthesis between bone marrow and peripheral blood cells in heterozygotes might be due to the increased rate of breakdown in the red cell of messenger RNA for β-chain (28) either from the thalassemic or the normal β-chain locus. The factors responsible for balanced globin synthesis in some of the Negro heterozygotes may act by protecting the messenger RNA for β-chain from an excessive rate of decay. In heterozygotes with decreased mean cell hemoglobin values and either balanced or unbalanced globin synthesis in the peripheral blood, the total amount of α-chain per cell is decreased as well as the amount of β-chain suggesting that there may be depression of synthesis of α-chain in the bone marrow in some patients as part of the compensatory changes occurring in the nucleated erythrocyte.

The difference in severity of clinical disease in the Negro and Caucasian homozygotes is not reflected in differences of peripheral blood or bone marrow (7) β/α ratios. Further studies are needed to clarify the relationship of the biochemical differences in the heterozygotes to the clinical differences in the homozygotes.

ACKNOWLEDGMENTS

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