Two Cases of Sickle Cell Disease Presumably Due to the Combination of the Genes for Thalassemia and Sickle Cell Hemoglobin

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In recent years our knowledge of the etiology of sickle cell disease has increased considerably. As a rule, both parents of a child with this disease show the sickle cell trait. This has led to the hypothesis that usually sickle cell disease is due to homozygosity for a gene which, when heterozygous, results only in the sickle cell trait. Occasionally, however, families are encountered in which the blood of only one of the parents of a child with clinical sickle cell disease can be induced to sickle. The study of such families has thus far resulted in the recognition of 3 other genetic types of sickle cell disease. In each of these 3 other types, the nonsickling parent is heterozygous for a gene responsible for another hematologic abnormality. The children who develop sickle cell disease in these families receive the gene responsible for the sickling phenomenon from one parent and 1 of the 3 other genes mentioned above from the other parent. The 3 other genes in question are: 1) the gene responsible for thalassemia,6 2) the gene responsible for hemoglobin-c (hemoglobin-III),7-8 and 3) the gene responsible for hemoglobin-d.9

By now, approximately 18 families have been described in Italy, South America, and the United States in which sickle cell disease was presumably due to the first of the 3 less common mechanisms mentioned above, i.e., homozygosity for both the sickle cell and the thalassemia genes.5-6 It is the purpose of the present report to describe still another such family. The justification for so doing is that the bloods of the 2 presumed “double heterozygotes” in this family have been subjected both to electrophoretic and alkaline denaturation studies, with results of some theoretic interest.

Description of Family

The index case for this family is S. J. L., a 36 year old white married woman of Greek descent who, in September of 1950, was referred to one of us (J. S. L.) because of a persistent anemia accompanied by the sickling phenomenon. The anemia had first been discovered in 1935, during her only pregnancy. Sickling was first noted in 1943. Episodes of fever and jaundice had occurred for many years. The chief findings on physical examination included a palpable and audible plural friction rub over the lower right anterior chest. The liver
edge was questionably palpable 2 finger breadths below the costal margin. The edge of the spleen was also readily palpable 2 finger breadths below the costal margin. The upper border of splenic dullness was 4 finger breadths above the costal margin. There was moderate edema of the feet and ankles. The chief laboratory findings are summarized in Table 1.

These findings plus the clinical history and the results of the physical examination suggested the diagnosis of sickle cell anemia, although, as is well known, the spleen is not usually palpable in patients with sickle cell anemia after the second or third decade.

This patient was first seen shortly after the publication of the initial case report in the English language concerning the appearance of sickle cell disease in persons heterozygous for both the sickling and thalassemia genes. Because of her nationality, it was surmised that the disease in this patient might also be on this basis rather than on the more usual basis of homozygosity for the sickle cell gene. The family studies necessary to a decision were somewhat complicated since the patient lived in Los Angeles whereas most of her relatives resided in Buffalo, New York. However, it was possible to obtain hematologic data on both parents, the only child, the husband, 5 siblings, 2 nieces, and a nephew of the patient. These studies consisted of a determination of the number of erythrocytes and leukocytes per cu.mm. of blood, Gm. of hemoglobin per 100 cc. of blood, the hematocrit, the differential blood count, and a reticulocyte count. Stained films were graded for the occurrence of oval, target, and sickle cells. The findings on the patient's husband were entirely within normal limits. The patient's son exhibited only the sickle cell trait. The findings on the patient's husband were entirely within normal limits. The patient's son exhibited only the sickle cell trait. The findings on the patient's husband were entirely within normal limits. The patient's son exhibited only the sickle cell trait.

One nucleated erythrocyte was observed in 1 of 3 blood films obtained on 3 different visits. 12.5 nucleated erythrocytes per 100 WBC.

The patient's father, J. J., was born in Lumona, Greece in 1881 and came to this country at age 22. The patient's mother was born in Sparta, Greece in 1889, and emigrated to the United States at age 18. The father and mother are not known to be related. Eleven children were born to this marriage. There were 2 deaths in infancy and 1 stillbirth. A fourth child is described as appearing to be normal until about 1 year of age, at which time he became pale, listless, and chronically ill. He died at home at age 2½ after a brief febrile illness. In retrospect, it seems quite possible that he had a more severe form of the disease present in his sister, S. J. L. Seven members of this family reached adult life. In addition to the patient, 2 members of the family had noteworthy medical histories: G. J., the oldest of the children, developed signs of thyrotoxicosis at about age 36. At age 39 he underwent a subtotal thyroidectomy. This was followed by a local recurrence, and when seen by us he was about to receive a course of radioactive iodine. T. J., a male aged 34, had been found...
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To have splenomegaly at age 9, when he was briefly hospitalized. He apparently enjoyed average health during his youth, and entered the Navy at age 25. While in the Navy he developed an acute polyarthritis, for which he was hospitalized. A diagnosis of rheumatic fever was made, and he was discharged from the service. At age 33 he developed a pneumonitis. This was the first time he was informed of the presence of a severe anemia. When seen by us he was a thin, sallow white male who appeared chronically ill. Circumstances did not permit a physical examination. The hematologic findings in this individual were very similar to those in the original patient. His sickle cells were of the filamentous type seen in typical sickle cell anemia. Erythrocyte agglutination was marked in the counting chamber at room temperatures; this disappeared with gentle heating.

From Table 1 and Figure 1 it can be seen that the mother and a sister of the patient had normal hematologic values but a positive sickling test. The father and 2 brothers were found to have an elevated erythrocyte count and a significantly decreased MCV and MCH. One of these brothers (C. J.) showed a slight increase in oval cells, to an extent that would not usually arouse comment. The other brother (G. J.) exhibited a significant increase in the number of target cells. The father was found to have a very minor increase in both target and oval cells. These findings are consistent with the diagnosis of thalassemia minor.

Fig. 1—The pedigree of the J.-L. kindred.

In view of the finding of thalassemia minor in one parent and the sickle cell trait in the other, the most probable explanation of the sickle cell disease present in the patient (S. J. L.) and her brother (T. J.) is that they received the sickle cell gene from one parent and the thalassemia gene from the other. From the fact that sickling of the red cells was present in 2 individuals, it is certain that the sickle cell gene was present. Final proof that the thalassemia gene was also present would depend on the observation of thalassemia minor in one or more of their children. In this family such proof was lacking, since the only child of S. J. L. showed the sickle cell trait and T. J. had no children. Tests for the A, A, BO, MN, and Rh (C, D, E, c) blood groups revealed no evidence of nonpaternity.

Biochemical Studies

The hemoglobins of the patient, 5 of her siblings, and the parents were examined by electrophoresis of carbonmonoxyhemoglobin in cacodylate buffer of pH 6.5 and ionic strength 0.1, and in 0.01 M Na2HPO4. The recent adaptation of the technic, introduced by Haurowitz, of partially separating alkali-denatured hemoglobin from undenatured material by one-third saturation with ammonium sulfate, was applied to the same hemoglobin specimens. Holden and
Freeman\textsuperscript{16} had previously applied a similar technic to the separation of denatured globin from globin.

\textit{Sickle cell trait} (S. P. J. and L. J. K.): Electrophoresis in cacodylate buffer revealed the presence of both normal adult and sickle cell hemoglobins in these individuals. Alkali denaturation tests showed the absence of significant amounts of an alkali resistant residue. The percentages of sickle cell hemoglobin, 44 and 45 per cent respectively, are among the highest which have been observed in sickle cell trait.\textsuperscript{14}

\textit{Thalassemia minor} (J. J., G. J., and C. J.): The alkali denaturation test resulted in 6, 5, and 2 per cent of an alkali resistant residue. Electrophoresis in cacodylate buffer revealed a one peak diagram in each of these individuals. A small shoulder, representing a component of slightly greater mobility than normal adult hemoglobin, was present in the scanning diagrams of the hemoglobins of G. J. and J. J.

\textit{Sickle cell–thalassemia disease} (S. J. L. and T. J.): Electrophoresis in cacodylate buffer revealed a preponderance of sickle cell hemoglobin. The apparent percentages, as measured from the scanning diagrams, were 61 and 84 per cent, respectively. The alkali resistant residue comprised 19 and 5 per cent, respectively, of the hemoglobins of S. J. L. and T. J. Electrophoresis in 0.01 M \(\text{Na}_2\text{HPO}_4\), in which resolution of mixtures of normal adult and fetal hemoglobins occurs,\textsuperscript{18} revealed the presence of normal adult hemoglobin in T. J.'s hemoglobin. S. J. L.'s hemoglobin has not been studied by this method.

A two peak electrophoretic pattern of carbonmonoxylhemoglobin in cacodylate buffer has been previously reported in an individual with sickle cell-thalassemia disease.\textsuperscript{12} A fast component, having the mobility of sickle cell hemoglobin, and a slow component, having the mobility of normal adult hemoglobin, were found. Electrophoresis in 0.01 M \(\text{Na}_2\text{HPO}_4\) of the hemoglobin from this individual resulted in a three peak pattern with components having the mobilities of normal adult, fetal, and sickle cell hemoglobins, respectively.\textsuperscript{20} This observation indicates that the slow component in cacodylate buffer at pH 6.5 includes both normal adult and fetal hemoglobins. The electrophoretic and denaturation results on the hemoglobins of the two sickle cell–thalassemia individuals in this study indicate that they also have the three forms, normal adult, fetal, and sickle cell.

The apparent percentages of sickle cell hemoglobin by electrophoretic analysis at pH 6.5 in the 4 cases of sickle cell–thalassemia disease studied by this method vary from 61 to 84 per cent.\textsuperscript{29} Under the same experimental conditions, the lowest apparent percentage of sickle cell hemoglobin which has been observed in the most common type of sickle cell disease is 75 per cent, and in the majority of cases this figure is greater than 90 per cent.\textsuperscript{29} Thus, the average fraction of sickle cell hemoglobin is higher in the most common type of sickle cell disease than in sickle cell–thalassemia disease.

The biochemical findings in the individuals with sickle cell trait and thalassemia minor are in agreement with the results of previous studies.\textsuperscript{14, 16, 21} No fetal hemoglobin is present in the individuals with the sickle cell trait, but significant amounts of the alkali resistant residue were found in 2 of the 3 individuals with thalassemia minor. The mobilities of normal adult and fetal
Table 2.—Biochemical Findings in the J.-L. Kindred

<table>
<thead>
<tr>
<th>Name</th>
<th>Diagnosis</th>
<th>Apparent Percentage by Electrophoresis</th>
<th>Per cent Alkali Resistant Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slow Component*</td>
<td>Fast Component†</td>
</tr>
<tr>
<td>S. J. L.</td>
<td>Sickle cell: thalassemia</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>S. P. J.</td>
<td>Sickle cell trait</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>J. J.</td>
<td>Thalassemia minor</td>
<td>100†</td>
<td>0</td>
</tr>
<tr>
<td>G. J.</td>
<td>Thalassemia minor</td>
<td>100†</td>
<td>0</td>
</tr>
<tr>
<td>T. J.</td>
<td>Sickle cell: thalassemia</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>L. J. K.</td>
<td>Sickle cell trait</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>C. J.</td>
<td>Thalassemia minor</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* Normal adult and fetal hemoglobins.
† A small shoulder, representing a component of slightly greater mobility than normal adult hemoglobin, was present.
§ The separation of native from denatured protein by one-third saturation with (NH₄)₂SO₄ is incomplete. The figures in this column should not be interpreted as representing the actual percentage of fetal hemoglobin present.
§ Sickled cell hemoglobin.

Discussion

It is possible at the present time to reach certain tentative conclusions concerning the overall relative frequencies of the various genetic types of sickle cell disease. There can be no doubt that by far the majority of cases of sickle cell disease, unlike the 2 described in this paper, develop on the genetic background of homozygosity for the gene responsible for the sickling phenomenon. In families in which this most common type of the disease appears, both parents have the sickle cell trait; 1 out of 4 of their children, on the average, receives a sickle cell gene from both parents and develops sickle cell anemia.

The second most frequent type of sickle cell disease would appear to be the type which develops in a child who receives the gene responsible for the sickling phenomenon from one parent and the gene responsible for a new and still poorly understood hemoglobin abnormality from the other parent. This new hemoglobin has been designated hemoglobin-III or hemoglobin-c, and the disease has been named sickle cell-hemoglobin-c disease. Electrophoretically, this hemoglobin differs from normal in its mobility even more than sickle cell hemoglobin. Its solubility behavior is similar to that of normal adult hemoglobin. Hemoglobin-III or c seems to be due to the action of a dominant gene. In a series of 48 families where it was possible to test both parents of a child who appeared to have clinical sickle cell disease, we discovered that in three of these families one of the parents had a sickling gene and the other had the gene responsible for this new abnormality. In other words, on the basis of these very preliminary figures, it would appear that in about 6 per cent of the Negro families studied thus far in...
which sickle cell disease was present, the disease was due to this combination of a sickling gene with the gene responsible for this new hemoglobin abnormality. Inasmuch as the frequency of the sickle cell gene is now known with considerable accuracy, we can, on the basis of simple proportionality, make some first crude estimates of the frequency of the new hemoglobin abnormality. It would appear that in the Negro population in the Detroit area, approximately 3 out of 1000 individuals should be heterozygous for the gene responsible for the new hemoglobin abnormality. Individuals who are homozygous for the gene responsible for this newly described type of hemoglobin would be expected to occur with a frequency of about 2.3 per 1,000,000 births; such individuals have not yet been recognized. Sickle cell–hemoglobin-c disease appears to run a milder clinical course than does the more usual variety. For this reason this type of the disease does not have the same probability of diagnosis as classical sickle cell anemia; accordingly, the frequency estimate given above may be regarded as minimum. The true frequency of American Negroes with hemoglobin-c may be between 0.5 and 1.0 per cent.

The third type of sickle cell disease, from the standpoint of relative frequency, appears to be the type described in this paper. As has been pointed out elsewhere, this type probably accounts for the majority of cases of sickle cell disease which have been described in presumably Caucasian individuals. It is more than coincidence that so many of these cases in Caucasians are found in individuals of Sicilian and southern Italian, or, like this family, Greek derivation. These are ethnic groups in which the thalassemia gene is especially frequent, and also in which the sickle cell gene is not infrequently encountered, possibly in consequence of racial admixture occurring over a period of several thousands of years, when Sicily and parts of Greece were the crossroads of the Mediterranean world.

Finally, what appears to be a fourth type of sickle cell disease, recognized thus far in only a single family, has recently been described by one of us. These cases, which involve two apparently Caucasian siblings, appear to be due to the simultaneous presence of a sickling gene and a previously undescribed gene which is also responsible for a hemoglobin abnormality. This latest type of hemoglobin, which has been tentatively designated as hemoglobin-d, is characterized electrophoretically by the mobility of sickle cell type hemoglobin, but unlike the sickle cell type of hemoglobin, is not associated with the phenomenon of sickling.

Attention has already been called to the fact that the proportion of sickle cell type hemoglobin present in the original patient and her clinically similar brother is very significantly higher than in the uncomplicated sickle cell trait, although less than is usually encountered in sickle cell anemia. This was also true of the case described by Sturgeon, Itano and Valentine. The presence of the thalassemia gene is associated with a subnormal amount of hemoglobin per erythrocyte. Recent biochemical studies have suggested that the primary action of the thalassemia gene is not to produce an abnormal hemoglobin but to provide a partial block to normal adult hemoglobin synthesis. It has, therefore, been proposed that the preponderance of sickle cell hemoglobin in an individual who is doubly heterozygous for the sickle cell and thalassemia genes might be due primarily to the diminished production of normal adult hemoglobin.
An absolute increase in the amount of sickle cell hemoglobin per erythrocyte also contributes to its preponderance in sickle cell-thalassemia disease. With sickle cell trait as the reference condition, a similar increase in the amount of abnormal hemoglobin per allele present has been observed in sickle cell anemia and sickle cell-hemoglobin-c disease. This increase could be interpreted as compensatory in nature, so that in the absence or impairment of the normal process some compensation on the part of the alternative, aberrant process occurs. An analogous hypothesis has been advanced to account for the presence of fetal hemoglobin in thalassemia major. In terms of relative rates of hemoglobin synthesis, this phenomenon may be ascribed to a longer period of hemoglobinization by a synthetic process which produces an aberrant hemoglobin at a subnormal rate.

Singer and Chernoff have suggested, on the basis of its inability to form tactoids, that the alkali resistant component in the hemoglobin in sickle cell anemia is fetal hemoglobin. The slowly migrating component observed on electrophoresis of sickle cell anemia hemoglobin in cacodylate buffer of pH 6.5 apparently corresponds to the alkali resistant component. This component has been found to have the same electrophoretic mobility and ultraviolet absorption spectrum as fetal hemoglobin. Its immunologic behavior likewise is similar to that of fetal hemoglobin. It is difficult to state with certainty the identity of protein molecules on the basis of a few tests; however, their similar behavior in each of these tests renders highly probable the assumption of the identity of fetal hemoglobin and the alkali resistant component.

Holden and Freeman observed that the separation of globin and acid-denatured globin by salt precipitation was not quantitative, particularly in the presence of hematin. Likewise, Haurowitz felt that one-third saturation with ammonium sulfate did not result in a quantitative separation of denatured and undenatured hemoglobin in alkali-treated hemoglobin specimens and considered the results which he obtained by use of this method to be approximations of the actual amount of denaturation. Thus, although the amount of alkali resistant residue is apparently an index of the relative amount of fetal hemoglobin present, the method of Singer et al. cannot at the present time be regarded as a method for the quantitative determination of fetal hemoglobin. Quantitative studies on both the precipitated and the dissolved material must be conducted in order to evaluate the degree of fractionation which the method achieves.

The 2 siblings with sickle cell-thalassemia disease differ from each other in the proportions of the 3 hemoglobins, and an especially large difference occurs in the fetal hemoglobin proportions. A difference in the fetal hemoglobin percentage has also been found in siblings with sickle cell anemia and sickle cell-hemoglobin-d disease. This phenomenon may possibly be related to the age at which severe anemia first occurs; if this is early in infancy, the fetal mechanism might be reactivated with greater efficiency than if crises appear after the fetal mechanism has been dormant for a period of time. Singer, Chernoff, and Singer noted the lack of any discernible correlation between the percentage of the alkali resistant component and the severity of anemia. It is probable, however, that the presence of a higher proportion of non-sickle cell hemoglobin than is generally
found in the most common type of sickle cell disease may result in a less severe anemia.22

Differences in adult hemoglobin proportions among siblings have been observed in the sickle cell trait.27 Familial data on these ratios suggest the existence of a genetic control. The data can be explained by postulating the existence of three allelic inherited rate modifications for normal adult hemoglobin synthesis which are responsible for the net synthesis within an erythroblast of the same molecule at different rates and a single rate for the synthesis of sickle cell hemoglobin.24 Alternatively, it has been suggested that it is the synthesis of the sickle cell hemoglobin which is subject to the rate modifications.27 A decision between these two possibilities is not possible at present. It has also been suggested that the observed gradations in the severity of thalassemia might be due to inherited differences in the effectiveness of normal adult hemoglobin synthesis,21 in combination with the apparently independent block to normal hemoglobin synthesis which has been attributed to the thalassemia gene.25 In the present instance, the occurrence of different proportions of normal adult and sickle cell hemoglobins in two individuals who apparently possess identical alleles for sickle cell hemoglobin synthesis and for thalassemia may, on the basis of these possibilities, be due to modifications of either normal or sickle cell hemoglobin synthesis.

A brief discussion of the terminologic problems presented by the growing list of abnormal hemoglobins seems appropriate at this point. Time and again during the early development of a field of investigation, conflicting terminologies have resulted in confusion, especially among those not intimately associated with the field. Singer, Chernoff, and Singer,16 writing at a time when only normal, fetal, and sickle cell hemoglobin had been recognized, suggested that these be designated N, F, and S hemoglobin respectively. Somewhat later, Kaplan, Zuelzer, and Neel8 proposed that as a temporary measure, until our knowledge of hemoglobin differences had reached the point where a rational system of nomenclature could be devised, the electrophoretically distinguishable hemoglobins of adults be given numbers in the order of their discovery, normal being referred to as I, sickle cell as II, and a then newly discovered type as III. It has subsequently become apparent that 1) there are demonstrable electrophoretic differences between normal and fetal hemoglobin in 0.01 M \( \text{Na}_2\text{HPO}_4 \),15 and 2) fetal-type hemoglobin occurs in some of the anemias of adults.16 Itano9 has suggested the use of letters, \( a \) representing normal adult hemoglobin, \( b \) the sickle cell hemoglobin, \( c \) the type referred to as III by Kaplan, Zuelzer and Neel,8 and \( d \) the most recently discovered type. Since both normal adult and fetal hemoglobin may be classified as normal hemoglobins—i.e., they are present at some time during the life of the great majority of healthy, normal individuals—the use of \( N \) could be confusing. It has been suggested that the initials, \( a \) and \( f \), be used to designate normal adult and normal fetal hemoglobins respectively.22 No abnormal forms of fetal hemoglobin have been detected.

It is apparent that an arbitrary system of numbering or lettering hemoglobins places more of a strain on the memory than a system in which the name of the hemoglobin is associated with some clinical aspect of that particular hemoglobin.
Thus, referring to the hemoglobins simply as normal adult, fetal, and sickle cell is certainly the simplest and in many ways the best procedure. Unfortunately, for the two most recently discovered hemoglobins (c and d) there are no associated salient clinical features which could serve as the source of convenient names. The results of homozygosity for the genes responsible for either hemoglobins c or d are still unknown. Under the circumstances, one is almost forced to adhere to a nonspecific type of nomenclature. There is no reason to use 2 systems, one involving letters and the other numbers; it is suggested that only the letter system proposed by Itano be retained. In time we shall probably discover the results of homozygosity for the genes responsible for hemoglobins c and d. There is a real probability, particularly in view of the increased numbers of target cells in individuals heterozygous for the gene for hemoglobin-c, that these homozygotes will correspond to already named hematologic entities. When this occurs, it should be possible to apply more rational names to the abnormal hemoglobins involved.*

**SUMMARY**

A family of Greek derivation is described in which 2 out of 6 children examined exhibited a sickle cell type of anemia. The father of these children was found to have thalassemia minor and the mother the sickle cell trait. It is presumed that the anemia in the two children was due to simultaneous heterozygosity for the sickling and thalassemia genes. Biochemical studies with reference to the occurrence and amounts of normal, sickle cell, and fetal hemoglobin were carried out on the parents and the 6 children. The theoretic interpretation of the biochemical findings is discussed.

**REFERENCES**

2. —: The inheritance of sickle cell anemia. Science 110: 64, 1949.

* On January 6, 1953 the Study Section on Hematology of the U. S. Public Health Service sponsored a Symposium on the Inherited Abnormalities of Hemoglobin. The participants in that Symposium drew up a series of recommendations concerning the terminology to be applied to these hemoglobins. These recommendations, which will shortly be published in BLOOD, supersede the considerations of the last two paragraphs.
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