Studies on Abnormal Hemoglobins

X. A New Syndrome: Hemoglobin C-Thalassemia Disease

By Karl Singer, Alfred P. Kraus, Lily Singer, Herbert M. Rubinstein and Seymour R. Goldberg

Thalassemia1 (Mediterranean hemopathic syndromes,2,3 Mediterranean anemia,4 hereditary leptocytosis5) is the general term frequently applied to a group of genetically conditioned disorders exhibiting all sorts of gradation from the severe Cooley’s anemia to an asymptomatic form which can only be recognized by special laboratory procedures.2,4,6-8

Recently, it has been demonstrated that the offspring of one parent with the sickle cell trait and one with thalassemia may develop a severe hemolytic anemia with some of the characteristics of both thalassemia and sickle cell disease, including microcytosis and numerous sickled erythrocytes in the blood film. This syndrome has been called microcrepanocytic,9 or sickle-cell-thalassemia, disease.10-12 Although only one gene for the abnormal sickle cell (S) hemoglobin is transmitted, the hemolysates prepared from the erythrocytes of patients with microcrepanocytosis contain 60 to 80 per cent S hemoglobin.12

In 1950, another abnormal hemoglobin, type C, was discovered.11,15 It has been encountered in association with normal adult (A) hemoglobin in the hemoglobin C trait,15-17 with S hemoglobin in sickle cell–hemoglobin C disease,16-17 and in the homozygous condition as pure hemoglobin C disease.15,19 This paper describes a Negro family in which several individuals have either thalassemia or the hemoglobin C trait; in two members the combined occurrence of both anomalies is demonstrable. Significantly, the hemolysates obtained from these two patients with hemoglobin C-thalassemia disease contain the pathologic pigment C in amounts of about 75 per cent.

Methods

Hemoglobin analyses were performed with the technics of alkali denaturation, electrophoresis in the Tiselius apparatus at pH 6.5, and paper electrophoresis at pH 8.6, as outlined in previous communications of this series.19-21 For computing the hematologic indices22 (MCV, MCH, MCHC) and for determining the various blood types, the conventional standardized procedures were employed.

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Proposition

George D., a 33 year old Negro, was admitted to Michael Reese Hospital in 1952 with the chief complaint of chronic ulcerations of both legs. Past history revealed that he had been suffering from severe "anemia" for many years and had received many blood transfusions in several other hospitals. The anemia had not responded to intensive treatment with iron or liver extracts. He had always been slightly jaundiced. Physical examination showed mild icterus of the sclerae, hepatosplenomegaly (4 cm. and 8 cm. respectively below the costal margins), and deep skin ulcers extending over almost the entire anterior aspects of both lower legs. The patient had not received any blood transfusions for several years prior to the present admission.

Laboratory findings. Hb: 8.8 Gm. per cent; RBC: 4.0 million; hematocrit (Wintrobe): 28; MCV: 70 cu.m.; MCH: 22 γγ; MCHC: 31 per cent; reticulocytes: 4.8 per cent (192,000/ cu. mm.); target cells: 17 per cent; numerous nucleated red cells and stippled erythrocytes in the film. The osmotic fragility showed a shift to the right; beginning hemolysis at 0.44 per cent NaCl, complete hemolysis at 0.12 per cent NaCl. Serum bilirubin: 1.8 mg. per cent. Urinalysis negative. Repeated tests failed to demonstrate sickling with the sodium metabisulfite method.

Fig. 1.—Standard electrophoresis pattern on propositus, George D.

Hemoglobin analysis. Alkali resistant (F) hemoglobin 25.3 per cent. Standard electrophoresis at pH 6.5 showed a major component which consisted of a mixture of A and F hemoglobin and a small "shoulder" of a second component with a faster mobility, the nature of which was not immediately apparent (fig. 1). This second component was not an artifact, since it was seen on repeated electrophoretic examinations of several freshly prepared hemolysates.

The clinical findings, the chronic microcytic anemia with low MCH and almost normal MCHC, and the nucleated and stippled erythrocytes in the film as well as the large amount of F hemoglobin established the diagnosis of a thalassemia syndrome.

Study of the Felsanthus D. Family

Felsanthus D., a brother of the propositus, is his only direct relative living in Chicago. He had no history of any significant illness, and physical examination revealed no relevant abnormality. Hemoglobin analysis, however, showed him to be a carrier of the hemoglobin C trait (fig. 2). The hematologic data on Felsanthus are compiled in table 1. There was some degree of leptocytosis present as evidenced by some target cells and an increased osmotic resistance of the erythrocytes. These features are not unusual in the hemoglobin C trait and do not necessarily indicate any weak expressivity of a thalassemia gene.

Felsanthus D. is married to a carrier of the sickle cell trait, who is otherwise in excellent health (fig. 2, table 1).
Their two sons, James and Albert, have sickle cell–hemoglobin C disease. On physical examination, the liver was found to be slightly enlarged in Albert; no enlargement of the spleen was demonstrable in either child. Hematologically, these two boys show only a mild anemia, but a high degree of leucoctosis (table 1); almost 70 per cent of their erythrocytes appear as target cells in the film and the osmotic fragility of their red cells is definitely decreased. An occasional sickled cell is seen in the smears.

Electrophoretic analyses of their hemolysates in the Tiselius apparatus at pH 6.5 revealed the presence of three different components exhibiting the mobilities of A or F, of S, and of C hemoglobin (fig. 2). Quantitatively, the percentages of the slowest moving components were 6.7 and 6.6, respectively. The alkali denaturation technic gave values of 7.6 and 5.7 per cent resistant hemoglobin.
The small differences of the values obtained with both methods are within the technical limits of error. Thus, the slowest components are entirely composed of F hemoglobin. These findings are further proof that the alkali denaturation technic can be used for quantitative determination of F pigment. F hemoglobin has been encountered previously in sickle cell–hemoglobin C disease.

As is the rule in the C variant of sickle cell disease, the C pigment constitutes the major component of the pathologic hemoglobins, and was present in the two hemolysates in amounts of 53.9 and 59.6 per cent (fig. 2). It should be noted that in C + S disease, although the patients are heterozygous for both pathologic pigments, the expressivity of the gene for C hemoglobin is frequently greater than that for hemoglobin S. The highest value for C pigment which we have encountered in C + S disease was 67 per cent of the total pigment in the hemolysate. Thus, the presence of the abnormal gene for S hemoglobin enhances the “activity” of the gene for hemoglobin C in this disorder. Furthermore, it may be worthwhile to emphasize that the findings of a low MCV and MCH with a normal MCHC in typical sickle cell–hemoglobin C disease have also been observed by Kaplan and associates and do not, in these instances, suggest the expression of a thalassemia gene.

Study of the Other Family Members

The existence of a severe thalassemia syndrome in the propositus, and of the typical hemoglobin C trait in his brother, prompted an investigation of the other family members who were living in Memphis, Tennessee. The propositus was sent to Memphis, a family reunion organized, and by this arrangement physical examinations and blood specimens of the entire living family obtained.

Of the parents, only the mother, Mrs. Anna D., is alive. She had given birth to four sons and seven daughters. Two of her sons had died, one of “liver disease” and the other in a train accident. The seven daughters were all located in Memphis and examined. The hematologic data and the abnormal findings of physical examinations of this kindred are summarized in table 2. The results of the differential hemoglobin analyses may be seen from figures 3 and 4. Figure 5 may be of aid in the interpretation of the variable expressivity of the thalassemia gene in this family.

The hemolysate prepared from Mrs. Anna D.’s red cells contained 77.4 per cent hemoglobin C, the remainder consisting of normal adult pigment. No alkali resistant hemoglobin was demonstrable. The red cell level of this patient was 6.1 million with a hemoglobin value of 12.7 Gm. per cent. The MCV and MCH were definitely decreased, but the MCHC was within normal range. The reticulocyte output was elevated (table 2). A few nucleated red cells, stippled cells, and a large number of target cells were seen in the film.

The microcytic erythrocytosis in this patient points to the presence of a milder thalassemia syndrome and makes it likely that the propositus inherited the thalassemia gene from his mother. The unusual amount of about 77 per cent hemoglobin C resembles similar values for hemoglobin S encountered in sickle cell–thalassemia disease. It is, therefore, postulated that Mrs. Anna D. is afflicted with a heretofore undescribed syndrome: hemoglobin C–thalassemia disease.
<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>ABO</th>
<th>CDE</th>
<th>MN</th>
<th>HGB (g/%)</th>
<th>RBC (mill.)</th>
<th>Hematocrit</th>
<th>MCV (cuµm)</th>
<th>MCH (py)</th>
<th>MCHC (%)</th>
<th>Retics.</th>
<th>Target Cells (%)</th>
<th>Nucleated RBC</th>
<th>Serum Bilirubin (mg.%)</th>
<th>Osmotic fragility; normal 0.48-0.32 (‰ NaCl)</th>
<th>Hepatomegaly</th>
<th>Splenomegaly</th>
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<tr>
<td>Anna D. (mother)</td>
<td>68</td>
<td>f</td>
<td>B</td>
<td>eDE/c</td>
<td>N</td>
<td>12.7</td>
<td>6.1</td>
<td>36</td>
<td>59</td>
<td>21</td>
<td>35</td>
<td>3.8</td>
<td>230</td>
<td>+</td>
<td>0.9</td>
<td>0.42-0.22</td>
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<td>0</td>
</tr>
<tr>
<td>Fanny D.</td>
<td>45</td>
<td>f</td>
<td>B</td>
<td>eDE/e</td>
<td>N</td>
<td>13.0</td>
<td>4.8</td>
<td>40</td>
<td>83</td>
<td>27</td>
<td>32</td>
<td>0.8</td>
<td>38</td>
<td>0</td>
<td>0.9</td>
<td>0.48-0.32</td>
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<td>0</td>
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<tr>
<td>Daisy D.</td>
<td>44</td>
<td>f</td>
<td>B</td>
<td>eDE/e</td>
<td>MN</td>
<td>9.7</td>
<td>5.0</td>
<td>29</td>
<td>50</td>
<td>19.5</td>
<td>34</td>
<td>3.6</td>
<td>180</td>
<td>17</td>
<td>1.8</td>
<td>0.44-0.24</td>
<td>marked</td>
<td>marked</td>
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<tr>
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<td>38</td>
<td>f</td>
<td>B</td>
<td>eDE/e</td>
<td>MN</td>
<td>13.3</td>
<td>6.3</td>
<td>46</td>
<td>72</td>
<td>21</td>
<td>29</td>
<td>2.8</td>
<td>176</td>
<td>2</td>
<td>0.8</td>
<td>0.44-0.26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Josephine D.</td>
<td>35</td>
<td>f</td>
<td>B</td>
<td>eDe/ee</td>
<td>N</td>
<td>12.5</td>
<td>4.4</td>
<td>37</td>
<td>86</td>
<td>28</td>
<td>34</td>
<td>1.5</td>
<td>66</td>
<td>12</td>
<td>0.3</td>
<td>0.42-0.28</td>
<td>0</td>
<td>0</td>
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<td>33</td>
<td>m</td>
<td>B</td>
<td>eDE/e</td>
<td>N</td>
<td>8.8</td>
<td>4.0</td>
<td>28</td>
<td>70</td>
<td>22</td>
<td>31</td>
<td>4.8</td>
<td>192</td>
<td>17</td>
<td>1.8</td>
<td>0.44-0.12</td>
<td>marked</td>
<td>marked</td>
</tr>
<tr>
<td>Felsahtus D</td>
<td>32</td>
<td>m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>15.3</td>
<td>4.8</td>
<td>43</td>
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<td>0.40-0.22</td>
<td>0</td>
<td>0</td>
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<td>31</td>
<td>f</td>
<td>B</td>
<td>eDe/ee</td>
<td>MN</td>
<td>11.2</td>
<td>5.5</td>
<td>38</td>
<td>68</td>
<td>20</td>
<td>29</td>
<td>2.1</td>
<td>116</td>
<td>14</td>
<td>0.6</td>
<td>0.44-0.28</td>
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<td>0</td>
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<td>29</td>
<td>f</td>
<td>B</td>
<td>eDe/ee</td>
<td>N</td>
<td>11.5</td>
<td>5.7</td>
<td>34</td>
<td>60</td>
<td>20</td>
<td>34</td>
<td>2.0</td>
<td>114</td>
<td>47</td>
<td>+</td>
<td>0.42-0.18</td>
<td>0</td>
<td>0</td>
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<td>Pearl D.</td>
<td>27</td>
<td>f</td>
<td>O</td>
<td>eDe/ee</td>
<td>N</td>
<td>11.1</td>
<td>4.7</td>
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<td>0.4</td>
<td>0.48-0.28</td>
<td>0</td>
<td>0</td>
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</table>
Fig. 3.—Standard electrophoresis patterns on D. family. The components are recorded in the sequence of their quantitative representation. “U.C.” = unidentified component (see text).

Fig. 4.—Results of paper electrophoresis studies on D. family
Among her children, only Bertha D. (#9, table 2, and figs. 3 and 4) showed almost identical hematologic and electrophoretic findings. Her red count was 5.7 million, with 11.5 Gm. per cent hemoglobin, composed of 74.1 per cent hemoglobin C, 23.2 per cent hemoglobin A, and—in contradistinction to her mother—a small amount (2.7 per cent) of F hemoglobin. It may be pointed out here that Bertha D., as well as her mother, seem to be heterozygous for hemoglobin C as well as for thalassemia. The thalassemia gene appears to enhance the production of C hemoglobin, just as the presence of the gene for S hemoglobin seems to increase the manufacture of C hemoglobin in typical sickle cell–hemoglobin C disease.

Besides Felsanthus D. (see above), only one other sibling, Josephine D. (#5, table 2, and figs. 3 and 4), showed the characteristics of the uncomplicated hemoglobin C trait.

Daisy D. (#3, table 2, and figs. 3 and 4) suffers from a more severe form of thalassemia, similar to the syndrome observed in the propositus. Study of her blood demonstrated a microcytic anemia (Hb 9.7 Gm. per cent, RBC 5.0 million, MCV 59 cu., MCH 19.5 g), increased reticulocyte level, hyperbilirubinemia and nucleated and stippled red cells in the film. There is also marked hepatosplenomegaly. The quantity of alkali resistant pigment in this 44 year old patient’s hemolysate was high (46.5 per cent).

The other siblings, Magnolia (#4, table 2) and Aura (#8) appear to have only mild thalassemic manifestations with low MCV and MCH, and some leucocytes. The finding of a mild hypochromic anemia in Pearl D. with a MCV of 81 cu., a MCH of 23.5 g, and a MCHC of 29 per cent, but an almost normal osmotic fragility are difficult to interpret, since the existence of iron deficiency anemia has not been definitely ruled out. Only in the hemolysate prepared from Aura’s red cells was an abnormal amount (2.4 per cent) of F hemoglobin detectable.

Fanny D. (#2, table 2) was the only member of the family who seemed to show no hematologic abnormalities.
**Other Observations**

It has been mentioned that, on standard electrophoresis in the Tiselius apparatus at pH 6.5, a second component with a mobility faster than that of A hemoglobin was constantly demonstrable in the hemolysate of the propositus. It first appeared as a small “shoulder,” but at longer runs its separation became more distinct (Fig. 3). Such a compound was also observed in the hemoglobin solutions prepared from the erythrocytes of Fanny, Daisy, Magnolia, and Aura (Fig. 3), and took up from 5.2 to 13.5 per cent of the electrophoretic patterns. Its mobility was determined as 2.7 to 2.8 X 10^{-5}/cm²/volt/sec. which is definitely less than that of C hemoglobin (3.2 X 10^{-5}/cm²/volt/sec.). The nature of this component is not clear. In about two hundred hemoglobin analyses in the Tiselius apparatus with a standardized technic a similar finding has been observed only occasionally in hemolysates prepared from various types of erythrocytes. Itano^{11} also reports on such observations in patients with thalassemic disease, and also in some normal hemolysates. The fact that almost all the hemolysates of the members of this family, which do not contain hemoglobin C, revealed this factor is noteworthy. It is not suggested that this erythrocyte constituent is necessarily a pigment compound. It certainly is not F hemoglobin as suspected by other investigators.\(^{11}\) Attempts to remove this factor by preparative ultracentrifugation were not successful. Unfortunately, no electrophoretic analyses with crystallized hemoglobin from these patients were feasible. With paper electrophoresis, this unidentified compound was not demonstrable beyond doubt, since the finding of such small amounts of a component is difficult to interpret with this technic. Further investigations into the nature and significance of this constituent of red cell hemolysates, particularly in the thalassemic syndromes, are indicated.

**Discussion**

It is now well recognized that thalassemia manifests itself as a group of hereditary syndromes with all sorts of gradations from the most severe Cooley's anemia to the asymptomatic “trait” which reveals only slight leptocytosis.\(^{2,4,6-8}\) The common denominator of these disorders is the production of structurally defective, thin (leptocytic) erythrocytes which, however, exhibit this anomaly to a varying degree in various patients. Hepatosplenomegaly, bone changes, skin ulcers, and the signs and consequences of marked hyperhemolysis are observed in the more severe syndromes only. For practical purposes we have found the following simple classification very useful; it is based on the alterations of the red cell and hemoglobin levels:

1. Thalassemia major (Cooley’s anemia): very severe microcytic hemolytic anemia.
2. Thalassemia intermedia, characterized by a less severe, but still marked anemia.
3. Thalassemia minor: mild anemia.
5. Thalassemia minima\(^{25}\): slight leptocytosis only.

Valentine and Neel\(^{26}\) have suggested that thalassemia major represents homozygosity for a genetic factor which is heterozygous in the other syndromes,
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grouped together by these authors as "thalassemia minor." These investigators were aware of the varying expressivity of the thalassemia gene, but considered markedly anemic (intermediate) types as being of very rare occurrence. It has, however, been pointed out in the recent literature\(^3\) that the more severe "minor" types of thalassemia are not unusual, and some authors have expressed doubt as to whether these intermediate forms of the disease are always heterozygous.\(^3\)

Be that as it may, there is general agreement that mating between any of the thalassemia types may result in offspring with thalassemia major.\(^2\)\(^9\)

Microdyserythrocytosis, or sickle cell–thalassemia disease results when an individual is heterozygous both for the thalassemia and the sickle cell gene. The interaction of these two genetic factors may produce a rather severe hemolytic anemia, characterized by microcytosis, low MCH, and numerous sickle cells in the smear.\(^9\)

In the family under consideration in this paper, two individuals, Mrs. Anna D. and her daughter Bertha D., reveal still another variant of thalassemia, which may be called hemoglobin C–thalassemia disease. In contradistinction to the combination sickle cell–thalassemia, the presence of hemoglobin C does not particularly enhance the severity of the clinical picture due to the thalassemia gene. These two individuals with hemoglobin C–thalassemia disease show only a microcytic erythrocytosis with a slight reduction of the hemoglobin levels. That the large amount (about 75 per cent) of hemoglobin C in their erythrocytes plays no significant role in the hematologic manifestations is illustrated by the fact that at least two other members of the same family, Magnolia and Aura D., exhibit similar hematologic aberrations without the presence of C hemoglobin. The two patients with hemoglobin C–thalassemia disease have, however, a conspicuously large number of target cells (43 to 47 per cent) in the film (table 2).

Aside from the two individuals with the new syndrome, the following thalassemic types were also recognized in this family. The propositus, George D., and his sister Daisy have symptoms and signs best fitting into the "intermediate" category according to our definition. Magnolia and Aura D., as mentioned before, reveal a microcytic erythrocytosis. The exact classification of Pearl D. is difficult, since it is not definitely established whether she is afflicted with a thalassemic syndrome. Although she shows a mild hypochromic anemia, the osmotic fragility test reveals no marked leptocytosis. Two of the children of Mrs. Anna D., Felsanthus and Josephine, harbor the hemoglobin C trait without any clear-cut signs of thalassemia. Fanny D. does not appear to have any hematologic abnormalities. These data establish evidence that the thalassemic gene and the hemoglobin C gene segregate independently.

Felsanthus D., a carrier of the hemoglobin C trait, married a carrier of the sickle cell trait. The two children of this marriage, James and Albert D., are examples of sickle cell–hemoglobin C disease. They show a mild anemia of a hemolytic nature, with elevated reticulocyte output and hyperbilirubinemia. Their red cells reveal a low MCV and MCH in one individual and borderline values in the other. The MCHC was normal in both instances.

It has been pointed out in the literature that the erythrocytes in the milder thalassemia syndromes have a low MCV and MCH, though the MCHC is either normal or only slightly decreased.\(^7\) These findings may be interpreted as re-
flecting the low volume of thin (leptocytic) red cells. The surface areas of such cells may be normal or even larger than normal, but a disproportion exists between their contents and their surfaces.\(^{(28)}\) The fact that the MCHC is at most only slightly decreased indicates that each cell, for its volume, is not very deficient in hemoglobin.\(^{(27, 28)}\) These observations raise doubt as to whether insufficient hemoglobin synthesis, a concept frequently invoked in the thalassemia disorders,\(^{(12, 28)}\) plays any truly significant pathogenetic role.

Kaplan and his associates have reported\(^{(15)}\) that similar alterations in the hematologic indices, as seen in the milder thalassemic syndromes, may also be found in cases of sickle cell–hemoglobin C disease. In this malady a definite tendency to leptocytosis is present. This phenomenon was also noted in the two children of Felsanthus D. (table 1) as well as in other patients with C + S disease whom we have encountered.\(^{(23)}\)

The same investigators have emphasized that numerous target cells are a common feature in conditions in which hemoglobin C is demonstrable.\(^{(14, 17)}\) In the uncomplicated milder thalassemic syndromes target cells are also found, though not always in very impressive numbers.\(^{(4, 17, 29-32)}\) Among the thirteen members of the family under consideration, the largest numbers of target cells were seen in the patients with hemoglobin C–thalassemia disease and with sickle cell–hemoglobin C disease (tables 1 and 2). Thus, the finding of an unusually large percentage of target cells in a case of thalassemia should create suspicion that one may be dealing with the hemoglobin C variant of the disease.

It has been stated that the only manifestation of the abnormal gene in thalassemia minima may be the increased resistance of the red cells to hypotonic saline solutions, which is related to the leptocytic shape of the erythrocytes.\(^{(4, 28)}\) Since it is now well known that leptocytosis may also occur in carriers of the hemoglobin C trait, the results of the osmotic fragility test are no longer conclusive, without being supplemented by a hemoglobin analysis. This is documented by the observations on Felsanthus D. who, as a carrier of the hemoglobin C trait, shows an increased osmotic resistance of his red cells, but does not harbor the thalassemia gene. From this example the necessity for hemoglobin studies in the genetic analysis of the hereditary hemolytic syndromes is apparent.

The hemolysates of the two patients with thalassemia intermedia (George and Daisy D.) contained large quantities of F hemoglobin (25.3 and 46.5 per cent), a frequent characteristic in the more severe types of this hereditary illness. In the milder manifestations of the thalassemia gene, alkali resistant pigment is inconsistently present and then in smaller amounts only.\(^{(20)}\) The hemolysates of Aura and Bertha D., who both showed only a microcytic erythrocytosis, contained not more than 2.4 and 2.7 per cent F pigment. The simultaneous occurrence of C hemoglobin and thalassemia did not particularly enhance the production of fetal pigment. Alkali resistant hemoglobin was, however, present in amounts of 5.7 and 7.6 per cent in the hemolysates of the two children with sickle cell–hemoglobin C disease.

A feature of sickle cell–thalassemia disease, which has occasioned much interest, is the finding that S hemoglobin amounts to 60 to 80 per cent of the total pigment in the hemolysate,\(^{(12, 13)}\) although all the evidence available indicates that these patients are heterozygous for the sickle cell gene.\(^{9}\) In other conditions
in which heterozygosity for the sickle cell gene exists, i.e., the A + S trait and C + S disease, the quantities of S hemoglobin do not exceed 50 per cent.13, 17, 24 It has been postulated that the high percentage of S hemoglobin in microdrepanocytic disease is due to the inefficient synthesis of normal adult hemoglobin in the presence of the thalassemia gene, whereas the production of S hemoglobin remains unaffected.17 As mentioned previously, it is, however, difficult to reconcile this hypothesis with the demonstration of a normal or only slightly depressed MCHC in both microdrepanocytic disease22 and the milder thalassemic syndromes.27

In our two patients with hemoglobin C–thalassemia disease, the pathologic pigment amounted to 77.4 and 74.1 per cent, the remainder being furnished by A, and A + F hemoglobins. The MCHC of their erythrocytes was within normal limits. There is no reason to assume that homozygosity for hemoglobin C existed in these persons. Thus, it appears from all these observations that, when an individual is simultaneously heterozygous for thalassemia and an abnormal hemoglobin (S or C), an usually large percentage of the pathologic pigment may be present in the hemolysate. This finding is not so exceptional when it is realized that in sickle cell–hemoglobin C disease the percentage of C hemoglobin may also amount to as much as 67 per cent24 when the expressivity of the associated gene for S hemoglobin is low. In this disorder the patient is heterozygous for both abnormal hemoglobins. What is remarkable, however, is the emerging fact that, at times, the thalassemia gene as well as the gene for S hemoglobin may modify the expression of the gene for hemoglobin C in an identical manner. That there may be also other unidentified modifiers at work can be deduced from the well established findings that in the A + C and A + S traits, which are heterozygous for both hemoglobins, the quantity of normal hemoglobin frequently exceeds the 50 per cent level. Thus, the diminished expressivity of a gene for a pathologic pigment enhances the expression of the gene for the companion pigment. The thalassemia gene promotes the expressivity of the pathologic hemoglobin gene if both abnormal genes are present in an individual.

It seems now established that the genes for thalassemia and for the pathologic hemoglobins (S or C) are inherited independently and located on different chromosomes. This mechanism was first considered by Powell, Rodarte, and Nee16 and later proven by Silvestroni and Bianco.8 The Italian investigators observed: (1) that a child of parents, one of whom had microdrepanocytic disease and the other thalassemia, was normal and (2) that a child of parents, one with microdrepanocytic disease the other being normal, also had microdrepanocytic disease. Similarly, in the family reported in this paper, one child (Fanny D.) is hematologically normal, although the mother is most likely heterozygous for hemoglobin C as well as for thalassemia. Regardless of the unknown genetic constitution of the other parent, it is clear that the genes for thalassemia and hemoglobin C are not allelomorphs and segregate independently.

The severe clinical picture in sickle cell–thalassemia disease suggests interaction of the two pathologic genes which, thus, exert a more pronounced detrimental effect. In hemoglobin C–thalassemia disease such an interaction manifests itself—at least in our two observations—only by an unusual number of target
cells and by the uncommonly high percentage of C hemoglobin in an heterozygous individual.

Why two of the children, George and Daisy D., have a much more severe type of thalassemia than does the mother or other siblings affected, is not understood. It is conceivable that the father may also have been a carrier of the thalassemia gene and these two individuals are homozygous for this abnormality. However, the literature contains a number of similar cases in which only one parent was found to be transmitting the abnormal gene. The varying expressivity of the thalassemia gene may be due to modifying genes the nature of which is not known.

The analysis of this family does not contribute any new information toward the understanding of the basic genetic mechanisms of the pathologic hemoglobins. Whether the genes for S and C hemoglobin are allelomorphs is not yet established. Neel et al. have pointed out that investigations of the children of individuals with sickle cell–hemoglobin C disease will aid in the clarification of this problem. If the offspring of the marriage of patients with sickle cell–hemoglobin C disease with normal individuals are either normal, or also have sickle cell–hemoglobin C disease, one may conclude that the two genes are not alleles. Such information is not yet available.

Since the recognition of the thalassemic syndromes, numerous instances of the occurrence of these clinical pictures in individuals of non-Mediterranean origin have been reported. Thalassemia is quite common in certain provinces in India, in Thailand, and has also been found in Chinese and Negroes, as well as occasionally in persons of “pure” English stock. The term Mediterranean anemia is, therefore, inappropriate. It would, indeed, be tempting to call the disease “hereditary leptocytosis.” But this is definitely not permissible, since hereditary leptocytosis is known to appear also in conditions with erythrocytes carrying hemoglobin C. For want of a better designation which should be related to the basic but yet unidentified alteration of the red cell structure in this disease process, we have employed the term thalassemia throughout this communication.

Hemoglobin C has been found so far in Negroes only. It will be of interest to find whether this pathologic pigment occurs also in “white” persons. If this should be the case, hemoglobin C–thalassemia disease will probably not remain a variant of thalassemia, confined exclusively to the Negro race.

**Summary**

1. A Negro family is described in which several individuals exhibit either the manifestations of thalassemia, or of the uncomplicated hemoglobin C trait; in two members the combined occurrence of these two anomalies is demonstrable. This combination is designated as hemoglobin C–thalassemia disease.

2. Hemoglobin C–thalassemia disease manifests itself in these two patients as a microcytic erythrocytosis. The red cells reveal a low MCV and low MCH, but a normal MCHC. About 45 per cent of the erythrocytes appear as target cells in the film. The amount of hemoglobin C in the hemolysates was about 75 per cent, the remainder being composed of A hemoglobin, and in one instance
also of a small quantity of F hemoglobin. This is analogous to the results of the
hemoglobin analyses in sickle cell-thalassemia disease, where 60 to 80 per cent
of the pathologic hemoglobin S are found, though these individuals are heterozy-
gous for both the pathologic hemoglobin and the thalassemia genes. The hy-
pothesis is advanced that the thalassemia gene modifies (enhances) the expres-
sivity of the gene for the pathologic pigment.

3. In genetic studies of families with thalassemia, hemoglobin analyses repre-
sent a necessary requirement. It is now established that thalassemia, as well as
disorders associated with hemoglobin C, reveals a tendency to leptocytosis and
thus may show erythrocytes with increased osmotic resistance.

4. The segregation of the thalassemia gene and of the genes for pathologic
hemoglobins take place independently of each other. These genes are not
allelomorphs.

SUMMARIO IN INTERLINGUA

(1) Es describite un familia negre in le qual plure individuos exhibi le mani-
festationes o de thalassemia o del non-complicate tracto de hemoglobina-C. In
duo membros del familia le occurrence comiminate de iste duo anomalias es
demonstrabile. Le combination es designate como “morbo a hemoglobina-C plus
thalassemia.”

(2) Le morbo a hemoglobina-C plus thalassemia se manifesta in iste duo
patientes como un erythrocytosis microcytic. Le erythrocytos revela basse
valores median del volumine corpuscular e del hemoglobina corpuscular sed un
normal valor median del concentration del hemoglobina corpuscular. Circa 45
pro cento del erythrocytos in le frottis ha tin apparenitia clypeiforme. Le quanti-
tate de hemoglobina-C in le hemolysatos es circa 75 pro cento; le resto es hemo-
globina-A o, in un caso, hemoglobina-A plus un parve procentage de hemoglobi-
na-F. Isto es analoge al resultados del analyse de hemoglobina in le morbo a
cellulas falciforme plus thalassemia, ubi le pathologic hemoglobina-S amonta a
inter 60 e 80 pro cento ben que individuos con iste disordimie es heterozyge al
genies de tanto le hemoglobina pathologica como etiam de thalassemia. Es formu-
late le hypothese que le gen de thalassemia modifica (e promove) le expressivitate
del gen del pigmento pathologic.

(3) In studios genetic de familias con thalassemia, analyses de hemoglobina es
un requirimento indispensabile. Il es hodie un facto establite que thalassemia—
como etiam disordinies associate con homoglobina-C—revela un tendentia a
leptocytosis e ergo pote esser characterisate per erythrocytos con augmentate
resistentia osmotic.

(4) Le segregation del gen de thalassemia e del genes del hemoglobinas patho-
logic occurre independentemente. Iste genes non es allelomorphic.

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