Studies on Abnormal Hemoglobins

XIII. Hemoglobin S–Thalassemia Disease and Hemoglobin C–Thalassemia Disease in Siblings

By Karl Singer,† Aaron M. Josephson, Lily Singer, Paul Heller and Hyman J. Zimmerman

SUFFICIENT FAMILY STUDIES have been reported demonstrating that microdripanocytic disease represents the double heterozygous state for the abnormal sickle cell hemoglobin and the thalassemia genes.1–4 The inheritance pattern is that of transmission of the non-allelic genes for thalassemia and sickle cell hemoglobin to the same individual, most frequently with interaction of these genes. The pattern seems to be the same in C-thalassemia and E-thalassemia diseases. Recently, case reports have appeared demonstrating the inheritance of these genes with no demonstrable interaction.4–5 These latter discoveries pose a problem of genetics and of diagnosis of the hemoglobinopathies which this paper will try to present and discuss.

Hemoglobin analysis in the usual case of sickle cell–thalassemia disease by means of the standard electrophoretic technics reveals a pattern which is often indistinguishable from that seen in patients with (homozygous) sickle cell anemia. The electrophoretic pattern is that of a major component furnished by Hb S, and a minor component of Hb A + F, Hb A, or Hb F alone.3 Hb F may be demonstrated by means of the alkali denaturation technic. Combining the technics of electrophoresis (preferably moving boundary) and alkali denaturation is necessary for demonstrating these various patterns. Classically, the pattern seen in microdripanocytic disease is that of the S + A + F grouping.3 Occasionally, however, the Hb A fraction is lacking and the differentiation of microdripanocytic disease from pure sickle cell anemia4,5 may be quite difficult, requiring genetic studies and perhaps evaluation of certain other hematologic features.

A most intriguing aspect of Hb S–thalassemia disease is the frequent finding of high percentages of Hb S in the hemolysate of the affected individual.3,4,12–14 This phenomenon has been interpreted to represent either suppression3,4 of Hb A formation by the thalassemia gene, or enhancement3 of production or of expressivity of the gene for the abnormal hemoglobin by the thalassemia gene.

In a preceding paper of this series,15 two Negro patients with Hb C–thalassemia disease were described. Hematologically, they showed a microcytic hypop
chronic erythrocytosis. Hemoglobin analysis revealed the presence of about 75 per cent of Hb C in association with Hb A, or a mixture of Hb A + F, respectively. Thus, a pattern for Hb C-thalassemia disease similar to that seen in Hb S-thalassemia disease was uncovered.

This paper reports the hematologic and genetic features of two siblings, one with Hb S-thalassemia and the other with Hb C-thalassemia disease. The abnormal hemoglobins were supplied by the father who was afflicted with Hb C + S disease, and the thalassemia gene by the mother who had a very mild although definite thalassemia.

METHODS

Hemoglobin analyses were performed with the techniques of alkali denaturation,\textsuperscript{16} electrophoresis in the Tiselius apparatus at pH 6.5,\textsuperscript{17} and paper electrophoresis at pH 8.6.\textsuperscript{18} The technique of starch electrophoresis was that of Kunkel and Wallenius as partially modified in this laboratory.\textsuperscript{19} Hematologic data were obtained with the conventional standardized procedures.\textsuperscript{20} Serum iron was determined by the method of Hamilton et al.,\textsuperscript{21} and iron binding globulin as described by Cartwright and Wintrobe.\textsuperscript{22}

CASE REPORTS AND HEMOGLOBIN ANALYSES

1.—Reuben W., a 34 yr. old colored male, had been diagnosed as having sickle cell-Hb C disease at another hospital. The nature of the hemoglobinopathy was established by electrophoretic hemoglobin analysis (figs. 1 and 2).\textsuperscript{*} He had been admitted to the hospital for a leg ulcer in 1948, and for a unilateral ophthalmic (vitreous) hemorrhage in 1951. P.E. revealed heart and lungs normal; liver, spleen, and kidneys not palpable. Radiographic bone survey was negative. Hematologically, the patient had a compensated hemolytic process (table 1) as evidenced by a reticulocyte count of 3.6 to 7.4 per cent with a red cell level of 4.6 mill. There was no evidence of bleeding. Numerous target cells were seen in the film. Investigation of the patient's family was prompted by the statement that his wife was normal, however one of his children had died of "sickle cell anemia."\textsuperscript{19}

2.—Bernice W., 27 yr. old Negress, wife of Reuben W., was an apparently healthy individual, except for a marked degree of deafness which developed after an episode of measles during her childhood. Her family history is non-contributory. No members of her immediate family were available for study. P.E. was essentially normal except for the deafness. As may be seen from table 1, the reticulocyte level is within normal limits. The sickling test was negative. The peripheral blood film revealed some ovalocytosis and the presence of numerous target cells (26 per cent). The osmotic fragility was markedly decreased. No fetal hemoglobin was demonstrable by the method of alkali denaturation. Electrophoresis in the Tiselius apparatus (fig. 1) demonstrated the presence of a minor component which migrated slightly faster than the main component, Hb A. This minor component, designated here as A,\textsuperscript{†} amounted to 10.2 per cent of the total hemolysate. The Tiselius electrophoresis was performed at a pH of 6.5, A, therefore faster with this type of electrophoresis, as compared with the main component A, than in the starch electrophoresis which was carried out at pH 8.6. Such a faster component had previously been observed in patients with thalassemic syndromes. It was referred to as the "unidentified component" (U.C.) in communication \textsuperscript{21} of this series.\textsuperscript{3} Recently, Kunkel and Wallenius demonstrated this hemoglobin component, in small amounts, in normal hemolysates by means of starch electrophoresis.

\* We are indebted to Mr. C. Schlutz at the Michael Reese Research Foundation for the determination of the blood groups.

\† The classification of the subtypes of normal adult Hb was given at the Panel on Genetics of Hemoglobinopathies at the 11th International Congress of Hematology, Boston 1956, as follows: symbol A1 for main component, A2 for slow component, and A3 for fast component.
Fig. 1.—Standard electrophoresis patterns on W. family

*Hb A₂ seems to be identical with the component "U.C." (Unidentified Component) previously encountered in some thalassemic syndromes (see text)

Fig. 2.—Results of paper electrophoresis studies on W. family
Tiselius was disemise equal amormrits, and from pure Hb C Hb F had remained unchanged.

Grade II systolic murmur over the base of the heart; x-ray of the chest showed the heart amid the hemmitologic indices and other particulars more recorded in Table 1. Hemoglobin capacity were within the range of the greatest number of target cells seen in and MCH are decreased although the MCHC is normal. His peripheral blood film reveals a few sickled cells were seen on the peripheral blood film (fig. 3). Hematologically, the picture of Hb S-thalassemia disease. However, hemoglobin analysis in the Tiselius apparatus as well as by paper electrophoresis uncovered a pattern indistinguishable from that found in classic sickle cell anemia (figs. 1 and 2). The moving boundary electrophoresis revealed that the major component (91\%)

Table 1. —Hematologic Data on W. Family

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Age yrs</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Hb Gum %</th>
<th>RBC mill</th>
<th>Hct %</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reuben (father)</td>
<td>34</td>
<td>m</td>
<td>Hb C + S disease</td>
<td>14.4</td>
<td>4.6</td>
<td>14</td>
<td>31.3</td>
</tr>
<tr>
<td>Bernice (mother)</td>
<td>27</td>
<td>f</td>
<td>Thalassemia minor</td>
<td>12.3</td>
<td>4.2</td>
<td>37</td>
<td>29.2</td>
</tr>
<tr>
<td>Patrice</td>
<td>5</td>
<td>f</td>
<td>Sickle cell trait</td>
<td>13.0</td>
<td>4.2</td>
<td>37</td>
<td>30.9</td>
</tr>
<tr>
<td>Sema</td>
<td>4</td>
<td>f</td>
<td>Sickle cell trait</td>
<td>12.0</td>
<td>4.8</td>
<td>39</td>
<td>26.9</td>
</tr>
<tr>
<td>Steve</td>
<td>3</td>
<td>m</td>
<td>Hb S-thalassemia disease</td>
<td>7.8</td>
<td>5.5</td>
<td>23</td>
<td>27.3</td>
</tr>
<tr>
<td>Gregory</td>
<td>1.5</td>
<td>m</td>
<td>Hb C-thalassemia disease</td>
<td>10.4</td>
<td>4.0</td>
<td>29</td>
<td>72.5</td>
</tr>
</tbody>
</table>

*All patients were blood type O eDe MN.

trophoresis and found it to be increased in thalassemia minor. In our own laboratory we have consistently found an increase in A\(_2\) in all patients with thalassemia minor so far studied. The amount of A\(_2\) as determined by starch electrophoresis in this patient was 9.2 per cent. This correlates very well with the amount of the fast moving component noted in the Tiselius electrophoresis. Because of these hematologic and electrophoretic findings it seemed warranted to assume that Mrs. W. had a mild form of thalassemia (thalassemia minima).

3.—Patrice W., 5 yrs., was clinically and hematologically normal except for having a sickle cell trait (table 1; figs. 1 and 2).

4.—Sena W., aged 4, was also a healthy child except for the demonstrable sickle cell trait pattern (table 1; figs. 1 and 2).

5.—Steven W., aged 3, showed a hypochromic microcytic anemia. Hb 7.8 Gm. per cent. The hematologic indices and other particulars are recorded in table 1. P.E. revealed a grade II systolic murmur over the base of the heart; x-ray of the chest showed the heart and lungs to be normal. Liver was not palpable, but the tip of the spleen could be felt below the left costal margin. A radiographic bone survey was normal. Serum iron and iron binding capacity were within the range of normal. A few sickled cells were seen on the peripheral blood film (fig. 3). Hematologically, the picture was that of Hb S-thalassemia disease.

However, hemoglobin analysis in the Tiselius apparatus as well as by paper electrophoresis uncovered a pattern indistinguishable from that found in classic sickle cell anemia (figs. 1 and 2). The moving boundary electrophoresis revealed that the major component (91 per cent) was provided by Hb S and the remainder by a slower moving non-S fraction. Fetal hemoglobin, as determined by alkali denaturation, amounted to 10.0 per cent. There is, therefore, excellent agreement between the non-S Hb fraction found in the Tiselius apparatus and the amount of alkali resistant hemoglobin. The slower moving fraction noted in the Tiselius was thus composed of Hb F only.

6.—Gregory W., age 18 months, was slightly anemic (Hb 10.4 Gm. per cent). The MCV and MCH are decreased although the MCHC is normal. His peripheral blood film reveals the greatest number of target cells seen in this family, 53.0 per cent (fig. 4). The sickle cell test was negative. Despite the hypochromia and microcytosis the serum iron and iron binding globulin were normal. P.E. was essentially normal. Hemoglobin electrophoresis by means of the moving boundary technic in conjunction with the alkali denaturation procedure uncovered a C + F + A pattern. Whether the large amount of Hb F found is partly due to the age of the patient cannot as yet be stated.* This pattern is readily differentiated from the Hb A + C trait in which both components are usually present in about equal amounts, and from pure Hb C disease where ordinarily only Hb C is found.\(^{1,10-11,31}\)

* At the time of writing this paper, this patient was 24 months old and the amount of Hb F had remained unchanged.
Microspherocytosis was noted on the peripheral blood smear. The number of these cells was apparently insufficient to affect the saline fragility study. A 24 hour incubated saline fragility did not reveal the presence of these spherocytes.

**Discussion**

The diagnosis of thalassemia minor is occasionally rather difficult to establish. It is often confused with that of iron deficiency anemia, and differential diagnosis frequently depends on serum iron studies, measurement of erythrocyte protoporphyrin, and genetic evaluation. Most often the differential diagnosis is based on the response to iron therapy. The technic of starch electrophoresis as described by Kunkel and Wallenius has provided the hematologist with a
relatively simple method for determination of the "accessory hemoglobins." These workers found that hemolysates of normal blood split into three fractions, a main component, A1; a slow component, A2; and a fast component, A3. We have confirmed this work in our laboratory and our results are essentially the same as those of Kunkel and Wallenius. The slow component, A2, amounts to less than 3 per cent in normal hemolysates. It is less than 1.5 per cent in most cases of iron deficiency anemia, and it is between 3 and 10 per cent in all cases of thalassemia minor so far studied. In the few cases of thalassemia major studied in our laboratory and also by Dr. Kunkel, no elevation of A2 was found. These findings in our patient, Mrs. Bernice W., were of great aid in establishing the presence of thalassemia minor.

In this family study we again have an instance of an S + F pattern which is not due to homozygous sickle cell disease. It may be safely stated that the phenotype S + F is not diagnostic for sickle cell anemia, and family studies may be necessary to rule out Hb S-thalassemia disease as the cause of this hemoglobin analysis pattern. Smith and Conley, Itano et al., and Zuelzer have previously reported this type of pattern in Hb S-thalassemia disease.

The double heterozygous state for the abnormal hemoglobin and the thalassemia is manifest in this family as one patient with Hb S-thalassemia disease and one with Hb C-thalassemia disease (fig. 5). In this family there is evidence of marked interaction of the abnormal hemoglobin gene with the gene(s) for thalassemia in each instance. The child with Hb S-thalassemia disease has 91 per cent of the abnormal hemoglobin in his hemolysate (fig. 1). The child with Hb C-thalassemia...
The above terminology is that which was accepted by the Panel on Genetics of Hemo-
globinopathies at the 6th International Congress of Hematology, Boston 1956.

**Fig. 6.**—Genotypes of W. family

![Genotypes of W. family diagram]

1. Suppression of sickle by thalassemia gene
2. Enhancement of abnormal hemoglobin by thalassemia gene
3. Modifiers, effect of other genes
4. Different thalassemia genes
5. Thalassemia and/or sickle cell disease one due to presence of multiple linked genes

**Fig. 7.**—Possible genetic theories for inheritance of abnormal hemoglobins in the presence of thalassemia.

disease has 81.3 per cent Hb C (fig. 1). These patients received only one gene for
the abnormal hemoglobin (fig. 6). Theoretically, the manifestation of this gene
in the individuals receiving just one gene should be either equal amounts of the
abnormal hemoglobin and Hb A, or less than 50 per cent of the abnormal hemoglobin,
as may be seen in all cases of the sickle cell trait.11, 11, 21 Obviously something has affected the expressivity of the gene for the abnormal hemoglobin in the
cases under consideration. Previous workers have postulated that the gene for thalassemia inhibits or suppresses the production of Hb A. Considering that the thalassemia gene is a single gene, one must attempt to explain the phenomenon of no suppression in the mother of this family, with almost complete suppression of the production of Hb A in the children manifesting the double heterozygous state. An explanation for this phenomenon may be the effect of modifying genes. Another way of stating the problem is that we may be dealing with diseases which have multiple genetic causations (fig. 7). A number of reports have appeared which have demonstrated the inheritance of an abnormal hemoglobin and thalassemia in the same individual with no evidence of interaction of the genes. Neel et al. report the occurrence of Hb S trait accompanied by a thalassemia minor. The amounts of Hb S in 3 such patients were 36.2, 27.7, and 22.3 per cent, respectively. Humble et al. from England have also reported a family in which three children had inherited a Hb S gene and a thalassemia minor, yet only one of the three had any evidence of interaction of the genes. Roche et al. report a family of Tunisian muselman in which one child showed interaction of the Hb S with the thalassemia gene and another had a simple sickle cell trait with thalassemia minor. Zuelzer reports a case of Hb C-thalassemia disease in which the patient had only 29.0 per cent Hb C. One of two prime inheritance patterns would seem to be manifest in the inheritance of the double heterozygous states. The thalassemia gene may be present in two different forms, i.e., a gene which has the intrinsic ability to interact with the gene for the abnormal hemoglobin or one which does not have this ability. The other possibility is the presence of multiple or modifying genes. No definite answer to these problems is presently available. The authors prefer the latter theory as this would help explain the marked clinical variations seen in the heterozygous form of thalassemia.

In sickle cell anemia there is also some evidence that there may be more than one gene involved. Zarafonetis et al. have recently shown that the hyposthenuria found in patients with homozygous sickle cell disease is present, to a larger degree, than in patients with the sickle cell trait. There seems to be no relation of the hyposthenuria to the amount of Hb S present. It is also quite possible that a single gene with pleiotropic effect might produce the renal concentration defect. A multiple gene theory would possibly also explain the fact that patients with sickle cell anemia have a marrow regulation different from that of normal individuals. Whereas the normal marrow can compensate or increase its erythropoiesis some six to eight times its normal level, the marrow of a patient with sickle cell anemia has only a fourfold increase of red cell production. A patient with sickle cell anemia who receives transfusions to a normal red cell and hemoglobin level will have a marrow showing very little or no red cell population, and the disappearance of radioactive iron from the plasma will show a curve similar to that seen in aplastic anemias. The causation of these various defects cannot be logically explained on the presence of Hb S alone. If then we are dealing with diseases which are controlled by more than one gene it becomes possible to explain the various inheritance patterns of the double heterozygous states on the basis of transmission of genes which are specific for interaction with the abnormal hemoglobins, or on the absence of transmission of such genes.
The majority of cases of Hb S- or Hb C-thalassemia disease so far studied have been manifest by amounts of the abnormal hemoglobin greater than 60 per cent. A high index of suspicion for the double heterozygous states may be necessary to determine those cases having small amounts of the abnormal hemoglobin. The use of starch electrophoresis should prove extremely helpful in uncovering those cases of very mild thalassemia, or patients having thalassemia together with abnormal hemoglobins.

Summary

A family study is reported in which the simultaneous presence of C-thalassemia and S-thalassemia disease is noted. Marked interaction of the genes for the abnormal hemoglobins and for thalassemia is evident.

The value of the accessory hemoglobins for the diagnosis of thalassemia minor is discussed.

The Hb S + F pattern can no longer be assumed to be diagnostic of sickle cell anemia. Evaluation of all hematologic data and the family background of the patient may be necessary to rule out the presence of the thalassemia gene.

A discussion of the genetics of the double heterozygous states for thalassemia and an abnormal hemoglobin is presented.

Summary in Interlingua

Es reportate un studio familial in que le presentia simultanea de morbo de hemoglobina C plus thalassemia e de morbo de hemoglobina S plus thalassemia es notate. Marcate grados de interaction del genes pro le hemoglobinas anormal con le genes pro thalassemia es evidente.

Es discutite le valor de hemoglobinas accessori pro le diagnose de thalassemia minor.

Il ha devenite impossibile considerar le configuration de hemoglobina S e F como diagnostico de anemia a cellulas falciforme. Il pote esser necessari evalutar omne le datos hematologic e le historia familial ante que le presentia del gen pro thalassemia pote esser negate.

Es presentate un discussion del genetica de duple status heterozygotic pro thalassemia e un hemoglobina anormal.

REFERENCES

STUDIES ON ABNORMAL HEMOGLOBINS

30 Unpublished observations.
Studies on Abnormal Hemoglobins: XIII. Hemoglobin S-Thalassemia Disease and Hemoglobin C-Thalassemia Disease in Siblings

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