Hemoglobin H–Thalassemia Disease

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THE FOLLOWING REPORT of the combination of hemoglobin H and thalassemia in a child of Italian extraction appears to be the fifth published documented occurrence of this genetic combination.

In 1955, Rigas, Koler and Osgood reported the discovery of a new hemoglobin variant in two patients who had been referred as cases of Cooley's anemia. These patients were siblings of Chinese parentage living on the western coast of the United States. Both had a history of easy fatigability, splenomegaly and severe hypochromic microcytic anemia. About 35 per cent of their hemoglobin had an electrophoretic mobility which was faster than that of normal adult hemoglobin types. It was pointed out that neither parent had this fast-moving component, which they named Hgb H.

A further study of this family revealed another affected sibling and three unaffected ones. The father and a daughter of one of the affected patients had morphologic changes consistent with thalassemia minor. The authors also noted red cell inclusions when the blood of the three affected siblings were stained with 2 per cent sodium metabisulfite or with brilliant cresyl blue. Hgb H was shown to be precipitated by either sodium metabisulfite or reticulocyte stain. They therefore postulated that these Heinz-like inclusions represented disintegrating Hgb H and probably heralded actual hemolysis.

There have been at least four subsequent reports dealing with new cases of Hgb H. Minnich has reported on a Thai family with this hemoglobin variant and Gouttas et al. described an affected Greek family. In both of these no Hgb H was found in either parent though one of the parents and one or more of the siblings in each family was found to have thalassemia minor. Motulsky reported that 18 to 20 per cent of the hemoglobin of two mildly anemic, unrelated Filipinos had been found to be of this fast-moving variety. After application of sodium metabisulfite or brilliant cresyl blue, red cell inclusions like those noted by Rigas were found in both Gouttas's and Motulsky's cases. Lehmann and Singh found an individual in Malaya whose blood was thought to contain Hgb H, but the studies on this patient were not published.

Three more recently discovered hemoglobin variants named Hgb J, Hgb J, and Hgb K have also been found to move faster than normal.

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The authors wish to thank Dr. Arno Motulsky of the University of Washington for his interest. He has confirmed the presence of hemoglobin H in the reported patient and its absence in other members of the family. He has also demonstrated the virtual absence of A2 Hemoglobin in the patient by means of starch electrophoresis.
hemoglobin when the buffer solution used in the electrophoresis apparatus is adjusted to a pH of 8.6. All previously described hemoglobin variants (i.e., S, C, D, E, F and G) move more slowly than does the normal adult type. At a pH of 6.6, however, the above fast-moving hemoglobins may easily be separated from Hgb H because Hgb I, Hgb J and Hgb K migrate as cations, while Hgb H migrates in the opposite direction as an anion. These hemoglobins also differ from H in that they have so far been reported only as “traits.” One of the parents in each case of I, J and K (where parents were available for study) was found to have the hemoglobin variant, and there has been no mention of an association with thalassemia.\footnote{Because of the rapid discovery of new hemoglobin variants and the insufficient time for comparative study, there has been some confusion in terminology. The “Hgb H” found in an Algerian child by Cahanncs has been found instead to be identical with the Hgb I found at about the same time in an American Negro family by Rucknagel. Furthermore, the hemoglobins discovered by Robinson et al. and named Liberian 1 and 2 apparently represent the same variants that were almost simultaneously reported in other parts of the world as hemoglobins J and K, respectively.}
**Fig. 2.—Paper electrophoretic patterns of all members of family at pH 8.6.**

**CASE REPORT**

T.C. is a ten-year-old male child of Italian parentage, living in New York City, who was first seen in the Hematology Clinic of the Babies Hospital in January, 1957. He was referred by his family physician for further evaluation of Cooley's anemia. The anemia was discovered at the age of 7 years during an investigation of transient abdominal pain. The child had been in excellent health up to that time with the exception of occasional fainting spells following exercise during the previous five months. Weekly injections of liver extract and oral medication with an iron preparation prescribed by his physician for a 6-month period did not influence the anemia. He was therefore transfused. He received three additional blood transfusions at approximately six-month intervals, the last one in November, 1956. The only symptoms had been mild headache, dizziness and pallor for a few weeks before transfusions, which were said to have been completely relieved by this procedure. There was no history of anemia or jaundice among members of the immediate family, all of whom were of Italian origin.

Physical examination revealed an active, alert, well nourished male child whose weight and measurements were within one standard deviation of the mean for his age. There was no apparent icterus or pallor. The spleen was palpable 3 cm. below the
left costal margin in the midclavicular line. The liver was palpable approximately 2 cm. below the right costal margin and was soft in consistency.

The hemoglobin level varied between 9.5 and 10 Gm. per 100 ml. and the red blood cell count between 3.5 and 4.5 million. The white blood cell count was normal. The blood smear (fig. 1) showed microcytosis, hypochromia, polychromasia and poikilocytosis. There were a few target cells and stippled cells, some fragmentation and many oval-shaped cells. Platelet count was 126,000 per cu. mm. Reticulocyte counts varied between 1 and 13 per cent. Serum bilirubin was 1.7 mg. per 100 ml., all indirect. Direct Coombs’ test was negative and the blood type was Group O-Rh negative.

Red cell fragility in hypotonic saline was markedly decreased. Hemolysis began at 0.35 and was not complete at 0.225. In a normal control blood, hemolysis began at 0.42 and was complete at 0.35. Hemoglobin paper electrophoresis at pH 8.6 revealed a spot in the H-I-J-K area moving faster than normal adult hemoglobin (fig. 2). Electrophoresis of the hemoglobin at pH 6.6 showed a spot which had moved toward the anode (fig. 3). Alkali denaturation showed 1.6 per cent fetal hemoglobin. Inspection of the red cells after addition of brilliant cresyl blue revealed intraerythrocytic inclusions seemingly identical to Heinz inclusion bodies. Roentgenograms of the skull and long bones were normal.

Investigation of the immediate family revealed the following information. The parents and all four siblings were asymptomatic and did not have splenomegaly. Both sisters had a mild hypochromic anemia. The mother and the two sisters had significantly de-
Fig. 4.—Blood smear of mother.

Fig. 5.—Blood smear of sister, F.C.
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Fig. 6.—Blood smear of sister, N.C.

Fig. 7.—Blood smear of father.
creased red cell osmotic fragility, hypochromia, anisocytosis, poikilocytosis, and ovalocytosis (figs. 4, 5 and 6). The father and the patient's two brothers had normal blood smears (figs. 7, 8 and 9).

**DISCUSSION**

The genetic aspects of the abnormal hemoglobins have been extensively studied. Hemoglobins S and C have been found to be transmitted by allelic genes on the same chromosome, and it is certainly plausible that other variants may be similarly interpreted. The heterozygous condition for a given hemoglobin variant results in an asymptomatic trait, whereas the homozygous state usually results in an hemolytic anemia. Combinations of hemoglobin variants may also cause hemolytic anemia. The simultaneous heterozygous condition for both Hgb S and Hgb C is an example of this.

The genetic nature of thalassemia is similar in some respects to that of the hemoglobin variants described above. In other words, thalassemia minor is the heterozygous form, whereas thalassemia major is the homozygous condition. The occasional intermediate type, or mild Cooley's anemia, has been difficult to explain genetically. Studies of families showing members with both sickle cell and thalassemia genes, indicate that the gene for thalassemia is found on a different locus from that on which the gene for Hgb S is found. There is reason to believe that linked genes explain the combination more satisfactorily than independent ones. The frequent association of Hgb...
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Fig. 9.—Blood smear of brother, M.C., Jr.

- = FEMALE
□ = MALE
■ = THALASSEMIA
□ = HEMOGLOBIN H

Fig. 10.—Pedigree of C. family.

H with thalassemia has not been explained. The absence of Hgb H in both parents of our patient (as well as in the parents of other patients similarly studied) is puzzling. However, there may be an increased penetrance of
hemoglobin H in the presence of the thalassemia gene. Larger amounts of hemoglobin S, C or E occur when these hemoglobins are found in association with the thalassemia gene than are present in the simple heterozygous condition for any of these hemoglobins. Hgb F, or fetal hemoglobin, is also present in large amounts in thalassemia major. In the family of our patient, it is suggested that the father may carry the gene for Hgb H, although it may not be expressed at all because of the absence of thalassemia (fig. 10).

The actual positions of genes for Hgb H and thalassemia are unknown. Their occurrence as allelomorphs would seem to be excluded by the fact that a child with this combination resulted from a marriage between a man with Hgb H-thalassemia and a normal female. If this is shown to be a consistent finding, linked or independent genes would be likely.

The clinical picture associated with Hgb H-thalassemia disease seems to be that of a mild to moderately severe hemolytic anemia. When other hemoglobin variants are associated with thalassemia (i.e., Hgb S-thalassemia, Hgb C-thalassemia, or Hgb E-thalassemia), the result is almost invariably a milder disease than that seen with typical thalassemia major.17 The group of patients with mild Cooley's anemia has been puzzling to both the clinician and the geneticist. It would now appear that some members of this group really represent combinations of thalassemia with certain hemoglobin variants including Hgb H.

SUMMARY

A child of Italian parentage is presented as an example of Hgb H-thalassemia disease. A family study is included. Previous documented reports have included individuals of Chinese, Thai, Greek and Filipino parentage. Genetic aspects of the association of Hgb H with thalassemia are discussed. It is suggested that the study of other patients who present a clinical picture resembling mild Cooley's anemia may reveal the presence of hemoglobin variants.

SUMMARIO IN INTERLINGUA

Es presentate le caso de un puero de ancestres italian como exemplo de morbo a hemoglobina H e thalassemia. Un studio familial es includite. Previe documentate reportos del condition ha concernite individuos de origine chines, thai, grec, e philippin. Aspectos genetic del association de hemoglobina H con thalassemia es discutite. Es signalate que le studio de altere patientes con aspectos clinic simile a anemia de Cooley in forma leve va possibilemente revelar le presentia de variantes de hemoglobina.

REFERENCES
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