The Effect of Iron Deficiency on the Expression of Hemoglobin H

By Richard T. O'Brien

Two brothers with hemoglobin H disease are described. In one boy, a concomitant iron deficiency was associated with complete suppression of hemoglobin H in the peripheral blood. The effects of iron on globin synthesis are discussed.

The effect of heme on globin synthesis has been the subject of much recent investigation and speculation. Two brothers with hemoglobin H disease have been recently studied. In one of these boys, concomitant iron deficiency was associated with complete suppression of the appearance of hemoglobin H in the peripheral blood.

CASE REPORTS

Case 1

A 6-yr-old Filipino boy was referred to the Yale-New Haven Hospital for evaluation of chronic hemolytic anemia of undetermined etiology that was first discovered during a routine examination at 2 yr of age. He had been entirely asymptomatic. His physical examination was within normal limits. Specifically, there was no growth retardation, jaundice, hepatosplenomegaly, or thalassemic physical features.

Laboratory data included: hemoglobin, 9.7 g/100 ml; hematocrit, 34.2%; reticulocyte count, 8.8%; red blood cell mean corpuscular volume (MCV) 70 cu μ; serum iron, 86 μg/100 ml; and total iron-binding capacity (TIBC), 213 μg/100 ml. Red blood morphology showed moderate polychromasia, microcytosis, hypochromia, and targeting. Numerous red blood cell inclusions were demonstrated after incubation of the blood with brilliant cresyl blue.1 Starch block hemoglobin electrophoresis at pH 7.0 (phosphate buffer)2 yielded 19.4%–20.4% fast-migrating hemoglobin on three separate occasions during the ensuing 6 mo. Hemoglobin A2 was 1.9% (starch block electrophoresis at pH 8.6, barbital buffer). Hemoglobin F by the alkali denaturation technique3 was 2.2%.

Case 2

The 2-yr-old male sibling of case 1 was referred with his brother for evaluation of anemia that was discovered for the first time a few weeks earlier by his private physician. Except for pallor and easy fatigability over the preceding few months, he had been asymptomatic. Physical examination was normal except for pallor and a systolic ejection murmur along the left sternal border. There was no jaundice, hepatosplenomegaly, or physical features suggestive of thalassemia.

Laboratory data included: hemoglobin, 5.2 g/100 ml; hematocrit, 24.5%; reticulocyte count, 2.4%; MCV, 71 cu μ; serum iron, 24 μg/100 ml; and TIBC, 580 μg/100 ml. Red blood cell morphology showed marked microcytosis and hypochromia and moderate
polychromasia and targeting. No red blood cell inclusions were seen after incubation of the blood with brilliant cresyl blue. Starch block hemoglobin electrophoresis, run simultaneously with his brother's sample, failed to demonstrate any fast-migrating hemoglobin at neutral or alkaline pH. He was treated with oral iron, 6 mg/kg/day.

When reevaluated 2 mo later, his mother admitted to giving the iron medication irregularly. He was still profoundly iron deficient with a serum iron of 35 μg/100 ml and a TIBC of 671 μg/100 ml. His hemoglobin was 7.4 g/100 ml and hematocrit was 32.2%. Starch block hemoglobin electrophoresis at pH 7.0 revealed 2.4% fast-migrating hemoglobin. Because of the persistence of the iron deficiency, the patient was given 200 mg of intramuscular iron (Imferon) in divided doses.

Reevaluation 4 mo after the intramuscular iron revealed the following: hemoglobin, 8.9 g/100 ml; hematocrit, 37%; reticulocyte count, 7.4%; MCV, 61 μM; serum iron, 185 μg/100 ml; and TIBC, 532 μg/100 ml. The peripheral blood smear showed moderate polychromasia, microcytosis, hypochromia, and targeting. Starch block hemoglobin electrophoresis at pH 7.0 showed 15.8% of fast-migrating hemoglobin corresponding to hemoglobin H. Hemoglobin A2 was 1.9% and hemoglobin F was 3.8%. Incubation of the blood with brilliant cresyl blue revealed numerous red blood cell inclusions.

**Family Studies**

Table 1 summarizes the hematologic data on all the family members available. The father was hematologically normal. The mother had mild anemia, microcytosis, hypochromia, and a few target cells in her peripheral blood. Neither parent had any fast-migrating hemoglobin demonstrated on starch block electrophoresis at neutral or alkaline pH. The mother's iron and TIBC were within normal limits.

**RESULTS AND DISCUSSION**

Hemoglobin H disease is a moderately severe form of α-thalassemia in which there is a marked deficiency of α-chain production and a resultant excess of β-chains, which form tetramers, beta-4, or hemoglobin H. Hemoglobin H disease usually is caused by the inheritance and interaction of two α-thalassemia genes, the severe α-thalassemia 1 gene and the “silent” or mild α-thalassemia 2 gene.

The diagnosis of hemoglobin H disease in the above cases was based on the presence of a hypochromic, microcytic hemolytic anemia, numerous red blood cell inclusions after incubation of the blood with brilliant cresyl blue, and a fast-migrating hemoglobin on starch block electrophoresis. Family studies showed one parent to have a mild microcytic, hypochromic anemia and the other to be hematologically normal. Concomitant with severe iron deficiency, case 2 initially had no hemoglobin H demonstrable on electro-

<table>
<thead>
<tr>
<th>Case</th>
<th>Hemoglobin (g/100 ml)</th>
<th>MCV (μM)</th>
<th>MCH (μg/L)</th>
<th>RBC Morphology</th>
<th>Hb A (%)</th>
<th>Hb A2 (%)</th>
<th>Hb F (%)</th>
<th>Hb H (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.3</td>
<td>71</td>
<td>18.0</td>
<td>Markedly abnormal</td>
<td>75.5</td>
<td>1.9</td>
<td>2.2</td>
<td>20.4</td>
</tr>
<tr>
<td>2</td>
<td>8.9</td>
<td>61</td>
<td>16.2</td>
<td>Markedly abnormal</td>
<td>78.5</td>
<td>1.9</td>
<td>3.8</td>
<td>15.8</td>
</tr>
<tr>
<td>Mother</td>
<td>11.9</td>
<td>67</td>
<td>22.3</td>
<td>Slightly abnormal</td>
<td>95.2</td>
<td>3.3</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>Father</td>
<td>16.5</td>
<td>83</td>
<td>29.0</td>
<td>Normal</td>
<td>95.5</td>
<td>2.4</td>
<td>2.1</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. Hematologic Comparison of Cases 1 and 2 Illustrating the Suppression of Hemoglobin H With Iron Deficiency

<table>
<thead>
<tr>
<th>Date</th>
<th>Hemoglobin (g/100 ml) Case 1</th>
<th>Hemoglobin H (%) Case 1</th>
<th>Hemoglobin H (%) Case 2</th>
<th>Serum Fe/TIBC (µg/100 ml) Case 1</th>
<th>Serum Fe/TIBC (µg/100 ml) Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 17, 1972</td>
<td>9.7</td>
<td>19.4</td>
<td>0</td>
<td>86/213</td>
<td>24/580</td>
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<tr>
<td>April 26, 1972</td>
<td>8.2</td>
<td>19.9</td>
<td>2.4</td>
<td>Not done</td>
<td>35/671</td>
</tr>
<tr>
<td>August 11, 1972</td>
<td>9.3</td>
<td>20.4</td>
<td>15.8</td>
<td>Not done</td>
<td>185/532</td>
</tr>
</tbody>
</table>

Phoresis, but following iron repletion the hemoglobin was present in large amounts. The known lability of hemoglobin H was not responsible for the failure to demonstrate it initially, because each electrophoresis was performed within hours of venipuncture and the fast hemoglobin was identified from the sibling’s blood on three separate occasions using the same starch block under identical conditions. Quantities of hemoglobin H as low as 2% can be readily detected by starch block electrophoresis at pH 7.0 (Table 2).

Pearson and McFarland\(^8\) reported an adult with hemoglobin H disease and iron deficiency, and with iron repletion the hemoglobin H rose from 9.8% to 18%. \(^{51}\)Cr red cell survival in their patient was normal while iron deficient and was shortened when the iron deficiency was corrected. Red cell survival data are difficult to interpret in hemoglobin H disease because of greater binding of radioactive chromium to hemoglobin H than to hemoglobin A.\(^7\) However, if the suppression of hemoglobin H formation is due to an inhibition of \(\beta\)-chain production, the effect would be more balanced peptide chain synthesis and increased survival of the red blood cells reaching the circulation.

The influence of iron deficiency on the hemoglobin composition of the peripheral blood has been well documented by its suppression of hemoglobin A\(_2\) in normal individuals and in those with \(\beta\)-thalassemia trait.\(^5,9\) Iron deficiency also lowers the percentage of abnormal hemoglobin in individuals heterozygous for \(\beta\)-chain hemoglobinopathies, such as hemoglobins E and S.\(^10\) The mechanism by which iron exerts this effect is not yet known. That heme has an effect on globin synthesis has been documented but is incompletely understood. Heme stimulates globin synthesis by stabilizing polysomes on messenger RNA\(^11\) and by coordinating the assembly of peptide chains.\(^12\) Reticulocytes of iron-deficient rabbits incorporate fewer \(\alpha\)-chains into hemoglobin yielding a large pool of \(\alpha\)-chains.\(^12\) Diminished synthesis of \(\alpha\)-chains relative to \(\beta\)-chains has also been found in iron-deficient patients and patients with sideroblastic anemia.\(^13\) In \(\alpha\)-thalassemia there is already a marked impairment of \(\alpha\)-chain production. However, the addition of heme-depleted \(\alpha\)- or \(\beta\)-chains has been reported to inhibit specifically the synthesis of the homologous peptide chain in a cell-free system in vitro.\(^14\) This could possibly be the mechanism of the suppression of the \(\beta\)-4 tetramer, hemoglobin H, observed in the present patient while iron deficient. Unfortunately, \(\alpha\)- and \(\beta\)-chain synthesis was not studied in this patient. Such studies would be
necessary to confirm the suggestion that iron deficiency lessens the polypeptide chain synthetic imbalance in hemoglobin H disease.

REFERENCES

1. Rigas DA, Koler RD: Decreased erythrocyte survival in hemoglobin H disease as a result of the abnormal properties of hemoglobin H. Blood 18:1, 1961


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