
CASE REPORT

Paroxysmal Nocturnal Hemoglobinuria with the Development of Aplastic Anemia

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THE DIAGNOSIS of paroxysmal nocturnal hemoglobinuria presents many difficulties. Although the main features of the syndrome are incorporated in the descriptive title "chronic hemolytic anemia with nocturnal hemoglobinuria and constant hemosiderinuria," it is usually not until the characteristic hemoglobinuria is noted that the diagnosis is considered clinically. However, during the course of this disease hemoglobinuria may be a very infrequent occurrence or it may be persistent rather than paroxysmal and its relationship to sleep may not be at all constant. The fundamental defect in this syndrome is an undue sensitivity of the red blood cells to the lytic action of an agent present in the fresh serum of the patient or of a normal individual, especially if the test is carried out in an acid medium.^{1, 2}

The case to be described presented as a chronic hemolytic anemia. Hemoglobinuria developed while the patient was in the hospital and during this phase he showed an undue sensitivity of his erythrocytes to the lytic action of serum in an acid medium. Following repeated blood transfusions he lost all evidence of *in vivo* hemolysis and there has been no recurrence of the hemoglobinuria. He is now suffering from aplastic anemia.

CASE REPORT

The patient, a male aged 53 years, with no family history of anemia or jaundice, had four healthy male children. He first noticed weakness, breathlessness, and palpitations in 1948 and had been under the care of his family doctor who had made a diagnosis of pernicious anemia. He was treated with adequate courses of parenteral liver and vitamin B₁₂ with iron therapy by mouth but despite this therapy his condition deteriorated. Consequently blood transfusions at intervals of two to three months were required to maintain the patient even in moderate health. In March 1951 he was admitted to hospital.

The skin and visible mucous membranes were pale, with slight icterus. The tongue showed normal papillae. The liver was enlarged three fingerbreadths below the right costal margin; no lymphadenopathy was present and the spleen was not palpable. Blood pressure was 110/55 and the pulse rate 72. The cardiovascular system, respiratory system, and central nervous system showed no abnormalities. The patient stated that on a few occasions before admission he had noticed his urine to be dark brown in color. This information was elicited only on close questioning and the color was apparently not remarkable.

The following investigations were carried out in hospital: hemoglobin 46 per cent (Haldane) = 6.8 Gm. per 100 ml.; erythrocytes 1.62×10^6 per cu.mm.; P.C.V. 19 per cent; M.C.V. 114 cu. μ ; M.C.C. 36 per cent; E.S.R. 12 mm. in 1 hr.; leukocytes 4750 per cu.mm.; differential leukocyte count, metamyelocytes 2 per cent, neutrophil polymorphs 62 per

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cent, lymphocytes 34 per cent, monocytes 2 per cent. The red blood cells showed macrocytosis, polychromasia, and punctate basophilia. Reticulocytes 16 per cent. Osmotic fragility of erythrocytes in hypotonic saline was normal. Coombs (anti human globulin) test was negative. The serum bilirubin 1.7 mg./100 ml.; serum proteins 7.8 Gm. per cent; serum albumin 5.0 Gm. per cent; serum globulin 2.8 Gm. per cent; serum alkaline phosphatase 9.8 King units; serum thymol turbidity 3.6 units. Serologic tests for syphilis, including the Wassermann and Kahn tests, were negative. Serum cold agglutinins positive to titer of 1/32. The bone marrow showed a well marked normoblastic hyperplasia and 56 per cent of the nucleated cells belonged to the erythrocytic series. A normal amount of free acid in the stomach was demonstrated by analysis of the gastric contents. The urine was amber in color and contained neither bile, albumin, nor blood. On quantitative estimation urobilinogen was found to be present in excessive amounts. Examination of the urinary deposit showed neither red blood cells nor casts but specific examination for hemosiderinuria was not made.

About one month after he was admitted to hospital, during the early afternoon, the patient passed dark colored urine for the first time. This hemoglobinuria lasted all through that day and then disappeared. It recurred three days later and for three days the hemoglobinuria was more marked in the mornings, although it was present throughout the day. It was not related to iron therapy. Once more the hemoglobinuria spontaneously subsided until a course of intravenous heparin was started. Hemoglobinuria then recurred in a more serious form, at first more marked in the day urine, but, after the drug was withdrawn and the hemoglobinuria became less marked, it was mainly nocturnal in character. During these episodes the urine contained a varying amount of hemoglobin, but no red blood cells, and on examination of the urinary deposit there was a constant and massive hemosiderinuria. At the periods of hemoglobinuria there was evidence of intravascular hemolysis. The serum, which was brown in color, showed the characteristic absorption bands of methemalbumin and gave a positive Schumm's test. The cold hemagglutinins were 1/32; the Wassermann and Donath-Landsteiner tests were negative.

Autohemolysis occurred when a freshly drawn specimen of blood was incubated at 37 C. for 2 hours. This heat sensitivity test of Hegglin and Maier³ is positive in both acquired hemolytic anemia and paroxysmal nocturnal hemoglobinuria and indicates either the presence of an autohemolysin in the serum or undue sensitivity of the patient's erythrocytes to his own fresh serum. Tests were then carried out according to the method of Dacie⁴ in an attempt to detect the presence of an agglutinin or hemolysin active at body temperature but no serum antibodies could be demonstrated. In this test fresh normal serum is used as a source of complement and the pH is optimum. To exclude the possibility of an incomplete antibody adsorbed onto the surface of the erythrocytes, the washed corpuscles were tested on three occasions with Coombs anti human globulin serum with negative results. The inability to demonstrate any circulating agglutinin, hemolysin, or incomplete antibody suggested that the process of autohemolysis or increased incubation fragility of the blood was due to some cellular defect.

Using the technic of Dacie⁴ the sensitivity of erythrocytes of the patient to the lytic action of his own serum and compatible fresh normal serum was tested in an acid medium. On two occasions the result was incomplete in that hemolysis of cells occurred in both the unacidified and acidified serum of the patient but not in either the unacidified or acidified normal serum. On another occasion hemolysis occurred in both the acidified serum of the patient and the acidified normal serum but no hemolysis was observed in the unacidified sera. All controls were negative, including the erythrocytes of the patient suspended in inactivated acidified normal serum and normal erythrocytes in the acidified serum of the patient. Tests to determine the sensitivity of the erythrocytes to heterohemolysins or to the isohemolysins in anti-A and anti-B sera were not made. Because of the association of a chronic hemolytic anemia of macrocytic type, the presence of hemoglobinuria, the massive hemosiderinuria, the positive acid hemolysis test, and the absence of any hemolysin or incomplete autoantibody, a diagnosis of paroxysmal nocturnal hemoglobinuria was made. As the intravascular hemolysis persisted and the hemoglobin continued to fall the patient required repeated transfusions. Reactions to transfusions with whole blood were frequent

and mounting in severity although they were not followed by immediate or massive hemoglobinuria. Transfusions of whole blood were therefore discontinued and washed red blood cells resuspended in saline to one half the original volume were used for transfusion and these produced no reaction. When the hemoglobin was 80 per cent = 11.8 Gm./100 ml., the patient was discharged from hospital.

When the patient was readmitted to the hospital in December 1951 he had a peripheral pancytopenia. The hemoglobin was 24 per cent (Haldane) = 3.5 Gm./100 ml.; erythrocytes 1.4×10^6 per cu.mm.; P.C.V. 12 per cent; M.C.V. = 83 cu. μ ; M.C.C. 30 per cent; leukocytes 2300 per cu.mm.; platelets 155,000 per cu.mm.; differential leukocyte count, neutrophil polymorphs 16 per cent, eosinophil polymorphs 2 per cent, lymphocytes 80 per cent, monocytes 2 per cent. Reticulocytes 0.6 per cent; serum bilirubin 0.6 mg./100 ml. The acid hemolysis test and sensitivity to lysis by accelerator globulin⁵ were negative. The bone marrow was examined on three occasions. It was hypoplastic with normoblastic erythropoiesis. The urine contained no hemoglobin and only minute amounts of hemosiderin. The peripheral pancytopenia and bone marrow hypoplasia have persisted during the whole year that the patient has continued under observation. A therapeutic trial of cortisone consisting of 100 mg. for seven days followed by 50 mg. for ten days produced no hematologic improvement either in the peripheral blood or bone marrow and repeated transfusions were required. It was noted that the sensitivity to whole blood transfusions had been lost and that the patient could now be transfused with whole blood without reaction. The patient has remained under observation for some twelve months, during which time he has been admitted to hospital on a number of occasions for blood transfusions. During this period his red cells were repeatedly tested for sensitivity to lysis by fresh serum in an acid medium and by accelerator factor, and a positive result was obtained on two occasions. In this stage it was found that the Crosby⁵ test using accelerator globulin was more sensitive than the acid hemolysis test for detecting the erythrocytic abnormality.

COMMENTARY

This case presented with severe macrocytic anemia which had been refractory to adequate courses of parenteral liver and vitamin B₁₂. When he was first seen in the hospital there was unequivocal evidence of acquired hemolytic anemia of macrocytic type with reticulocytosis, hyperbilirubinemia, increased urinary urobilinogen excretion, and normoblastic hyperplasia of the bone marrow. However, these changes were not associated with any evidence of spherocytosis or increased osmotic fragility of erythrocytes. Tests for the presence of iso- and autoantibodies in the serum of the patient were negative and no incomplete antibody adsorbed onto the surface of the erythrocytes could be demonstrated using Coombs antihuman globulin serum.

One afternoon while the patient was in the hospital he developed severe hemoglobinuria which appeared while he was warm in bed. This in itself would tend to exclude paroxysmal cold hemoglobinuria. The clinical picture was quite unlike that usually found in paroxysmal cold hemoglobinuria due to syphilis and this condition was excluded by the negative serologic tests for syphilis and the negative Donath-Landsteiner test for cold hemolysins in the serum of the patient. Hemoglobinuria may also be induced by exposure to cold in some patients with high titer cold hemagglutinins in their serum but is associated with signs of vascular insufficiency. In this case the hemoglobinuria was not related to cold, there was no evidence of Raynaud's phenomenon, and there was a low titer of cold hemagglutinins. Although hemoglobinuria may also occur in some unusual types of acquired hemolytic anemia, no mechanism for immune hemolysis

with either complete or incomplete antibody could be demonstrated. The abnormality appeared to be intracorpuseular and the sensitivity of the erythrocytes to lysis by his own and normal compatible serum, particularly in an acid medium, satisfied the criteria of Dacie⁶ and established the diagnosis of paroxysmal nocturnal hemoglobinuria. The reason for the incomplete results with the acid hemolysis tests may have been technical rather than actual. It has been noted that in the acid hemolysis test, lysis of corpuscles does not always occur in the tube containing the patient's cells and patient's serum although it is always present when the serum is acidified. However, failure of fresh serum to lyse the patient's cells may be due to a number of factors. These factors responsible for false negative acid hemolysis tests have been shown by Crosby⁵ to be: (1) not stoppering the tubes used in the tests, with consequent loss of CO₂ and rise in pH of the serum; (2) using plasma instead of serum for the test, where the presence of the anticoagulant will inhibit the reaction; (3) previous massive transfusions with a consequently small proportion of abnormal cells; (4) collection of the fresh normal serum used in the test through a wide-bore needle into a paraffined syringe, ejecting the blood into a dry tube, and allowing the blood to clot.

These factors, which might give false negative or incomplete results, were not fully appreciated when the early tests for acid hemolysis were made. Factors 1, 3, and 4 were all operative and were probably responsible for the incompletely positive acid hemolysis result. When due cognizance was taken of all details of technic, the erythrocytes of the patient were found to be sensitive to lysis by his own and normal compatible serum in an acid medium. Some workers have regarded complement as the agent responsible for the lysis of the abnormal cells in this disease but the biologic reactions of the lytic agent do not exactly correspond to that of complement. Recently Crosby and Dameshek⁷ have shown that the lytic effect is related to blood clotting and that the lytic agent is at least closely related to activated coagulation accelerator factor. A test for paroxysmal nocturnal hemoglobinuria based on the thrombin activation of this coagulation accelerator factor has been devised by Crosby.⁵

The hemoglobinuria in paroxysmal nocturnal hemoglobinuria is regarded as a necessary component of the symptom complex, whereas, in fact, it is an inconstant symptom which may be lacking for considerable periods.⁸ Indeed, Marks⁹ has shown that only half the cases present with a history of hemoglobinuria, and in unequivocal cases of this disorder hemoglobinuria may be entirely lacking or may occur for the first time while the patient is in the hospital, as in this case and that of Hickey and Malley.¹⁰ Emphasis on the paroxysmal and nocturnal nature of the hemoglobinuria may also be misleading, as persistent hemoglobinuria has been described in a case of paroxysmal nocturnal hemoglobinuria by McIlvanie and Beard.¹¹ In the present case hemoglobinuria occurred for the first time two years after the onset of the disease and while the patient was in hospital. Hemoglobinuria lasted continuously for two periods of three days and seventeen days respectively and it was noted that when the hemoglobinuria was severe it was present and persistent throughout the day, but as it became less severe its nocturnal and paroxysmal character became more pro-

nounced. During the period of gross intravascular hemolysis and hemoglobinuria the blood serum contained methemalbumin and there was constant and massive hemosiderinuria.

Because of the inhibition by heparin *in vitro* on the lytic action of the serum of the patient on his own corpuscles it was decided to treat the patient with a course of this drug. It was found that the concentration of heparin required *in vitro* to inhibit the reaction was beyond the normal therapeutic range obtainable *in vivo*. Undeterred by this and unaware of the warnings by Crosby and Dameshek⁷ that concentrations of heparin less than 1/15,000 enhanced rather than diminished hemolysis in paroxysmal nocturnal hemoglobinuria, the patient received, in divided doses, 60,000 units of heparin intravenously daily for three days. Two days after this therapy was commenced the patient had a recurrence of his hemoglobinuria in its most severe form and the therapy was discontinued. As intravascular hemolysis continued, with a catastrophic fall in the hemoglobin threatening the life of the patient, blood transfusions were given. These transfusions were associated with severe pyrexial reactions which were not due to blood group incompatibility. Following these reactions the patient appeared to be quite well for a period which varied from eight to twenty-four hours when an exacerbation of the hemoglobinuria recurred. This is compatible with the findings of Crosby and Dameshek⁷ who noted that there are two phases to the blood transfusion reaction in paroxysmal nocturnal hemoglobinuria. They noted that in the first phase intravascular hemolysis stopped and hemoglobinuria, if present, ceased. This was considered to indicate an inhibition of the hemolytic factor by the transfusion reaction. Several hours later, however, this inhibiting action ceased and the second phase was characterized by a return of the hemolysis and hemoglobinuria. The hemolytic response to the whole blood transfusions was considered to be biologic evidence of the *in vivo* lytic effect of fresh normal plasma on the cells of the patient. Consequently plasma-free transfusions of red cells in saline were given. These were obtained by thrice washing compatible red cells free from plasma and resuspending them in saline to one half the original volume. These plasma free transfusions produced no reaction and were followed by a satisfactory rise in the hemoglobin level. The importance of transfusion with saline-washed red cells in paroxysmal nocturnal hemoglobinuria has been noted by Dacie¹² and others.

When the patient was readmitted to hospital nine months later there was no history of any recurrence of hemoglobinuria and hematologic examination showed that the hemolytic process had been replaced by a peripheral pancytopenia and marrow hypoplasia. The urine was free from hemoglobin and contained only minute traces of hemosiderin. Tests for the presence of complete iso- and autoantibodies in the serum were again negative. Using Coombs anti human globulin serum no incomplete antibodies could be detected on the erythrocytes of the patient. The pancytopenia with marrow hypoplasia has persisted for the twelve month period that the patient has been under observation. He has been maintained by frequent whole blood transfusions which now do not produce any reaction. His erythrocytes have been tested at intervals for sensitivity to acid-hemolysis and lysis by accelerator globulin and a positive result to these tests has been obtained on two occasions. This would suggest that the

hypoplastic marrow is still capable of producing cells with the erythrocytic defect and that the reduced production of abnormal cells, which usually form only a proportion of the whole, is responsible for the technically negative results. Although the treatment of cases of paroxysmal nocturnal hemoglobinuria by blood transfusion has occasionally been followed by a diminution of the hemolytic process^{13, 14} with a marked decrease in the rate of intravascular hemolysis, this has only been a transient phase which was followed in a matter of days or weeks by a return of the hemolysis. In this case blood transfusions were followed for a period of twenty months by complete absence of any evidence of increased blood destruction or active regeneration.

The association of the erythrocytic defect of the paroxysmal nocturnal hemoglobinuria type with aplastic anemia was noted by Dacie and Gilpin,¹⁵ who described the case of a 12 year old boy with a refractory anemia of the Fanconi type who had a severe reaction to whole blood transfusion and whose red cells gave a positive acid-hemolysis test. A similar erythrocytic defect in a case of aplastic anemia in an adult was described by Letman¹⁶ who suggested that other cases of aplastic anemia might prove to be atypical forms of paroxysmal nocturnal hemoglobinuria. In the present case there were two distinct phases in the disease. One was characterized by active intravascular hemolysis, hemolytic anemia, hemoglobinuria, marrow hyperplasia, and undue sensitivity of the erythrocytes to lysis in an acid medium and the other by marked subsidence of the various indicators of the hemolytic process, by peripheral pancytopenia with a hypoplastic marrow and by a great diminution in the sensitivity of the erythrocytes to acid hemolysis. Pancytopenia is one of the characteristic manifestations in paroxysmal nocturnal hemoglobinuria but it has been suggested by Crosby and Dameshek⁷ that this is due to a pancytolytic effect brought about by the same destructive mechanism which is responsible for the lysis of red cells. It is not considered to be caused by a diminished formation of blood cells from a hypoplastic marrow as occurred in phase two of this present case. This present case demonstrates the evolution from an active hyperplastic marrow producing defective erythrocytes which were rapidly destroyed in the peripheral circulation due to an erythrocytic defect of paroxysmal nocturnal hemoglobinuric type, to a hypoplastic marrow producing limited numbers of abnormal erythrocytes which are only occasionally a sufficient proportion of the whole to be susceptible in vitro to tests for acid hemolysis and which, in vivo, are associated with the loss of the normal evidence of active erythrocytic destruction.

SUMMARY

1. A case of paroxysmal nocturnal hemoglobinuria is presented which developed hemoglobinuria for the first time while in the hospital and showed severe reactions to whole blood transfusion but none to washed red cells.
2. Therapeutic administration of heparin induced severe hemoglobinuric episodes which were followed by a remission in the transfusion reactions to whole blood.
3. Later, aplastic anemia developed with a marked subsidence in the various indicators of the hemolytic process although the erythrocytic defect found in paroxysmal nocturnal hemoglobinuria was still present.

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