Hemoglobin H Associated with an Uncommon Variant of Thalassemia Trait

By W. A. Dittman, A. Haut, M. M. Wintrobe and G. E. Cartwright

In 1955 Rigas et al. and Gouttas et al. independently reported the discovery of a new hemoglobin characterized electrophoretically at pH 8.6 by a more rapid anodal mobility than that of normal adult hemoglobin. This hemoglobin has subsequently been identified by the letter “H.” More recently, “fast” hemoglobins other than “H” have been described. These include hemoglobins I, J, K, N and two others found in infants but not identified by letters.

The anodal migration rate of hemoglobin H, on paper electrophoresis at pH 8.6, is distinct from all others except hemoglobin I. At pH 6.5 the anodal migration of hemoglobin H distinguishes it from hemoglobin I. The spontaneous denaturation of hemoglobin H within intact erythrocytes in vitro, results in the formation of multiple inclusion bodies. This process, which is accelerated by the incubation of red cells at 37°C with brilliant cresyl blue, or with the reducing agent, sodium dithionite, results in a characteristic appearance which is sufficient to enable detection of the anomaly.

The familial transmission of most abnormal hemoglobins has been illustrated by the demonstration of the hemoglobin in question in at least one parent of the propositus if adequate family studies were performed. The inheritance of hemoglobin H differs from this pattern. Apparently, hemoglobin H is only manifest if accompanied in the same individual by another genetic anomaly pertaining to hemoglobin formation. Thus, it has been demonstrated only when associated with thalassemia (or similar) trait, except for one instance and in that case it was associated with a new abnormal hemoglobin identified as “Q” by Vella et al. In some instances, therefore, hemoglobin H has been inherited from a parent in whom the presence of the abnormal hemoglobin could not be demonstrated.

The occurrence of hemoglobin H in a 26 year old American woman of Sardinian descent is reported in this paper. In this instance the associated “thalassemia trait” in relatives was unusual because of the absence of the characteristic elevation of the A2 component.

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A 26-year-old white housewife of Sardinian ancestry was seen at the Salt Lake County General Hospital on December 3, 1957 because of lifelong fatigue. Anemia, first noted in childhood, was diagnosed as "Mediterranean anemia" when she was 17 years old. At the age of 17 years she had "hepatitis." At age 25, because of right-sided and epigastric pain, "green" jaundice, vomiting, light stools, dark urine and intolerance to fatty foods a cholecystectomy was done and multiple black pigment stones were removed. In the last ten years she received 16 whole blood transfusions in treatment of her anemia. Each of these transfusions had produced a sense of well being for about two weeks. She was never incapacitated by anemia.

Her father died at age 59 of heart disease and diabetes. Her mother is living and is 50 years old. She had had "hepatitis" and gall stones; anemia was not known. Of two sisters, ages 24 and 22, one is anemic. The patient's only child, an infant, was found to have hypochromic leptocytes. All other members of the family live in Sardinia and no information concerning them is available.

Physical examination.—The patient was a small, olive-skinned white female. Except for multiple pigmented nevi and a midline abdominal scar, her skin was normal. Hair and nails were normal. There was slight scleral icterus. The heart was normal. Neither the liver, spleen nor abdominal masses were felt. The neurologic examination was normal. There were no abnormalities of the facies, head or hands.

On laboratory examination the findings were as follows: hemoglobin, 10 Gm. per 100 ml.; volume of packed red cells, 40 ml. per 100 ml.; red blood cells, 5.94 million per cubic millimeter; mean corpuscular volume, 67 μ3; mean corpuscular hemoglobin, 17 μg; mean corpuscular hemoglobin concentration, 25 per cent; reticulocytes, 15.4 per cent; erythrocyte sedimentation rate, 4 mm. per hour; platelets, 286,000/cu.mm.; white blood cells, 9,500 cu.mm. with 62 per cent polymorphonuclear leukocytes, 1 per cent basophils, 30 per cent lymphocytes and 7 per cent monocytes. The morphologic changes in the red cells were striking, there being marked anisocytosis, tear drop cells, fragmented cells, target...
cells, occasional Howell-Jolly bodies, stippling and polychromatophilia (fig. 1). The majority of the red cells were hypochromic and many were microcytic. A metabisulfite preparation was negative for sickle cells. Urinalysis was normal. The total plasma protein was 8.0 Gm. per cent with an albumin of 5.1 Gm. per cent and a globulin of 2.9 Gm. per cent. The plasma iron was 119 μg. 100 ml. The bilirubin was 0.1 mg. per cent direct and 1.4 mg. per cent indirect. The urine urobilinogen was 0.88 Ehrlich units in two hours. The Coombs’ test was negative. The serologic test for syphilis was negative. An L.E. test was negative.

Material aspirated from the bone marrow was cellular. The myeloid:erythroid ratio was 1:2. The leukocyte series and the megakaryocytes were normal and erythroid maturation was normoblastic in appearance.

There was decreased osmotic fragility of the patient’s red blood cells (fig. 2). A methyl violet preparation was negative for Heinz Bodies. Inclusion bodies were seen after incubation of the patient’s red blood cells with brilliant cresyl blue (fig. 3). As determined by the alkali denaturation test, fetal hemoglobin was 2.1 per cent. Hemoglobin electrophoresis was performed on paper for four hours at 350 volts employing a veronal buffer of pH 8.6 ionic strength 0.05. A “fast” component having a greater anodal mobility than normal adult hemoglobin was seen. This corresponded to the mobility of the “H” fraction in a sample of blood provided by Dr. Rigas116 (fig. 4). The findings were the same on starch block electrophoresis at pH 8.6 (fig. 5). After elution from the starch, the H hemoglobin was found to comprise 15.2 per cent of the total hemoglobin in our patient and 20 per cent in Rigas’ patient. The A2 constituent was reduced to less than 1 per cent in both samples containing H hemoglobin (table 1). At pH 6.5, with a 0.05 M phosphate buffer a component migrated toward the anode together with the “H” of Rigas whereas the normal adult hemoglobin migrated to the cathode.

Family Study (fig. 6).—Samples of blood from the patient’s mother, two sisters and only child were examined in our laboratory (table 1). Conclusive evidence of a hypochromic microcytic anemia with hyperferremia was found in one sister. Her hemoglobin electrophoretic patterns were normal on paper and starch block; hemoglobin H was not present and the A2 component was quantitatively normal. In the case of the patient’s mother, the

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![Fig. 2.](image-url) Osmotic fragility curve demonstrating the decreased fragility of the patient’s erythrocytes.
Fig. 3.—Photomicrograph of the patient's blood, demonstrating the erythrocyte inclusion bodies which appeared after incubation with brilliant cresyl blue for twenty minutes at 37 C. Counterstained with Wright's stain.

Fig. 4.—Pattern after paper electrophoresis, comparing the propositus with Rigas' patient and a normal. Hemoglobin H migrated toward the anode at pH 6.5 and 8.6.
Fig. 5.—Hemoglobin electrophoresis on a starch block, at pH 8.6, comparing the propositus with Rigas' patient and a normal. The presence of a "fast" component and the reduction in the A₂ fraction are demonstrated.

Fig. 6.—Pedigree reported herein. The propositus is II₂; II₁ and II₄ are normal. See table 1 for hematologic values.

blood smear revealed hypochromic leptocytes and target cells; however, a blood sample could not be obtained for the confirmatory measurements of the red cell indexes and serum iron. Her electrophoretic patterns and A₂ value were normal; hemoglobin H was not present. Examination of the blood of the patient's son revealed hypochromia, microcytosis and leptocytes and, at 3 months of age, anemia. Electrophoresis of the hemolysate was performed on paper, starch block and starch gel, at pH 8.6 and 6.5. The sample obtained at 3 months of age was electrophoretically normal; the A₂ content was quantitatively normal. Alkal-
Table 1

<table>
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<tr>
<th>Subject*</th>
<th>V.P.R.C. (mL/100 mL)</th>
<th>Hgb. (Gm./100 mL)</th>
<th>M.C.H.C. (%)</th>
<th>M.C.V. (µ)</th>
<th>Hypochromic leptocytes on blood smear</th>
<th>Plasma iron (µg./100 mL)</th>
<th>F.E.P.† (µg./100 RBC)</th>
<th>Inclusion bodies†</th>
<th>Electrophoresis</th>
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<td>Hb. H-Thal.**</td>
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<tr>
<td>I₁ (Mother)</td>
<td>30.3</td>
<td>7.9</td>
<td>26</td>
<td>61</td>
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<td>Yes</td>
<td>32–177</td>
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<tr>
<td>II₁ (Propositus)</td>
<td>40</td>
<td>10.0</td>
<td>25</td>
<td>67</td>
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<td>No</td>
<td>119</td>
<td>10,14,14</td>
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<td>II₁ (Sister)</td>
<td>36</td>
<td>10.6</td>
<td>30</td>
<td>74</td>
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<td>No</td>
<td>302</td>
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<tr>
<td>III₁ (Son)</td>
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<td>Newborn</td>
<td>55.5</td>
<td>10.1</td>
<td>29</td>
<td>96</td>
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<td>3 mos.</td>
<td>35.0</td>
<td>9.9</td>
<td>28</td>
<td>74</td>
<td>Yes</td>
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*See figure 6.
†Free erythrocyte protoporphyrin.
‡Inclusion bodies after incubation at 37 C. with brilliant cresyl blue.
†Reference 26.
**Analyses performed in our laboratory on a specimen generously supplied by Dr. Rigas; Case 10, reference 16.
resistant hemoglobin was 2 per cent of the total. Thus, the child appeared to have inherited the hypochromic microcytic anemia without the hemoglobin H.

**DISCUSSION**

The features which were of particular interest in our patient with chronic hypochromic anemia were: (1) the demonstration of intraerythrocytic inclusion bodies after incubation with brilliant cresyl blue; (2) electrophoretic migration of hemoglobin at pH 8.6 and 6.5 identical with the reference sample containing hemoglobins A and H; (3) the spontaneous denaturation and precipitation of some of the hemoglobin from the refrigerated hemolysates and (4) an A2 hemoglobin fraction less than one per cent. These findings are consistent with the previous reports of hemoglobin H occurring in Chinese, Greeks, Filipinos, Algerians, Thais, Malayans, Trans-Jordanians, Nepalese and a single case of an American child of Italian extraction. The demonstration of a very small A2 component in individuals with hemoglobin H-thalassemia disease has been reported by others and is in contrast to the occurrence of increased amounts of A2 in typical uncomplicated thalassemia minor.

In addition to hemoglobin H, the propositus probably carried either the thalassemia trait or some disorder closely akin to it. The distinction between the last two rests upon the significance one places upon the normal A2 values found in the patient’s son and sister, who certainly carried the “trait,” and in the mother, who was most probably a carrier of the same “trait.”

In a survey of the A2 values in 34 mothers of children exhibiting Cooley’s anemia, Kunkel reported that only two mothers had a normal A2 value, although in both the hematologic picture was compatible with thalassemia trait. Thus, about 5 per cent of otherwise typical instances of thalassemia trait lacked elevated A2 values.

Gerald, in a report of an occurrence of hemoglobin H noted the presence of microcytosis (without hemoglobin H) in the father and two other paternal relatives of the propositus; the maternal side was normal. The three microcytic relatives had a normal level of the A2 component. On the basis of this finding, it was considered that nonthalassemic hereditary microcytosis was present, and it was suggested that the term “pseudo-thalassemia” be applied.

Although the occurrence of hemoglobin H has been reported only in conjunction with the “thalassemia trait,” the description of this trait in near relatives of the propositus has been supported by information on the A2 values in only two instances: the report of Gerald and the present report. In both of those pedigrees the associated “thalassemia trait” was found to lack the elevated A2 values reported in typical cases.

Information on the A2 values in the “thalassemia trait” relatives in other hemoglobin H pedigrees is needed to determine the significance of this observation. Furthermore, this may have bearing upon the relative paucity of cases of hemoglobin H-thalassemia reported in persons of Italian ancestry. In the latter group the thalassemia-trait has been described with elevated A2 values in at least 95 per cent of the cases.
In the present case, the presumptive source of the hemoglobin H gene was the patient's father (deceased).

Summary

A 26 year old American housewife of Sardinian extraction with chronic hypochromic anemia was found to have a hemoglobin component identical to hemoglobin H. The A₂ hemoglobin fraction was decreased. Relatives were found to exhibit the hematologic features of thalassemia trait. In this pedigree, in contrast to the usual finding in the "thalassemia trait," the A₂ values were not increased. The same observation had been made in the only other comparable report.

Summario in Interlingua

Un menagera american de 26 annos de etate de descendentia sardinian con anemia hypochromic esseva studiate con le resultato que il esseva constatate que illa possede un componente hemoglobinic que es identic con hemoglobina H. Le fraction hemoglobinic A₂ esseva reducite. Esseva constatate que consanguineos del paciente habeva le characteristicas hematologic de tracto de thalassemia. In iste grupo de consanguineos, per contrasto con le constata- tion usual in casos de "tracto de thalassemia," le valores de A₂ non esseva augmentate. Le mesme observation se trova in le unic altere reporto de typo comparabile.

References

13. —, and —: Observations on some “fast”


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Four cases are reported in whose blood Heinz bodies were found following the administration of dianminodiphenyl sulphone ("Dapsone") for dermatitis herpetiformis. Three of the patients developed hemolytic anaemia. The regular examination of the blood for Heinz bodies is recommended for the control of patients receiving this drug.—R. M. H.
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