Further Studies on Hemoglobin C

I. A Description of Three Additional Families Segregating for Hemoglobin C and Sickle Cell Hemoglobin

*By James V. Neel, M.D., Ph.D., Eugene Kaplan, M.D., and Wolf W. Zuelzer, M.D.*

In previous communications the biochemical, genetic, and clinical aspects of a newly recognized inherited abnormality of hemoglobin were briefly described. The new hemoglobin, which was independently designated hemoglobin-III by Kaplan, Zuelzer, and Neel, and hemoglobin-ε by Itano, will, in this and subsequent publications from our group, be termed hemoglobin C in keeping with recent terminologic recommendations. The presence of hemoglobin C is readily demonstrated by electrophoretic studies of hemoglobin suspensions, and may be suspected hematologically in the presence of abnormal numbers of target cells. The tendency to form hemoglobin C appears to depend on a single mendelian factor. Approximately 30 to 40 per cent of the hemoglobin of individuals heterozygous for this factor is electrophoretically abnormal. The combination of this factor with the genetic factor responsible for the sickling phenomenon results in a hemolytic anemia of varying degrees of severity.

The original studies referred to above were based on the investigation of two American Negro families. It will be the purpose of this and the following paper to describe three more American Negro families in which hemoglobin C has been demonstrated, and to present additional information concerning the two original families. These three families were recognized in the course of a systematic survey of approximately 100 families in which there were one or more persons with sickle cell disease. The present paper will be concerned primarily with the identification and genetic patterns of the further families, while the second paper will attempt to synthesize the hematologic observations. Particular attention will be directed toward the significance of the target cell.

**Terminology**

Recent studies on the hemolytic anemias associated with, and presumably due to, the sickling phenomenon have led to the recognition of at least four genetically distinct sub-types. In this paper we will use the term “sickle cell disease” as a generic term including all hemolytic anemias due to, or associated with, the sickling phenomenon. The most common type of sickle cell disease is due to homozygosity for the gene responsible for the sickling phenomenon; we shall refer to this as sickle cell anemia. The second most common type of sickle cell disease among the American Negro appears to be that due to the simultaneous

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presence in an individual of the sickle cell gene and the gene responsible for hemoglobin C; we shall refer to this as sickle cell-hemoglobin C disease. The third type is due to the simultaneous presence of the sickle cell gene and the thalassemia gene, and has been termed sickle cell-thalassemia disease. The fourth and apparently rarest type is due to the concurrence of the sickle cell gene and still another gene responsible for an inherited hemoglobin abnormality, and is termed sickle cell-hemoglobin D disease.

METHODS

The hematologic methods employed have been described in a previous paper. The electrophoretic studies followed the methods described by Wells and Itano.

FIG. 1.—The segregation for the sickling phenomenon and hemoglobin C observed in the five families discussed in this paper. Legend: left half of symbol horizontally lined, presence of hemoglobin C; right half of symbol solid black, presence of sickle cell hemoglobin; vertically lined symbol, no abnormal hemoglobin present; plain white symbol, presence of abnormal hemoglobin not known.

DESCRIPTION OF MATERIAL

The Y. Family (Fig. 1a, Table 1)

Summary. The index case for this family was an 8 year old Negro boy with chronic anemia and erythrocyte sickling. Although his clinical course simulated that of sickle cell anemia, with repeated attacks of musculoskeletal pain and swelling of the joints, his disease appeared hematologically distinct from sickle cell anemia. Both hemoglobin C and sickle cell hemoglobin could be demonstrated electrophoretically in the patient. A portion of the hemoglobin of the father and of two siblings was of the C type. The mother had the sickle cell trait, and the two remaining siblings were essentially normal.

Case histories. J. Y., an 8 year old Negro boy, has been observed since early childhood for what appeared to be true sickle cell anemia. He was first studied at 2 years of age when, during hospitalization for acute pneumonia, he was found to have a mild normocytic, normochronic anemia associated with erythrocyte sickling, reticulocytosis, and splenomegaly. Since then the anemia has remained mild. The splenomegaly has disappeared, but there have been recurrent, approximately annual, attacks of pain and swelling in his elbows and
<table>
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<th>Family</th>
<th>Name</th>
<th>Age</th>
<th>Blood group (phenotype)</th>
<th>Electrophoretic findings</th>
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<th>Hemoglobin, Gm. %</th>
<th>E% reticulocytes</th>
<th>Occurrence of sickling</th>
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<td>8.0 10.8</td>
<td>0.7 5.2 62.0</td>
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The hemoglobin levels have varied between 7 and 9.6 Gm. per cent, the red cell count between 2.8 and 4.4 million, and the reticulocytes between 4.6 and 5.8 per cent. There was erythrocyte sickling in moist blood films. In fixed preparations sickled forms were only rarely present but more than 50 per cent of the red cells were target cell forms. The percentage of siderocytes (erythrocytes containing cytoplasmic granules staining with potassium ferrocyanide and dilute hydrochloric acid) was within normal limits. The serum bilirubin was 0.6 mg. per cent. The bone marrow was hypercellular, with an M:E ratio of 1:2.5. Electrophoretically the hemoglobin was a mixture of two types. On the basis of the appearance of the electrophoretic curve, the above described findings in the patient, and the detection of hemoglobin C in the father, it is assumed that both hemoglobin C and sickle cell hemoglobin were present in the patient.

The patient’s father (A. Y.) was treated for syphilis five years ago but has otherwise been in excellent health, and on physical examination had no splenomegaly, pallor, icterus, or other significant abnormality. Although not examined by us, nine of his siblings were alive and reported to be in good health, as were their children. Two siblings had died in infancy of unknown causes. Examination of A. Y.’s blood revealed hemoglobin levels of 12 to 13.9 Gm. per cent, red cell counts of 4 to 5.3 million, reticulocytes between 0.2 and 1.0 per cent, and a serum bilirubin of 0.2 mg. per cent. There was no sickling of the erythrocytes, but in the fixed blood films up to 6 per cent of the red cells were target cell forms. An electrophoretic analysis of his hemoglobin showed it to be a mixture of two types (fig. 2a). When analyzed electrophoretically, a 1:1 mixture of his hemoglobin with that of J. W. (figs. 1d and 3b), the prototype for the uncomplicated hemoglobin C trait, resolved into two components comparable to those seen in A. Y. or J. W. alone, thus rendering it very probable that the electrophoretically abnormal component in A. Y. was hemoglobin C. A normal recipient was transfused with A. Y.’s erythrocytes and their survival followed for twenty days, when 75 per cent of the transfused cells still remained in the recipient’s circulation. This is entirely within the normal range of erythrocyte survival.

The patient’s mother (C. H. Y.) was a healthy Negro female whose blood had the characteristics of the sickle cell trait. Less than 1 per cent of the red cells were target cell forms.

The patient was one of five children. An older sister, L. Y., has never appeared ill, and on physical examination had no significant abnormalities. Hematologic studies revealed a hemoglobin of 10 to 12 Gm. per cent, erythrocyte count of 3.3 to 4.1 million, and reticulocytes of 0.6 to 1.4 per cent. The erythrocytes did not sickle, but 2 to 10 per cent of the red cell population were target cells. The serum bilirubin was 0.1 mg. per cent. Electrophoresis resolved her hemoglobin into two components (fig. 2b). These were assumed to be the same two components observed in her father’s hemoglobin.

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C. Y. was a 4 year old Negro child whose history and physical examination were entirely normal. Her hemoglobin was 10.5 Gm. per cent, red cell count 3.8 million, and reticulocyte count 0.1 per cent, with 0.3 per cent target cells, and no erythrocyte sickling. Serum bilirubin was 0.2 mg. per cent. Electrophoretic analysis of her hemoglobin yielded the unimodal curve characteristic of normal (fig. 2d).

W. Y. was a normal appearing 2 year old child with a mild hypochromic microcytic anemia of nutritional origin. The hemoglobin levels were 7.0 to 8.3 Gm. per cent, erythrocyte counts 4.5 to 5.1 million, and reticulocyte count 0.4 per cent. The red cells were typical of iron deficiency anemia, and they did not sickle. Target cells comprised approximately 5 per cent of the red cell population. Electrophoretic studies were not carried out. The signifi-
enee of the target cell count in the presence of iron deficiency anemia is difficult to evaluate. Until further study this child is therefore presumed to be normal with respect to hemoglobin C.

The P. Family (Fig. 1b, Table 1)

Summary. The index case was a 10 year old Negro girl with a mild hemolytic anemia and erythrocyte sickling. Clinically and hematologically her disease was distinct from sickle cell anemia. Electrophoretically her hemoglobin consisted of a mixture of two types of hemoglobin which are assumed to be sickle cell hemoglobin and hemoglobin C. A sister presented identical hematologic and electrophoretic findings. The mother and one sibling had the sickle cell trait. One brother was entirely normal clinically and electrophoretically, while another brother by electrophoresis had a mixture of two types of hemoglobin demonstrated to be normal and hemoglobin C. The father did not sickle but had numerous target cells and was assumed to be the source of the hemoglobin C present in three of his children; electrophoretic studies were not carried out.

Case histories. M. P., a 10 year old Negro girl, was referred to the Anemia Clinic of the Children's Hospital of Michigan one year ago because of splenomegaly and occasional episodes of mild pain in the elbows and knees. Except for an infrequent respiratory or skin infection, she had always appeared in excellent health. During the year of our observation she has remained entirely free of symptoms. The patient was an alert, happy child, well nourished and developed. Except for a persistent enlargement of the spleen, which extended 2 cm. below the costal margin, the physical findings were essentially within normal limits. X-ray studies revealed no significant cardiac enlargement, essentially normal long bones, and slight hypertrophy of the frontal bones.

The hemoglobin levels varied between 7.2 and 11.0 Gm., the red cell count between 3.1 and 4.5 million, the reticulocytes between 0.6 and 7.6 per cent. The erythrocytes sickled in sealed moist blood films, and in fixed films more than half were target cells with no sickled forms visible. Siderocytes were not increased. The osmotic fragility began at .37 per cent saline and was complete at .21 per cent. The serum bilirubin varied between .0.3 and 1.3 mg. per cent. The marrow aspirate revealed a moderate erythroid hyperplasia with an M:E ratio of 1:1.6. Electrophoretic analysis of her hemoglobin resulted in a pattern indicating a mixture of two types which, from the appearance of the curve, the patient's course, and the demonstration of hemoglobin C in a brother, were assumed to be sickle cell hemoglobin and hemoglobin C.

The patient was one of five children by this marriage, the mother having had five children by an earlier marriage. These half-siblings were not available for study, but they were reported to be in excellent health. The mother (Z. M. P.) has been followed in a cardiac clinic for many years because of mild rheumatic heart disease. Examination of her blood revealed a hemoglobin level of 10 Gm. per cent, a red cell count of 4.4 to 5 million, and a reticulocytosis of 3.4 to 4.2 per cent. The erythrocytes appeared slightly hypochromic on smear, and they sickled in sealed moist blood films. The elevated reticulocyte count observed in this woman on two occasions has not been satisfactorily explained.

The father, F. P., was a large, vigorous man in apparently excellent health, who had been free of any significant illness except for syphilis treated ten years before. There had been no pallor or icterus. There was no palpable enlargement of either liver or spleen. Examination of his blood revealed a hemoglobin level of 14.3 Gm. per cent, a red cell count of 4.3 million, and a reticulocytosis of 4.3 per cent. The erythrocytes appeared slightly hypochromic on smear, and they sickled in sealed moist blood films. The elevated reticulocyte count observed in this man on two occasions has not been satisfactorily explained.

Ja. P., a 9 year old brother of the patient, was entirely well, with no splenomegaly, anemia, or erythrocyte sickling. The average frequency of target cells on stained blood films was 1.5 per cent. Electrophoretic analysis revealed his hemoglobin to be entirely homogeneous.

Je. P., a 7 year old brother, has always been in excellent health, and likewise had no splenomegaly, anemia, or erythrocyte sickling. Target cells comprised 3 per cent of his red
HEMOGLOBIN C

Fig. 2. (Above.) See legend on opposite page.

Fig. 3. (Opposite.) See legend on opposite page.
cell population. The serum bilirubin was 0.3 mg. per cent. Electrophoretic analysis revealed his hemoglobin to consist of a mixture of two types (fig. 3a). Electrophoretic analysis of a 1:1 mixture of his hemoglobin with that of J. W. (figs. 1b and 3b), the prototype of the hemoglobin C trait, resulted in a bimodal curve sufficiently comparable to the two originals that identity of the two peaks in Je. P. as normal and hemoglobin C was considered established.

A. P., a 6 year old sister, had never appeared acutely or chronically ill and had been free of pallor, icterus, or any episodes of pain or soft tissue swelling. She was a well developed child in apparently excellent health, with no abnormalities save for a firm spleen which extended 2 cm. below the costal margin. X-ray studies revealed no significant changes in the cardiac shadow, long bones, or skull. The hemoglobin levels have been 9.3 to 10 Gm. per cent, red cell counts 3.5 to 4.3 million, and reticulocyte counts 2 to 8 per cent. The erythrocytes sickled in sealed moist blood films, and in the fixed stained smears over 50 per cent were target cell forms, whereas there were no sickled forms. Siderocytes were not increased. Osmotic fragility began at 0.37 per cent saline and was complete at 0.18 per cent. Electrophoretic studies on this girl were unsatisfactory.

D. P., a 4 year old brother, was entirely well, without splenomegaly, icterus, or pallor. His blood counts revealed values within the normal limits. There was erythrocyte sickling in sealed moist blood films, and in fixed films the target cell frequency averaged 1 per cent. Electrophoresis of his hemoglobin revealed a mixture of two types, assumed to be normal and sickle cell hemoglobin.

The M. Family (Fig. 1c, Table 1)

Summary. The index case, a 5 year old Negro boy with mild hemolytic anemia and erythrocyte sickling, exhibited the electrophoretic pattern characteristic of a mixture of two types of hemoglobin, which are assumed, on the basis of the family findings and mobilities, to be hemoglobin C and sickle cell hemoglobin. The clinical and hematologic features of his disease were distinct from sickle cell anemia. The child's father had the sickle cell trait. The child's mother had the asymptomatic hemoglobin C trait. The maternal grandfather showed increased numbers of target cells and was assumed also to have the hemoglobin C trait.

Case histories. G. M. is a 5 year old Negro boy who was considered a well baby up to 18 months of age when he was found to have a mild normochromic, normocytic anemia, moderate hepatosplenomegaly, and erythrocyte sickling. Except for infrequent mild pains in his legs and an episode of pneumonia for which he was hospitalized, he has remained free of complaints. The child was well developed and intelligent. There was no pallor or icterus. There was slight hepatomegaly up to age 2 years; this has since disappeared. The spleen has remained moderately firm and nontender, and has been palpable 2 to 4 cm. below the costal margin since 1 1/2 years of age. The heart and skeleton were not unusual on physical or roentgenologic examination.

The hemoglobin varied between 8 and 10.8 Gm. per cent, the red cell count between 3.1 and 4.8 million, and the reticulocyte count between 0.7 and 5.2 per cent. Target cells were always prominent in his peripheral blood films and comprised 50 to 85 per cent of the red cell population. His erythrocytes sickled in sealed moist blood films, but sickle forms were not present in the fixed stained films. Siderocytes were not increased. The bone marrow aspirate revealed a moderate erythroid hyperplasia with an M:E ratio of 1:2.2. The hemolytic index at 2 years was 5.5 units and at 5 years was 11 units. The serum iron was 150 μg., and the serum bilirubin 1.3 mg. per cent. The patient's erythrocytes were rapidly eliminated after transfusion into a normal recipient. Erythrocytes from a normal donor, as well as from the patient's mother, were eliminated at the normal rate when transfused into the patient.

Fig. 2.—The results of an electrophoretic analysis of the hemoglobin of four members of the Y. family. (a) A. Y., (b) L. Y., (c) R. Y., and (d) C. Y.

Fig. 3.—Electrophoretic findings with respect to the hemoglobin of (a) Je. P., (b) J. W. (prototype for the hemoglobin C trait), and (c) a 1:1 mixture of hemoglobin from Je. P. and J. W.
Electrophoretic studies were not entirely satisfactory but suggested a mixture of two components, thought, on the basis of the findings in his parents, to be sickle cell hemoglobin and hemoglobin C.

The patient was an only child. His father was normal save for the sickle cell trait. His mother was a vigorous young woman in apparently excellent health, with no anemia, icterus, or splenomegaly. Her erythrocytes did not sickle, but in fixed blood films 2 to 5 per cent were distinct target cell forms. Their osmotic fragility was within the normal range. Electrophoretic analysis of her hemoglobin revealed the presence of two types of hemoglobin which, by calibration with the blood of J. W., could be shown to be hemoglobin C and normal. Her erythrocytes, when transfused into both a normal infant and her son, were eliminated at the normal rate.

The maternal grandmother was a healthy Negro woman aged 48, with no hematologic abnormality. The maternal grandfather was a Negro, aged 52, in excellent health who had no anemia, icterus, or visceral enlargement. Whereas his red cells did not sickle, 5 to 10 per cent were target cells in ordinary blood films. It was not possible to obtain a sample of this man's blood for electrophoretic studies, but from the presence of the increased number of target cells and the occurrence of hemoglobin C in his daughter, it was suspected that he was heterozygous for the hemoglobin C gene.

Further Observations on Families Previously Described

The two families previously described have been followed during the two year interval which has elapsed since our earlier report. In family W. (fig. 1d), two of three children have sickle cell-hemoglobin C disease. Ro. W., who had a mild hemolytic anemia, erythrocyte sickling, and persistent hepatosplenomegaly is now 14 years old, and remains in apparent good health with no change in her hematologic status. The liver is no longer palpable, and the spleen has likewise decreased in size, now extending only 1 cm. below the costal margin. T. W., with a similar mild hemolytic anemia, sickling, and visceral enlargement is now 12 years old, moderately underweight, but otherwise in good health. His liver is no longer palpable, and the spleen has remained essentially unchanged, extending 3 cm. below the costal margin. J. W., the father, who has the hemoglobin C trait, remains entirely asymptomatic.

We have now had the opportunity to examine seven additional blood relatives in this family, not previously reported. Erythrocyte sickling was uniformly absent. Among these individuals there were three with definitely increased target cells, 33 per cent, 22 per cent and 5 per cent respectively. These are thought to have the hemoglobin C trait. A fourth adult with 3.6 per cent target cells has been tentatively designated as normal. The hematologic findings in all these people and their relationship to the original two cases in the family are indicated in figure 1d and table 1. The red cells of the two individuals with 33 per cent and 22 per cent target cell forms also had increased osmotic resistance. No other hematologic or clinical abnormality could be demonstrated in the group. Unfortunately, electrophoretic studies could not be performed.

In family C. (fig. 1e), the youngest of three children has sickle cell-hemoglobin C disease; the father and one brother have the hemoglobin C trait. P. C., the patient, has a mild hemolytic anemia and erythrocyte sickling. She is now 5 years old, and since our first report has had a single mild episode of abdominal pain not associated with icterus or change in the hematologic picture. The previously noted enlargement of liver and spleen has now completely receded. Both the father and brother with hemoglobin C trait remain entirely asymptomatic.

DISCUSSION

In each of the three new families described in this paper, the presence of hemoglobin C was definitely established in at least one member of the family. This was accomplished by combining a hemoglobin suspension derived from one of the originally described hemoglobin C carriers (J. W.) with a hemoglobin sus-
pensions from a suspected individual and demonstrating that the mixture separates into two components during electrophoresis in a manner comparable to the results of electrophoresing either suspension separately. The available hematologic, familial, and electrophoretic data on these families can best be explained by the interpretation given in the pedigrees of figure 1, a–c. The further data obtained on the W. kindred is thought to fit into the pattern indicated in figure 1d.

The five pedigrees contain information regarding the offspring from two types of marriages, namely, hemoglobin C trait × sickle cell trait, and hemoglobin C trait × normal. We will consider the results of the former type of marriage first. This comes to attention because of the occurrence among the issue of at least one case of sickle cell-hemoglobin C disease. Disregarding the index case of sickle cell-hemoglobin C disease for each sibship, and assuming there is only one index case per sibship, then among the five sibships thus far studied from the mating sickle cell trait × hemoglobin C trait the ratio of persons with hemoglobin C to sickle cell trait to normal to sickle cell-hemoglobin C disease is 4:1:5:2. There are thus six children with hemoglobin C and six without. The children of the single marriage known with a high degree of probability to be hemoglobin C × normal (fig. 1d) consist of two normal and two with hemoglobin C. Wherever it has been possible to study both parents of a child with hemoglobin C, at least one has also possessed the abnormal hemoglobin. These facts lend further support to our previous suggestion that hemoglobin C is the result of the action of a single gene. There is no evidence of sex-linkage. From the experience to date, this gene would appear to have a high degree of penetrance.

As was pointed out previously, and as will be discussed in some detail in the following paper, the frequency of target cells is increased in the hemoglobin C trait. In our previous paper it was reported that the highest percentage of target cells thus far encountered in the hemoglobin C trait was in case 5, the J. W. of figure 1d in this paper, who had 22 per cent. It is of some interest that the highest frequency of target cells encountered in the additional cases of hemoglobin C trait reported in the present paper occurred in H. W. and Dv. W., brother and nephew of J. W., who averaged 33 and 22 per cent respectively. This finding suggests that the frequency of target cells is in part controlled by intrafamily factors.

It is not possible at present to reach conclusions concerning the genetic relationship between the genes responsible for hemoglobin C and sickle cell hemoglobin. A decision depends on the results of an analysis of the kinds of children obtained from marriages of persons with sickle cell-hemoglobin C disease × normal. If such marriages produce either normal children or children with sickle cell-hemoglobin C disease, it may be concluded that the two genes are not allelomorphs. Failure to produce such children, on the other hand, suggests allelomorphism or close linkage. The absence of such children will reach the 5 per cent level of significance with regard to linkage or allelomorphism when, among five children, none is of the parental types. In view of the fact that sickle cell-hemoglobin C disease is apparently no great rarity, the data necessary to a decision will, no doubt, be accumulated within the next few years in hematologic clinics which have a somewhat more mature case roster than our own.
Hemoglobin C

Summary

Three families are described in which there occur one or more children with sickle cell-hemoglobin C disease. Additional data are presented concerning two previously described families.

References

Further Studies on Hemoglobin C: I. A Description of Three Additional Families Segregating for Hemoglobin C and Sickle Cell Hemoglobin

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