The Hemoglobin D Syndromes

By Amoz I. Chernoff

HGB D has been shown to be far more prevalent among the American Negro than had heretofore been believed. An incidence of 0.4 per cent has been reported from this laboratory and confirmed by a study of hemoglobin types carried out by Conley and associates. Foci of appreciable Hgb D prevalence have also been encountered in Algerian Moslems and Indians of North Central India. To date, Hgb D has been found as the heterozygous Hgb D trait, in the mixed heterozygous state in combination with Hgb S (Sickle cell-Hgb D disease), and possibly as homozygous Hgb D disease. A fourth combination, that with thalassemia, may have been encountered by Lehmann and associates.

The study to be reported below was undertaken to delineate more fully the clinical, hematologic, and genetic characteristics of the Hgb D trait and homozygous Hgb D disease. No hematologic abnormality could be defined for the Hgb D trait, while homozygous Hgb D disease presented as a virtually asymptomatic, extremely mild hemolytic anemia.

METHODS AND MATERIALS

Routine hematologic studies were performed by standard methods as previously described. Fecal urobilinogen determinations were based on a modification of the method of Watson and the hemolytic index was calculated by the formula of Miller, Singer, and Dameshek. The Coombs' test was performed with commercial antiglobulin sera in the usual manner. The technic of Daland and Castle employing sodium metabisulphite, was used for determining the presence of sickle.

Paper electrophoresis was carried out as previously described and the amount of fetal hemoglobin determined by the alkaline denaturation procedure.

Survival times of the red cells were determined using Cr tagged erythrocytes autotransfused into the patients according to a modification of the method of Weinstein et al. Solubility determinations of the reduced hemoglobins were carried out by a modification of the technic of Itano. A description of the method will be the subject of a subsequent report.

The patients included in this study were selected from a group of individuals with Hgb D encountered in a survey of the Negro population of Barnes and Homer G. Phelps Hospitals, St. Louis. In addition, a white patient at the Durham VA Hospital, Durham, N. C. and two members of his family were found to have the Hgb D trait and were included. The subject with homozygous Hgb D disease was referred for investigation by Drs. George Smith and Ruth Steinkamp, St. Louis.

From the Departments of Internal Medicine, Washington University School of Medicine, St. Louis 10, Mo.; Duke University School of Medicine, and the Durham Veterans Administration Hospital, Durham, N. C.

Supported by Grant No. A-1615 from the National Institutes of Health, Bethesda, Maryland, and by a grant from the Muscular Dystrophy Associations of America, Inc.

Submitted July 28, 1957; accepted for publication Oct. 4, 1957.

The author wishes to thank Pauline C. Farr and Peggy Horton for technical assistance.
RESULTS

Characteristics of Hgb D

The chief identifying characteristics of Hgb D are its electrophoretic mobility and degree of solubility in the reduced state. Hgb D and Hgb S have identical electrophoretic patterns on paper electrophoresis at pH 8.8 or in free electro-
phoresis at pH 6.5, and may be separated from other human hemoglobins by these technics (fig. 1). Hgb D differs from Hgb S by its greater solubility in the reduced state in phosphate buffer and hence by the inability of Hgb D to form tactoids or cause sickling of the erythrocytes. In all other respects studied, Hgb D resembles the normal adult compound Hgb A. These include spectrum determinations, resistance to alkali denaturation, and immunologic reactivity.

**Heterozygous Hgb D Trait**

Eleven individuals whose blood contained Hgb A plus Hgb D were available for study. Five of these were extensively investigated. Genetic studies carried out in several of their families suggest that the pattern Hgb A plus Hgb D represents the heterozygous state of Hgb D and therefore may properly be referred to as the Hgb D trait. Although no quantitative determinations were made of the relative amounts of Hgb A and Hgb D, gross inspection of the paper electrophoresis pattern always revealed a darker spot of hemoglobin in the position of Hgb A. Analogous findings are recorded in all other known heterozygous hemoglobin trait states, with the possible exception of the Hgb J trait, where Hgb A invariably forms the major pigment type.

None of the eleven individuals with the Hgb D trait manifested any historical, physical, or hematologic abnormalities specifically related to the genetic abnormality of hemoglobin synthesis. Present in this group of patients were such incidental diseases as hypertensive cardiovascular disease (four cases), pneumonia, and chronic lymphocytic leukemia. A summary of the pertinent hematologic data is presented in table 1. Bone marrow examinations were performed in three of the subjects; two were entirely normal, while in the individual with leukemia approximately 60 per cent of the marrow cells were small mature lymphocytes. Hemolytic index determinations in three patients were within normal limits. The osmotic fragility determinations of the erythrocytes revealed no alterations from normal. Bilirubin levels were consistently normal. Red cell survival time determinations by the Cr51 method revealed a t/2 of from 25 to 35 days in the three individuals in whom this procedure was carried out (normal values, 27 ± 3 days). Coombs’ tests and sickling tests were persistently negative.

Paper electrophoretic studies of hemoglobin solutions prepared from the erythrocytes of this group of individuals revealed the pattern of Hgb A plus Hgb D (fig. 1). One of these specimens was also studied by Tiselius electrophoretic technics and the abnormal pigment was found to have a mobility at pH 6.5 of $2.65 \times 10^{-2}$ cm./volt/sec. Comparable mobilities are observed with both Hgb S and Hgb D. The amount of fetal hemoglobin in these specimens was within normal limits. The solubility of the reduced hemoglobin solutions in 2.58 M phosphate buffer was found to range from 1.3 to 1.6 Gm./L., a value considerably greater than that found with sickle cell trait hemoglobin solutions. The Hgb D trait specimens were completely soluble in 2.24 M phosphate buffer, whereas sickle cell trait blood is partially insoluble in this system. The gelling phenomenon could not be elicited in concentrated reduced solutions of the hemoglobin.
### Table 1.—Clinical and Laboratory Data in Patients with the Hgb D Trait

<table>
<thead>
<tr>
<th>CASE</th>
<th>AGE</th>
<th>SEX</th>
<th>RACE</th>
<th>RBC</th>
<th>HGB</th>
<th>PCV</th>
<th>MCV</th>
<th>MCHC</th>
<th>RETICS</th>
<th>TARGET CELLS</th>
<th>OSMOTIC FRIAGILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.B.</td>
<td>69</td>
<td>F</td>
<td>N</td>
<td>3.95</td>
<td>12.2</td>
<td>35</td>
<td>89</td>
<td>35</td>
<td>0.6</td>
<td>Few</td>
<td>0.46–0.34</td>
</tr>
<tr>
<td>L.J.</td>
<td>45</td>
<td>F</td>
<td>N</td>
<td>4.35</td>
<td>14.2</td>
<td>40</td>
<td>90</td>
<td>35</td>
<td>2.0</td>
<td>Few</td>
<td>0.44–0.36</td>
</tr>
<tr>
<td>J.J.</td>
<td>11</td>
<td>F</td>
<td>N</td>
<td>5.19</td>
<td>15.2</td>
<td>42</td>
<td>81</td>
<td>36</td>
<td>0.5</td>
<td>Rare</td>
<td>0.44–0.34</td>
</tr>
<tr>
<td>L.M.</td>
<td>58</td>
<td>F</td>
<td>N</td>
<td>4.55</td>
<td>13.9</td>
<td>41.5</td>
<td>91</td>
<td>34</td>
<td>1.0</td>
<td>0</td>
<td>0.44–0.34</td>
</tr>
<tr>
<td>A.T.M.</td>
<td>30</td>
<td>F</td>
<td>N</td>
<td>4.85</td>
<td>16.2</td>
<td>45</td>
<td>93</td>
<td>36</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>J.W.</td>
<td>44</td>
<td>M</td>
<td>W</td>
<td>5.16</td>
<td>16.6</td>
<td>46</td>
<td>90</td>
<td>36</td>
<td>0.6</td>
<td>0</td>
<td>0.46–0.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CASE</th>
<th>HEMOLYTIC INDEX</th>
<th>Cr²⁺/₂</th>
<th>BONE MARROW (E.M.)</th>
<th>VAN DEN BERGH</th>
<th>HGB F</th>
<th>SOLUBILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.B.</td>
<td>10</td>
<td>37 days</td>
<td>1:4</td>
<td>&lt;0.8</td>
<td>N</td>
<td>—</td>
</tr>
<tr>
<td>L.J.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>&lt;0.8</td>
<td>N</td>
<td>1.5 Gm./L</td>
</tr>
<tr>
<td>J.J.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>&lt;0.8</td>
<td>N</td>
<td>—</td>
</tr>
<tr>
<td>L.M.</td>
<td>15</td>
<td>25 days</td>
<td>1:2</td>
<td>&lt;0.8</td>
<td>N</td>
<td>1.35 Gm./L</td>
</tr>
<tr>
<td>A.T.M.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>J.W.</td>
<td>6</td>
<td>25 days</td>
<td>1:2</td>
<td>—</td>
<td>N</td>
<td>1.66 Gm./L</td>
</tr>
</tbody>
</table>

Symbols used in Tables 1 and 2: Age in years; RBC = red cell count per cu. mm. X 10⁶; Hgb in Gm. per 100 ml.; PCV = packed cell volume in per cent; MCV = mean corpuscular volume in cu. microns; MCHC = mean corpuscular hemoglobin concentration in per cent; retics = reticulocytes in per cent; osmotic fragility; first figure refers to concentration of saline with 10% hemolysis, second figure 90% hemolysis; Cr²⁺t/₂ = half time of Cr²⁺ disappearance in autotransfused red cells (Normal = 27 ± 3 days); Bone Marrow (E.M.) = erythrocytic-myelocytic ratio; van den Bergh in mg. per cent; Hgb F in percent, N = normal amount; 0 = absent; — = no information.
Genetic studies, carried out in several of the kinships, revealed a pattern comparable to that seen in the inheritance of Hgb S and other alleles of Hgb A. (fig. 2). With the evidence at hand, however, it was not possible to prove that the gene responsible for Hgb D is an allele of Hgb A. Four of the families with the Hgb D trait claimed American Indian blood in their ancestry. These data, however, are insufficient to permit one to draw any conclusions regarding the importance of the American Indian as a reservoir of Hgb D.

**Homozygous Hgb D Disease**

One individual was encountered whose hemoglobin pattern suggested homozygous Hgb S disease, but whose erythrocytes could not be induced to sickle. Further study suggested that the patient was homozygous for Hgb D and could, therefore, be classified as an example of pure or homozygous Hgb D disease.

The patient was a 40-year-old Negress whose history was unremarkable except for occasional episodes of dizziness, some fatty food intolerance, and a vague feeling of lack of energy since early adulthood. Her career as a nurse brought her in contact with diagnostic laboratory procedures, and at the age of 26 a diagnosis of "anemia" was made. She was treated with a variety of iron preparations and HCl which were taken intermittently until shortly before she was seen in our laboratory; there had been no apparent relief of her easy fatigability or alteration in her hematologic status. Except for minor arthralgia of her fingers in recent months, no other symptoms referable to possible hematologic diseases were obtained. The past history was likewise unrevealing. In 1939 she underwent an uneventful appendectomy. Her menstrual periods were entirely normal but she had never become pregnant.

Physical examination revealed a well developed, rather heavy set woman of somewhat greater than average height (fig. 3). Her weight was 168 pounds. Her features were not characteristically Negroid and her lips were somewhat thin. Her complexion was light, her hair dark brown and curly, but not kinky. The remaining examination was entirely within normal limits, particularly as related to pallor, icterus, hepatosplenomegaly, adenopathy, and bone, joint or muscle involvement.
The hematologic data are summarized in table 2. Red counts varied from 5.5 to 6.5 million per cu. mm.; hemoglobin from 12 to 13 Gm. per cent; mean corpuscular volume 67 cu. microns; mean corpuscular hemoglobin 23 γγ and mean corpuscular hemoglobin concentration 35 per cent. White cell, platelet, and reticulocyte counts were normal. The stained blood film revealed only microcytosis, some hypochromia, and 50 to 80 per cent target cells (fig. 4). No nucleated red cells were seen. Osmotic fragility curves indicated a marked symmetrical shift to the right, 10 per cent hemolysis being apparent in 0.34 per cent saline and 90 per cent in 0.20 per cent saline (fig. 5). Bone marrow examination revealed moderate erythrocytic hyperplasia, the E:M ratio being 2:3. Although the daily fecal excretion of urobilinogen was 94 mg. and the hemolytic index was found to be 12, Cr2 tagged red cells revealed the half-time survival of her erythrocytes to be 21 days.
indicating a somewhat shortened life span for her red cells. Other laboratory data included a serum iron of 79 γ per cent, a total iron binding globulin of 232 γ per cent; van den Bergh less than 0.8 mg. per cent; total protein 6.7 Gm. per cent of which 4.6 Gm. was albumin and 2.1 Gm. globulin; cholesterol 191 mg. per cent; alkaline phosphatase 1.8 B.U.; cephalin cholesterol flocculation, negative; thymol turbidity 5.7. Gastric analysis showed small amounts of free and combined acid after histamine. Stools were negative for blood and the urine examination was normal. Basal metabolic rate was -9. Coombs’ and sickling tests were negative. Roentgenograms of skull and long bones revealed no abnormalities.

Paper electrophoretic studies of hemoglobin solutions prepared from her erythrocytes revealed a single spot migrating in the position of Hgb S (fig. 1). The alkali denaturation test for Hgb F showed no increase in the quantity of this pigment. Reduced solutions of hemoglobin were completely soluble in 2.24 M phosphate buffer, while in 2.58 M phos-

---

**Fig. 4.—Photomicrograph of peripheral blood film in case of homozygous Hgb D disease (X 1250).**

**Table 2.—Data in Case of Hgb D Disease**

<table>
<thead>
<tr>
<th>RBC</th>
<th>5.5–6.5 million/cu.mm.</th>
<th>Bone Marrow–E.M</th>
<th>2:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>12–13 Gm. / 100 ml.</td>
<td>Osmotic Fragility</td>
<td>0.34%–0.20% saline</td>
</tr>
<tr>
<td>PCV</td>
<td>35–38%</td>
<td>Fecal Urobilinogen</td>
<td>94 mg./day</td>
</tr>
<tr>
<td>MCV</td>
<td>67 cu. μ</td>
<td>Hemolytic Index</td>
<td>12</td>
</tr>
<tr>
<td>MCH</td>
<td>23 γγ</td>
<td>Gr^a/ Survival Time (t 2)</td>
<td>21 days</td>
</tr>
<tr>
<td>MCHC</td>
<td>35%</td>
<td>Serum Fe</td>
<td>79γ %</td>
</tr>
<tr>
<td>WBC</td>
<td>8,600/cu.mm.</td>
<td>Fe Binding Capacity</td>
<td>232γ %</td>
</tr>
<tr>
<td>Platelets</td>
<td>550,000/cu.mm.</td>
<td>Total Protein</td>
<td>6.7 Gm.%</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1.0–1.5%</td>
<td>A/G</td>
<td>4.6/2.1</td>
</tr>
<tr>
<td>Differential</td>
<td>Normal</td>
<td>Alk. Phosphatase</td>
<td>1.8 BU</td>
</tr>
</tbody>
</table>

indicating a somewhat shortened life span for her red cells. Other laboratory data included a serum iron of 79 γ per cent, a total iron binding globulin of 232 γ per cent; van den Bergh less than 0.8 mg. per cent; total protein 6.7 Gm. per cent of which 4.6 Gm. was albumin and 2.1 Gm. globulin; cholesterol 191 mg. per cent; alkaline phosphatase 1.8 B.U.; cephalin cholesterol flocculation, negative; thymol turbidity 5.7. Gastric analysis showed small amounts of free and combined acid after histamine. Stools were negative for blood and the urine examination was normal. Basal metabolic rate was -9. Coombs’ and sickling tests were negative. Roentgenograms of skull and long bones revealed no abnormalities.

Paper electrophoretic studies of hemoglobin solutions prepared from her erythrocytes revealed a single spot migrating in the position of Hgb S (fig. 1). The alkali denaturation test for Hgb F showed no increase in the quantity of this pigment. Reduced solutions of hemoglobin were completely soluble in 2.24 M phosphate buffer, while in 2.58 M phos-
HEMOGLOBIN D SYNDROMES

OSMOTIC FRAGILITY

Fig. 5.—Osmotic fragility curves in Hgb D syndromes.

Fig. 5.—Osmotic fragility curves in Hgb D syndromes.

phate buffer the solubility was 1.3 Gm. L, far greater than the 0.1-0.3 Gm./L. seen in cases of sickle cell anemia. The visible spectrum of Hgb D was identical to that of Hgb A.

Family studies revealed the kinship as diagrammed in figure 6. The patient is one of four sisters, all of whom have Hgb D, her three siblings having the Hgb D trait. All were of light complexion, two had blonde hair, another was a brunette. Two half brothers and two children of another half brother had only Hgb A. The only other member of the family available for study was a paternal uncle with Hgb A. The paternal side of the family is stated to have an admixture with American Indian blood, while the patient’s maternal grandfather and great grandfather were said to be white Englishmen.

DISCUSSION

The widespread distribution of Hgb D has only recently become apparent with the discovery of foci of significant Hgb D prevalence in the American Negro (0.4 per cent), the Algerian Moslems (2.0 per cent), and Sikhs of North-Central India (2.0 per cent). Sporadic examples of Hgb D have also been detected among Caucasian individuals in England and the United States. Other examples of Hgb D have been encountered in a British family of Spanish and Austrian extraction, in a mulatto child of an English mother, and in a Turkish family. Another reservoir may be located among the American Indian as is suggested by several of the family studies carried out in this
investigation. To date, however, no such focus has been uncovered, possibly because extensive hemoglobin surveys of the American Indian have not yet been made. It is thus apparent that Hgb D has a wider distribution among the races of the world than does any other known type of abnormal hemoglobin. This observation suggests that the genetic mutation responsible for Hgb D may, in fact, have arisen in several different ethnic groups rather than having been disseminated from a single focus. Hgb D may consequently be of much less use as an anthropologic tool than Hgbs C, E or S which seem to be more limited in their racial distribution. On the other hand, the widespread distribution of Hgb D may suggest anthropologic relationships not seriously considered before.

The family studies reported in this communication support, but do not prove, the concept that Hgb D is an allele of Hgbs A, S and C. Theoretically, Hgb D may therefore exist in heterozygous or homozygous forms or in the mixed heterozygous state with another abnormal hemoglobin. Furthermore, combinations with other genetic hematologic abnormalities are to be expected. As previously mentioned, four syndromes involving Hgb D may have already been observed. It is to be expected that other combinations, such as Hgb C-Hgb D disease, Hgb E-Hgb D disease, etc., will be encountered particularly because Hgb D is found in groups which harbor a significant incidence of other
abnormal hemoglobins or live in close proximity to other peoples who have a high prevalence of an abnormal hemoglobin.

A number of reports of the Hgb D trait have appeared in connection with family studies of patients with sickle cell-Hgb D disease. The patients reported in this study differ in no way from the patients already described; the association of Hgb D trait with chronic lymphocytic leukemia in a Cauca-sion individual is unique, but seems to be purely coincidental. The number of patients with hypertensive cardiovascular disease among our patients with the Hgb D trait (four of eleven) seems high but can probably be explained on the basis of the number of Negro clinic patients with cardiovascular disease.

No specific abnormalities directly related to the presence of the abnormal hemoglobin are associated with the Hgb D trait. The hematologic picture is entirely normal and hemolytic phenomena are absent. Similar findings are reported in all other heterozygous forms of the abnormal hemoglobins with the possible exception of the occurrence of hematuria and splenic infarction in the sickle cell trait.

The heterozygous hemoglobin traits can usually be detected only by the electrophoretic determination of the hemoglobin types. Exceptions are, of course, the presence of sickling in sickle cell trait and the occurrence of target cells in many individuals with the Hgb C trait. The identification of the Hgb D trait must, however, depend on electrophoretic study and would be supported by the high solubility of the reduced hemoglobins and the absence of sickling.

Two cases presumed to be homozygous Hgb D have now been described. The patient of Lehmann and associates manifested essentially the same findings as did our patient. The data presented by Lehmann are not sufficiently complete for one to be certain of the diagnosis of pure Hgb D disease since these authors were unable to carry out family studies without which a definitive diagnosis of homozygous Hgb D disease cannot be made. The parents of the patient presented in this communication were both dead. Although specific genetic proof of homozygosity is also lacking in this instance, the presence of Hgb D in the four siblings resulting from this union is so strongly suggestive of the presence of Hgb D in each of the parents that this is a likely assumption. It must, however, be emphasized that an electrophoretic pattern of almost 100 per cent Hgb D does not alone prove homozygosity for this pigment in either of the two examples recorded. Thalassemia-Hgb S disease, and presumably thalassemia-Hgb D disease, may demonstrate up to 100 per cent of the abnormal hemoglobin in spite of presumed heterozygosity for the pathologic pigment. The absence of any definite hematologic stigmata of thalassemia in the patient presented in this report or in the available members of her family adds further support to the belief that she represents an example of pure Hgb D disease.

In comparison with other homozygous hemoglobin diseases, pure Hgb D disease seems to be characterized by the mildest of symptoms and a virtual absence of clinical findings. In marked contrast are the usual severe findings in sickle cell anemia. Homozygous Hgb C and homozygous Hgb E diseases
are characterized by much milder symptoms and findings than is sickle cell anemia, with Hgb C disease usually more severe than Hgb E disease. Hgb D disease, on the basis of the only known examples of this syndrome, is much less of a problem than any of the previously described pure hemoglobin diseases.

From the hematologic standpoint, homozygous Hgb D disease may be characterized as a very mild hemolytic anemia. The red cells are microcytic and normochromic and, as in homozygous Hgbs C and E diseases, present many target cells on the stained blood film. Erythrocytosis may at times be seen, but hemoglobin levels are normal or low normal. No other hematologic abnormality, except for the shift of the osmotic fragility curve, is apparent.

The electrophoretic findings of the hemoglobins in homozygous Hgb D disease exactly simulate those in homozygous Hgb S disease. However, gelling and tactoid formation are absent and the sickling phenomenon cannot be elicited. The solubility of the reduced hemoglobins in 2.58 M phosphate buffer is approximately 1.3 Gm./L., considerably higher than that found for Hgb S.

**SUMMARY**

The clinical and hematologic manifestation of the Hgb D trait and homozygous Hgb D disease have been presented. Family studies suggest that Hgb D is transmitted in a manner identical to that of Hgb A.

**SUMMARIO IN INTERLINGUA**

Es presentate le manifestation clinic e hematologic del tracto de hemoglobina D e del morbo de hemoglobina D homozygotic. Studios familial suggere que hemoglobina D se transmitte in un maniera identic con illo de hemoglobina A.

**REFERENCES**

2. Conley, C. L. Personal communication.
10. Miller, E. B., Singer, K., and Dameshek, W.: Use of the daily fecal output of urobi-
HEMOGLOBIN D SYNDROMES


The Hemoglobin D Syndromes

AMOZ I. CHERNOFF

Updated information and services can be found at:
http://www.bloodjournal.org/content/13/2/116.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml