The Effect of Iron Therapy in Paroxysmal Nocturnal Hemoglobinuria

By Wendell F. Rosse and Lorenz A. Gutterman

Two patients with paroxysmal nocturnal hemoglobinuria who were iron deficient were treated with parenteral iron compounds. In both instances, a marked increase in hemoglobinuria followed, beginning 4 days after the first administration of the iron compound. The rise in hemolysis paralleled the rise in reticulocyte count. When erythropoiesis was suppressed with transfusion, the administration of iron did not bring about an increase in hemolysis. This indicates that the effect of iron in bringing about hemolysis in patients with paroxysmal nocturnal hemoglobinuria is due to an increase in erythropoiesis, resulting in the production of excessive numbers of complement-sensitive cells.

Paroxysmal Nocturnal Hemoglobinuria (PNH) is characterized by intravascular hemolysis, resulting in hemoglobinemia and a loss of iron in the urine, either as hemosiderin or as hemoglobin. As a result, patients, with the illness are often iron deficient, either at initial presentation or at some point in their clinical course. The erythroid hypoplasia seen in this syndrome may, in part, be due to iron deficiency.

Strebing first noted, in 1882, that the administration of iron sometimes caused exacerbation of hemoglobinuria in patients with paroxysmal nocturnal hemoglobinuria, and the phenomenon was well documented by Hickey and Malley. More recently, the phenomenon has been noted by Mengel and his associates, who have attributed the increased hemolysis to a direct, oxidative, toxic effect of iron on the PNH cell. However, Hartmann and Jenkins have suggested that the increase in hemoglobinuria may, in fact, be due to an increase in erythropoiesis, resulting in the production of more defective cells.

In order to distinguish between these two possible causes of hemoglobinuria following iron therapy in paroxysmal nocturnal hemoglobinuria, we have carefully studied two patients with PNH and iron deficiency following the intravenous administration of iron. We have shown that the increase in hemolysis did not begin until the increase in reticulocyte count had occurred. The degree of hemolysis paralleled the height of the reticulocyte count. When erythropoiesis was diminished by transfusion, the administration of iron did not increase the rate of hemolysis despite the fact that the same total number

From the Departments of Medicine, Duke University Medical Center, Durham, N.C.
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Wendell F. Rosse, M.D.: Associate Professor, Departments of Medicine and Immunology, Duke University Medical Center, Durham, N.C.; recipient of a Research Career Development Award 1-K4-CA-38,862 from the National Cancer Institute. Lorenz A. Gutterman, M.D.: Department of Medicine, University of Maryland, Baltimore, Md.
of PNH cells were present. These findings are consistent with the hypothesis that the hemoglobinuria seen following iron administration in paroxysmal nocturnal hemoglobinuria is due to the increase in erythropoiesis with the consequent production of a large number of complement-sensitive cells.

**METHODS**

Routine examination of the blood including determination of hemoglobin concentration, white blood cell count, hematocrit and reticulocyte count were by standard methods.

**Plasma Hemoglobin**

Plasma hemoglobin was measured by a modification of the method of Hanks et al. By this technique, normal values are 0.3 to 0.6 mg. per cent.

**Urine Hemoglobin**

Urine was collected for 24-hour periods and stored at 4°C under toluene. Aliquots were tested for hemoglobin by a modification of the benzidine method. The amount of hemoglobin in the urine was expressed as milligrams of hemoglobin excreted per day.

**Serum Iron and Iron Binding Capacity**

Serum iron and iron binding capacity were determined by Biosciences Laboratories, Los Angeles, Calif. Normal values for this laboratory are: serum iron, 65-175 mg. percent; iron binding capacity 250-410 mg. per cent.

**Complement Lysis Sensitivity**

Complement lysis sensitivity tests were performed according to the method of Rosse and Dacie, using anti-I for sensitization. The percentage of complement-sensitive cells present was determined by inspection from the graphs.

**Case Histories**

Patient A.G. (previously reported by Mengel et al.) is a 46-year-old Negro, former baseball player, who was first diagnosed as having paroxysmal nocturnal hemoglobinuria during an evaluation for chronic anemia and hemoglobinuria. In 1954, he first noted dark urine and anemia which were attributed to "hepatitis," following an operation. Between 1954 and 1960, he had occasional bouts of dark urine and mild anemia but did not seek medical advice. In 1960, he was seen for the first time in the Durham Veterans Administration Hospital with a hemoglobin of 7.9 Gm. per cent, reticulocyte of 13 per cent, white blood count of 7800 with a normal differential count. The diagnosis of paroxysmal nocturnal hemoglobinuria was made on the basis of a positive acidified serum lysis test (Ham's test) and, during the next few years, he was treated with folic acid, Halotestin, oral iron compounds, Dilantin and other medications. It was noted that during this time hemoglobinuria occurred following intramuscular injection of Imferon. He did not tolerate iron given by mouth because of stomach cramps, usually associated with darkening of the urine. During this period, he had received transfusions approximately every 3-6 months.

In June 1968, he was admitted to the Clinical Research Unit at Duke University Medical Center for evaluation of parenteral iron therapy. At this time, his hemoglobin was 6.3 Gm. per cent, reticulocyte count was 6.0 per cent and the white blood count was 5400 with a normal differential. On peripheral film, the red cells appeared hypochromic and a few bizarre forms were present. Serum iron was 15 µg. per 100 ml., and the unsaturated iron binding capacity 385 µg. per 100 ml. Bone marrow revealed no stainable iron and erythroid hyperplasia. Fifty-five per cent of the red cells in the peripheral blood were complement sensitive and the sensitivity of these cells was approximately 15 times that of normal cells. Plasma hemoglobin was 11.5 mg. per cent and no hemoglobin was present in the urine.
Fig. 1.—The clinical course of patient A.G. following iron therapy.

After initial evaluation, he was given iron dextrin (Astrafer) each day for 8 successive days (see Fig. 1). The reticulocyte count and urine and plasma hemoglobin remained at pretreatment levels until the fourth day when they all began to rise. The reticulocyte count reached a peak on day 10. During this same period, the urine and plasma hemoglobin both rose. At this time, the patient experienced severe abdominal pain and was transfused with 5 units of packed red cells on 2 successive days with prompt relief of his abdominal pain. The total hemoglobin rose to 14.1 Gm. per cent and the reticulocyte count fell to 7.4 per cent. Concurrently, the plasma hemoglobin and urine hemoglobin fell. When they had again reached baseline levels or below, he was given a further dose of intravenous iron. No increase in plasma or urine hemoglobin was noted over the next 7 days.

Over the next 3 months, he was given fluoxymesterone (Halotestin), 20 mg./day, and ferrous sulfate, 200 mg. twice a day. During this time, his hemoglobin was maintained at 15 Gm. per cent but he gradually began to pass large amounts of dark red urine. He did not tolerate well the oral iron because of gastrointestinal discomfort, but he continued to take 300 mg./day of ferrous sulfate until late January, 1969. During this time, 75 per cent of his red cells were complement-sensitive. In March of 1969, his hemoglobin was 11.5 Gm. per cent and he was readmitted to the Clinical Research Unit. Iron was present in the bone marrow in both granular and nongranular forms at this time. Fluoxymesterone was discontinued and 500 mg. of iron was administered intravenously. No hemolytic response was seen.

During the next 6 months, he took fluoxymesterone but no oral iron and his hemoglobin gradually fell to 8 Gm. per cent. He was admitted to the Clinical Research Unit in November 1969, for further evaluation. The red cells appeared to be hypochromic, the serum iron was 18 mg. per cent with a serum iron binding capacity of 500 μg. per cent. Bone marrow examination revealed large amounts of granular iron, the granules ranging from 1 to 3 μ in diameter; no nongranular iron was present. The reticulocyte count was
18 per cent. He was given 500 mg. of iron dextran (Imferon) with a repetition of the sequence previously noted: elevation of reticulocyte count, followed by plasma hemoglobin elevation and hemoglobinuria.

Patient M. C. is a 48-year-old lumberman who had had a 2-year history of anemia and intermittent abdominal and back pain. In September 1967, the diagnosis of paroxysmal nocturnal hemoglobinuria was made at Charlotte Memorial Hospital on the basis of a positive acidified serum lysis test. He was noted to be iron deficient but iron was withheld because of the possible "toxic" effect of iron. His hemoglobin gradually declined to 5.5 Gm. per cent and his performance status likewise declined.

In October 1968, he was admitted to the Clinical Research Unit at Duke University Medical Center for evaluation for treatment with parenteral iron (Fig. 2). At this time, his hemoglobin was 5.9 Gm. per cent, his reticulocyte count was 8.4 per cent, white blood count was 5300 with a normal differential. The red cells were hypochromic with some bizarre forms present. Bone marrow aspiration showed erythroid hyperplasia and a lack of stainable iron. Complement lysis sensitivity tests showed that 40 per cent of the cells present were sensitive to complement. These cells were approximately five times as sensitive as normal cells to the lytic action of complement. Plasma hemoglobin was 9.9 mg. per cent and urine hemoglobin was not detectable. Hemosiderin was present in the urine. Serum iron was 20 μg. per cent with an iron binding capacity of 380 μg. per cent.

After baseline values had been obtained, 500 mg. of iron dextran (Imferon) were given on 3 successive days. The reticulocyte count began to rise on subsequent days and plasma hemoglobin likewise rose. The peak of reticulocyte response was reached on day 13, at which time 3.2 Gm. of hemoglobin was being excreted in the urine per day. During this time the hematocrit rose from 22.1 per cent to 24 per cent and the percentage of complement-sensitive cells likewise increased from 40 per cent to 45 per cent. However, at the peak of the hemolytic reaction, the patient experienced abdominal pain and was transfused with 5 units of packed red cells on 2 successive days. Immediately thereafter, the reticulocyte count and urine and plasma hemoglobin decreased. When the reticulocyte
count had reached low values, he was given a further 500 mg. of iron dextran but no increase in urine or plasma hemoglobin was noted.

After discharge, the patient was given 300 mg. of ferrous sulfate per day and 20 mg. of fluoxymesterone (Halotestin) per day. On this regimen he has maintained a hemoglobin between 12 and 15 mg. per cent and has noted no hemoglobinuria. The percentage of complement-sensitive cells rose to 68 per cent. He has returned to 100 per cent performance status.

He returned to the Clinical Research Unit in January 1970, at which time the hemoglobin concentration was 15.2 Gm. per cent, reticulocyte count 6.0 per cent, serum iron 65 µg. per cent, and serum iron binding capacity 310 µg. per cent. The plasma hemoglobin concentration was 9.5 mg. per cent and no hemoglobin was detectable in the urine. Bone marrow aspiration demonstrated iron which was not present in large granules.

He was given 500 mg. of iron dextran intravenously. No change in the reticulocyte count, plasma hemoglobin, urine hemoglobin or symptoms were noted in the next 10 days.

**Discussion**

The patients presented conclusively demonstrate that the effect of iron in bringing about hemoglobinuria in patients with paroxysmal nocturnal hemoglobinuria who are iron deficient is a consequence of the increase in erythropoiesis. In both patients, the increase in plasma and urine hemoglobin did not occur until 3 to 4 days after the administration of iron and was simultaneous or parallel with the increase in reticulocytes. The hematocrit subsequently rose rather than fell and the percentage of complement-sensitive cells also increased. When erythropoiesis was suppressed by a transfusion, no hemoglobinuria resulted upon the administration of intravenous iron, despite the fact that the total number of circulating complement-sensitive PNH cells and their sensitivity to complement was unchanged. These findings strongly suggest that iron per se does not have a direct lytic effect on the PNH cell.

The red cells of patients with PNH consist of two populations: one which is markedly sensitive to the lytic effect of complement, and another which is more nearly normal in its sensitivity to complement. The proportion of complement-sensitive cells varies from patient to patient; both of the present patients had large circulating complement-sensitive populations. The life span of the complement-sensitive cells is markedly less than that of the complement-insensitive cells. Therefore, the percentage of complement-sensitive cells produced per day is very much larger than the percentage of cells circulating in the peripheral blood at any given time. In patient A.G., the proportion of C-sensitive cells produced per day was greater than 90 per cent at a time when 60 per cent of his circulating cells were complement-sensitive. Any increase in erythropoiesis will increase the production of both complement-sensitive and complement-insensitive cells. The total number of complement-sensitive cells produced will be increased since the number of precursors of complement-sensitive cells is usually greater than of the complement-insensitive cells unless the circulating complement-sensitive population is very small. Since the complement-sensitive cells which are produced are subject to immediate attack by the processes leading to lysis, the increase in the total amount of hemolysis occurring will be rapidly apparent. It is presumably hemolysis of these cells which appears as hemoglobinuria during the early
repletion phases of patients with paroxysmal nocturnal hemoglobinuria with iron deficiency.

The amount of hemoglobinuria following the administration of iron in patients with PNH will depend upon several factors: (1) the presence of iron-deficient erythropoiesis (as defined by Bainton and Finch since it is apparent that when iron-deficient erythropoiesis is not present [patient M.G., second study], the administration of iron does not result in increased hemolysis, (2) the proportion of complement-sensitive cells which are present, since this may vary greatly among patients with PNH. If this population is large, more hemolysis is likely than if it is small, (3) degree of anemia, since severely anemic iron-depleted patients will respond to therapy with a greater erythropoietic rate than those less anemic, and (4) the rate at which iron is administered and utilized. Both the present patients had marked hemoglobinuria on their first treatment with parenteral iron, since both were severely iron deficient, both had large complement-sensitive populations, both were anemic, and in both, the iron was administered rapidly. As a result of the hemolytic episode, both patients developed abdominal pain, a frequent complication following severe hemolytic episodes in patients with PNH. It would, therefore, appear that the parenteral administration of iron might be harmful to some patients with PNH despite the fact that the patient is iron deficient and iron is not toxic to the cell itself.

All the iron given parenterally may not be readily available for erythropoiesis. Patient A.G. had the signs of iron-deficient erythropoiesis in September 1969 (hypochromia and a decreased serum iron) despite the fact that large amounts of stainable iron were present as large granules in his bone marrow. When given intravenous iron, he responded in exactly the same fashion as he had when iron had been absent from his marrow. The granular iron in the marrow did not appear to be available readily enough to be incorporated into the red cells, a finding noted by Henderson and Hillman in iron-deficient subjects. This is probably due in part to the excessive call on iron stores in patients with paroxysmal nocturnal hemoglobinuria because of the extremely high rates of erythropoiesis and in part to the loss of the iron in the urine as hemoglobin or hemosiderin, which renders unavailable a pool of iron usually rapidly reincorporated into hemoglobin. When iron is taken daily by mouth, the replenishment of the daily loss may prevent iron-deficient erythropoiesis.

Because of these considerations, it would appear to be preferable to treat patients with paroxysmal nocturnal hemoglobinuria with a form of oral iron. If oral iron compounds are not well tolerated because of gastrointestinal intolerance, small amounts of parenteral iron may be given, provided that the hematocrit is not excessively low or that the patient does not have a large complement-sensitive population. If either of these conditions are present, the hemolytic reaction may be prevented by the prior administration of washed red cells. From the present studies, it would appear to be unwise to treat anemic, iron-deficient patients with paroxysmal nocturnal hemoglobinuria with intravenous iron preparations.
REFERENCES


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