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23. The authors are indebted to the Medical Research Council of Canada and the Canadian Cystic Fibrosis Foundation for financial support, to Dr. David Kells of the Department of Biochemistry, University of Toronto, for performing analytic ultracentrifugation studies, and to Mrs. Cecilia Feng for technical assistance.
24. Requests for reprints should be addressed to: J. F. Forstner, M.D., Research Institute, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario M5G 1X8 (Canada).
25. Accepted for publication December 18, 1975.

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Pediat. Res. *10:* 613-620 (1976)

Erythrocytes sickle cell anemia
hemoglobin α-thalassemia

Sickle Cell Syndromes. I. Hemoglobin SC-α-Thalassemia

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Extract

Hematologic and globin synthesis studies were performed in a black American family in which the genes for α-thalassemia and hemoglobins (Hb) S and C were segregating. The following distribution of these abnormalities was found: father, sickle cell trait + α-thalassemia; mother, HbC trait + α-thalassemia, propositus, HbSC + α-thalassemia; older sibling, α-thalassemia trait; and younger sibling, hemoglobin H disease.

The child with HbSC-α-thalassemia demonstrated more severe anemia and a more hemolytic picture than is typical of HbSC disease. Her erythrocytes exhibited decreased osmotic fragility in comparison with HbSC erythrocytes, but had an indistinguishable oxygen equilibrium curve and 2,3-diphosphoglycerate (2,3-DPG) level. Erythrocyte sickling in the patient, however, was significantly reduced, with less than 35% sickle forms observed at nearly complete oxygen desaturation.

The sibling with hemoglobin H disease exhibited 26% Bart's (γ_4) hemoglobin at birth, a level comparable with that seen in infants with HbH disease in Far Eastern populations. At age 5 months typical findings of mild hemoglobin H disease appeared, with HbH making up 6.5% of the total hemoglobin.

Speculation

The presence of α-thalassemia in the proband of this study appeared to modify her HbSC disease so as to reduce its clinical severity as well as its pathologic potential. The ameliorative effect of α-thalassemia would appear to be related to a reduction in the intracellular hemoglobin concentration in the patient's erythrocytes, but other factors may also be responsible for these changes. Further study of genetically modified sickle hemoglobinopathy syndromes may ultimately aid in the development of effective means for the treatment of these disorders.

The clinical expression of sickling disorders may vary widely, even among individuals having an apparently identical form of sickle cell disease. This variability has stimulated investigation of the role of genetic as well as nongenetic factors in the hematologic

and clinical expression of the sickle hemoglobinopathies. In a number of atypical sickling disorders specific modifying factors have been identified that seem to alter the ability of the erythrocyte to undergo sickle transformation. Other genetic traits appear to modify the clinical expression of sickling disorders by different, but thus far unidentified mechanisms.

Abnormalities that have a genetic basis and are known to modify sickle cell diseases include: associated hemoglobin α-chain (19, 40) and β-chain (4, 23, 24) structural abnormalities, thalassemias and thalassemia-like disorders (1, 28, 35, 46); variability in the production of fetal hemoglobin (2, 17); and the presence of other associated hematologic disorders (22, 38).

In this report we describe the clinical and hematologic findings of a child having an apparently modified form of hemoglobin SC disease related to the association of α-thalassemia.

METHODS

HEMATOLOGIC AND HEMOGLOBIN STUDIES

Hematologic measurements were made with a Coulter model S electronic cell counter which was standardized daily using a commercial standard. Other determinations were performed by standard methods (5). Osmotic fragility tests were carried out with fresh samples of defibrinated blood as described by Dacie (8).

Blood samples for hemoglobin analysis were collected in EDTA. The erythrocytes were isolated by centrifugation, washed three times in isotonic saline, and lysed with 2–3 volumes of water without the addition of an organic solvent. Stroma-free lysates were prepared and subjected to electrophoresis on cellulose-acetate strips in Tris-EDTA-borate buffer at pH 8.6. For quantitative estimation of individual hemoglobins, the bands were excised after electrophoresis and the hemoglobins eluted and measured as previously described (15). Each indicated value represents the mean of determinations done at least in triplicate. Alkali-resistant hemoglobin was estimated according to the procedure of Betke *et al.* (3). DEAE-Sephadex column chromatography of carbon monoxide-saturated hemoglobins was performed as described by Dozy and coworkers (9).

GLOBIN SYNTHESIS STUDIES

Blood samples obtained for the globin synthesis determinations were collected in heparin and kept at ice temperature before the incubations. Washed cells, 1.0-ml packed cell volume, were incubated for 2 4 hr in medium containing 10 μCi of L-[^{14}C]leucine (specific activity 250 mCi/mmol). The incubation procedure was as previously described (16). Globin chromatography on carboxymethyl cellulose columns was performed by the method of Clegg and coworkers (7). Equal aliquots of the chromatographic effluent fractions were taken for determinations of incorporated radioactivity (16); $\alpha/\beta + \gamma$ radioactivity ratios were calculated using the total incorporated radioactivity recovered from the chromatographic fractions corresponding to each of the globin chains.

OXYGEN AFFINITY AND SICKLING STUDIES

Oxygen equilibrium curves were determined with a model 217 blood gas analyzer from Instrumentation Laboratories Inc., Lexington, Mass. Blood samples were equilibrated in the tonometer with varying mixtures of 5.60% CO_2 in air and 5.60% CO_2 in nitrogen. After equilibration with each gas mixture, aliquots of the blood were withdrawn for measurement of the pO_2, pH, and percentage of oxygen saturation. A normal alkaline Bohr factor was applied whenever necessary for correction of pO_2 values to correspond to pH 7.40.

Determinations of 2,3-DPG were performed using protein-free trichloroacetic acid filtrates prepared from whole blood samples. A spectrophotometric assay procedure was employed as described by Keitt (18).

For enumeration of the percentages of sickled erythrocytes in relation to oxygen saturation, aliquots of equilibrated blood samples also were drawn into syringes containing 5% formalin and 0.01 M Na_2HPO_4 in isotonic saline. The formalin-fixed erythrocytes were examined in a hemocytometer counting chamber and classified according to the criteria of Rampling and Sirs (31). For each sample, 1,000 cells were counted.

All clinical studies were carried out with informed consent according to the provisions of the Declaration of Helsinki.

RESULTS

CASE REPORTS

The patient, *RC*, a 7-year-old girl, was referred for hematologic evaluation in preparation for an adenoidectomy. She had had multiple episodes of otitis media, and had developed severe nasopharyngeal obstruction with persistant mouth-breathing. She was noted to be anemic, and was found from a hemoglobin electrophoresis study to have hemoglobins S and C. Apart from the problems attributable to her tonsillar hypertrophy, the child had generally been healthy. She had never been noted to have jaundice, fatigue, pain crisis, or other clinical manifestation of sickle cell disease. She appeared somewhat pale and her spleen was enlarged extending 2 cm below the costal margin.

After completion of the hematologic evaluation the child was given a transfusion of packed red cells sufficient to increase her blood hemoglobin concentration to 11.0 g/dl. An adenoidectomy under general anesthesia was performed without complication, and her recovery and subsequent course were uneventful.

The parents of the child and her 9-year-old sister had been in good health, and had not been known to have anemia or other hematologic abnormality. During the time that the evaluation of the patient was in progress, her mother gave birth to a full term infant after an uneventful pregnancy. The infant did well during the neonatal period and showed no evidence of anemia or jaundice. Her subsequent course was without apparent complication. Hematologic studies of the infant were performed at birth and subsequently at the age of 5 months.

A family pedigree, indicating the diagnosis ultimately established for each of the family members, is shown in Figure 1.

HEMATOLOGIC AND HEMOGLOBIN FINDINGS

The results of hematologic studies of the patient and her family are presented in Table 1. The patient (*II-2*) was moderately anemic with a persistently elevated reticulocyte count. Her mean corpuscular volume (MCV) and hemoglobin (MCH) indices were markedly reduced in spite of a normal serum iron level and total iron-binding capacity. Her blood smear (Fig. 2*C*) demonstrated many of the features typical of hemoglobin SC disease erythrocytes, including the presence of target cells and "safety-pin" forms. The cells appeared more flattened, however, and thin, symmetrical-appearing target forms made up a majority of

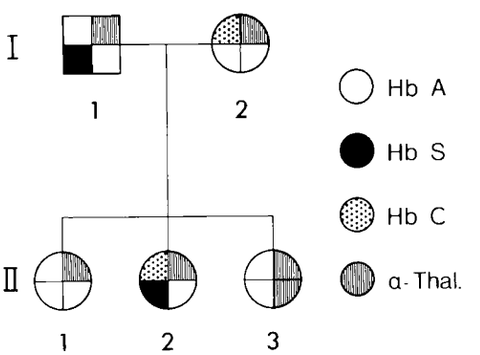

Fig. 1. Pedigree diagram of the family indicating hemoglobin findings. The proband is represented as *II-2*. *α-Thal.*: α-thalassemia; *Hb:* hemoglobin.

Table 1. *Hematologic values*[1]

Pedigree no.	Age, yr	Hemoglobin, g/dl	Packed cell volume, %	RBC/μl, $\times 10^{-6}$	MCV, fl	MCH, pg	MCHC, %	Reticulocytes, %	Serum iron, μg/dl	Total iron binding capacity, μg/dl
I-1	28	13.4	40.2	5.67	71	23.8	33.6	1.6	65	297
I-2	28	9.1	28.6	5.01	57	18.1	31.6	4.0	109	358
II-1	9	11.0	31.1	4.27	73	25.8	35.2	0.3	59	398
II-2	7	7.9	25.8	4.91	53	16.2	30.6	7.2	98	302
II-3	Newborn	16.9	57.1	6.91	83	24.3	29.5	11.2		
	5 months	8.5	26.5	5.05	53	16.6	31.5	4.6		

[1] MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

the erythrocytes seen in stained smears of her blood. Contracted, densely staining cells that resembled spherocytes were also regularly seen, but sickle forms were never observed.

Hemoglobin electrophoresis of stroma-free hemolysates from the patient demonstrated approximately equal quantities of hemoglobins S and C (Table 2). There was no significant elevation of the level of her alkali-resistant hemoglobin.

Both parents of the patient and her older sister (*II-1*) also were mildly anemic with microcytic erythrocyte indices in the absence of demonstrable iron deficiency. Blood smears from the father (Fig. 2*A*) and the sister (*II-1*) demonstrated microcytosis but generally unremarkable erythrocyte morphology otherwise. The blood smear of the mother showed anisocytosis, poikilocytosis, target and "pencil" forms, and "safety-pin" cells (Fig. 2*B*).

Electrophoresis of hemoglobin from the father demonstrated an AS pattern with HbS representing 26.0% of the total. His HbA_2 and alkali-resistant hemoglobin fractions were normal (Table 2). The mother's hemoglobin electrophoresis study demonstrated hemoglobins A and C. Hemoglobins A_2 + C made up 24% of her total hemoglobin; her alkali-resistant fraction was also within the normal range. The older sister's hemoglobin electrophoresis pattern was normal, as were the levels of HbA_2 and alkali-resistant hemoglobin.

Freshly drawn blood samples from the patient, her parents, and her older sister were incubated with brilliant cresyl blue, and a careful search was made for erythrocyte inclusion bodies, but none were identified.

The patient's younger sister (*II-3*), who was born during the course of the family study, was not anemic at birth (Table 1), but her MCV and MCH indices were significantly reduced (normal values at birth: MCV 104–118 fl; MCH 33.5–41.4 pg). When she was restudied at age 5 months she was found to have anemia with an elevated reticulocyte count and microcytosis. Her stained blood smear (Fig. 2*D*) demonstrated hypochromia, anisocytosis, poikilocytosis, and many target cells.

Hemoglobin studies of the infant were performed at birth and were repeated at age 5 months, according to the methods described above. In addition, stroma-free hemolysates were prepared from her washed erythrocytes and the hemoglobin was subjected to DEAE-Sephadex column chromatography. The chromatographic analysis at birth (Fig. 3) demonstrated peaks of HbA and HbF which accounted for 23.0% and 50.6%, respectively, of the total hemoglobin. These peaks were followed by a late-eluting peak that contained 26.3% of the recovered hemoglobin. This hemoglobin fraction exhibited a more rapid electrophoretic mobility at pH 8.6 than that of HbA, and on further analysis was found to consist exclusively of hemoglobin γ-chains. A similar study done when the infant was 5 months old demonstrated less than 19% HbF, with HbA making up 83.1% of the total. The late-eluting fraction was considerably smaller, representing 8.0% of the recovered hemoglobin; this fraction contained no α-chain globin and consisted mainly of hemoglobin β chains.

When blood obtained from the infant at birth was incubated with brilliant cresyl blue for 2 hr and a careful search made for erythrocyte inclusion bodies, none were found. A similar study performed when the infant was 5 months old demonstrated large numbers of typical hemoglobin H erythrocyte inclusions.

GLOBIN SYNTHESIS STUDIES

To obtain a quantitative assessment of the balance between the synthesis of α and non-α-globin proteins, globin synthesis studies were performed with peripheral blood red cells from each of the family members.

Globin synthesis by blood reticulocytes from the mother (*I-2*) was significantly deficient in α chain synthesis as compared with the synthesis of β chains (Fig. 4). The globin chromatogram from this study also demonstrated a relatively small quantity of β^C globin in relation to that of β^A recovered from the chromatography column. This finding substantiates the relatively small percentage of HbC (24%) that was found in the mother's blood, as compared with the range of 35–45% HbC that is usually seen in hemolysates of hemoglobin C-trait individuals. A very similar relationship was seen in the globin synthesis study of the father with regard to the synthesis of the α and non-α-globins, as well as the quantitative representation of the β chains of hemoglobins A and S.

The globin synthesis study performed with blood from the patient (*II-2*) (Fig. 5) also demonstrated a relative deficiency of L-[^{14}C]leucine incorporation into the α-globin fraction as compared with the incorporation into β^S + β^C. The globin synthesis study of the infant (*II-3*) demonstrated a reduced synthesis of α chains as compared with that of the β^A + γ chains, both when she was studied at birth and at 5 months of age.

The α/β + γ synthesis ratios from each of the family members are shown in Table 2. In every case the ratio was significantly less than those of nonthalassemic control subjects (1.04 ± 0.05). From these findings, in the absence of demonstrable iron deficiency, we conclude that each member of the family had α-thalassemia. These results, taken together with the other hematologic and hemoglobin findings described above, formed the basis for the diagnoses indicated in Figure 1.

SPECIAL HEMATOLOGIC STUDIES

Osmotic fragility was determined with freshly prepared erythrocytes from the patient, and compared with similarly prepared samples from normal subjects and from patients with HbSC disease (Fig. 6). The patient's cells exhibited decreased osmotic fragility as compared to that of the HbSC erythrocytes, which in turn showed decreased osmotic fragility relative to the normal control subjects.

The oxygen equilibrium curve of fresh whole blood from the patient (Fig. 7) was shifted to the right of the normal curve, with the pO_2 at 50% oxygen saturation being approximately 6 mm Hg higher than that of the normal. Her blood oxygen equilibrium curve did not differ significantly, however, from those obtained in

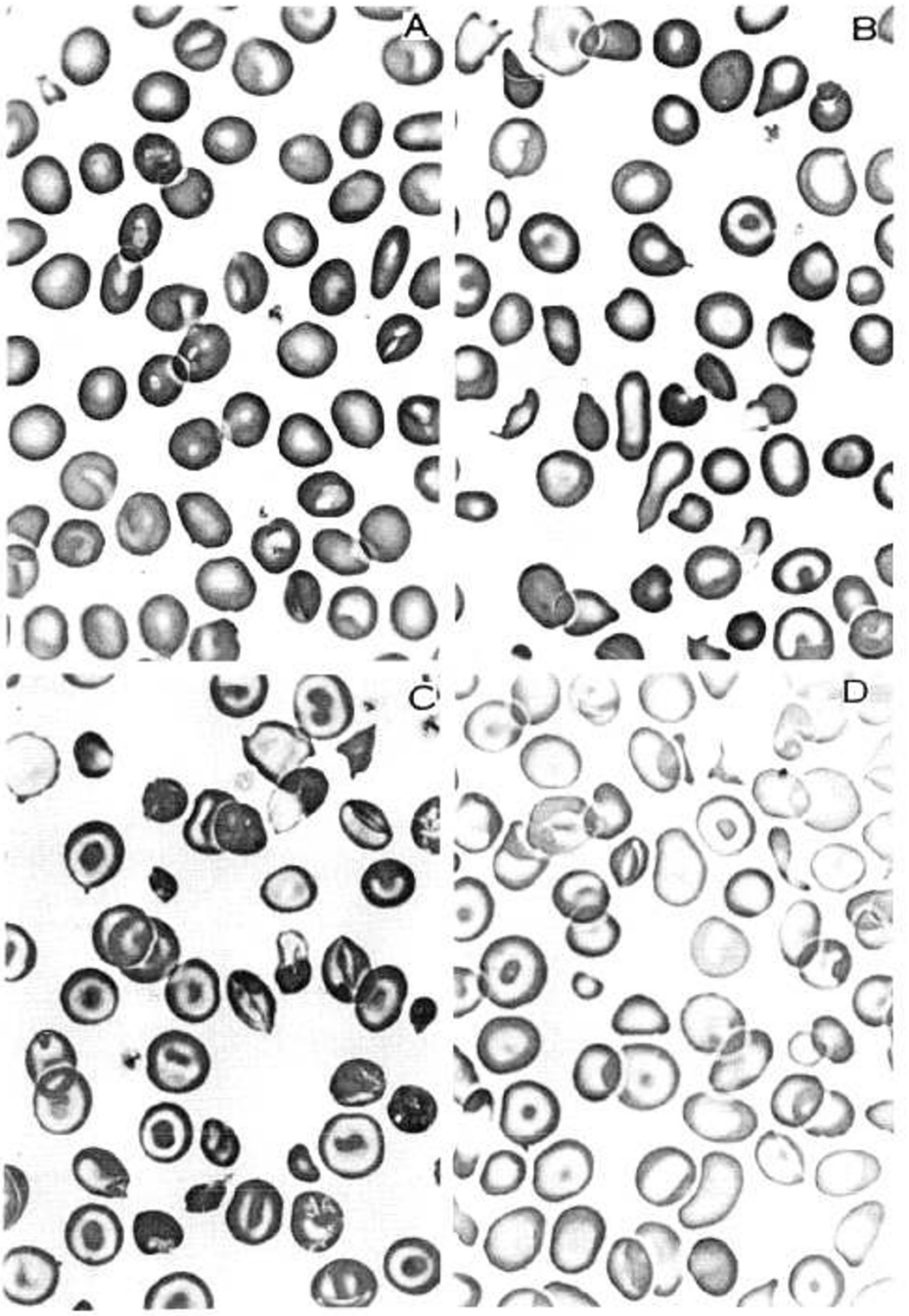

Fig. 2. Photomicrographs of peripheral blood smears of selected family members. *A: I-1* (AS-α-thalassemia); *B: I-2* (AC-α-thalassemia); *C: II-2* (SC-α-thalassemia); *D: II-3* (hemoglobin H disease).

Table 2. *Hemoglobin values and results of globin synthesis studies*

Pedigree no.	Age, yr	HbA, %	HbS, %	HbC, %	HbA_2, %	Alkali-resistant hemoglobin, %	$Hb\gamma_4$, %	$Hb\beta_4$, %	$\frac{\alpha}{\beta + \gamma}$ (L-[^{14}C]Leu)
I-1	28	69.1	26.9		3.5	0.5			0.68
I-2	28	75.0		24.0		1.0			0.77
II-1	9	97.5			2.3	0.2			0.80
II-2	7		50.5	47.7		1.8			0.68
II-3	Newborn	23.0			0.1	50.6	26.3		0.61
	5 months	83.1			0.7	9.7	1.5	6.5	0.69

experiments performed in a similar manner with blood samples from five patients with HbSC disease. The level of 2,3-DPG in fresh blood from the patient was 18.5 ± 1.1 μmol/g of hemoglobin. The level of 2,3-DPG in a group of HbSC patients was 16.9 ± 1.9 μmol/g Hb, and in a group of normal controls was 15.0 ± 2.4 μmol/g Hb.

In an effort to evaluate the sickling ability of the patient's erythrocytes, samples of her blood were equilibrated with gas mixtures of varying oxygen content to achieve a range of oxygen saturation values, and at each point the number of sickled cells present was determined. The results of this study are shown in Figure 8 together with those obtained with blood samples from five patients with HbSC disease. At oxygen saturation levels of greater than 50% both the patient and the control subjects exhibited less than 20% of sickle cell forms. At saturation levels of less than 50%, the HbSC blood samples showed a nearly linear increase in the percentage of sickle cells as the oxygen saturation decreased, and reached more than 90% as total desaturation was approached. At oxygen saturation levels below 30%, the percentages of sickle cells in the HbSC-α thalassemia patient's blood were significantly less than those at corresponding oxygen saturation levels in the control subjects. Moreover, at the lowest oxygen saturation level achieved (about 4%) the patient's blood contained less than 35% sickle cell forms.

DISCUSSION

Several examples have previously been described of α-thalassemia occurring in combination with abnormal hemoglobins with β-chain substitutions. These have included hemoglobins S (39, 46), C (36), E (43), and J (41). To our knowledge the patient described in this report represents the first known example of α-thalassemia occurring in combination with hemoglobins S and C.

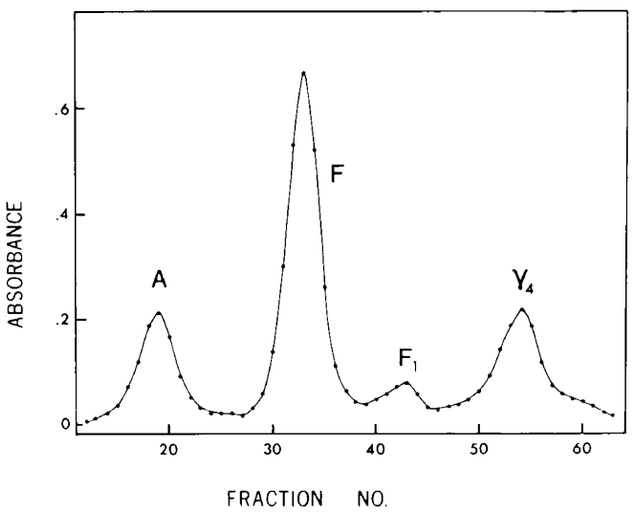

Fig. 3. DEAE-Sephadex chromatography of hemoglobin prepared from cord blood erythrocytes of *II-3* (hemoglobin H disease).

The asymptomatic clinical course of this patient may have been related to an ameliorative effect of the α-thalassemia on her hemoglobinopathy; however, it has not been uncommon in our own experience for children with HbSC disease to be free of any symptom attributable to sickle cell disease over a period of many years of observation. On the other hand, this patient demonstrated a more severe degree of anemia than we have usually encountered in children with HbSC disease, and her persistently elevated reticulocyte counts suggest a more hemolytic process than is typical of hemoglobin SC disease.

The oxygen equilibrium curve of the patient's blood was indistinguishable from that seen in HbSC disease, indicating a comparable degree of hemoglobin oxygen saturation in the two conditions at the same partial pressure of oxygen. However a direct enumeration of the numbers of sickled cells at varying oxygen tensions demonstrated a significantly reduced ability of the patient's erythrocytes to undergo sickle transformation. The apparent beneficial effect of the α-thalassemia in this patient is consistent with previous observations (39, 46) that α-thalassemia reduced the severity of the clinical course of patients with sickle cell anemia. The reduced ability of the child's erythrocytes to sickle may be related to the diminished concentration of hemoglobin in her cells, in accordance with the observation of Seakins *et al.* (33) that sickling is strongly influenced by the intracellular concentration of sickle hemoglobin. It is difficult to provide an explanation for the apparently more severe hemolytic process in this child in the presence of diminished erythrocyte sickling. Conceivably, the increased hemolysis is primarily related to some other cause.

From available evidence, α-thalassemia appears to occur with high frequency in American blacks. Cord blood electrophoresis studies of black infants have suggested an incidence of α-thalassemia trait of between 2 and 7% (12, 25, 44). These studies all were based on the association between α-thalassemia and the presence of elevated levels (greater than 2%) of Bart's (γ_4) hemoglobin detected at or near the time of birth. Although an increased level of Bart's hemoglobin in the neonatal period has been shown to correlate well with the presence of α-thalassemia, both by hematologic and globin biosynthesis criteria (12), recent studies by Esan (10, 11) did not support an association of this kind in black infants in Nigeria. Most importantly, the author observed several pairs of monozygotic twins in which only one twin had an elevated level of Bart's hemoglobin. A similar example of hemoglobin Bart's disparity in identical twins was described by Pembrey and coworkers (30) from a study of a Saudi Arabian population. These observations suggest that at least in some cases elevated levels of Bart's hemoglobin at birth may be related to other, nongenetic factors.

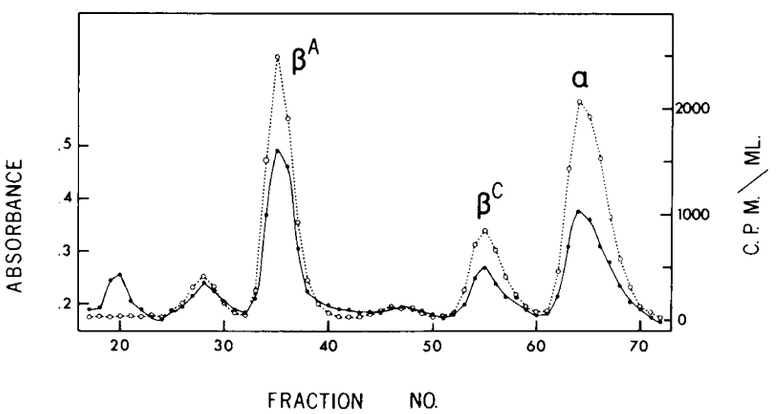

Fig. 4. Chromatogram of globin from red cells of *I-2* (Ac-α-thalassemia). ●——●: absorbance at 280 nm; O· · · O: incorporated L-[^{14}C]leucine.

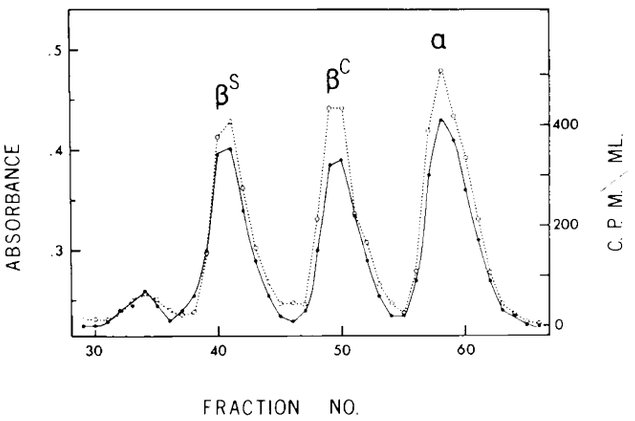

Fig. 5. Chromatogram of globin from red cells of the proband, *II-2* (SC-α-thalassemia). ●——●: absorbance at 280 nm; O· · · ·O: incorporated L-[^{14}C]leucine.

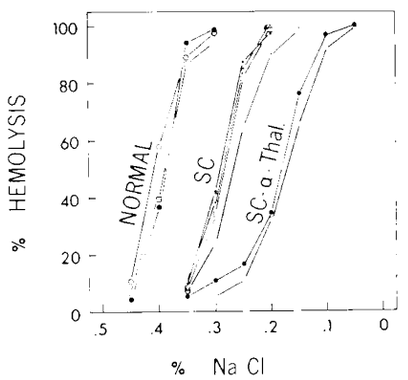

Fig. 6. Osmotic fragility of fresh blood samples from normal controls, subjects with hemoglobin SC disease, and the patient with hemoglobin SC-α-thalassemia. The results shown from the patient represent separate determinations performed several weeks apart.

Beyond the neonatal period heterozygous forms of α-thalassemia are particularly difficult to identify, and consequently few attempts have been made to determine the frequency of this disorder in older children and adults. Whereas elevated levels of hemoglobin A_2 and F serve to identify most heterozygous forms of β-thalassemia, these hemoglobins are characteristically present in normal amounts in α-thalassemia heterozygotes. Even microcytosis, a feature shared by most forms of thalassemia, seems not to be reliable criterion for the identification of α-thalassemia trait. In a Greek population individuals with hematologic and genetic features of α-thalassemia consistently had reduced MCV values (29), but studies of black infants (12), children (6), and adults (37) with α-thalassemia showed that the MCV indices may overlap considerably with the range of normal values.

When α-thalassemia occurs in combination with sickle cell trait or HbC trait, however, the percentage of HbS or HbC becomes significantly reduced in comparison with the usual amounts (35–45%) seen in heterozygous individuals (6, 37). In the absence of iron deficiency (21) or megaloblastic disease (13), this finding provides a reliable means for establishing a diagnosis of α-thalassemia in adult life. The lower than usual percentages of hemoglobins S and C found in the parents of the proband in this study provide further confirmation of this relationship, and together with the globin synthesis determinations established in both of them the presence of α-thalassemia.

The proband, with HbSC-α thalassemia, apparently inherited the gene for hemoglobin S from her father, hemoglobin C from her mother, and α-thalassemia from one of her parents. The older sister (*II-1*) derived a normal β-chain gene from her mother as well as from her father, and α-thalassemia from one parent. The hematologic findings of the proband, the older sister, and both of her parents are characteristic of α-thalassemia trait, although certain differences are apparent that can be related to the association with the different hemoglobin β-chain abnormalities present in each of them. The hematologic findings of the infant (*II-3*), particularly from the study performed when she was 5 months of age, are diagnostic of hemoglobin H disease, a more severe form of α-thalassemia.

Hemoglobin H disease is characterized by anemia, microcytosis, and erythrocyte inclusions, and represents an α-thalassemia abnormality in which the α-chain biosynthetic defect is of sufficient magnitude to produce a gross imbalance between α-chain and β-chain synthesis, with the formation of measurable quantities of β-chain tetramer molecules (hemoglobin H) and intracellular precipitates composed of β-chain protein. This form of α-thalassemia has been identified in many parts of the world with the highest frequencies reported in Mediterranean, Middle Eastern, and South East Asian populations (45); it appears to occur quite uncommonly in black populations in Africa and in the United States. Hemoglobin H disease in American blacks has been studied in detail by Schwartz and Atwater (32), who found that although the disorder shared most of the features of hemoglobin H

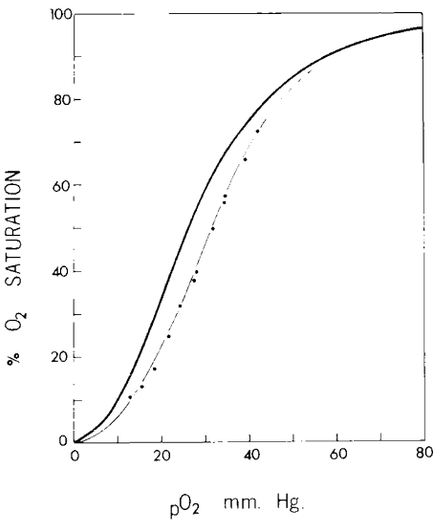

Fig. 7. Oxygen equilibrium curve of fresh whole blood from the SC-α-thalassemia patient. The determinations were performed at 37°, pH 7.4. The solid line (——) represents the whole blood curve of normal hemoglobin A subjects.

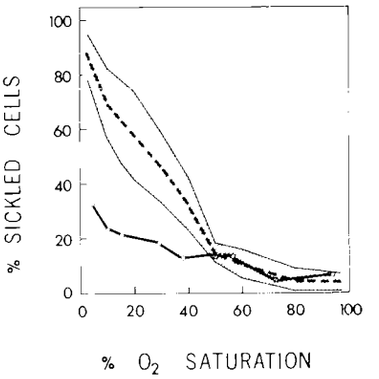

Fig. 8. Sickling of the SC-α-thalassemia patient's erythrocytes in relation to oxygen saturation of the blood. O——O: results obtained with blood from the patient. The *shaded area* indicates the mean and standard deviation of the curves obtained from five subjects with hemoglobin SC disease.

disease as it occurs in other populations, it is a significantly milder condition, both clinically and in terms of the α-chain biosynthetic defect, as determined by globin synthesis studies *in vitro*.

In Far Eastern populations in which α-thalassemia occurs with high frequency, four hematologically distinct forms of α-thalassemia have been identified. These include a "silent-carrier" state, characterized by mild microcytosis but otherwise normal findings; α-thalassemia trait, a condition characterized by abnormal erythrocyte morphology and hematologic features of mild thalassemia; hemoglobin H disease; and α-thalassemia hydrops fetalis, a disorder that has been uniformly fatal in the newborn period and is accompanied by severe anemia and a total absence of hemoglobins A and F, with Bart's hemoglobin and HbH making up all of the hemoglobin present in the blood (45). Family studies of individuals with hemoglobin H disease in these populations (42) have shown that in most instances one parent has α-thalassemia trait and the other the α-thalassemia silent carrier state. On the other hand, the parents of infants with the hydrops fetalis form of α-thalassemia both were shown to have the hematologic features of α-thalassemia trait (41).

These observations led to the conclusion (41) that two kinds of α-thalassemia genes exist in Far Eastern populations: a severe gene (α-thal$_1$) and one causing a lesser degree of impairment of α-chain synthesis (α-thal$_2$). The hydrops fetalis syndrome was envisioned as representing the homozygous form of α-thal$_1$. Inasmuch as infants having the hydrops fetalis form of α-thalassemia have been shown to be totally lacking the gene for hemoglobin α-chains (27), it follows that the α-thal$_1$ gene is an α^0 gene with no discernable gene product. The form of HbH disease seen in this population is accordingly thought to represent double heterozygosity for α-thal$_1$ and α-thal$_2$ (27), the latter being an α^+ gene that produces only partial suppression of α-chain synthesis.

The foregoing interpretation of genetic information was based on a single-locus model which assumes the existence of single pair of α-chain genes. Recent evidence has been presented in support of the existence of two pairs of α-chain loci (14), at least in some populations, and using a four gene model, Lehmann (20) suggested an alternative interpretation of the α-thalassemia syndromes. His hypothesis assumed a single type of α-thalassemia gene, which presumably caused complete suppression of α-chain synthesis. Using this model, the presence of 1, 2, 3, or 4 α-thalassemia genes corresponds, respectively, to the silent carrier, α-thalassemia trait, HbH disease, and the hydrops fetalis forms of the disease.

Either of the genetic models described appears adequate to account for the expression of α-thalassemia as seen in Thai and other Far Eastern populations, but it has not been possible to account for the forms of α-thalassemia that have been described in other population groups. In American blacks, Yemenite and Iraqui Jews (47), and in Arabs in Saudi Arabia (30, 46), the gene frequency of α-thalassemia is substantial and HbH disease has been described; however, the hydrops fetalis α-thalassemia syndrome has never been identified.

In family studies of Yemenite and Iraqui Jewish families with HbH disease, involving determinations of both hematologic features and globin synthesis (47), an attempt was made to identify mild and severe α-thalassemia genes. The experimental findings did not, however, support the existence of more than a single type of α-thalassemia gene and suggested that only two forms of α-thalassemia could be identified in this population, *viz.* α-thalassemia trait and HbH disease. It was proposed that these disorders could most readily be explained as representing, respectively, the heterozygous and homozygous expression of a single α-thalassemia gene.

Pembrey and coworkers (30), in their study of Arabs in Saudi Arabia, concluded that the findings in this population represented a form of α-thalassemia that is expressed as a disorder of a severity intermediate between that of α-thalassemia trait and HbH disease as they are known to occur in the Far East. As a possible explanation for these observations the authors postulated that six α-chain loci might be present in this population, allowing the expression of an intermediate fraction of the α-chain genes as α-thalassemia.

The genetics of α-thalassemia in blacks remains unclear, but from available evidence seems to follow a pattern similar to that observed in the Yemenite and Iraqui Jews (47), with α-thalassemia trait and HbH disease apparently being the only clearly discernable forms of α-thalassemia in this population. The findings of a detailed study of black families with HbH disease, as reported by Schwartz and Atwater (32), were consistent with this genetic pattern, but because of the mild degree of abnormality found in the globin synthesis studies of heterozygotes, rigorous proof of this hypothesis was lacking. An attempt has also been made to distinguish between mild and more severe forms of α-thalassemia trait in blacks (34), but a distinction of this kind remains unconvincing with the limited family studies that are available. The difficulty in identifying heterozygotes in adult life has been a major obstacle to the study of this condition and has caused a heavy reliance to be placed on the analysis of Bart's hemoglobin in the newborn period. The findings described by Esan (10, 11) raise doubts about the total validity of this method as a means for the identification of α-thalassemia in black populations and emphasize the need for confirming the diagnosis of α-thalassemia by other methods whenever possible.

Both of the parents of the infant with HbH disease described in this report appeared from the globin synthesis studies (Table 2) to have a form of α-thalassemia of a similar degree of severity. This is consistent with each parent being heterozygous for a relatively mild α-thalassemia gene (32), although it is impossible from the evidence available to exclude a double dose of a still milder α-thalassemia gene in either or both parents.

The limited genetic information now available about HbH disease in blacks, including the present case, seem clearly not to be explainable by the genetic mechanisms established in Far Eastern populations (41, 42). Two possible mechanisms that are consistent with the available genetic information are: (*1*) the presence of three or more α-chain loci in this population, with α-thalassemia genes of an α^0 type occupying an intermediate fraction of the total α-loci in HbH individuals, to produce a thalassemic disorder less severe than that of HbH disease as seen in the Far East (30); or (*2*) the presence of a milder (α^-) type of α-thalassemia gene which does not produce complete suppression of α-chain synthesis. The latter possibility does not necessarily exclude the existence of more than one type of α-thalassemia gene (with respect to severity) in this population, and is compatible with either a 2-locus or 4-locus model. Inasmuch as β-thalassemia in blacks in the United States is mainly of the β^+ variety, α-thalassemia may likewise cause incomplete suppression of α-chain synthesis in this population.

In spite of the unquestionably milder expression of hemoglobin H disease in blacks as compared with other populations, it is of interest that the infant with HbH disease described in this report had a level of Bart's hemoglobin at birth that was within the range of values seen in newborns with HbH disease in the Far East (26, 41). To our knowledge this infant represents the only known example of hemoglobin H disease in a black infant who was studied at birth.

Hemoglobin H disease in American blacks was only first described in 1972 (32, 34). The present case brings the total number of documented cases to nine. The apparent rarity of this condition seems surprising in view of evidence that the incidence of α-thalassemia among blacks is substantial, but several characteristics of the disorder as it occurs in the black population may serve to obscure its presence. Anemia is seldom severe in this form of HbH disease, and hemoglobin values may fall well within the range of normal values in affected individuals (32, 34), allowing its presence to be overlooked. The now widespread application of hemoglobin electrophoresis testing for the detection of sickling disorders among blacks in the United States might also be expected to demonstrate the fast moving hemoglobin H fraction in otherwise asymptomatic and nonanemic individuals, but this finding is also likely to be missed; the preparation of hemolysates for electropho-

resis testing in most hemoglobin screening laboratories includes the addition of toluene or chloroform to aid in the removal of stromal material, but these chemicals have the unfortunate property of quantitatively precipitating hemoglobin H and thereby completely obscuring its presence in the electrophoretic analysis. Because of these and other diagnostic difficulties, a true assessment of the incidence of α-thalassemia and the various forms of its clinical expression in blacks will clearly require a prospective study employing multiple types of laboratory assessment and more definitive diagnostic criteria than heretofore have been employed.

SUMMARY

A 7-year-old black child with hemoglobin SC disease and microcytosis was found by hematologic and globin synthesis studies to have concomitant α-thalassemia. Genetic studies of the family supported this diagnosis and, in addition, disclosed the presence of hemoglobin H disease in an infant sibling.

The child with Hb SC-α-thalassemia demonstrated more severe anemia and a more hemolytic picture than is typical of HbSC disease. Her erythrocytes exhibited decreased osmotic fragility in comparison to HbSC erythrocytes, but an indistinguishable oxygen equilibrium curve and level of 2,3-DPG. Erythrocyte sickling, however, was significantly reduced, with less than 35% sickle forms present at nearly complete oxygen desaturation.

The sibling with hemoglobin H disease exhibited 26% Bart's (γ_4) hemoglobin at birth, a level comparable with that seen in infants with HbH disease in Far Eastern populations. At age 5 months typical findings of mild HbH disease appeared, with HbH making up 6.5% of the total hemoglobin.

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48. We thank Rose Johnson and Sharon Williams for excellent technical assistance.
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51. This research was supported by Comprehensive Sickle Center Grant no. HL-15168 and Research Grant no. AM-12895 from the National Institutes of Health, United States Public Health Service.
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53. Accepted for publication December 18, 1975.

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