Multiple Inherited Erythrocyte Abnormalities in an American Negro Family: Hereditary Spherocytosis, Sickling and Thalassemia

By Flossie Cohen, Wolf W. Zuelzer, James V. Neel and Abner R. Robinson

IN THE DEVELOPMENT of our knowledge concerning the genetic relationships among the various inherited abnormalities of the red blood cell, the careful study of certain critical pedigrees provides the only decisive approach. The family which forms the subject of this report is a case in point. This American Negro family first came to our attention in 1950, in connection with a systematic study of approximately 100 families in which the sickling phenomenon was present (Family 60). The hematologic examination revealed, in several members of the family, microcytosis similar to thalassemia minor although in some instances it was not entirely typical. The examination further suggested that several persons possessed both the gene responsible for this thalassemia-like trait and that for sickling. Since, however, reports available at that time indicated that a moderately severe or moderate hemolytic anemia resulted when these two genes were brought together, and since in this family the two individuals thought to have both genes showed no striking hematologic abnormality, it seemed unwise at the time to term the hereditary microcytosis in this family thalassemia minor.

When later observations on the results of combining a thalassemia and a sickling gene indicated that major hematologic abnormality was not always observed, this family was reinterpreted as in fact exhibiting segregation for the thalassemia gene. With this reinterpretation it became apparent that the family provided evidence for the genetic independence of the loci responsible for thalassemia and for the sickling phenomenon, and the family has been so quoted.

With the growing evidence that the gene responsible for thalassemia does not in all families segregate independently of that responsible for sickling, it was decided to reinvestigate this family. One of the first members of the family studied was a six year old boy not previously available for study, who unexpectedly showed the classic picture associated with the presence of the gene producing hereditary spherocytosis. Restudy of the family has now made it evident that several other members of the family show a mild degree of hereditary spherocytosis. There are, therefore, actually three inherited abnormalities of the erythrocyte present in various combinations in this kindred, namely, hereditary spherocytosis, thalassemia and the sickling phenomenon.

If the reader will recall the rarity with which, 10 years ago, thalassemia...
was thought to occur in the Negro, and the relative rarity of hereditary spherocytosis in this race, not to mention earlier concepts of the thalassemia-sickling interaction, the reasons for the time lag in what now seems the correct interpretation of this family will be apparent. The results of this re-examination appear to have a number of very significant genetic implications. More specifically, the study of this pedigree indicates independent segregation of the genes for sickling and hereditary spherocytosis. In confirmation of the earlier interpretation, it also provides evidence for the independent segregation of the gene producing the sickling trait and thalassemia—at least the particular thalassemia gene represented in this family. Further evidence was obtained that the presence of the gene for hereditary spherocytosis masks the expression of this thalassemia gene. Lastly, the absence in this Negro family of an elevation of the A₂ component in the individuals exhibiting the hematologic evidence of thalassemia minor taken in conjunction with the apparent non-interaction between the genes for sickling and thalassemia suggest that the absence of increased amounts of A₂ may be of value in the distinction between “subtypes” of thalassemia.

PROCEDURES OF INVESTIGATION

Venous blood collected in ammonium and potassium oxalate was used for the following determinations: (1) hemoglobin concentration (with the use of the Beckman DU spectrophotometer); (2) red blood cell count (with the use of certified pipettes and counting chamber); (3) sickling test (method of Daland-Castle); (4) hematocrit determination (with the use of Wintrobe tubes; spun for 30 minutes at 3000 rpm); (5) osmotic fragility (method of Suess, Limentoni, Dameshek and Dolloff); (6) hemoglobin types (hemoglobin electrophoresis was run on paper at pH 8.4 with Veronal buffer and on starch block using a modification of the Kunkel and Wallenius' method to determine the A₂ content. This was done only in individuals who had no S hemoglobin. In our laboratory, the upper limit for normal values for the A₂ component is 3.2 per cent. The agar technic was also used to determine unusual components); and (7) alkali-resistant hemoglobin or fetal hemoglobin by the method of Singer et al. Glotted blood collected in iron-free syringes and tubes was used for serum iron determination by Ramsay's method.

Blood smears were made from finger prick blood and stained with Wright's stain. Blood grouping was done on all the members and failed to indicate any paternity exclusion.

In certain instances indicated in table 1 in which it was not possible to restudy certain members of the family, or to repeat special studies previously done, the data of the earlier studies have been utilized in the final interpretation of the family. All of the preserved blood smears were reviewed together with those currently obtained.

OBSERVATIONS

Table 1 and figures 1 and 2 summarize the findings in 17 members of this family. Information concerning physical findings and detailed histories was not obtained as it was difficult enough to reach most of these persons even once for the purpose of obtaining blood specimens. In presenting the members of this family, we will follow the sequence which in our opinion is most likely to minimize confusion, rather than the usual sequence of first describing the patient and then the members of each generation studied, generation by generation.

III 6, a 6 year old male reported to have been clinically well, was found
**Table 1.** - Hematologic and Biochemical Findings of the Pedigree

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<th>Hgb (g/100 ml)</th>
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<th>MCV</th>
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†On two separate occasions in 1950, mcv's of 85.4 and 89.9 were obtained for this person. The peripheral blood film obtained in 1957 fails to reveal a macrocytosis of the degree suggested by the cell constants and we are inclined to attribute the high mcv to a laboratory error.
†Homozygote.
to have a hemoglobin concentration of 9.2 Gm. per cent; the peripheral blood smear showed marked spherocytosis, polychromasia and anisocytosis. The sickling test was negative. The osmotic fragility of the erythrocytes was considerably shifted to the left, and the electrophoretic analysis of the hemoglobin showed a normal adult pattern. The A2 component was 1.8 per cent. A diagnosis of hereditary spherocytosis was made in this case.

III 4, a 9 year old sister of the above, was found to have a hemoglobin concentration of 10.6 Gm. per cent. The peripheral blood film showed spherocytosis, microcytosis, anisocytosis and mild polychromasia. The osmotic fragility of the red cells was increased. In addition, the sickling test was positive and the hemoglobin showed an AS pattern. Thus the evidence indicates that both the sickling gene and the gene for spherocytosis are present in this individual.

III 5, the third member of this sibship, was found to be clinically and hematologically normal, as was the father of these children, II 7. The hemoglobin of both individuals was A.

II 6, the mother of these three children, had a hemoglobin concentration of 11.9 Gm. per cent, and the peripheral blood film showed considerable spherocytosis, microcytosis, anisocytosis and mild polychromasia. The osmotic fragility of the erythrocytes was markedly increased. The sickling test was positive, and hemoglobin electrophoresis showed an AS pattern. Like her daughter, III 4, this woman is regarded as possessing both the genes for sickling and for spherocytosis.

In the process of further tracing the genes responsible for the two hematologic abnormalities observed in this branch of the family, I 1, a 59 year old male,
Fig. 2.—Photomicrographs of peripheral blood smears of pertinent members of the family. Pedigree designations are the same as in figure 1.

Note spherocytosis in I 1, II 3, II 6, III 4, and III 6; target cells and poikilocytes in I 2, II 8, III 1, III 3; and sickle cells in III 2.
was found to have considerable spherocytosis and some polychromasia in smears of his peripheral blood. The osmotic fragility of the erythrocytes was markedly increased. This man had been in good health all his life and was found to have a hemoglobin concentration of 12.5 Gm. per cent and MCV of 79.0 cu. µ. The sickling test was negative, and hemoglobin electrophoresis showed the usual pattern of A. The A₂ component was 2.0 per cent, and fetal hemoglobin was less than 1 per cent. This individual clearly appears to be the source of the gene for the spherocytosis observed in II 6 and her children, III 4 and III 6, as well as two other members of the family to be described below.

I 2, a 55 year old woman, wife of I 1, was found to account for the sickling gene in her daughter, II 6, and granddaughter, III 4, but she also presented evidence for the presence of a thalassemia or thalassemia-like gene. This woman had been in excellent health all her life. At the time of our study she had a hemoglobin concentration of 12.5 Gm. per cent with a red blood count of 6,070,000/cu.mm., MCV 79.0/cu.µ.; the serum iron was 114.0 µg. per cent. The peripheral blood film showed marked hypochromasia, microcytosis, anisocytosis, ovalocytosis and considerable numbers of target cells. The osmotic fragility of the red cells was markedly decreased, that is, the curve was shifted considerably to the right. The sickling test was positive. Hemoglobin electrophoresis showed an AS pattern.

The hematologic findings were in agreement with those noted in the first description of this individual in 1950. In the light of our present knowledge, they must be interpreted as indicative of both the sickling trait and thalassemia.

In studying the other offspring of this couple, I 1 and I 2, one of whom has hereditary spherocytosis and the other sickling and thalassemia, further confirmation for the maternal thalassemia gene was obtained in the case of II 8, a 28 year old male, who had a hemoglobin concentration of 12.2 Gm. per cent, RBC 6,150,000/cu.mm., MCV 78 cu. µ., serum iron 183.0 µg. per cent. His peripheral blood film resembled that of his mother with hypochromasia, microcytosis, anisocytosis, target cells and some ovalocytes. The osmotic fragility of the erythrocytes was decreased and the curve considerably shifted to the right. He had a negative sickling test and hemoglobin electrophoresis showed a normal adult pattern. The A₂ component was 2.5 per cent, and the fetal hemoglobin was less than 1 per cent. This individual thus manifests the hematologic features of uncomplicated thalassemia minor. The significance of the low A₂ component will be commented on later.

II 10, a 16 year old male, another son of I 1 and I 2, was found to exhibit the same stigmata of hereditary spherocytosis as his father, I 2, his sister, II 6, his niece, III 4, and his nephew, III 6. He showed no evidence for either the sickling or the thalassemia gene. Two other siblings in the second generation, II 5 and II 9, were both clinically and hematologically normal. II 1, a 35 year old male, proved to have a simple sickle cell trait.

The study of the remaining portion of this kindred proved to be of particular interest. II 3, a 34 year old woman, the remaining child of I 1 and I 2, showed the following pertinent findings: hemoglobin concentration of 12.4
Gm. per cent, RBC 5,950,000/cu.mm., MCV 74.4 cu. μ. and serum iron of 80.0 μg. per cent. The peripheral blood smear showed distinct spherocytosis, anisocytosis, polychromasia and mild ovalocytosis. The presence of spherocytosis was further confirmed by a shift to the left in the osmotic fragility of the red cells. The sickling test was positive and hemoglobin analysis showed an AS pattern. No sickle forms were found in the stained films. On the surface, this woman did not appear to differ from her sister, II 6, in that both showed clear-cut evidence for the possession of a sickling gene and a gene for spherocytosis. However, the study of the children of II 3 indicated a more complex situation.

She had been married twice, but her first spouse, II 2, had died several years ago, and consequently was never studied by us. They had a child, III 1, a healthy 15 year old male who had a hemoglobin concentration of 11.9 Gm. per cent, RBC 5,680,000/cu.mm., MCV 76.0 cu. μ. and a peripheral blood picture which showed unquestionable hypochromasia, microcytosis, ovalocytosis, anisocytosis and target cells. The serum iron was 110 μg. per cent; the osmotic fragility of the erythrocytes showed a marked shift of the curve to the right. The sickling test was negative, and the hemoglobin analysis showed a normal adult pattern. The A2 component was 1.5 per cent. On hematologic grounds, the diagnosis of thalassemia minor was made in this case. We will return to the probable origin of this thalassemia gene after consideration of III 1's half sibling, III 3.

II 3's second husband, II 4, father of III 2 and III 3, proved to be a healthy 35 year old Negro male whose only abnormality was the sickling trait. Their offspring, III 2, an 8 year old male, the propositus of the original study in 1950, has classic sickle cell anemia, and may be presumed to be homozygous for the sickle cell gene, each of his parents having been shown to carry one such gene. Whether he also has a thalassemia gene we have no way of deciding, but this point, in any case, is not pertinent to our presentation. His hemoglobin concentration has ranged from 6.5 to 7 Gm. per cent; he has large numbers of sickle cells in his peripheral blood smear. Hemoglobin analysis shows a single spot in the S position, and the fetal hemoglobin is 2.9 per cent.

III 3, a 3½ year old male and the younger child of II 3 and II 4, had a hemoglobin concentration of 10.5 Gm. per cent, RBC 4,720,000/cu.mm., MCV 74.5 cu. μ. These results are the average of determinations done in triplicate on three different occasions. Serum iron was 131.0 μg. per cent, and the peripheral blood smear showed mild but distinct microcytosis, hypochromasia and anisocytosis. The osmotic fragility of his red cells was decreased and the curve shifted to the right. A sickling test was positive, and hemoglobin analysis showed an AS pattern. These findings were interpreted as indicative of the presence of both the sickling and the thalassemia genes much as in the maternal grandmother, I 2. Thus two of the children, III 1 and III 3, of this woman, II 3, by different fathers, had the hematologic stigmata of thalassemia. The father of one failed to show any evidence of thalassemia, while the father of the other was deceased. It appears likely under the circumstances that both children inherited the thalassemia gene from the mother who is known to be the child of a parent with thalassemia, I 2, and to have herself a sibling.
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with thalassemia, II 8. It must be kept in mind, however, that in the mother herself, II 3, the hematologic characteristics of thalassemia were not demonstrable. Rather, her phenotype was that of spherocytosis and sickling. It is true there was a slight degree of ovalocytosis, but this was not sufficient to establish a diagnostic impression. If the assumption made above regarding the origin of the thalassemia gene in her two children is valid, it follows that she must herself be the possessor of a thalassemia gene, and that the expression of this gene is over-shadowed or suppressed by the simultaneous presence of the gene for spherocytosis, or possibly by the combination of spherocytosis and sickling. It is obvious from the observations in the remainder of this pedigree and from the literature that the sickling gene per se does not suppress the thalassemia gene. We have postulated then, that this individual, II 3, is the possessor of three abnormal genes, two of which are phenotypically expressed, while the presence of the third one can be inferred from its occurrence in two of her children.

GENETIC IMPLICATIONS

Three conclusions of some genetic significance can be drawn from the study of this family:

1. In the second generation of this pedigree, there are three instances in which the children of a mother with the thalassemia and sickle cell genes and a father with hereditary spherocytosis have received either both the thalassemia and sickle cell genes (II 3), or neither of these genes (II 5, and II 9). The occurrence of these three children would appear to provide conclusive evidence for the non-allelism of the particular thalassemia gene present in this family with the sickling gene. *

We have elsewhere presented both hematologic2 and genetic3,4 grounds for postulating the existence of at least two different genes resulting in the thalassemia phenotype, one probably an allele of the genes responsible for hemoglobins S and C, the other non-allelic to these genes and not yet established as allelic to any of the other genes responsible for abnormal hemoglobins. Kunkel and Wallenius7 first drew attention to the occurrence of relatively increased amounts of hemoglobin A2 in individuals with thalassemia minor. The thalassemia gene present in the family which forms the subject of our paper was not associated with increased amounts of the A2 component in the only two individuals in the pedigree with uncomplicated thalassemia minor, the average value observed in these individuals being 2.0 per cent. By contrast, in the "usual" case of thalassemia minor, in individuals of Italian or Greek ancestry, A2 values in the range of 3 to 6 per cent of the total hemoglobin are observed in this laboratory. If, now, a normal A2 level is frequently encountered in that type of thalassemia which segregates independently of the S-C locus, then the observation that A2 levels are usually elevated in persons of Italian and Greek ancestry with thalassemia minor suggests that the type

*In addition, II 10 may also lack both these genes, but in view of the experience with II 3, in whom the gene for spherocytosis effectively masked the presence of the thalassemia gene, it seems unwise to base any inferences on this individual.
of thalassemia present in this latter group will usually be the variety due to a gene which is an allele of the S-C locus. The situation with respect to thalassemia in the Negro is not yet sufficiently explored to permit generalizations.

Kunkel et al.\textsuperscript{12} and Gerald and Diamond\textsuperscript{13} observed that $A_2$ values were elevated in at least 95 per cent of Caucasians with thalassemia minor, and Gerald and Diamond have suggested that an elevated $A_2$ level should be a prerequisite for the diagnosis of thalassemia minor. They mention a patient with hemoglobin H disease whose mother showed the picture of thalassemia minor but without an elevated $A_2$ level. In our laboratory, we too have observed that the $A_2$ levels are usually elevated in Caucasians (Greek and Italian) with thalassemia minor, more specifically, 32 out of 34 individuals in 10 families. However, our experience with thalassemia in Negroes has been strikingly different. Thus, of 15 Negroes with thalassemia, distributed in 6 families, 10, representing 4 families, failed to show $A_2$ elevation. Accordingly, we are unable to accept an elevated $A_2$ level as the sine qua non of a diagnosis of thalassemia minor—unless one wishes to redefine the entity, a point to which we will return shortly. In this connection, in a study of 6 families, Gerald and Diamond\textsuperscript{13} have demonstrated striking intrafamilial similarities as regards the amount of hemoglobin $A_2$ in individuals with thalassemia minor, over a range of from 3.6 to 5.8 per cent of $A_2$, and have suggested on this basis the existence of “genetically different groups of thalassemia trait.”

In the great majority of individuals possessing both the thalassemia and sickle cell genes who have been studied to date, the amount of sickle cell hemoglobin present has been strikingly elevated over that encountered in individuals with the simple sickle cell trait, falling in the range of 70 to 100 per cent, exclusive of fetal hemoglobin. By contrast, no such “factor interaction” is observed in the family being described. Thus, it so happens that this family was included in the study of intrafamilial similarities in the amount of sickle cell hemoglobin present in individuals with the sickle cell trait, carried out by one of us in collaboration with Wells and Itano some years ago.\textsuperscript{14} The proportion of sickle cell hemoglobin present in I 2, who possesses both the sickle cell and thalassemia genes, was 27.7 per cent, while in I 3, who in addition to possessing these two genes also has a spherocytosis gene, the proportion was 22.3 per cent. Interestingly enough, in the two members of the family who possess the sickle cell gene in the absence of the thalassemia gene, II 1 and II 6, the latter also with a spherocytosis gene, the amount of sickle cell hemoglobin was greater (35.0 and 36.2 per cent, respectively). While the presence of the spherocytosis gene complicates the interpretation, the results suggest that, if anything, the presence of this particular thalassemia gene tends to decrease the amount of sickle cell hemoglobin.

2. In the third generation of this pedigree there occur, in two different sibships (one resulting from a thalassemia-sickle cell-spherocytosis x presumed normal marriage, and the other from a sickle cell-spherocytosis x known normal marriage) children who either have received both the sickle cell and spherocytosis genes, or who have received neither. This speaks for the non-allelism of the spherocytosis and sickle cell genes. Although several individuals who appeared to possess both the sickle cell and spherocytosis genes have
been reported previously, this is, to our knowledge, the first opportunity to study the segregation relationships of these two genes. From the clinical standpoint, the observations made on this family are in conformity with those of previous investigators, namely, that when the sickle cell and spherocytosis genes are combined, the clinical findings are determined by the type of spherocytosis present in the family. Thus, in this family the spherocytosis is of the clinically mild variety, and this is, by and large, the picture observed in the individuals with both the sickling and spherocytosis genes.

3. In an individual, II 3, who on hematologic and genetic grounds may be presumed to possess all three of the abnormal genes present in this family, the cytologic findings, including the osmotic fragility of the erythrocytes, are those of spherocytosis rather than thalassemia. This suppression or masking of the thalassemia effect provides an example of genetic epistasis on the cellular level. Whatever the fundamental defect responsible for spherocytosis, it has prevailed in the leukocyte of thalassemia, to all intents no less than in the normocyte.

**What is Thalassemia?**

This family tends to call forth, in an acute form, the question “What is thalassemia?” Hitherto, thalassemia has been defined as an iron-refractory, hypochromic microcytosis and leucocytosis. Should the term henceforth be restricted to those cases in which an elevation of the $A_2$ component is demonstrable, as suggested by Gerald and Diamond? We have summarized above the findings to date with respect to $A_2$ levels in thalassemia. In our present state of ignorance concerning the biochemical processes involved, it would seem unwise to eliminate the “low $A_2$” cases from the category of thalassemia and premature to establish a new nomenclature based on a single criterion at this time. In keeping with this view, Gerald has lately referred to “classical” and “non-classical” thalassemias. Our observations suggest that there may be more than one kind of thalassemia. Until the precise nature of the metabolic defect in thalassemia can be defined in terms of enzymatic reactions or abnormalities in protein structure, this argument would be doomed to remain in the realm of semantics were it not for evidence of a different kind. This thalassemia gene, whether or not its effect is regarded as a hemoglobinopathy sensu strictiori, has the remarkable property of interacting with some of the genes responsible for such true hemoglobinopathies as sickling, hemoglobin C and E and probably others. In persons heterozygous for the sickling gene, for example, the simultaneous presence of a thalassemia gene may so modify both the clinical-hematologic picture and the hemoglobin pattern that they resemble those of the homozygous state for sickling, i.e. sickle cell anemia, more than they do those of the ordinary heterozygote or sickle trait. It is now generally admitted that in such persons the production of hemoglobin A may be altogether lacking.

It is of interest that in the family described here and in one other Negro family in whom there was no increase in the $A_2$ fraction in members with hematologic stigmata of thalassemia, there was also no apparent interaction in those individuals who simultaneously possessed the gene for sickling
or for hemoglobin C. The A₂ fraction may thus discriminate between two kinds of thalassemia which also differ in their genetic behavior. The fact that these “non-interacting” cases occurred in Negroes while most of the “interacting” forms have been reported in Caucasians may also be significant.

We have, however, observed both “interacting” thalassemia and “high A₂” thalassemia in Negroes. Nevertheless, the question whether different thalassemia genes are concentrated in the many different populations now shown to have an appreciable incidence of thalassemia deserves study.

We do not have an answer to the question proposed: What is thalassemia? But perhaps we can replace the question with a more realistic one: What are the thalassemias? The answer must come from the biochemist, who will have to demonstrate the specific metabolic defect or defects involved and verify the current belief that it or they involve hemoglobin synthesis.

**SUMMARY**

An American Negro family has been described in which there are found the genes responsible for three inherited abnormalities of the erythrocyte, namely, the genes resulting in spherocytosis, a thalassemia-like trait and the sickling phenomenon. The study of this family provides evidence for the independent segregation of the genes responsible for this type of thalassemia and the sickling phenomenon, and for spherocytosis and the sickling phenomenon. It is noteworthy that the thalassemia gene present in this family is not associated with an increased proportion of hemoglobin A₂, and fails to exhibit “factor interaction” with the sickle cell gene, and the question is raised whether these attributes characterize a type of thalassemia gene non-allelic to the sickle cell gene.

**SUMMARIO IN INTERLINGUA**

Es describite un familia negre american in que esseva trovate le genes responsabile pro tres anormalitates hereditari dcl erythirocytos, i.e. le genes que resulta in spherocytosis, in un tracto thalassembioide, e in le phenomeno del falciformation. Le studio de iste familia provide datos comprobatori pro le conception de un segregation independente del genes responsabile pro iste typo de thalassemia e le phenomeno del falciformation e pro spherocytosis e le phenomeno del falciformation. Es notabile que le gen pro thalassemia presente in iste familia non es associate con un plus alte proportion de hemoglobina A₂ e non exhibi “interaction de factores” con le gen pro celularas falciforme. Assi le question es sublevate si iste attributos characterisa un typo de gen de thalassemia que es non-allelic al gen pro celularas falciforme.

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Multiple Inherited Erythrocyte Abnormalities in an American Negro Family: Hereditary Spherocytosis, Sickling and Thalassemia

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