Chronic Hemolytic Anemia Associated with Thalassemia and Sickling Traits

By Phillip Sturgeon, M.D., Harvey A. Itano, M.D., Ph.D. and William N. Valentine, M.D.

Powell, Rodarte and Neel recently described in detail a family of Sicilian ancestry displaying both the sickle cell trait and thalassemia minor; the first family displaying both of these traits described in this country. In one member of this family exhibiting a severe chronic hemolytic anemia, which was clinically indistinguishable from sickle cell anemia, both traits were present. In this paper attention was also directed to the reports from Italy by Silvestronsi and Bianco of five families exhibiting the same phenomenon.

In the course of the study in this clinic of a case of chronic hemolytic anemia in a 6 year old girl of Italian and Mexican ancestry a similar family was discovered. This represents the second family of this nature recognized in the United States. The purpose of this report is (1) to describe this chronic hemolytic anemia as it was observed in a child, (2) to present data which further characterize the anemia and (3) to describe the unique electrophoretic pattern of this patient’s hemoglobin.

Methods

Leukocyte and erythrocyte counts were made by routine methods and hemoglobin was determined as oxyhemoglobin with a photoelectric colorimeter. Bone marrow was aspirated from the iliac crest and studied by a volumetric technic. Red cell fragility to hypotonic saline was measured in a photoelectric colorimeter after centrifugation to remove unhemolyzed cells. In our hands with normal bloods, this method shows beginning hemolysis at 0.50 ± 0.02 per cent saline and complete hemolysis at 0.40 ± 0.02 per cent saline. Tests for sickling were carried out by the sealed cover slip method; the specimens were observed periodically for forty-eight hours. In vivo sickling tests were performed on the patient and her mother by the method of Sherman.

Serum iron was determined according to the method of Schales. Erythrocyte protoporphyrin was determined by the method of Grinstein and Watson, and urine and stool urobilinogen excretion was measured on single urine or stool samples as described by Watson and Hawkinson. In our hands the erythrocyte protoporphyrin method gives an approximate normal value of 23 μg. per 100 cc. of packed red cells. The urobilinogen methods give a normal mean of 936 Ehrlich.

From the Department of Research, Los Angeles Children’s Hospital, and the Department of Pediatrics, University of Southern California School of Medicine, Los Angeles; the Gates and Crellin Laboratories of Chemistry (Contribution No. 1602), California Institute of Technology, Pasadena; and the Department of Medicine, School of Medicine, University of California Medical Center, Los Angeles.

This study was supported in part by a grant from the U. S. Public Health Service.

Submitted August 20, 1951; accepted for publication November 7, 1951.

We are very grateful to Mrs. E. F. G., mother of this patient, for her cooperation in this study.
Units for the stool determination and 9.9 E.U. for the urine, with 80 per cent ranges of 108 to 1920 E.U. and 0.3 to 20.0 E.U. respectively.

Serologic studies for blood groups A, Ai, B, the Rh factors (C, D, E, c), M, N, S, and P were carried out with commercial reagents.* Other serologic studies included direct antiglobulin (Coombs)* and Donath-Landsteiner* tests.

Electrophoretic analyses (H.A.I.) of the patient's and her mother's hemoglobin were performed with 1.0 per cent solutions of carbonmonoxyhemoglobin in the Tiselius apparatus. Details of the preparation and analysis of these solutions were identical with those used in earlier studies.10, 11

CASE HISTORY

L. G. (F), *39534, a 6 year old female of Mexican and Italian ancestry, first visited Los Angeles Children's Hospital April 25, 1950. Her chief complaint was frequent attacks of sore throat during the preceding few months.

She was the product of a full term normal pregnancy in a 22 year old white female. The birth weight was 7 pounds 10 ounces. The neonatal period was uneventful as was the child's entire past history until the onset of her chief complaints. Her development was normal; she walked at 12 months of age, and talked at 14 to 18 months of age. She had had uncomplicated rubella and varicella during her third year, and other than the present complaints, was considered a healthy child.

Physical examination revealed a normal appearing, 6 year old female; height, 40 inches; weight, 35 pounds (fig. 1). The tonsils were large but not red, and there was moderate cervical lymphadenopathy. Examination of the heart and lungs was negative. Blood pressure was 110/68. The spleen edge was easily palpable into the left lower quadrant. The liver edge was palpable 2 cm. below the right costal margin. Jaundice was not evident and the rest of the physical examination was within normal limits.

The initial studies of peripheral blood are recorded in table 1, and are representative of repeated determinations. No quantitative or qualitative abnormalities of the white blood cells or platelets were noted. Reticulocyte counts on three occasions were 16, 24 and 9 per cent, and a maximum of 3 normoblasts per 100 white cells were recorded. Repeated examination of stained peripheral blood smears revealed hypochromasia, polychromasia, basophilic stippling and target cells, but no sickle cells were noted.

Simultaneous sickling tests of the patient's and her mother's blood prepared in a uniform manner revealed that the great majority of the patient's cells became sickled in 3 hours. The sickled cells were of the long filamentous type. In contrast, the mother's cells required 24 to 48 hours to sickle, and the sickled cells were predominantly of the "holly-leaf" variety. An in vivo sickling test revealed sickling of 11.6 per cent of the patient's cells and no sickling of her mother's cells.

Red cell fragility studies showed beginning hemolysis at 0.42 per cent saline with a very gradual increase with decreasing salt concentrations. Maximum hemolysis was obtained at 0.10 per cent saline; increased hemolysis was not present in the greater dilutions.

On aspiration of the left iliac crest, 3 cc. of fluid were removed. Volumetric studies revealed no fat (average normal, 0.95 ± 0.65 mm.), a 14 mm. myeloid erythroid layer (average normal, 3.97 ± 1.58 mm.), and many large particles. Fifty-eight per cent of the nucleated cells were normoblasts (average normal, 24.2 ± 7.8 per cent). Histologic examination of the particles revealed 100 per cent cellularity (average normal, 45 per cent). X-rays of the skull and long bones were negative.

Total serum bilirubin was determined on two occasions; values of 1.6 and 1.2 mg. per 100 ml. of serum were found. Of this, 0.6 and 0.8 mg. respectively were indirect reacting. Urobilinogen excretion determined on a portion of a 24 hour urine specimen was 2.5 Ehrlich

* The authors wish to express their thanks to the Certified Blood Donor Service of Jamaica, N. Y., which generously provided the antisera for the performance of the above tests.
Twenty-three living members of the family have been studied to date and the findings recorded in table 1. Results of the serologic studies are recorded in the genealogic chart (fig. 2).
The patient's mother is of Mexican ancestry; both of her parents were born in Mexico and are, as far as is known, of Mexican descent. The patient's father is of Italian ancestry; both of his parents are natives of southern Italy. The appearance of all members of the family is that of Caucasians.

L. G. (F)'s mother's (Mrs. E. F. G.) cells sickled, whereas the father's (B. G.) blood picture was found to be characteristic of thalassemia minor. A 6 month old sister's (S. G. (F))

Table 1.—Summary of Peripheral Blood Findings from the G and F Families

<table>
<thead>
<tr>
<th>Name</th>
<th>Age yrs</th>
<th>Sex</th>
<th>HGB (Gm.)</th>
<th>RBC (10^6/min)</th>
<th>P.C.V. %</th>
<th>Hct M.M.</th>
<th>M.C.H. pug</th>
<th>Stained Smear</th>
<th>Sickling Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. G. (F)</td>
<td>5</td>
<td>F</td>
<td>9.0</td>
<td>4.2</td>
<td>29</td>
<td>69</td>
<td>21.5</td>
<td>Target Cells† Basophilic Stippling*</td>
<td>positive</td>
</tr>
<tr>
<td>B. G.</td>
<td>27</td>
<td>M</td>
<td>12.8</td>
<td>5.88</td>
<td>45</td>
<td>76</td>
<td>22.0</td>
<td>Basophilic Stippling*</td>
<td>negative</td>
</tr>
<tr>
<td>E. G. V.</td>
<td>20</td>
<td>F</td>
<td>12.5</td>
<td>5.47</td>
<td>42</td>
<td>77</td>
<td>23.0</td>
<td>Basophilic Stippling*</td>
<td>negative</td>
</tr>
<tr>
<td>S. V. (G)</td>
<td>27</td>
<td>M</td>
<td>11.2</td>
<td>5.28</td>
<td>37</td>
<td>71</td>
<td>21.0</td>
<td>Target Cells* Slight Ovalocytosis</td>
<td>negative</td>
</tr>
<tr>
<td>A. P. G.</td>
<td>55</td>
<td>F</td>
<td>12.0</td>
<td>5.93</td>
<td>38</td>
<td>64</td>
<td>20.2</td>
<td>Target Cells* Basophilic Stippling*</td>
<td>negative</td>
</tr>
<tr>
<td>P. G.</td>
<td>67</td>
<td>M</td>
<td>15.6</td>
<td>4.38</td>
<td>47</td>
<td>106</td>
<td>36.0</td>
<td>Target Cells* Basophilic Stippling*</td>
<td>negative</td>
</tr>
<tr>
<td>S. G. (F)</td>
<td>27</td>
<td>F</td>
<td>9.6</td>
<td>4.51</td>
<td>not done</td>
<td></td>
<td>21.5</td>
<td>Normal</td>
<td>negative</td>
</tr>
<tr>
<td>E. F. G.</td>
<td>26</td>
<td>F</td>
<td>9.5</td>
<td>3.30</td>
<td>24</td>
<td>103</td>
<td>29.0</td>
<td>Normal</td>
<td>positive</td>
</tr>
<tr>
<td>V. F.</td>
<td>24</td>
<td>F</td>
<td>13.2</td>
<td>4.50</td>
<td>45</td>
<td>100</td>
<td>29.5</td>
<td>Normal</td>
<td>negative</td>
</tr>
<tr>
<td>R. F.</td>
<td>17</td>
<td>M</td>
<td>13.3</td>
<td>4.50</td>
<td>47</td>
<td>104</td>
<td>30.0</td>
<td>Normal</td>
<td>negative</td>
</tr>
<tr>
<td>L. F. M.</td>
<td>30</td>
<td>F</td>
<td>11.9</td>
<td>4.73</td>
<td>38</td>
<td>82</td>
<td>25.5</td>
<td>Normal</td>
<td>positive</td>
</tr>
<tr>
<td>M. M. (F)</td>
<td>2</td>
<td>F</td>
<td>14.7</td>
<td>5.28</td>
<td>43</td>
<td>82</td>
<td>28.6</td>
<td>Normal</td>
<td>positive</td>
</tr>
<tr>
<td>B. M. (F)</td>
<td>9</td>
<td>M</td>
<td>15.0</td>
<td>5.53</td>
<td>45</td>
<td>86</td>
<td>26.5</td>
<td>Normal</td>
<td>negative</td>
</tr>
<tr>
<td>M. F.</td>
<td>22</td>
<td>M</td>
<td>15.8</td>
<td>5.20</td>
<td>48</td>
<td>92</td>
<td>29.5</td>
<td>Normal</td>
<td>positive</td>
</tr>
<tr>
<td>J. F.†</td>
<td>2</td>
<td>M</td>
<td>10.6</td>
<td>5.52</td>
<td>37</td>
<td>68</td>
<td>19.5</td>
<td>Normal</td>
<td>negative</td>
</tr>
<tr>
<td>T. F.†</td>
<td>1</td>
<td>M</td>
<td>7.5</td>
<td>5.77</td>
<td>31</td>
<td>54</td>
<td>13.0</td>
<td>Target Cells*</td>
<td>positive</td>
</tr>
<tr>
<td>J. F.</td>
<td>63</td>
<td>M</td>
<td>14.3</td>
<td>4.71</td>
<td>45</td>
<td>96</td>
<td>29.5</td>
<td>Normal</td>
<td>positive</td>
</tr>
<tr>
<td>P. A. F.</td>
<td>54</td>
<td>F</td>
<td>13.1</td>
<td>5.62</td>
<td>49</td>
<td>88</td>
<td>27.5</td>
<td>Normal</td>
<td>negative</td>
</tr>
</tbody>
</table>

* Rare target cell or rare cell showing basophilic stippling.
† Moderate number of target cells.
‡ Currently under iron therapy.

Cells also showed characteristics of thalassemia minor. Three months of intensive iron therapy has not altered these findings. The sickling trait was found to be present in L. G. (F)'s maternal grandfather (J. F.), and one of her maternal aunts (L. F. M.), an uncle (M. F.), and two cousins [T. F., M. M. (F)]. The thalassemia trait inherited from the father's side was found in her paternal grandmother (A. P. G.), an aunt (E. G. V.) and a cousin, [S. V. (G)].

The majority of individuals in the F. family whose cells sickled are also type X. However, M. F. is type MM and also showed the sickle trait, while B. M. F. is type NN but his cells did not sickle.
CHRONIC HEMOLYTIC ANEMIA

Fig. 2.—Genealogic chart of the G and F family. The letters refer to the A-B-O blood groups, the Rh system (C, D, E, e), M, N, P and S systems.

Fig. 3.—Longsworth Scanning Diagrams of the ascending boundaries in the electrophoretic analyses of the patient's hemoglobin and her mother's and father's, compared to normal, sickle cell anemia and trait hemoglobin.

DISCUSSION

Powell, Rodarte and Neel's patient, a 38 year old male, gave a history of recurrent pain and aching in his bones and joints dating back to early childhood. He also had bouts of fever and yellow discoloration of the skin of four days to
several weeks duration. The 6 year old patient described in this paper has shown none of the above symptoms. To date, her disease remains subclinical. Physical findings in the case presented by Powell et al. were those of a barely palpable spleen and slight icterus, while in our case the spleen is markedly enlarged and there is no icterus.

Hematologically however, the 2 cases were found to be very similar. In both cases the resistance of erythrocytes to hemolysis in hypotonic saline was increased. Normoblasts, stippled cells and target cells were noted in both, but our case did not show sickled cells on direct smear.

It is of considerable interest that the case presented by Powell et al. originally presented itself clinically and hematologically as a case of sickle cell anemia. On the other hand, our case, having none of the symptoms of sickle cell disease, and no sickle cells on direct smear, but having a peripheral blood picture suggestive of thalassemia with normoblastosis, and a large spleen, was given the tentative diagnosis of thalassemia major (unusually mild). The failure to demonstrate thalassemia trait on the mother’s side of the family led to the recommendation by one of us (W. N. V.), in view of the case of Powell et al., that sickling tests be performed, whereupon the true nature of the disease became apparent. It is suggested, therefore, that cases of hemolytic anemia in children that might be diagnosed as mild cases of thalassemia major or severe cases of thalassemia minor may involve a genetic situation comparable to the one presented in this report.

Powell et al. demonstrated that 100 per cent of their patient’s red cells sickled, and that they sickled more readily than did the red cells obtained from the other members of the family. They also observed that their patient’s cells appeared to be qualitatively different in the type of sickling produced when compared to the sickled cells from other members. Our observations also indicated that all of the cells in the double heterozygote contain sickle cell hemoglobin. This has also been shown in sickle cell trait. Differences similar to those noted by Powell et al. were noted between the sickling of L. G. (F)’s cells and the sickling of her mother’s cells. In addition, a distinct difference was noted between the electrophoretic pattern of the patient’s hemoglobin and that of her mother’s E. F. G.

Electrophoretic analysis of the patient’s [L. G. (F)]’s hemoglobin reveals the presence of about 70 per cent of sickle cell hemoglobin and 30 per cent of a hemoglobin having the mobility of normal hemoglobin. Individuals with the sickle cell trait have between 24 and 45 per cent sickle cell hemoglobin, and those suffering from sickle cell anemia have 80 to 100 per cent sickle cell hemoglobin. Electrophoretic analysis of the patient’s mother’s hemoglobin, on the other hand, shows only 38 per cent sickle cell hemoglobin. Numerous samples of sickle cell trait and sickle cell anemia hemoglobin have been studied electrophoretically by one of us (H. A. I.), but L. G. (F)’s intermediate pattern has been encountered previously only in one other patient. The latter, a married woman of pure Greek extraction, had clinical manifestations resembling sickle cell disease. Electrophoresis of the patient’s hemoglobin shows that her mother’s intermediate pattern is similar to the pattern usually found in patients with sickle cell trait.

* Or of the hemoglobin characteristic of thalassemia. The similarity in electrophoretic behavior of the hemoglobin in thalassemia to that in normal individuals does not preclude its difference in other properties.20, 21
Phoretic analysis of her hemoglobin revealed a pattern similar to that described above. She may represent another instance of heterozygosity for thalassemia and sickling traits. Studies of the patient's family are being conducted by Dr. J. V. Neel.

Based on the above observations, further consideration can be given to the pathogenesis of the findings observed in individuals heterozygous for both thalassemia and sickling. It is well accepted that typical cases of either trait show little evidence of hemolytic anemia. Erythrocyte survival studies in both conditions are normal. However, the simultaneous presence of the two traits in both the case of Powell et al. and ours resulted in evidence of a substantial hemolytic process.

It is becoming increasingly apparent that the clinical manifestations of sickle cell disease bear a good correlation to the percentage of sickle cell hemoglobin in the corpuscles. Thus, cases with the fully developed picture of sickle cell disease show electrophoretically 80 to 100 per cent abnormal hemoglobin, while individuals with the trait ordinarily possess 24 to 45 per cent abnormal hemoglobin. It appears reasonable to assume, therefore, that intravascular sickling is dependent to a considerable degree upon the percentage of abnormal hemoglobin present, and in turn, that the hemolytic process and much of the clinical symptomology is related to the degree of intravascular sickling. Hence, it is not surprising that an individual, such as reported here, possessing 70 per cent abnormal hemoglobin should have evidence of a brisk hemolytic anemia. In fact, it can be suspected that the sickling phenomenon is responsible for the severity of the hemolytic process and in effect that she has mild sickle cell disease.

The question then presents itself, why does 70 per cent sickle cell hemoglobin exist in a subject who also has thalassemia minor, whereas sickle cell trait is usually associated with 24 to 45 per cent sickle cell hemoglobin?

It should be recalled, in the usual case of sickle cell trait, that the amount of normal hemoglobin present is presumably related to the influence of the normal gene. In thalassemia, however, the fundamental defect appears to be an inability to synthesize hemoglobin in adequate amounts. This results in the characteristic findings of a refractory hypochromic microcytic anemia associated with low erythrocyte protoporphyrin and with an abundance of body iron stores. Thus the sickle cell gene is not associated in this case with a gene capable of producing normal hemoglobin in normal amounts, but with a gene which results in defective hemoglobinization of cells. One would expect, therefore, that the percentage of sickle cell hemoglobin in each cell would be greater in the presence of the thalassemia gene than in the normal gene. In effect, if the above hypothesis is correct, the increase in the percentage of sickle cell hemoglobin is due to reduced formation of "normal" hemoglobin in the individual heterozygous for both sickling and thalassemia traits.

**Summary and Conclusions**

The family history, case history and genealogy of a 6 year old girl suffering from a chronic hemolytic anemia is presented. The disease, resulting from her

* Studied at request of Dr. John S. Lawrence as the result of a personal communication with Dr. J. V. Neel.
inheritance of both the gene for sickle trait and that for thalassemia trait, is compared to a similar case in a 38 year old male reported by Powell, et al.

To date the child has had no clinical evidence of an hemolytic anemia, except for an enlarged spleen. Hematologically, however, all findings indicate the presence of a brisk hemolytic process.

Electrophoretic analysis of the patient’s hemoglobin reveals a unique pattern intermediate between the usual sickle cell trait and sickle anemia patterns.

REFERENCES

3 Sturgeon, P.: The volumetric and microscopic pattern of bone marrow in normal infants and children. IV. A statistical analysis. Blood (Submitted for publication).
17 Dameshek, W.: Personal communication.
Chronic Hemolytic Anemia Associated with Thalassemia and Sickling Traits

PHILLIP STURGEON, HARVEY A. ITANO and WILLIAM N. VALENTINE