Clinical Manifestations of Inherited Abnormal Hemoglobins

I. The Interaction of Hemoglobin-S with Hemoglobin-D

By Phillip Sturgeon, Harvey A. Itano and William R. Bergren

INTRODUCTION

WITH THE USE OF ELECTROPHORETIC STUDIES in addition to routine clinical hematological tests, three varieties of abnormal human hemoglobin were characterized.1 The first abnormal hemoglobin discovered, hemoglobin-S, is characteristically found in sickle cell anemia and in sickle cell trait.2 Hemoglobin-C,3 electrophoretically different from normal hemoglobin (hemoglobin-A) and from hemoglobin-S, does not cause sickling. Target cell formation is a prominent characteristic of hemoglobin-C.4 The simultaneous presence of hemoglobins-S and -C results in sickling, target cell formation and a hemolytic anemia resembling, but less severe than, sickle cell anemia.4 Hemoglobin-D6 also does not cause sickling, but it has an electrophoretic mobility indistinguishable from hemoglobin-S. Its interaction with hemoglobin-S likewise results in sickling and a hemolytic process. A fourth abnormal hemoglobin (hemoglobin-E) is to be discussed in the succeeding paper.

This report deals with the clinical and hematologic manifestations of hemoglobin-D in the only family thus far known to possess this variety. Included are several members who are carriers of the D trait and two members who have inherited hemoglobin-S from one parent and hemoglobin-D from the other.

METHODS

The technics employed in this laboratory for hemoglobin determinations, red blood cell counts, packed cell volume, serum iron, serum copper and free erythrocyte protoporphyrin determinations were reported recently.7 The report included an analysis of the accuracy of the methods in our hands, and normal values were given for children and adult males from this area. Reticulocyte counts were performed by the “dry” method. Tests for erythrocyte sickling were carried out by the sealed cover slip method and also the sodium metabisulfite technic of Daland and Castle.8 The presence of intravascular sickling was determined by the
in vitro sickling test of Sherman. Red cell fragility to hypotonic saline was determined by a method referred to previously. Serum bilirubin was measured photoelectrically as azobilirubin.

Hemoglobin electrophoretic analyses were performed with 1.0 per cent solutions of carbon monoxymohemoglobin in the Tiselius apparatus. The preparation and analysis of these solutions were identical to those used in earlier studies. Solubility of the hemoglobins studied was determined in 2.24 and 2.58 M phosphate as amorphous ferrohemoglobin; details of this method were published recently.

The nomenclature employed in this report for the various forms of hemoglobin is that agreed upon at the symposium on hemoglobin abnormalities.

**CASE HISTORY**

**Family H.: Propositus R. H., Sickle Cell Hemoglobin-D (S/D) Disease**

Family H. was first reported in 1934 by Cooke and Mack as the second example of a white American family having sickle cell disease. In addition to the ethnologic interest in this family, the above authors drew attention to the slowness with which the cells of the propositus sickled compared to the rate of sickling observed in sickle cell disease in Negroes. The mother is of Irish, English, and American Indian ancestry and the father of Irish and English ancestry.

R. H., a 21 year old Caucasian, was first noted to be pale at two months of age, and four months later received his first blood transfusion. At that time, physical examination revealed the spleen to be enlarged below the umbilicus. The anemia persisted, and several additional blood transfusions were administered. The child's spleen was removed when he was one year old. At that time no sickling tests had been done and no definitive diagnosis established. Later, examination of blood from one of his sisters, prompted by her symptoms of fever and drowsiness, revealed anemia and a slow type of sickling. Subsequent tests also revealed slow sickling in the blood of the propositus (R. H.) and of his father. Blood from the rest of the members of the family (J. H., G. H. Jr., H. H. D., A. M. H.) including the mother (M. H.) did not sickle.

At 5 years of age, R. H. (C. H. 3521) was first seen in this clinic where he has been followed to date. He has been admitted to the hospital 22 times for attacks of severe pain in the extremities or abdomen. These episodes have followed a course typical of that seen in sickle cell crises. Blood transfusions have been administered on many occasions for relief of pain but not for anemia. For attacks of less severity he has visited the outpatient department on innumerable occasions. His clinical course, except for the absence of anemia, has not differed from that of the usual case of sickle cell anemia.

Physical examination at this time reveals an individual of small stature* and normal proportions. There are scars on the abdomen at the sites of incisions for splenectomy and exploratory laparotomy. The sclerae are not icteric and the liver is not palpable.

B. H., a 23 year old female, is one of R. H.'s sisters. Since 10 years of age, she has been clinically well; recently she has been gainfully employed as an airline hostess. Prior to the age of 10 years, however, she had experienced relatively infrequent bouts of anemia and pain in the extremities. These required transfusions on a few occasions. Physical examination at present reveals no abnormality other than the small stature; the spleen is not palpable. Figure 1 is a photograph of R. H., B. H. and their mother, M. H. It shows the general appearance of small stature but good proportions.

Repeated routine studies of R. H.'s blood have revealed a positive sickling test, a mild degree of anemia and a persistent reticulocytosis. Table 1 lists pertinent laboratory data. It shows that R. H. and his sister B. H. have a moderate anemia with a distinct increase in red cell volume, a moderate reticulocytosis and approximately 20 per cent intravascular sickling. The amount of intravascular sickling is quantitatively comparable to that noted in some instances of frank sickle cell anemia during quiescent phases. Qualitatively, however, the individual sickled cells do not show the degree of sickling seen in a control S/S sickle cell anemia case. The M. C. V. of 113 and 118 cubic micra in these cases is consider-

* The small stature of R. H. and B. H. was commented upon by Cooke and Mack.
Fig. 1.—Members of the H. family. Each line in the background represents 6 inches in height. At left is M. H., carrier of D trait. Next is the daughter, B. H., who is asymptomatic but has S/D disease. At right the propositus, R. H., who also has S/D disease.

Table 1.—Summary of Hematologic Data: Sickle Cell Hemoglobin-D Disease, and Hemoglobin-D Trait

<table>
<thead>
<tr>
<th>Clinical laboratory test</th>
<th>Sickle cell-D disease</th>
<th>D trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, Gm. %</td>
<td>9.1</td>
<td>9.9</td>
</tr>
<tr>
<td>Red blood cells, 10^6/mm³</td>
<td>2.66</td>
<td>2.82</td>
</tr>
<tr>
<td>Packed cell volume, %</td>
<td>30.0</td>
<td>33.0</td>
</tr>
<tr>
<td>Mean cell volume, μ³</td>
<td>113.1</td>
<td>118.0</td>
</tr>
<tr>
<td>Mean cell Hb. conc., %</td>
<td>30.1</td>
<td>30.0</td>
</tr>
<tr>
<td>Reticulocyte, % of RBC</td>
<td>8.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Sickling*</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>In Vivo Sickling, % of RBC</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Target cells, % of RBC</td>
<td>1.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Serum bilirubin, mg. %</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Fragility, range, % saline</td>
<td>0.44-0.30</td>
<td>0.48-0.32</td>
</tr>
</tbody>
</table>

*H = Holly leaf, or slow variety of sickling; F = filamentous or rapid variety; Neg. = No sickling.
SICKLE CELL HEMOGLOBIN-D, DISEASE AND TRAIT

A/A

P?C,

I "U

S/D QC,

#{149}-Qt4

Fig. 2.—Photomicrograph X400 of blood smears from A) Normal; B) Hemoglobin-D trait (M. H.); C) Propositus (R. H.); D) Sister of propositus (B. H.), also having sickle cell hemoglobin-D disease.

ably greater than the mean value of 96.7 reported by Neel in 6 patients over 16 years of age with sickle cell disease.17 The hematologic findings of M. H. (the mother) and G. H., Jr., (a brother) are also listed in the table. They show no quantitative abnormalities, but both are carriers of hemoglobin-D. The morphology of the red blood cells from R. H., B. H. and G. H. are compared to normal cells in figure 2. The increased size in both instances of S/D disease and a tendency for the cells to assume partially sickled shapes can be noted. In the trait condition morphologic abnormalities are not noted.

It can be concluded, therefore, that the propositus and his sister B. H. have a hemolytic process with mild anemia but that clinically R. H. is frequently incapacitated by painful sickle cell crises, while B. H. is essentially symptom-free.

SPECIAL STUDIES

R. H. from the time of his first visit was carried in the records of this clinic with a diagnosis of sickle cell anemia. However, in the course of studies of abnormal hemoglobins in sickle cell disease by one of us (I.), it was noted that the mother's (M. H.'s) blood did not sickle. This finding confirmed the similar observation previously recorded by Cooke and Mack.15 Electrophoretic studies of the mother's hemoglobin revealed, however, the same pattern as that noted in sickle trait.6 Therefore, it was concluded that a new hemoglobin was involved, one which has the same electrophoretic mobility as sickle hemoglobin but does not cause sickling. This is now termed “hemoglobin-D”. The H. family, to date, is the only one known to carry this type of hemoglobin.

Figure 3 illustrates on the right the electrophoretic patterns of the mother (M. H.), the propositus (R. H.) and the sister (B. H.). These are compared respectively on the left to instances of sickle cell trait and sickle cell anemia.

The small component having a mobility slightly less than that of normal hemoglobin in the pattern for R. H. and B. H. is comparable in position to a similar
component in an instance of sickle cell anemia known to have a relatively large amount of fetal hemoglobin. Further details pertaining to this aspect of the patient's electrophoretic patterns and to increased ferrohemoglobin solubilities have been dealt with by Itano. Recently the father's blood was obtained; a positive sickling test, confirming the report of Cooke and Mack, was found. The electrophoretic and solubility analyses gave results also characteristic of sickle trait.

The genealogy of this family is depicted in figure 4. It illustrates that the father is a carrier of hemoglobin-S and the mother of hemoglobin-D. Two children, R. H. and B. H., have inherited both abnormalities and are the ones afflicted with a hemolytic process resembling sickle cell disease. We have also made studies on G. H., Jr. and H. H. D. As reported by Cooke and Mack, their blood does not sickle, but on electrophoretic studies they show the presence of hemoglobin-D. We have not studied J. H. or A. M. H. but Cooke and Mack reported a nega-

SICKLE CELL
HEMOGLOBIN-D DISEASE

![Sickle cell disease diagram](image)

Fig. 3.—On the right from top to bottom are moving boundary electrophoretic patterns of hemoglobin-D trait and two samples of sickle cell hemoglobin-D disease. At top on the left is a normal pattern and below, for comparison, a sickle trait, and two samples of sickle cell anemia. Fetal hemoglobin is also present in small amounts in the patterns labeled "S/S" and "R. H. (S/D)."
Fig. 4.—Genealogy of the H. family. The numbers refer to the age of the individuals. The letters refer to the A-B-O blood groups; the most probable genotype of the Rh system is also given; these are based on the reactions with anti-C, -D, -E and -e.

DISCUSSION

Itano has considered ways in which intravascular sickling could result from the interactions of hemoglobin-S and -D. Because of identical mobilities of the two components, the possibility that hemoglobin-S is produced in disproportionately large quantities in the presence of -D cannot be studied electrophoretically. If, however, both hemoglobins are produced in nearly equal quantities, the possibility was suggested that hemoglobin-D has a greater stabilizing effect on hemoglobin-S in the sickled (insoluble) state than does hemoglobin-A. In support of this latter theory is the clinical observation on stained smears of numerous partially sickled cells. Usually, in our hands, stained smears do not reveal more than a few, if any, sickled cells, except in severe cases of sickle cell anemia in crises.

The present study substantiates that in vivo intravascular sickling does occur in the presence of these two genes. It also has been demonstrated that there is a hemolytic process. The relative mildness of the hemolytic anemia correlates well with the incomplete type of intravascular sickling. The marked differences between the two siblings in their clinical symptomatology, on the other hand, does not correspond to the slight differences in their electrophoretic or hematologic findings. Although B. H., the asymptomatic sibling, produces more fetal hemoglobin, the difference in intravascular sickling is negligible. The rate of hemolysis as judged by their reticulocyte counts and hemoglobin levels appears to be the same. The stained smear, however, does reveal more sickling in the case of R. H. Whether this is a constant difference cannot be concluded from the few comparative studies thus far completed. However, it has been reported
previously that the milder forms of sickle cell disease are associated with higher total ferrohemoglobin solubilities. It may be significant, therefore, that the solubility of B. H.'s hemoglobin is distinctly higher than that of R. H.'s. On the other hand, it appears plausible also that vasomotor, anatomical or other mechanisms may be responsible for this clinical difference.

The genetic pattern of the H. family is similar to that in families having individuals afflicted with sickle cell thalassemia disease and sickle cell hemoglobin-C disease. In each instance there appears to be an exception to the genetic system proposed by Neel for the transmission of sickle cell disease, in that the patient with the clinical sickle cell anemia has but one parent, instead of both, with sickle cell trait. Electrophoretic studies of the non-sickling parent, however, reveal that he is the carrier of a different abnormal gene. This gene interacts with the sickle (S) gene in the afflicted individual, resulting in a clinical syndrome closely resembling sickle cell anemia. The possible genetic modes for the interactions of these genes have been reviewed by Neel.

Several members of this family are carriers of hemoglobin-D trait, and two of them have been studied in detail. No changes have been detected in their red cell morphology or routine peripheral blood hematology suggestive of their hemoglobin molecular abnormality. This is unlike the situation in carriers of hemoglobin-C. In the latter entity, Kaplan et al. have emphasized that the presence of abundant target cells is highly suggestive of the hemoglobin-C anomaly.

It appears, therefore, that the presence of hemoglobin-D only can be suspected clinically when it is associated in the same individual with hemoglobin-S. The absence of sickling in one parent of an individual having a mild form of sickle cell anemia, which may also be macrocytic, should lead to the suspicion of hemoglobin-D. Confirmation of this in the non-sickling parent can be obtained through electrophoretic studies of his blood. These studies should reveal a pattern like that found in sickle cell trait. It is also evident that a screening program to detect abnormal hemoglobins cannot rely on either the sickling test or electrophoresis alone. In the first case, it will fail to distinguish carriers of hemoglobin-D from normal individuals, and in the latter, it will fail to distinguish the hemoglobin-D carrier from individuals having sickle cell trait.

The possibility that a homozygous D/D individual may reveal some clinical or hematological abnormalities indicative of the presence of hemoglobin-D must remain conjectural for the present. The results of interaction of hemoglobin-D with hemoglobin-C and thalassemia are also unknown.

**Summary and Conclusions**

A clinical and hematologic description is presented of the family demonstrating the existence of the genetically determined hemoglobin-D originally described by Itano.

The interaction of hemoglobin-D with hemoglobin-S in two members of this family has resulted in a hemolytic process and a mild anemia. Clinically, one of the patients is asymptomatic. The other has repeated painful episodes characteristic of those seen in sickle cell crises.
Clinical and hematologic evaluations of two members of the family inheriting normal hemoglobin and hemoglobin-D revealed no abnormalities.

The only feature of hemoglobin-D which distinguishes it from normal hemoglobin is its electrophoretic mobility which is similar to that of hemoglobin-S. Hemoglobin-D is distinguished from hemoglobin-S by a higher solubility and by a failure to sickle and from hemoglobin-C by a lack of target cell formation and a different electrophoretic mobility.

**Summario e Conclusiones in Interlingua**

Es presentate un description clinic e hematologic del familia in qui se demonstrava le geneticamente determinate hemoglobina D primo reportate per Itano. Le interaction de hemoglobina D con hemoglobina S in duo membros de iste familia ha resultate in un processo hemolytic e un leve anemia. Del puncto de vista clinic, un del patientes exhibi nulle symptomas. Le altere ha repetite episodios penose del typo characteristic del crises de morbo a cellulas falciforme.

Nulle anormalitates esseva revelate in le evaluation clinic e hematologic de duo altere membros del mesme familia qui hubeva hereditate hemoglobina normal e hemoglobina D.

Le sol characteristica de hemoglobina D que distingue lo de hemoglobina normal es su mobilitate electrophoretic que es simile a lo que se observa in hemoglobina S. Hemoglobina D se distingue de hemoglobina S per su plus alte solubilitate e per le facto que illo provoca nulle falciformation. Hemoglobina D se distingue de hemoglobina C per un differente mobilitate electrophoretic e per non provocar cellulas corolliforme.

**Addendum**

Since submission of the present communication, J. C. White and G. H. Beaven have reported (J. Clin. Path. 7: 175-200, 1954) a family with members having hemoglobin-D. This second occurrence of hemoglobin-D suggests that surveys of populations for abnormal hemoglobins may throw more light on its distribution. Clearly this abnormal hemoglobin is not the private property of the family discussed in the present communication.

**II. Interaction of Hemoglobin-E and Thalassemia Trait**

**Introduction**

In a previous report, the clinical manifestations of the interaction of sickle cell and thalassemia traits in the same individual were described. It was suggested that the abnormal clinical and laboratory findings were primarily the consequence of hemoglobin-S. Despite the fact that the individual was only a carrier of the sickle trait, hemoglobin-S was demonstrated electrophoretically in increased amount. The excessive proportion of hemoglobin-S compared to normal hemoglobin was assumed to result from the selective retardation of normal hemoglobin synthesis by the thalassemia gene.

The above, plus the fact that hemoglobins-C, -D, and -E do not cause sickling, might lead one to reject the idea that the theoretically possible, but heretofore unreported, combinations of the thalassemia gene with the above genes
TABLE 2.—Summary of Hematologic Data: Hemoglobin-E Thalassemia Disease, Thalassemia Trait

<table>
<thead>
<tr>
<th>Clinical laboratory tests</th>
<th>Hb-E thal.</th>
<th>Thal. trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, Gm. %</td>
<td>5.9 - 7.4</td>
<td>12.5</td>
</tr>
<tr>
<td>Red blood cells, 10^6/mm.³</td>
<td>2.98 - 3.83</td>
<td>5.99</td>
</tr>
<tr>
<td>Packed cell volume, %</td>
<td>21.0 - 24.0</td>
<td>43.2</td>
</tr>
<tr>
<td>Mean cell volume, µ³</td>
<td>63.0 - 72.0</td>
<td>72.0</td>
</tr>
<tr>
<td>Mean cell Hb, µg/m³</td>
<td>18.0 - 21.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Mean cell Hb conc., %</td>
<td>28.0 - 30.5</td>
<td>29.0</td>
</tr>
<tr>
<td>Reticulocytes, % of RBC</td>
<td>2.0 - 5.6</td>
<td>—</td>
</tr>
<tr>
<td>Normoblasts/100 WBC</td>
<td>3.0 - 16.0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Values are given in range observed during the course of four observations over a 3½ year period.

would result in a hemolytic anemia. In this report an instance of such a genetic combination will be described. The clinical laboratory data demonstrate the presence of an anemia, in part hemolytic but to a greater extent due to inhibition of hemoglobin synthesis.

METHODS

The laboratory methods employed are those referred to in the preceding paper. In addition, analyses of hemoglobins by closed plate paper electrophoresis were carried out at pH 9.0. This method has been described in detail by Bergren, et al.24

CASE HISTORY


M. M.'s father is of Guatemalan, Spanish and Hindu ancestry. Her mother's parents are both Italian.

The mother's pregnancy with this child was normal; it terminated March 9, 1947 in a full term girl who had an uncomplicated neo-natal period. The child seemed well until the age of 3 years, when, on one occasion, some dark urine was passed. Her pediatrician* was consulted, and he, after physical examination and the performance of X-ray and blood studies, made a tentative diagnosis of Cooley's anemia.

On November 18, 1950, examination in this laboratory revealed a well-nourished, pale child with yellow-tinted skin and icteric sclerae. The facial appearance was suggestive of that noted in some cases of Cooley's anemia. Other positive physical findings included enlargement of the spleen to 4 cm. below the left costal margin; the liver edge was palpable 3 cm. below the right costal margin.

Roentgenologic studies revealed the heart to be at the upper limits of normal in size. The trabecular pattern of the bones was abnormally coarse and the diploe of the cranial bones was relatively expanded.

The results of routine blood examination of the patient and of two relatives available for

* The authors wish to thank Dr. Donald Shelby who originally referred the patient to this laboratory for confirmatory studies. They also wish to express their gratitude to Dr. Lloyd P. Tainter, the family physician, for his cooperation in following the child and obtaining specimens from other members of the family for study.
study are recorded in table 2. The patient’s red blood cell morphology is compared in figure 5 to normal cells and to those from a carrier of the hemoglobin-C trait and a homozygous C/C individual.*

These studies illustrate that the patient has a chronic moderate anemia with slight but persistent reticulocytosis. The red cells are microcytic and hypochromic. There is marked anisocytosis, slight polychromasia and only a moderate number of target cells. The red cells do not show the extensive target cell formation characteristic of the hemoglobin-C anomaly. The mother’s and maternal grandmother’s cells also, as would be expected in thalassemia minor, are microcytic and hypochromic. To date it has not been possible to study the father’s red cell morphology.

Other laboratory studies revealed an icterus index of 42 units on one occasion and 50 on another. The total serum bilirubin was 6.8 mg. with 1.1 mg. of direct reacting pigment; the urine urobilinogen was positive in 1/2 dilution. A direct antiglobulin test was negative. Repeated sickling tests have been negative.

Studies of serum iron, copper, and free erythrocyte protoporphyrin revealed respectively values of 142, 148 and 33 micrograms per cent. These latter tests performed on the mother’s blood revealed values of 92, 128 and 53 micrograms per cent.

In view of the patient’s Italian-Spanish ancestry, physical, X-ray and laboratory findings, a diagnosis of Cooley’s anemia of mild or “intermediate” severity was made. Following this the patient was observed at infrequent intervals; she remained quite well and asymptomatic. She has never required a blood transfusion and has grown and developed relatively normally, despite the fact that her hemoglobin level fluctuates between 5.9 and 7.4 grams per cent.

Recently during the course of studying iron and porphyrin metabolism in thalassemia, we added to the methods employed, paper electrophoresis of hemoglobins. These studies revealed the unusual nature of the propositus’ disease.

**Special Studies**

Upon inspection of the migration of the patient’s hemoglobin on paper, the initial impression one gained was that hemoglobin-C must be present. On the

---

* The authors wish to thank Dr. William Valentine who generously provided the hemoglobin-C specimens.
Fig. 6.—At the bottom of the figure are the moving boundary and paper electrophoretic patterns of propositus’ hemoglobin (M. M.). These are compared to hemoglobins from instances of S thalassemia disease, S/C disease, S trait and to the normal A/A hemoglobin.

Based on clinical evidence, the second component was assumed to be fetal hemoglobin, an impression later substantiated by further study. These initial conclusions are congruent with comparisons evident in figure 6, in which are shown migration patterns of hemoglobins from instances of sickle cell thalassemia disease, sickle cell hemoglobin-C disease and sickle cell trait. Under the conditions of migration on paper at pH 9.0, all of the hemoglobins act as anions, with hemoglobin-C as the slowest component, -S as intermediate in mobility, and -A as the fastest. The mobility of the slower component in the patient’s hemoglobin most closely approximates that of hemoglobin-C. Although previous experience had shown that fetal hemoglobin usually migrates relatively rapidly, assuming a position intermediate between hemoglobins -A and -S, it had been noted in some instances that the migration of the fetal component is retarded in the presence of a slower moving hemoglobin. The mobility of the faster component in the present instance would not be inconsistent, therefore, with identification as fetal hemoglobin.

Subsequently, a routine examination of M.M.’s hemoglobin by the moving
boundary method at pH 6.5 forced us to revise our original thinking concerning the abnormal hemoglobin involved. The pattern obtained is illustrated at the bottom of the left hand column in figure 6. At the acid pH, all of the hemoglobins migrate as cations, with an order of mobility reverse to that described above for alkaline conditions. However, the abnormal component was found in a position approximating that of sickle (S) hemoglobin instead of having the expected mobility of hemoglobin-C. The other component, the slow one under these conditions, was identified as fetal hemoglobin by its electrophoretic mobility, resistance to alkali denaturation and ultraviolet spectrum.

The discrepancy in migration between the acid and alkaline sides of isoelectric point precluded the possibility of the abnormal hemoglobin in this case being hemoglobin-C. Further studies showed that a new hemoglobin, hemoglobin-E, was present. A preliminary report of these findings has been published. Analysis for the proportions of the two components showed 41 per cent of the mixture to be fetal hemoglobin and 59 per cent to be the new hemoglobin. The solubility, as ferrohemoglobin, of the hemoglobin-E/fetal hemoglobin mixture was determined, and the solubility of hemoglobin-E was deduced to be similar to that of the normal hemoglobin-A. This finding is consistent with the failure of -E to cause sickling.

A specimen of the father's blood adequate for a sickling test and for electrophoretic studies was obtained recently. It does not sickle. The electrophoretic pattern at pH 6.5 by the moving boundary method resembles that of a sickle cell trait with a relatively large hemoglobin-A peak. Analysis of the pattern showed 72 per cent hemoglobin-A and 28 per cent -E. Mixture experiments demonstrated that the mobility of -E at pH 6.5 is slightly less than that of -S. On filter paper at pH 9.0 a result was obtained which resembled an A/C pattern with the hemoglobin-A spot predominating. The -E component in the father's blood migrated slightly faster than the corresponding component in M. M.'s blood. This apparent difference is undoubtedly a secondary effect due to different second components, -A and -F, respectively, in the two bloods.

A satisfactory method for the migration of hemoglobins on filter paper under acid
conditions has not been established in our laboratories to date. Results to date, however, show different mobilities for hemoglobins-E and -C at pH 7.0, but denaturation interferes with a distinct separation. In the Tiselius apparatus on the acid side, the ascending boundary separations are not obscured by denatured products. A more extensive description of the chemical and physical properties of hemoglobin-E will be published elsewhere.

The genealogy of this family, including recent findings from studies of the father's blood, is depicted in figure 7. It illustrates that the mother and maternal grandmother are carriers of thalassemia minor, that the father is the carrier of the hemoglobin-E abnormality, and that the only child has inherited both genes.

**DISCUSSION**

On the basis of the above observations, it is reasonable to assume that the patient has inherited a gene for the production of hemoglobin-E from her father and a thalassemia gene from her mother. Her condition can be called, therefore, hemoglobin-E thalassemia disease. An essentially analogous condition, sickle cell thalassemia disease, is found in the individual heterozygous for the sickle cell and the thalassemia genes. The latter individual has predominantly a hemolytic type of anemia. Previous observations have indicated that hemoglobins-A, -S, and -F are present in the latter condition, presumably due to an incomplete inhibition of hemoglobin-A production by the thalassemia gene, but that hemoglobin-A is absent in individuals homozygous in the sickle cell gene. Recently, however, we have observed a patient (J. G.) with sickle cell thalassemia disease whose blood contains hemoglobins-S and -F but no detectable hemoglobin-A.* Hemoglobin-A is likewise absent in the presence of hemoglobins-E and -F in our present propositus. In light of these results, the presence of measurable amounts of hemoglobin-A does not appear to be an inevitable consequence of double heterozygosity in alleles for thalassemia and one of the abnormal hemoglobins, and hemoglobin analysis alone may not suffice to differentiate sickle cell thalassemia disease from sickle cell anemia. A similar variability in the amount of hemoglobin-A has been encountered in thalassemia major, in which the inhibition of hemoglobin-A production may range from moderate to complete.

Although red cell survival studies have not been performed with this patient's blood, the presence of a slight hemolytic process is indicated by the mild but persistent relative reticulocytosis and the raised level of indirect acting bilirubin. The red cell morphology, like that in Cooley's anemia, is microcytic and hypochromic with marked anisocytosis and slight normoblastosis. There is, however, a relatively marked degree of anemia in comparison to the reticulocyte level. This, too, is characteristic of Cooley's anemia; it is consistent with Crosby's calculations that the rate of hemoglobin production in Cooley's anemia is about one half that in sickle cell anemia.

Other findings in this case compatible with a diagnosis of Cooley's anemia are the X-ray changes in the bones, the high serum iron and copper, the relatively low erythrocyte protoporphyrin, and the negative antiglobulin and sickling tests. The question then presents itself, "Why does this rather typical Cooley's anemia exist in an individual who is only a carrier of the trait?"

* Unpublished observations.
It is well known that the presence of a single thalassemia allele usually results in clinically insignificant hematologic abnormalities. On the other hand, the presence of the second allele results in a profound anemia characterized by marked suppression of adult hemoglobin synthesis and a mild hemolytic component. Individuals heterozygous for thalassemia trait and sickle trait exhibit a selective retardation of adult hemoglobin synthesis with an apparently compensatory production of hemoglobin-S. Consequently, they have predominantly a sickle type of hemolytic anemia, somewhat attenuated in nature.

The instance of hemoglobin-E thalassemia disease presented in this report more closely resembles thalassemia major than sickle cell thalassemia disease. This resemblance leads us, therefore, to the assumption that hemoglobin-E production is the result of a gene-controlled process, and that its pathogenic mechanism is one of relative retardation or inhibition of hemoglobin production. On the one hand this inhibition is not as severe as that ordinarily seen in the presence of another thalassemia gene, but on the other hand, the E gene does not permit the rate of hemoglobin synthesis associated with an S or A gene. The relatively reduced percentage of hemoglobin-E in the father's electrophoretic pattern is quite consistent with this assumption.

A possible racial origin of this abnormal gene cannot be ascertained as yet. However, in view of the recent reports by Lehman of a high frequency of the sickle cell trait in the Vedians of South India and the low incidence of the Rh chromosome cDe, it is no longer necessary to assume an African origin of the sickle gene. Studies of African and Indian populations for other abnormal hemoglobins have not been reported. Inasmuch as hemoglobin-C also has a high frequency (2-3 per cent) in American Negroes, it is plausible that it and other hemoglobin abnormalities might have their origins in the same regions as hemoglobin-S. If such should prove to be the case, it may be significant that in the family reported here the father, who carries the hemoglobin-E trait, is in part of Hindu ancestry.

The above considerations also raise the possibility that the cases of Mediterranean anemia reported from the Orient by Minnich, et al. might include instances of interactions of hemoglobin-E and thalassemia. It is also apparent, in view of the data presented in this and the preceding paper, that clinically or genetically atypical cases of Cooley's anemia or sickle cell anemia will have to be analysed for the interactions of hemoglobin abnormalities with thalassemia or each other to clarify the mode of their inheritance.

SUMMARY AND CONCLUSIONS

Clinical, hematologic and special hemoglobin studies are presented of a family exhibiting a new abnormal hemoglobin (hemoglobin-E). Its interaction with thalassemia trait in one of its members also is described.

Hemoglobin-E does not sickle; under alkaline conditions it migrates slowly at a rate comparable to hemoglobin-C, while at an acid pH it migrates at a rate similar to hemoglobin-S.

The result of its interaction with thalassemia is a microcytic, hypochromic anemia clinically resembling thalassemia major of an intermediate degree of severity. The major defect appears to be in hemoglobin synthesis, although the slight persistent reticulocytosis suggests a mild hemolytic process.
Summario e Conclusiones in Interlingua

Es presentate studios clinic, hematologic, e specialmente hemoglobinic de un familia qui exhibi un nive hemoglobina anormal (hemoglobina E). Es etiam describite le interaction de hemoglobina E con le tracto de thalassemia in un del membros de iste familia.

Hemoglobina E non provoca falciformation. Sub conditiones alcalin illo migra lentemente, a un tempo comparabile a illo de hemoglobina C. In le presentia de un acide pH illo migra a un tempo comparabile a illo de hemoglobina S.

Le resultato del interaction con thalassemia es un anemia microcytic, hypochromic. Del puncto de vista clinic isto resimila thalassemia major de un grado intermediari de severitate. Le major defecto de iste hemoglobina pare concerner su synthese ben que le leve reticuloctosis persistente es indicative de un leve processo hemolytic.

Addendum

Subsequent to our first report on hemoglobin-E, the authors learned by personal communication from A. I. Chernoff that independently he had under investigation atypical cases of Cooley's anemia exhibiting paper electrophoretic patterns similar to those described for hemoglobin-E. A sample of blood from one of his subjects was sent to these laboratories for comparison. There was found, on the basis of migration on paper at alkaline pH, a reasonable correspondence between hemoglobin-E and the sample furnished by Dr. Chernoff. Due to a change in assignment of one of the present authors (H. A. I.), Tiselius equipment temporarily was not available. Since, to date, a satisfactory method for the migration of hemoglobins on paper under acid conditions has not been established in our laboratories, it was not possible to establish to our full satisfaction complete correspondence of the two hemoglobins. The designation by Chernoff, Minnich, and Chongchareonsuk (Science 120: 605–6, 1954) of their hemoglobin as -E seems reasonable on the grounds of their clinical, hematologic, and paper electrophoretic data. Full confirmation of the identity necessarily must rest on moving boundary electrophoresis at acid pH.

References

9. SHERMAN, I. J.: The sickling phenomenon, with special reference to the differentiation
404 CLINICAL MANIFESTATIONS OF INHERITED ABNORMAL HEMOGLOBINS


6 STURGEON, P.: Personal observations.


Clinical Manifestations of Inherited Abnormal Hemoglobins

PHILLIP STURGEON, HARVEY A. ITANO and WILLIAM R. BERGREN