A New Hereditary Hemoglobinopathy (the Lepore Trait) and Its Interaction with Thalassemia Trait

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This report details our experiences with a new hemoglobinopathy which we have named the “Lepore trait.” This condition was discovered during a recent survey of the relatives of thalassemia major children. The mother of a child with clinical thalassemia major was found to possess a new abnormal hemoglobin (which we have named the Lepore hemoglobin) demonstrable only by starch block electrophoresis. Since the father of the child did not differ from the majority of thalassemia heterozygotes previously studied, it was presumed that the propositus was doubly heterozygous for the Lepore trait and for classical thalassemia trait—a belief strengthened by the subsequent detection of the Lepore hemoglobin in his blood. The Lepore trait itself was identified in four other members of the mother’s family.

This new hemoglobinopathy differs in several respects from the abnormal hemoglobin traits previously described. It is characterized by an erythrocyte morphology closely resembling that seen in classical thalassemia trait and by the presence of a new abnormal hemoglobin (the Lepore hemoglobin) occurring in low concentration (10-12% of the total pigment). In these properties, and in the clinical picture of its interaction with classical thalassemia trait, it seems to be more closely allied to thalassemia than to the abnormal hemoglobin syndromes. The present communication describes the clinical aspects of the Lepore trait, the syndrome resulting from its interaction with classical thalassemia trait, and the physicochemical properties of the Lepore hemoglobin.

METHODS

Conventional hematologic methods were used for the determination of erythrocyte and reticulocyte counts. Total hemoglobin was estimated spectrophotometrically as the oxyhemoglobin derivative. Hematocrit values were obtained using either a macro or a micro...
method. The alkali-resistant hemoglobin fraction was determined by the method of Singer et al., except where otherwise specified. Sickling tests were done with sodium metabisulfite as the reducing agent.

The starch block electrophoretic technic utilized has been described in a separate publication. When this technic is applied, normal adult hemoglobin can be fractionated into three parts: a major component, a "fast" minor component, and a "slow" minor component (hereafter called Hgb A₂). When quantitated, the A₂ component is expressed as per cent of the total hemoglobin present in the electrophoretic pattern. In a study of 20 healthy adults, the normal range for the Hgb A₂ content was found to be 1.7 to 3.1%, with an average of 2.4%. Values greater than 3.1% have been found only in association with thalassemia and with pernicious anemia in relapse.

CASE REPORT

The propositus, M.L. (CMC 45–91–32), was an 8-month-old infant referred to one of us (L.K.D.) for investigation of a severe anemia and splenomegaly. The child had been thought to be normal until one month earlier when routine examination disclosed the presence of the aforementioned abnormalities. At this first visit the following findings were noted: enlargement of the liver and spleen (4 cm. and 3 cm., respectively, below the costal margins), and slight enlargement of the heart. The results of examination of the peripheral blood were: Hgb, 8.0 Gm./100 ml.; RBC, 4.01 millions/cumm.; WBC, 32,000/cumm.; platelets, 181,000/cumm.; reticulocytes, 12.1%; RBC morphology, marked aniso- and poikilocytosis resembling that seen in thalassemia major; nucleated erythrocytes, 53/100 WBC. A preliminary diagnosis of thalassemia major was made, subject to confirmation by hemoglobin analysis and family study.

One month later, investigation of the immediate family was performed. The abnormal erythrocyte morphology characteristic of thalassemia trait was demonstrated in the mother and in the father (both were of Italian ancestry). The one sibling was found to be normal. The propositus' hemoglobin was 92% alkali-resistant (determined by a modification of Jonxis' technic). As part of a study of thalassemia then in progress, starch block electrophoresis of the hemoglobin from the members of this family was done. Contrary to expectation, an abnormal hemoglobin was demonstrated in the mother's blood, the details of which are given later. Initially the hemoglobin electrophoretic pattern of the propositus could not be distinguished from that seen in thalassemia major. Later a trace amount of the abnormal pigment was identified after optimal conditions for electrophoresis had been determined (fig. 1).

The propositus' course continued to be uneventful, except for chronic anemia and moderate irritability. No transfusions were given, the hemoglobin level remaining between 6 and 8 Gm.%. At 16 months of age the rapidly increasing splenomegaly was felt to be interfering with development and nutrition. In hopes of alleviating these complaints, as well as possibly improving the hematologic status, splenectomy was performed at 17 months of age. Following the operation, the child's appetite and disposition improved markedly. The period of follow-up is not yet sufficiently long to assess the effect on the hematologic course.

Examination of the spleen: The spleen weighs 291 grams, compared to a normal weight of 30 grams for the age. The splenic white pulp is relatively reduced in amount, the malpighian bodies are small with a scant rim of mature cells and a moderate sized secondary center. The red pulp is increased in amount and is crowded both in sinusoids and extravascular spaces with lymphoid, erythroid and myeloid forms. In both the latter two series there is a marked predominance of immature elements. Megakarocytes are rare. There is an increased number of both mature and immature plasma cells. Eosinophils are also increased in number. No erythropagocytosis or hemosiderosis is recognized. None of the features present serve to distinguish this spleen from others of Cooley's anemia."

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Hemoglobin studies. Hemoglobin fractionation by starch block electrophoresis was utilized to determine the Hgb A₂ content in the members of this family. The father (A.L.) was found to have an elevated Hgb A₂ content, consistent with the usual findings in the parents of thalassemia major children.¹

The mother (H.L.), however, exhibited a normal proportion of the A₂ component as well as a small amount of abnormal hemoglobin (11% of the total pigment). This abnormal hemoglobin was found to differ in its physicochemical properties from the hemoglobins which have been described previously.

The abnormal hemoglobin was observed to migrate at pH 8.6 with a mobility indistinguishable from that of Hgb S (fig. 2). After electrophoretic separation as the cyanmethemoglobin, the abnormal pigment was recovered from the starch block by elution. Comparison of the spectral absorption curve
with that of normal cyanmethemoglobin failed to reveal any differences over the range investigated (320 m\(\mu\) to 2000 m\(\mu\)). The minimal elevation of the alkali-resistant fraction indicates that the abnormal hemoglobin is at least as easily denaturable as normal hemoglobin.

Further specimens of the blood of the mother (H.L.) were sent to other laboratories in an effort to differentiate the abnormal factor from the known hemoglobins of similar electrophoretic behavior (hemoglobins S, D, G, and
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Lö. Through the kindness of Dr. A. R. Robinson (The Child Research Center of Michigan) we have been informed of the following properties of H.L.'s hemoglobin. When electrophoresed in free solution (Tselius electrophoresis) at pH 6.5 (cacodylate buffer of ionic strength 0.1, 16-hr. run) a single peak in the “A” position was found. The electrophoretic pattern in agar (citrate buffer of pH 6.2 and ionic strength 0.05, 16-hr. run) consisted of a major component in the “A” and a minor component in the “F” position, and between “F” and “A,” a pattern indistinguishable from that obtained with normal bloods. Solubility of the hemoglobin was infinite in 2.24 M phosphate buffer, and 1.6 Gm. per liter in 2.58 M phosphate buffer. Chromatography on ion exchange resin columns was not successful in demonstrating the presence of any hemoglobin other than Hgb A.

A second specimen of H.L.'s blood and a specimen from A.L. were sent to Dr. J. H. P. Jonxis and Dr. T. H. J. Huisman (University of Groningen, The Netherlands). Their studies yielded the following results. Moving boundary electrophoresis (phosphate buffer, 0.01 M, of pH 6.75 with 0.03 M sodium chloride added) produced a similar pattern with each showing, in addition to the “A” hemoglobin peak, a small peak moving with mobility similar to that of Hgb F and less than Hgb S or D. Additional studies with paper electrophoresis (veronal buffer, pH 8.8, ionic strength 0.06) and column chromatography (Amberlite IRC-50) were interpreted as indicating only the presence of Hgb A.8

In summary, the preceding reports illustrate the inability of technics other than starch electrophoresis to demonstrate the presence of the abnormal hemoglobin in H.L. Inasmuch as the technics cited have sufficed to demonstrate the known abnormal hemoglobins of similar electrophoretic mobility at pH 8.6 (hemoglobins S, D, G, and L), the abnormal hemoglobin of H.L. may be said to be distinguished from all previously reported hemoglobins.

If we were to follow established custom in naming this new hemoglobin, we would select the next letter of the alphabet after that used in naming the most recently described abnormal hemoglobin. The rapid pace of discovery in this field, however, has so outstripped the rate of formal announcement that we are aware of several new hemoglobins presently awaiting selection of a suitable designation. To avoid any possible duplication in terminology, we have chosen the name “Lepore” for our new hemoglobin, in imitation of the practice of blood group investigators of adopting the name of the family in which a new factor is first discovered. A specific designation with clinical, biochemical or genetic implications will be chosen in the future.

Inheritance of the “Lepore” hemoglobin. Electrophoretic investigation of the relatives of H.L. demonstrated the presence of the “Lepore” hemoglobin in four other adults (fig. 3). The distribution of the abnormal hemoglobin among the members of this family is such as to suggest strongly that its inheritance is under the control of a single gene.

The hematologic and electrophoretic data available on the members of this family are given in table 1. It is evident that the presence of the Lepore hemoglobin is associated with abnormal morphology of the erythrocytes. The similarity of the morphology to that found in classical thalassemia trait
is apparent from the photomicrographs of figure 4. The degree of poikilo- and anisocytosis and the frequency of target cells varied considerably among the affected individuals. The reduced mean cell volume seemed to be the most consistent feature.

We have chosen the name, the "Lepore trait," for the clinical syndrome characterized by the presence of the Lepore hemoglobin and associated with a thalassemia-trait-like morphology of the red cells, in view of its inheritance as a single gene defect.

**Discussion**

In a separate communication we have given the results of our hematologic and electrophoretic studies of adult thalassemia heterozygotes, selected on
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Fig. 4.—Upper left, classical thalassemia trait (A.L.); note the hypochromia, the poikilocytosis (including ovalocytes and cells with "tails"), and the microcytes. Upper right, "Lepore trait" (H.L.); note the similarity to classical thalassemia trait and in addition the greater degree of the target cell formation. Lower left, normal (M.L.) for comparison. Lower right, double heterozygote for the "Lepore trait" and classical thalassemia trait (M.L.); note the nucleated erythrocyte, the extreme degree of anisocytosis and the marked variation in extent of hemoglobinization of the cells. Wright's stain, x 668.

the basis of being parents of children with thalassemia major. In that investigation a reduced mean cell (erythrocyte) volume and an increased Hgb A2 content were found to be present in all of the thalassemia heterozygotes examined. In the original publication, these two criteria were used to define "thalassemia trait." Because of the common tendency to associate the word "thalassemia" with certain clinical and morphologic characteristics, irrespective of etiology, it has since been decided to use the amplified designation of classical thalassemia trait in place of the shorter phrase (see page 835, footnote). The adjective "classical" is deemed justified in view of the predominating frequency of this particular variety of thalassemia trait. Such subdivision of the syndrome "thalassemia trait" thus prepares the way for the recognition of further varieties—rather than excluding them from the general category of thalassemia as the earlier terminology was interpreted by some to do.

A.L., the father of the propositus, exhibits a reduced mean cell volume and an increased A2 content (table 1). On the basis of these findings, it is presumed that he is an example of classical thalassemia trait. H.L., the mother of the propositus, is quite evidently affected with a different condition, despite
the similarity in erythrocyte morphology. Her disease is a new, genetically determined entity which has been termed the "Lepore trait."

The propositus, M.L., differs markedly from either parent in his clinical state and presumably possesses the combination of their defects. The electrophoretic identification of the abnormal hemoglobin in his blood is conclusive evidence of the presence of the gene for the Lepore trait (fig. 1). The proof of the coexistence of classical thalassemia trait is less certain but is suggested by the quantitation of the \( A_2 \) component. The slightly subnormal level of \( A_2 \) in association with an alkali-resistant fraction of 74% is decidedly abnormal. Studies of newborns and young infants have shown that the \( A_2 \) fraction is inversely proportional to the alkali-resistant hemoglobin content. In thalassemia major children a similar relationship is present, although the ratio of Hgb \( A_2 \) to the nonalkali-resistant fraction is considerably greater than is the case for normal infants. The reduction of the \( A_2 \) content in the propositus to one-half of the normal average in the face of the reduction of the nonalkali-resistant fraction to one-fourth of normal, represents a relative increase of the Hgb \( A_2 \) fraction analogous to that found in thalassemia major. This relative increase is visually apparent when the density of the \( A_2 \) spot for M.L. is compared to that of a cord blood specimen containing a similar amount of alkali-resistant hemoglobin (fig. 1). This we interpret as indicating the presence of classical thalassemia trait.

The syndrome of the propositus is thus a new hemoglobinopathy due to simultaneous heterozygosity for classical thalassemia trait and for the Lepore trait. This interaction syndrome differs in several respects from the known combinations of thalassemia with the abnormal hemoglobins. Whereas the classical thalassemia trait-Lepore trait combination was accompanied by a preponderance of alkali-resistant hemoglobin, the highest levels reported in other thalassemia-abnormal hemoglobin diseases have been less than 50%. Again, the Lepore hemoglobin is present in trace amounts, while in the other examples of double heterozygosity an augmentation of the abnormal hemoglobin concentration has usually been demonstrated.11

The Lepore trait itself shows several features not heretofore observed in an abnormal hemoglobin trait. The heterozygotes for the previously described abnormal hemoglobins have not been associated with distinctive morphologic changes, except target cell formation (as in Hgb E and C traits).12 The microcytosis and aniso- and poikilocytosis present in the Lepore trait are in marked contrast to this. In the previously published hemoglobinopathies, the concentration of the abnormal hemoglobin component in adult heterozygotes has never been found to be less than 20%,11 while the Lepore hemoglobin comprises only 10 to 12% of the total pigment. It is conceivable that these discrepancies reflect a fundamental distinction between the Lepore hemoglobin and the known abnormal hemoglobins. In view of the similarity between the erythrocyte morphology of the Lepore trait and classical thalassemia trait, and between the electrophoretic and the clinical aspects of homozygous classical thalassemia trait and the double heterozygote for classical thalassemia trait and the Lepore trait, we are led to believe that the Lepore trait may be more closely related to thalassemia than to the abnormal hemoglobin syndromes.
Although there are no explicit descriptions in the literature of an abnormal hemoglobin resembling the Lepore hemoglobin, we would like to call attention to the family reported by Humble et al. In this pedigree, the propositus exhibited a moderately severe hemolytic anemia associated with a Hgb S content of nearly 100%. The propositus was considered to be an example of thalassemia-Hgb S disease since the mother (Mrs. H.) appeared to have thalassemia minor. It is noteworthy that on paper electrophoresis, the hemoglobin of Mrs. H. was observed to show some "tailing," as if a small amount of an S-like hemoglobin was present. Except for the normal MCV reported, Mrs. H. parallels closely the Lepore trait.

The addition of the Lepore trait to the family of hemoglobinopathies introduces the possibility of a number of new syndromes. Included among these are the combination of the Lepore trait with the various abnormal hemoglobin traits, and the homozygous state for the Lepore trait. Considering the similarity between the Lepore trait and classical thalassemia trait, these syndromes are expected to resemble, respectively, the known combinations of thalassemia with the abnormal hemoglobins, and thalassemia major. Since the Lepore heterozygote cannot be distinguished from classical thalassemia trait by paper electrophoresis, it is possible that examples of these conditions have already been encountered but have not been recognized.

SUMMARY

A new hemoglobinopathy, termed the "Lepore trait," is described. The Lepore trait is characterized by an altered erythrocyte morphology, resembling classical thalassemia trait, and by the presence of a hitherto unreported abnormal hemoglobin occurring in low concentration (10-12% of the total hemoglobin). The trait is transmitted as if due to a single gene defect. Simultaneous inheritance of the Lepore trait and classical thalassemia trait occurred in one member of the pedigree studied and resulted in a severe hemolytic anemia clinically indistinguishable from thalassemia major.

On the basis of the erythrocyte morphology, the clinical findings, and the electrophoretic studies, it is conjectured that the Lepore trait may be more closely related to thalassemia than to the other abnormal hemoglobin syndromes.

SUMMARIO IN INTERLINGUA

Es describite un nove hemoglobinopathia, designate como "tracto Lepore." Illo es characterisate per un alterate morphologia crythrocytic, resimilante illo del classic tracto de thalassemia, e per le presentia de un usque nunc non reportate hemoglobina anormal que occurre in basse concentrationes (10 a 12% del hemoglobina total). Le transmission del tracto pare indicar que illo es causate per un sol defecto genic. Le simultanee transmission hereditari del tracto Lepore e del classic tracto de thalassemia occurreva in un del membros del familia studiate e resultava in un sever anemia hemolytic que esseva clinicamente indistinguibile ab thalassemia major.

Super le base del morphologia erythrocytic, del constatationes clinic, e del

*See footnote (p. 835) for definition of "classical thalassemia trait."
studios electrophoretic, il es conjecturate que le tracto Lepore es possiblemente plus intimemente relationate a thalassemia que al altere syndromes de hemoglobina anormal.

REFERENCES
A New Hereditary Hemoglobinopathy (the Lepore Trait) and Its Interaction with Thalassemia Trait

PARK S. GERALD and LOUIS K. DIAMOND