Hemoglobin I: An Inherited Hemoglobin Anomaly

By D. L. Rucknagel, E. B. Page and W. N. Jensen

Studies of hemoglobin by the various techniques of electrophoresis and alkali denaturation have resulted in a striking development in the knowledge of abnormal hemoglobins and in the hereditary hemolytic anemias, some of them never previously recognized.

During the course of the examination of the electrophoretic properties of the hemoglobins of Negro patients admitted to the medical service of the hospital, a previously undescribed abnormal hemoglobin characterized by a rapid electrophoretic mobility at pH 8.6 was detected. The abnormal hemoglobins already described include types S, C, D, E, G, and H. It is proposed that this seventh abnormal pigment be designated as hemoglobin J.

METHODS

Hemoglobin samples were prepared according to a modification of the technic of Drabkin and paper electrophoresis carried out in accord with the method of Larson and Ranney. Electrophoresis of the hemoglobin in the Tiselius apparatus, after conversion to CO hemoglobin was carried out in a cacodylic acid-sodium cacodylate buffer of pH 6.5 and ionic strength of 0.1. Ultracentrifuge sedimentation determinations were made in the Spinco Model E analytical ultracentrifuge. Ferrohemoglobin solubilities were determined by the method of Itano. Hematologic studies were performed in accord with methods previously described. A modification of the method of Barkan and Walker was used for the determination of plasma iron content.

MATERIAL

The propositus, G. H., a 25 year old male Negro was admitted to the hospital in September, 1954, with the complaint of recurrent epigastric discomfort of five years’ duration. Available hospitalization records showed three previous admissions for similar complaints from 1950 to 1951. The history was compatible with a diagnosis of peptic ulcer and although previous gastrointestinal x-ray series had demonstrated a deformity of the duodenum, x-ray studies at the time of his last hospitalization were normal. There was no history suggestive of anemia, hemolysis, cholelithiasis, leg ulcers, nor arthralgia. Past history revealed only the usual childhood illnesses which were without apparent sequelae.

Physical Examination: Temp. 98.6 F., Pulse 80, B.P. 118/68 mm. Hg. The patient was a well-developed, muscular, well-proportioned Negro with a chocolate brown skin color. The patient had physical features which suggested American Indian ancestry. There was a straight nose, moderately thin lips, and wavy, rather than kinky, black hair. There was epigastric tenderness to palpation, liver and spleen were not palpable, lymph nodes were

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normal and there was no bony tenderness. Neurologic examination was within normal limits.

*Laboratory Findings:* Urine analysis was normal. Examination of the peripheral blood showed a hemoglobin of 15.4 Gm. per cent, R.B.C. 5.10 million per cu. mm., W.B.C. 8,700 per cu. mm. and a normal differential leukocyte count. Examination of wet preparations and dried stained smears showed normocytic normochromic erythrocytes without visible abnormalities. Repeated reticulocyte counts, sickle cell preparations, and stains for siderocytes were normal. Platelets were present in normal numbers on the smear and by direct count (260,000 per cu. mm.). Bleeding time, coagulation time, clot retraction, prothrombin content and tourniquet tests were normal.

Erythrocyte osmotic fragility tests were normal, the bilirubin level was 0.8 mg. per cent and fecal urobilinogen was not increased. Permission for bone marrow examination was refused. X-ray examination of the long bones, pelvis, vertebral column, skull and chest were normal.

*Family*

This family, which with the exception of the propositus had lived only in North Carolina, was of low economic status and was extremely suspicious of the entire clinical investigation. Clinical and hematologic examinations were made in 17 of the 19 living members of the family. The propositus was the second of 10 children, all of whom appeared to be in good health. The mother and father were living and well. The father initially denied living

![Fig. 1.—Paper electrophoretic patterns of hemoglobins C, S, A, and I at pH 8.6 in veronal buffer.](from www.bloodjournal.org)
sibling but it was later determined that he had two living siblings who were not available for examination. Although there was no definite history of Caucasian or American Indian ancestry, it was apparent that the mother, who had normal hemoglobin, had physical features characteristic of the American Indian. Attempts to contact the living members of the mother's family were not made.

Five of seven individuals of the third generation in this family were examined. One of these children (M. H.), the daughter of C. H., was found to have the combination of A and I hemoglobin.

There was no history suggestive of hemolytic phenomena, anemia, arthritis or arthralgia in those members of the family who were found to have the abnormal hemoglobin.

Brief descriptions of the five additional members of the family who have the combination of types A and I hemoglobins are presented below.

G. H., Sr., the father, age 54, a well-developed Negro with classic Negroid features. He denied previous or current illness. Physical examination was normal.

C. H., the elder brother, age 26, had pneumonia at age 24, which was treated with antibiotics, without complication. Otherwise, he had always enjoyed good health. Physical examination was normal.

M. H., the sister, age 23, denied illness or hospitalizations and the physical examination was normal.

O. M. H., the sister, age 15, denied illness and on physical examination was found to be normal.

M. H., the niece, age 2, has had no illness, and abnormalities were not found on physical examination.

Fig. 2. Paper electrophoretic patterns of hemoglobins from three individuals with combinations of type A hemoglobin and a rapidly moving abnormal component (pH 8.6).
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RESULTS

Hemoglobin Analyses: Values for alkaline resistant hemoglobin of 0.5 per cent, 0.7 per cent and 0.7 per cent were found in patients G. H., Jr., G. H., Sr., and C. H., respectively. This is within the normal range observed in our laboratory for this method.

In figure 1, the pattern obtained on electrophoresis of the hemoglobin from the propositus may be compared with that of hemoglobin C, sickle hemoglobin and normal hemoglobin.

Although hemoglobin I is easily distinguished from types A; C and S at pH 8.6, it is necessary to separate type I from type H at a lower pH. Results of the paper electrophoresis at pH 8.6 of the hemoglobin obtained from our patient, a patient with hemoglobin H who was reported by Rigas* and a third patient who is to be reported by Bergren* are presented in figure 2. In each of these subjects, there is the combination of normal hemoglobin and a second more rapidly moving component. At pH 8.6, differences in electrophoretic mobilities of the abnormal components are not apparent. However, at pH 6.5, hemoglobins A and I

* These hemoglobin samples were supplied by Dr. D. A. Rigas, Department of Medicine, School of Medicine, University of Oregon, Portland, Oregon, and Dr. W. R. Bergren, Childrens Hospital Society of Los Angeles, Los Angeles, California.

Fig. 3.—Paper electrophoretic patterns of hemoglobins from three individuals with combinations of type A and abnormal hemoglobin (pH 6.5).
Fig. 4.—Tiselius electrophoresis results, showing ascending boundary diagrams of normal type A hemoglobin (lower) and the hemoglobin of the propositus (upper) at pH 6.5.

are seen to migrate toward the cathode, whereas type H and type “X” (Bergren) migrate toward the anode (fig. 3). Thus, hemoglobin H and the hemoglobin found by Bergren, which have similar electrophoretic properties at pH 8.6 and 6.5, are clearly distinguishable from hemoglobin I.

Tiselius apparatus analysis of the hemoglobin from the propositus and of his sister, O. M. H., gave identical results. In figure 4, the ascending boundary diagram of the hemoglobin from the patient, G. H., Jr., is compared to that of a normal subject. There are two components present, one which has the characteristic mobility of type A, which constitutes 80 per cent of the total, and the second, type I, which has a mobility of $1.7 \times 10^{-6}$ cm$^2$/volt/sec, and comprises the remaining 20 per cent of the total.

In figure 5, the hemoglobin paper electrophoretic patterns at pH 8.6 of the propositus, the mother, the father, and three siblings are shown. In each of the affected members of this family, the hemoglobin has resolved into the two components, type A and type I. It should be emphasized that in each of the affected members, there was approximately the same ratio of normal to abnormal hemoglobin.
Ultracentrifugation of the patient's prepared hemoglobin showed a single peak, indistinguishable from that of normal hemoglobin, a finding which would indicate that the normal and abnormal pigments are of identical molecular weights. Absorption spectrum and oxygen carrying capacity of type AI hemoglobin were the same as that of type A hemoglobin.

**Genetic Studies:** All members of the immediate family are living and were available for study. Six individuals were found to have AI hemoglobins, the remaining 10 members of the family have normal A hemoglobin. In figure 6, the composition of the hemoglobin of the members of this family is depicted. It will be noted that the father, four of the nine members of the second generation and a niece were found to have type AI hemoglobin. Thus, the abnormality is present in three generations of this family.

**Hematologic Studies:** The results of the hematologic examinations of the propositus have been described. In the other members of the family who were found to have AI hemoglobin, anemia was not observed; examination of dried stained blood smears, wet preparations, siderocyte preparations and sickle preparations were normal (table 1). Total and differential leukocyte counts were normal in
five of the six individuals. In the sixth individual (O. M. H.) there was an unexplained lymphocytosis present.

Serum iron levels were normal in all patients with the hemoglobin AI. Bone marrow examinations and tracer iron studies which were proposed were refused by the family.

**Discussion**

To date, six (S, C, D, E, G, and H) abnormal hemoglobins which occur in a heredito-familial fashion have been described. These, together with normal (type
Table 2.—Electrophoretic properties of hemoglobins

<table>
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<tr>
<th>Type</th>
<th>Symbol</th>
<th>Solubility</th>
<th>Mobility pH 6.5</th>
<th>*Relative Mobility pH 8.6</th>
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<tr>
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<td>-</td>
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<tr>
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<td>-</td>
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* The numbers refer to mobility in decreasing order on paper electrophoresis.

A) and fetal hemoglobin (type F) constitute the various described hemoglobins (table 2). In addition, Battle has described a hemoglobin, found in members of a white family, which is associated with a type of hereditary hemolytic anemia. This abnormal component was detected on Tiselius electrophoresis at pH 7.8, but was not identifiable on paper electrophoresis at pH 8.6.

Of these hemoglobins, types A, C, E, and G may be identified by their electrophoretic mobilities in the Tiselius apparatus or on paper electrophoresis. Hemoglobins S and D exhibit the same mobility at pH 6.5 and pH 8.6. Although electrophoresis does differentiate types S and D from the other hemoglobins, they themselves must be separated by their different solubility characteristics and the failure of hemoglobin D to produce the sickling phenomenon. Hemoglobin F is best detected by its resistance to alkali denaturation, electrophoretic mobility and immunologic properties. Hemoglobin H exhibits a migratory rate at pH 8.6 in excess of that of hemoglobin A. The paper electrophoretic migratory rates of hemoglobins H and I, at pH 8.6, are indistinguishable and cannot be clearly differentiated. That these two hemoglobins are, however, different and do possess different isoelectric points is demonstrable when they are compared on paper electrophoresis at pH 6.5. At this pH, hemoglobins A and I migrate toward the cathode, whereas type H migrates toward the anode. These findings have been confirmed on Tiselius electrophoresis at pH 6.5 and pH 8.6.

Additional evidence that hemoglobin A1 and hemoglobin A1 differ is afforded by the different inheritance patterns as well as the different hematologic findings in the two diseases.

The clinical syndromes associated with the various abnormal hemoglobins have been adequately described in numerous reports.

Studies on the incidence of the various types of abnormal pigments indicate that sickle cell trait (SA) occurs in approximately 9 per cent of the American Negro population. Sickle cell anemia (SS) occurs in about 2.3 per thousand indi-

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individuals in this same population. Hemoglobin C trait has been estimated to occur in from 1 to 3 per cent of American Negroes and to date there have been reported 13 cases of hemoglobin C disease. The incidence of the latter disease has been estimated to be 1 per 6000 American Negroes. Only one case of hemoglobin D in combination with type S has been reported. In the Thai population hemoglobin E has been found in approximately 12.5 per cent of 590 individuals. There were two individuals of the 590 examined who had homozygous E hemoglobin. Hemoglobin G was described by Eddington and Lehman, but clinical examinations were not reported. Schwartz and Spaet have described the occurrence of a hereditary hemolytic anemia in an Italian family, wherein one member had a homozygous hemoglobin with electrophoretic properties similar to that described by Lehman and Eddington.

Rigas has reported the existence of hemoglobin H, which has a migratory rate greater than that of type A, in two siblings with hemolytic anemia. Definition of an inheritance pattern in this family was not possible.

Although clinical and routine hematologic examinations of the subjects with the combination of type A and I failed to elicit abnormalities, this abnormality is recognizable upon electrophoresis of the hemoglobin. While a definite description of the mode of inheritance must await further studies, it would seem that the abnormal hemoglobin is due to a gene whose effect is recognizable in the heterozygous state and this determinant may thus be treated as a dominant type of transmission. It is evident from this study that sex linkage is not present. Since all of the affected members of the family have similar quantities of type A and type I hemoglobins, it may be assumed that a similar gene is responsible in each case. Evidence of genetic or environmental modification of this gene has not been demonstrated. Thus, the presence of a single gene for hemoglobin A on each of two allelic chromosomes produces the trait or heterozygous state. The heterozygous state, (AI), is similar to that observed in sickle trait and in hemoglobin C trait in that overt clinical disease is not present. Hemoglobin I trait differs from these trait states in that no abnormalities such as sickling, target cells, intraerythrocytic crystals, siderocytes or other discernible erythrocytic malformations have been found.

It can only be assumed that the homozygous hemoglobin I state, as yet unrecognized, does occur. One can only speculate whether the homozygous state would be asymptomatic, associated with hematologic or other abnormalities, or perhaps, comprises part of a previously recognized syndrome.

Summary

A hitherto undescribed hemoglobin anomaly has been detected in a North Carolina Negro family. This new hemoglobin, designated type I, which has been electrophoretically characterized, was found in combination with normal adult A hemoglobin in 6 of 17 members of one family. In each, the ratio of normal to abnormal hemoglobin was similar. Hematologic abnormalities were not observed in any of the affected individuals. Ultracentrifugation sedimentation constants, absorption spectra, solubility and oxygen carrying capacity studies showed no differences from hemoglobin A.

It is proposed that the I hemoglobin gene is an allele of hemoglobin A and is
transmitted as a simple dominant character in a manner similar to that of sickle cell hemoglobin. The homozygous form of I hemoglobin has not as yet been observed.

**SUMMARIO IN INTERLINGUA**

In un familia negre de Nord-Carolina un non previemente describite anomalia hemoglobinic esseva detegite. Iste novo hemoglobina—designate como typo I—se distingue per su characteristicas electrophoretic. Illo esseva trovate in combination con normal adulte hemoglobina A in 6 inter 17 membros de un familia. In omnes le proportiones de normal e anormal hemoglobina esseva simile. Anormalitates hematologic non esseva observate in ulle del involvite individuos. Investigationes in re le constantes de sedimentation ultracentrifugational, le spectros absorptional, le solubilitate, e le capacitate oxygenifere revelava nulle differentias ab hemoglobina A.

Nos opina que le gen de hemoglobina I es un allelo de hemoglobimin A e que illo es transmitite como un simple character dominant in un maniera simile al transmission del hemoglobina de cellulas falciforme. Le forma homozygote de hemoglobina I ha non ancora essite observate.

**ADDENDUM**

A part of this study was reported at the January, 1955, meeting of the Southern Section of the American Federation for Clinical Research. Also, an abstract, under the title "Hemoglobin H: A Hereditary Hemoglobin Anomaly," appeared in Clinical Research Proceedings 5: 93, 1955. To avoid confusion, the designation of the above described hemoglobimi has beemi changed from hemoglobin H to hemoglobin I. This was necessary since the hemoglobin differs from that described by Rigas.

**REFERENCES**

12. ITANO, H. A.: Personal communication with the author.


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