Combinations of Hemoglobin G, Hemoglobin S and Thalassemia Occurring in One Family

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The rapid expansion of knowledge concerning the inherited abnormalities of human hemoglobin has raised numerous complex and fascinating questions concerning the genetic, biochemical and clinical relationships of the genes involved. Many of these problems can best be resolved by the intensive study of families in which two or more of the genes responsible for abnormalities in hemoglobin formation are present simultaneously. As a result of such studies, it has been shown that the genes responsible for hemoglobins C and S are very probably alleles,1 and that the genetic locus involved in the production of these two types of abnormal hemoglobin is independent of the thalassemia locus,2,3 although the latter conclusion is complicated by the growing realization that there may be several thalassemia loci.4 Thus far, family studies which would permit conclusions concerning the genetic relationships of the genes responsible for hemoglobins D, E, G, H, I and J, to one another and to the above mentioned genes, have not been presented.

This paper is concerned with a family in which there appear to be present three genes resulting in abnormalities in hemoglobin production, namely, the thalassemia gene, the gene responsible for hemoglobin S, and the gene for an abnormal hemoglobin component which in its properties meets the definition of hemoglobin G.5,6 Because of the fortunate combinations (genetically speaking) in which these genes occur in various members of the family, a number of conclusions can be drawn concerning the genetic, biochemical and clinical relations of the genes. In addition, certain other basic questions concerning the relation between genotype and hemoglobin patterns are raised. A preliminary account of this family has already been published.7

Materials and Methods

The hemoglobin solutions were prepared by the method described by Itano.8 The paper electrophoresis apparatus used was that described by Zuelzer et al.9 The buffers employed for paper electrophoresis were veronal buffer, pH 8.6 and ionic strength 0.075, and phosphate buffer, pH 6.5 and ionic strength 0.05. All runs were of 16 hours duration at 8 ma. and 200 volts.

Moving boundary electrophoresis was carried out in a Klett Tiselius apparatus (Longsworth modification). The buffer used was cacodylic acid—NaCl, pH 6.5 and ionic strength 0.1. The time of the run was 1000 minutes, the field strength 3.55 volts/cm. The hemoglobin solutions were first converted to carboxyhemoglobin prior to dialysis and maintained under

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CO tension throughout dialysis. The solubility determinations were carried out as described by Itano on reduced hemoglobin solutions.

The red cell survival studies carried out on two individuals were performed with Cr as described by Read, Wilson and Gardner. One hundred ml. of heparinized patient's blood withdrawn into a plastic bag was incubated with approximately 100 μc. of Cr. Fifty mg. of ascorbic acid was added, and the blood transfused back to the patient. Specimens withdrawn at weekly intervals were then tested for radioactivity.

Fecal urobilinogen was measured on a three day collection using the method of Watson. Plasma iron was determined as described by Hamilton, Gubber, Cartwright and Wintrube.

The osmotic fragility was determined by the method of Waugh and Asherman.

Fetal (alkali resistant) hemoglobin was determined by the method of Singer, Chernoff and Singer.

Blood grouping including A, B, O, D, C, E, c, M, N, S, s, Jk, Fy, K and Le, was done through the courtesy of Dr. Philip Levine, Ortho Laboratories. There were no combinations which would exclude the claimed parentage of the offspring.

**DESCRIPTION OF FINDINGS**

A pedigree of the family which summarizes some pertinent findings of the study is shown in figure 1. There follows a brief description of each of the 15 individuals on whom it was possible to make first hand observations. Certain of the hematologic and biochemical data are also summarized in table 1 and figures 1 and 2.

*The propotitus* (III-1, V. S.), a 28 year old male who had been seen intermittently at the Stanford University Hospitals since the age of 4 when, because of fever, recurrent joint pains, and a systolic murmur, he was thought to have rheumatic fever. Until the age of 20, he had crises from 2 to 4 times per month, characterized by painful joints, particularly the wrists, knees, elbows and ankles. At times these would be swollen, hot, and red, at other times merely tender. The crises were accompanied by fever to 104 F, frequent epistaxis, weakness and malaise. The attacks usually lasted 7 to 10 days. He had frequent hospital admissions, and was usually found to be acutely ill and febrile. He had a low blowing systolic murmur over the entire precordium and his spleen extended below the umbilicus. His joints were at times acutely inflamed. He was frequently transfused because of persistent anemia (Hb. 6-8 Gm. %). His height and weight were normal.
Table 1.—Summary of Hematologic and Biochemical Findings

<table>
<thead>
<tr>
<th>Pedigree Designation</th>
<th>Individuals</th>
<th>Sex</th>
<th>Age</th>
<th>Thalassemia Trait</th>
<th>Sickle Cell Trait</th>
<th>Anemia</th>
<th>Hemoglobin Type</th>
<th>Alkali Resistance %</th>
<th>Serum Iron µg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-3</td>
<td>F.C.</td>
<td>M</td>
<td>76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A A</td>
<td>&lt;1.0</td>
<td>112</td>
</tr>
<tr>
<td>I-4</td>
<td>F.H.</td>
<td>F</td>
<td>45</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>G G</td>
<td>&lt;1.0</td>
<td>114</td>
</tr>
<tr>
<td>I-6</td>
<td>M.H.</td>
<td>M</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A A</td>
<td>1.2</td>
<td>85</td>
</tr>
<tr>
<td>II-1</td>
<td>V.S.</td>
<td>M</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>S GS</td>
<td>3.9</td>
<td>241</td>
</tr>
<tr>
<td>II-2</td>
<td>C.S.</td>
<td>F</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A A</td>
<td>&lt;1.0</td>
<td>-</td>
</tr>
<tr>
<td>II-3</td>
<td>G.S.</td>
<td>M</td>
<td>26</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>GS GS</td>
<td>1.2</td>
<td>96</td>
</tr>
<tr>
<td>II-4</td>
<td>M.S.</td>
<td>F</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>A A</td>
<td>&lt;1.0</td>
<td>-</td>
</tr>
<tr>
<td>III-1</td>
<td>Fr.H.</td>
<td>F</td>
<td>14</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A A</td>
<td>1.8</td>
<td>121</td>
</tr>
<tr>
<td>IV-1</td>
<td>S.S.</td>
<td>M</td>
<td>1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG AG</td>
<td>4.1</td>
<td>-</td>
</tr>
<tr>
<td>IV-2</td>
<td>L.S.</td>
<td>F</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG AG</td>
<td>1.4</td>
<td>130</td>
</tr>
</tbody>
</table>

The severity of his illness subsided from the age of 20 to the age of 24; the attacks occurred less than once per month, there were no longer objective joint signs, and he did not require further hospitalization or transfusions. After the 24th year his attacks again increased in severity, accompanied by throbbing, generalized headache, aching epigastric pain and occasional vomiting. He became addicted to Dilaudid on three occasions. Though the attacks were usually not anticipated, emotional upset, excessive alcoholic indulgence and especially flexion of the knees for a prolonged period would precipitate them.

Occasional attempts had been made to raise the hemoglobin to normal levels and on one occasion, in 1954, he had received 11 units of blood within a two week period.

On physical examination he was well developed, poorly nourished, and appeared chronically ill. There was a yellow tinge to his skin and sclerae. The heart was not enlarged. A grade iii blowing systolic murmur was best heard at the pulmonic area, with P2 louder than A2. The liver was palpable at the costal margin. The spleen was hard and the edge easily palpable two fingers below the costal margin. The remainder of the physical examination was not remarkable.

On laboratory examination the findings were as follows: WBC 13,250/cu. mm.; differential leukocyte count: total neutrophils 42, banded 1, segmented 41, eosinophils 2, lymphocytes 44, monocytes 12; platelets, 270,000/cu. mm.; RBC 4,070,000/cu. mm.; hemoglobin, 10.7 Gm. %; hematocrit, 34.5%. MCV 84 cu. microns, MCH 26 µg., MCHC 31%. On peripheral smear there was marked anisocytosis, numerous target cells, occasional sickled cells, spherocytes, many macrocytes, occasional microcytes, slight to moderate hypochromia, occasional Howell-Jolly bodies, and 11 normoblasts/100 WBC's. Reticulocyte count was 12%. Osmotic fragility was decreased.

The direct bilirubin fraction was 0.06 mg.% and the total was 0.4 mg.%. Urine and urine urobilinogen were normal. Fecal urobilinogen was 500 mg./day. Serum iron was 241 µg%. Bone marrow showed erythroid hyperplasia. Red cell survival with Cr⁴ was one-third normal. Sickle cell preparation was positive.

The hemoglobin on paper electrophoresis showed a single spot in the position of S. Moving boundary electrophoresis, however, showed separation into two components, one which had the mobility normally assigned to S, namely 2.7 X 10⁻¹ cm.²/sec./volt, the other with the mobility of 2.5 X 10⁻¹ cm.²/sec./volt, the proportions being 48% S and 52% for the second component. The solubility of the hemoglobin solution in 2.24 phosphate buffer varied from 2.16 to 2.48 Gm./liter, with 3.9% of the hemoglobin alkaline resistant. The solubility values were definitely well beyond those established for solutions of hemoglobin S alone or in combination with up to 20% F. They fell within the range encountered in individuals heterozygous for the S gene, whose hemoglobins contain S in mixture with some...
Fig. 2.—Electrophoretic patterns: (a) Paper patterns of all members of the family. Note that III-1 appears as a single spot and has been labeled "S" because it is in the position of S hemoglobin. Conditions of run: pH 8.6, 0.75 μ; time of run: 16 hours at 7 ma. (b) The free electrophoresis patterns of all family members exhibiting hemoglobin G. Note the appearance of a small pigmented peak in IV-2 and the shoulder visible in the pattern of II-5. These components are as yet not identified and are probably artifacts. Conditions of the run: Cacodylate buffer, pH 6.5, 0.1 μ; time of run: 16 hours at 14 ma.

other component. The solubility values thus support the results of the moving boundary electrophoresis which demonstrates the presence of two components, S and a second component which, from the above findings, can be neither A nor F.

Description of other members of the kindred. I-1, I-2, I-3 and I-4, the paternal and maternal grandparents of the propositus, had immigrated to the United States from Calabria, Italy.
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There were no known Negro relatives. Only I-3 (F.C.), the maternal grandfather, age 76, was still alive. He had considered himself in good health all his life. Laboratory studies showed him to be hematologically normal. Red cell values were as follows: RBC 5,400,000/cu. mm.; hemoglobin 14.6 Gm.%; hematocrit 44%; MCV 81 cu. microns; MCH 27 μg.; MCHC 33%. On peripheral smear there was slight hypochromia. Alkali resistant hemoglobin was 0.3%.

Electrophoretic studies of I-3's hemoglobin done on paper and in the Tiselius apparatus gave normal results (hemoglobin A) as did the solubility tests.

II-1 (C.S.J.), II-2 (J.S.B.), and II-3 (T.S.), paternal uncles and aunts of the propositus, all had positive sickling tests. The hemoglobin levels were within the normal range in all three individuals, and the stained blood film showed no abnormalities.

Paper and moving boundary electrophoresis of the hemoglobin of each of these individuals revealed an AS pattern with a small unidentified pigmented component on the Tiselius apparatus, moving slightly faster than S.

The solubility tests gave results in the range for AS.

II-4 (F.S.), father of the propositus, age 52, had been well all his life. His past history and physical examination were not remarkable. Laboratory examination revealed the following: WBC 10,500/cu. mm.; differential leukocyte count: neutrophils 59%, banded 2%, segmented 57%, basophils 1%; lymphocytes 34%; monocytes 6%; RBC 4,590,000/cu. mm.; hemoglobin 14.8 Gm.%; hematocrit 46%; MCV 100 cu. microns; MCH 35 μg.; MCHC 32%; peripheral smear normal. Serum iron was 85 μg%.

The electrophoretic and solubility studies gave results identical with those of his siblings, II-1, II-2, and II-3, compatible with AS.

II-5 (F.C.S.H.), mother of the propositus, age 45, had complained of generalized weakness all her life and occasionally vague pain in her lower back and legs. She did not know of any anemia until the age of 43 when she was rejected as a blood donor. Referred to a private physician she received "iron injections" twice a week. There was no history of bleeding tendencies. Her past history and system review were not remarkable, except for vague growing pains at age 13.

On physical examination she was essentially normal. On laboratory examination the findings were as follows: differential count, normal; RBC 5,890,000/cu. mm.; hemoglobin, 11.3 Gm.%; hematocrit 42%; MCV 71 cu. microns; MCH 20 μg.; MCHC 27%; peripheral smear, normal. Serum iron was 114 μg%. Alkali resistant (fetal) hemoglobin was 0.5%. Sickle cell preparation was negative. Bone marrow showed erythroid hyperplasia. Red cell survival as judged by the Cr51 technic was approximately two-thirds normal.

Paper electrophoresis at pH 8.6 revealed a single spot in a position intermediate between A and S; with the moving boundary technic, two peaks were clearly distinguishable, one with a mobility of 2.5 x 10⁻⁴ cm.²/sec./volt, the other with a mobility of 2.7. The first peak contributed 80% of the pattern. The solubility demonstrated absence of hemoglobin S.

II-6 (M.H.), age 45, husband of the maternal aunt, had always considered himself healthy. Sickle cell preparation was negative. The hemoglobin level was normal, as were the appearance of a stained blood film, and the outcome of all pertinent tests relating to the composition of his hemoglobin.

II-7 (B.C.H.), age 39, maternal aunt of the propositus, had been well all her life, except for occasional nondescript pains in the legs and thighs at age 17. Her past history and system review were not remarkable.

On physical examination she appeared to be healthy. The liver and spleen were not palpable.

The laboratory findings were as follows: differential count normal; RBC 5,590,000/cu. mm.; hemoglobin, 10.8 Gm.%; hematocrit, 38%; MCV 68 cu. microns; MCH 19 μg.; MCHC 28%. The blood smear showed hypochromia, anisocytosis, poikilocytosis, occasional target cells, and basophilic stippling. Reticulocyte count was 2.5%. Osmotic fragility was decreased.

Bilirubin direct was 0.05 mg.%, and total was 0.5 mg.%. Fecal urobilinogen was 256 mg./day. Serum iron was 114 μg%. Alkali resistant (fetal) hemoglobin was 0.5%. Sickle cell preparation was negative. Bone marrow showed erythroid hyperplasia. Red cell survival as judged by the Cr51 technic was approximately two-thirds normal.

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Paper electrophoresis revealed a single spot in the position between A and S identical with that shown by the sister (II-5, F.C.S.H.). Free electrophoresis showed essentially a single peak with the mobility of $2.5 \times 10^{-4}$ cm$^2$/sec./volt, having a small shoulder with a mobility of 2.7.

III-2 (C.S.), age 24, wife of the propositus, had always seemed healthy. Hemoglobin level and appearance of stained blood film were within normal limits. Sickle cell preparation was normal. All further studies likewise yielded normal results.

III-3 (G.S.), age 26, brother of the propositus, had been well all his life. His past history and physical examination were not remarkable.

Laboratory examination revealed the following: RBC 5,300,000/cu. mm.; hemoglobin, 14.8 Gm.%; hematocrit, 46%; MCV 87 eu. microns, MCH 28 $\mu$g., MCHC 32%. Sickle cell preparation was positive. Fecal urobilinogen was 22.5 mg/day. Alkali resistant (fetal) hemoglobin was 0.6%. Serum iron was 96 $\mu$g.%

Paper electrophoresis of this individual’s hemoglobin showed a single spot extending from the position of S to F or G, without separation. The Tiselius patterns revealed two distinct components, one with the mobility of $2.5 \times 10^{-4}$ cm$^2$/sec./volt, the second 2.8, the latter being the mobility of hemoglobin S. The solubility of the hemoglobin in 2.24M buffer was 1.74 Gm./liter which falls in the range of the sickle trait.

III-4 (M.S.), age 25, wife of III-3, was a healthy woman with no anemia and a negative sickling preparation. The hemoglobin gave normal findings in every respect.

III-5 (F.S.), age 14, the daughter of II-6 and II-7, the maternal cousin of the propositus, was asymptomatic. Past history was not remarkable. Physical examination was normal. Laboratory examination revealed the following: WBC 7,050/cu. mm., with a normal differential. Blood smear showed hypochromia, rare stippled cells, rare target cells, and anisocytosis. The RBC was 5,300,000/cu. mm.; hemoglobin, 11.5 Gm.%; hematocrit, 42%; MCV 79 eu. microns, MCH 22 $\mu$g., MCHC 27%. Serum iron was 121 $\mu$g.%

Electrophoretic analysis of her hemoglobin was normal. The alkali resistant hemoglobin was 1.8%.

IV-1 (S.S.), age 1, son of the propositus had been a healthy infant. Physical examination was not remarkable. His hemoglobin was 12.0 Gm.%. Peripheral smear showed marked hypochromia, anisocytosis and poikilocytosis, stippled cells and 8% target cells. Sickle cell preparation was negative. Serum iron was determined. Fetal hemoglobin was 4.1%. Electrophoretically the hemoglobin gave the pattern of A.

IV-2 (I.S.), age 5, daughter of III-3 and III-4, was a healthy child. Physical examination revealed moderate axillary adenopathy, probably secondary to recent, infected, eczematous, lesions on her arms. On laboratory examination the findings were as follows: RBC 4,440,000/cu. mm.; hemoglobin, 12.7 Gm.%; hematocrit, 43.5%; MCV 90 eu. microns; MCH 28 $\mu$g.; MCHC 29%. Her peripheral smear was normal. Sickle cell preparation was negative. Alkali resistant hemoglobin was 1.4%. Serum iron was 130 $\mu$g.%

Paper electrophoresis showed a poorly differentiated pattern extending from the position of A to the position of F or G. The moving boundary electrophoresis showed two poorly separated peaks with mobilities of 2.38 and 2.48 $\times 10^{-4}$ cm$^2$/sec./volt, respectively, in addition to a small shoulder of greater mobility.

DISCUSSION

It is evident that at least three inherited abnormalities relating to the production of hemoglobin are present in this family. (1) One of these, hemoglobin S, is well established, and its demonstration in several members of the family requires no comment. (2) The second abnormal hemoglobin encountered in this pedigree has already been designated as hemoglobin G in a preliminary report on the basis of the then available characterization of that hemoglobin by Edington and Lehmann. The original hemoglobin G of these authors has since been described in greater detail by Edington, Lehmann and Schneider. A comparison
of their description of the physical properties of hemoglobin G with our findings 
shows no discrepancies other than the solubility in 2.58 M buffer. The hemo-
globin of II-7 has a solubility of 1.22 as opposed to 0.6 Gm/liter reported by 
Edington et al., for homozygous G. Lehmann after comparing the blood of II-7 
(B.C.H. of the pedigree) with that of his original patient, has expressed doubt 
concerning the identity of the two hemoglobins, finding on paper the former 
"moves more slowly than AA and just a fraction faster than GG." Whether 
these distinctions are an adequate basis for characterizing a separate hemoglobin 
the face of similarities in all other respects remains to be seen. For one thing, 
Edington’s and Lehmann’s patient is assumed by these authors to represent 
 homozygosity for the gene for hemoglobin G, whereas our patient II-7, as will 
be shown, cannot be a homozygote though her hemoglobin appears 
 homogeneous. For the time being it seems injudicious to assign a new designation and for 
these reasons the preliminary identification of the abnormal component as hemo-
globin G will be continued. (3) In addition, several members of the family show 
a blood picture which meets the usual criteria for thalassemia, namely hypo-
chromia, ovalocytosis, leptoerytosis, anisocytosis, poikilocytosis, stippling, and a 
variable degree of polycythemia in the absence of demonstrable iron deficiency. 
The very fact that the blood picture is abnormal in five members of three genera-
tions indicates an inherited trait that is comparable in its mode of transmission 
with thalassemia, thereby strengthening this interpretation. The thalassemia-
like picture is clearly independent of the presence or absence of either or both 
of the abnormal hemoglobins G and S. 

In the interpretation of the precise genetic basis for the findings in this family, 
it is convenient to begin with III-5. The hematologic findings in this 14 year 
girl are consistent with thalassemia minor, the normal serum iron ruling out a 
possible mild iron deficiency. The girl's mother, II-7, exhibits in a more marked 
form the cytologic findings of thalassemia minor; however, on electrophoresis, 
only hemoglobin G appears to be present. Hematologically speaking, II-7’s 
father is completely normal, both cytologically and electrophoretically. These 
facts make it clear that despite the electrophoretic homogeneity of the hemoglobin 
in Case II-7, only G appearing to be present, this patient must be considered to be 
heterozygous for the gene responsible for hemoglobin G. Since II-7’s husband is 
normal, she must also be the source of the thalassemia gene present in her daughter. 
II-7 may thus be assumed to be heterozygous for both the thalassemia gene and 
the gene responsible for hemoglobin G. 

Let us turn now to II-5, sister to II-7, whose findings differ from those of her 
sister only in the presence of a minor hemoglobin component which migrates 
with a speed of $2.7 \times 10^{-5}$ cm$^2$/sec./volt, the rate shown by hemoglobins D and 
S. With respect to the presence of the thalassemia and the hemoglobin G genes, 
it seems reasonable to conclude that II-5 resembles her sister in possessing both. 
We are unable at the present time to interpret the minor peak. Whether, as seems 
possible, it is an artifact or reflects still another complicating genetic factor, 
we are unable to say. In any event, the presence of this component does not 
affect our principal argument. 

II-5’s husband, II-4, by all criteria is an uncomplicated example of the sickle 
cell trait, as are all three of his siblings available for testing. Thus, the marriage
of II-4 and II-5 would seem to involve at least three different genes responsible for erythrocytic variations, namely, the genes responsible for hemoglobins G and S, and the thalassemia gene. This marriage resulted in two sons. One, III-3, exhibits the sickling phenomenon in the absence of an anemia and on electrophoresis possesses hemoglobins G and S. Married to a normal woman, III-4, he has produced one child who on electrophoresis shows a mixture of hemoglobins A and G. The most likely genetic interpretation of III-3 would appear to be that he has inherited the sickle cell gene from his father and the hemoglobin G gene from his mother, and has in turn transmitted the latter gene to his daughter.

III-1, the propositus, like his brother, III-3, exhibits on electrophoresis a mixture of hemoglobins G and S. Unlike his brother, however, he has a severe, chronic, hemolytic anemia. His only son, IV-1, exhibits morphologic abnormalities of his erythrocytes consistent with the diagnosis of thalassemia minor, although an iron deficiency anemia cannot entirely be excluded. Since III-2, the mother of this child, is hematologically normal, the thalassemia gene, if such it is, must have been inherited from his father, the propositus. The presence of a thalassemia gene in the propositus would also explain why his hematologic findings differ so strikingly from those of his brother III-3, even though both possess a comparable mixture of hemoglobins G and S. These considerations, together with the occurrence of the thalassemia gene in III-1’s mother, almost inescapably lead to the conclusion that III-1 possesses three different genes resulting in hematologic abnormality, namely, those responsible for thalassemia and hemoglobins G and S.

This interpretation of the pedigree leads to a number of important conclusions concerning the formal genetic relationships of these three genes, as follows.

1. The genes responsible for hemoglobins G and S cannot be alleles, otherwise IV-1, the son of the propositus, would have received either the gene responsible for hemoglobin G or the gene responsible for hemoglobin S—he has apparently received neither.

2. The genes responsible for hemoglobin G and for thalassemia cannot be alleles, otherwise the propositus could not have received both these genes from his mother, II-5, as analysis of the pedigree demonstrates.

3. Although this family yields no critical data on this specific point, other evidence indicates that the genes responsible for hemoglobin S and thalassemia are not allelic. It is therefore necessary to postulate at least three different genetic loci involved in hemoglobin production.

We may now proceed to construct a pedigree based on genotypes, in contrast to the purely descriptive pedigree of figure 1. We will designate the gene responsible for hemoglobin S as $h^s$, and its normal allele as $h^s$; the gene responsible for hemoglobin G will be designated as $h^G$, and its normal allele as $h^g$. Finally, the thalassemia gene will be designated as $h^T$, and its allele as $h^t$. The genotypes

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*This method of designating the genes follows the suggestions of Neel, except that for purposes of convenience the gene symbols which he suggested have been shortened. However, since this paper went to press, a modified system of terminology has been proposed by one of us (J.V.N., in press) at the Sixth International Congress of the International Society of Hematology. This system, advanced after consultation with a number of the active investigators in the field, appeared to find a provisional acceptance and supersedes that employed in the present paper.
of the various members of this pedigree, as we have interpreted this pedigree, are shown in figure 3.

If this pedigree accurately represents the facts, two further conclusions of importance to biochemical genetics can be drawn.

III-3 of this pedigree is heterozygous for the non-allelic genes responsible for hemoglobins G and S, yet he possesses no demonstrable hemoglobin A. Itano originally suggested the genes responsible for hemoglobin C and S were allelic because of the absence of normal hemoglobin in individuals heterozygous for both genes. Subsequent genetic data made the allelism of C and S probable. Nevertheless, if our interpretation of our pedigree is correct, then the absence of any hemoglobin A in individuals heterozygous for two “hemoglobin genes” does not provide critical evidence concerning the allelic relations of such genes.

One may speculate that the presence of the thalassemia gene in II-7 is responsible for the total suppression of production of hemoglobin A. This reasoning is supported by the observations in the case of II-5, an individual also apparently possessing the genes for G and thalassemia, and likewise showing no hemoglobin A. By contrast the only individual in this pedigree who appears to be a simple heterozygote for the G gene in the absence of the thalassemia trait, IV-2, shows both hemoglobins A and G in her electrophoretic pattern. It has already been shown that II-7, despite the fact that her hemoglobin is entirely type G, is not homozygous for the hG2 gene. The determination of the genotype of individuals with abnormal hemoglobins is a complicated problem and conclusions based on electrophoretic evidence alone are apt to be misleading. Physical-chemical homo- 

Fig. 3.—Pedigree of genotypes.
surveys for the frequency of the genes responsible for the various hemoglobin abnormalities are revealing an apparent excess of "homozygotes" on the basis of Hardy-Weinberg equilibrium expectation. We have already drawn attention to this fact in connection with hemoglobin C. The same finding emerges in recent surveys of hemoglobins D and E. Case III-1, that of the propositus, in addition illustrates a fallacy evident in the method of paper electrophoresis. The paper pattern shown by this individual's hemoglobin could not be interpreted other than S on paper alone, obviously because there was inadequate separation between S and G under these conditions. The solubility determination, however, belied this interpretation and the heterogeneity of this hemoglobin could be demonstrated by the moving boundary method. The results of the latter method satisfy our genetic interpretation.

The clinical hematologic significance of the presence of hemoglobin G in this family presents an interesting problem. The evidence with respect to the combination of genes resulting in other abnormalities of hemoglobin production in so-called double heterozygotes, for example h^A/h^S and h^G/h^A (sickle-thalassemia), suggests that the presence of two abnormal genes, each of which by itself is relatively or completely harmless, can produce clinically significant disturbances. When one analyzes the clinical and hematologic pictures exhibited by the various individuals of our pedigree who possess hemoglobin G, no indication appears that this variant is of clinical significance either in the simple trait (IV-2) or in the various combinations in which it is found in this remarkable family.

Hemoglobin G in combination with hemoglobin A is present in IV-2, an individual without any demonstrable hematologic handicap. Simple traits of this kind are usually benign, e.g., AS, AC, AD, and AE, so that the lack of abnormal findings in this individual is not surprising. More remarkable is the complete absence of symptoms or hematologic abnormalities other than sickling of the erythrocytes in III-3. This individual, heterozygous for the genes for both hemoglobins S and G, and possessing no hemoglobin A, nevertheless behaves in every respect like a simple, uncomplicated sickle cell trait. It would appear, therefore, that unlike the gene for C or thalassemia, the hemoglobin G gene does not enhance the pathologic effects of the S gene. Hemoglobin G serves the individual in this situation as well as does hemoglobin A. The proportions of G and S were comparable to those of A and S found in sickle cell trait. It seems remarkable in itself that in contrast to SC, where the proportions are in the neighborhood of 50:50, SG shows the proportions of 40:60. Since the solubility of pure hemoglobin G (II-7) is the same as that of A, and the solubility of SG (III-3) is comparable to that of the typical AS, one explanation for the lack of untoward effects in an SG individual may lie in the physical properties of this particular mixture, namely the presence of less than 50% S.

Where the gene for hemoglobin G is present in combination with a thalassemia gene, there is again no conclusive evidence that it contributes significantly to the hematologic or clinical manifestations of the latter. When one makes allowance —admittedly a subjective judgment—for the range of manifestations of thalassemia minor, then the cases of II-5 and II-7 are not clearly distinguishable from ordinary thalassemia minor either morphologically or as regards the severity of the hemolytic process or any disability. Nor, when they are compared to the
cases of simple thalassemia minor without hemoglobin G in the same family (III-5 and IV-1), does any difference become apparent.

Finally, the condition of the propositus himself, in whom all three abnormalities were found, namely sickling, thalassemia and hemoglobin G, was in no way different from the very well documented picture of sickle-cell-thalassemia disease. It is of interest that unlike the majority of cases of the latter which exhibited hemoglobin S in proportions ranging up to 80% and the remainder being entirely F, the combination G-S-thalassemia showed only approximately 50% S, the rest being almost entirely G with only a small fraction of F. Apparently the presence of the thalassemia gene does not necessarily depress or suppress the formation of hemoglobin G in a manner analogous to its usual effect on the production of hemoglobin A in ordinary sickle-cell-thalassemia.

It appears then that in the production of disease states in this family, the sickle trait and above all the thalassemia trait are the significant factors, and that the gene for hemoglobin G, either alone or in the various combinations, is not associated with a physiologic disturbance. Its appearance in this pedigree was, so to speak, accidental, the clinical disturbances presented by the propositus and his parents being satisfied by the demonstration of sickling and thalassemia features. The occurrence of G was brought to light by the application of electrophoresis with at first confusing and actually misleading results. The question arises whether hemoglobin G should be considered as an abnormality or a normal variant of human hemoglobin. Only further observations on larger case material can decide this question.

**Summary**

1. A Caucasian family is described in which, on the basis of clinical, hematologic and biochemical findings, it is postulated that the genes responsible for hemoglobins S and G and for the thalassemia defect are present.

2. On the basis of the study of this family, it is concluded that:
   a. The genes responsible for hemoglobins G and S cannot be alleles.
   b. The genes responsible for hemoglobin G and thalassemia cannot be alleles.
   c. The absence of hemoglobin A in individuals heterozygous for two "hemoglobin genes" does not provide critical evidence concerning the allelic relations of such genes.
   d. In this family, heterozygosity for the gene responsible for hemoglobin G results in an asymptomatic trait condition, in which some 40% of the hemoglobin is abnormal. When the gene responsible for G is combined with a hemoglobin S gene or a thalassemia gene, or both, the presence of hemoglobin G does not significantly alter the expression of these genes or their combinations. For example, an individual of the phenotype SG, whose hemoglobin contained no demonstrable A, was clinically a sickle cell trait, in that he showed no evidence of physiologic handicap.
   e. Individuals heterozygous for both the G and thalassemia genes may show on electrophoresis only hemoglobin G. This illustrates the unreliability in some cases of diagnosing genotype on the basis of electrophoretic findings.
   f. On the basis of these findings, hemoglobin G should probably be regarded as a normal variant of hemoglobin rather than as an abnormal type of hemoglobin.
SUMMARIO IN INTERLINGUA

1. Es describite un familia de caucasianos in que le presentia del genes responsible pro hemoglobina S, hemoglobina G, e le defecto de thalassemia es postulate super le base de constatationes clinic, hematologic, e biochimic.

2. Le studio de iste familia supporta le conclusion que:
   a. Le genes responsable pro hemoglobina G e homoglobimia S non pote esser allelos.
   b. Le genes responsable pro hemoglobina G e pro thalassemia non pote esser allelos.
   c. Le absen lui de hemoglobina A in individuos heterozygotic pro duo “genes de homoglobina” non representa un criterio concernente le relationes allelic de tal genes.
   d. In iste familia, heterozygia pro le gen responsable pro hemoglobina G resulta in un condition a tracto asymptomatic in que circa 40 pro cento del hemoglobina es anormal. Quando le gen responsable pro hemoglobina G es combine con un gen a hemoglobina S o un gen a thalassemia o ambes, le presentia de hemoglobina G non altera significativamente le expression de iste genes o lor combinationes. Per exemplo, un individuo del phenotypo SG con hemoglobina sin demonstrabili tracia de A eseva clinicamento un caso del tracto falciformante in tanto que ille exhibiva nulle signo de impedimento physiologic.
   e. Individuos qui es heterozygotic pro le genes a hemnoglobina C e thalasssemia exhibi a vices solmente hemoglobina G in le configuration electrophoretic. Isto illustra le risco de basar le diagnose del genotypo super constatationes electrophoretic.
   f. Super le base de iste constatationes il pare probable que hemoglobina G debe esser considerate como un variante normal de hemoglobina plus tosto que un typo anormal de hemoglobina.

REFERENCES

HEMOGLOBIN G, HEMOGLOBIN S AND THALASSEMIA IN ONE FAMILY

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Combinations of Hemoglobin G, Hemoglobin S and Thalassemia Occurring in One Family