Paroxysmal Nocturnal Hemoglobinuria: a Case Report with a Negative Ham Presumptive Test Associated with Serum Properdin Deficiency

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Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon form of chronic hemolytic anemia characterized by an acquired abnormality of the erythrocytes. The diagnosis is usually suspected when there is a history of the passage of dark urine after sleep and it is established by demonstration of the susceptibility of the red cells to hemolysis in acidified serum under special conditions.

Ham has proposed a simple “presumptive test” for detection of this disease. A positive result is cause for the performance of the more demanding “complete acid hemolysis test” for verification of the diagnosis. However, a negative presumptive test has been regarded as adequate for exclusion of a diagnosis of PNH.

We have recently encountered a case of PNH that displayed several features of interest. The presumptive test was negative, accounting for the failure to make the correct diagnosis initially. The basis for the false negative presumptive test and the atypical results of the complete acid hemolysis test appeared to be a peculiarity of the patient's serum. Furthermore, the existence of acidosis related to renal failure afforded an opportunity to study the effect of this alteration of acid-base equilibrium on PNH hemolytic activity in vivo. Finally, the necropsy observations provided a basis for consideration of the pathogenesis of the kidney disease noted not uncommonly in this hemolytic disorder.

Case Report

A 51-year-old Negro woman was first seen at the University of Chicago Clinics in August 1955, complaining of palpitation, breathlessness and swelling of the feet of 2 months' duration. In 1935, she noted persistent easy tiring after a 2-week febrile illness and was told she had sickle cell anemia. During the succeeding years she experienced periodic weakness and vague abdominal discomfort. She admitted passing dark urine occasionally but this did not seem to bear any relationship to the episodes of weakness, abdominal distress, or to sleep. In 1955, study at another hospital revealed an obscure anemia with a negative bone marrow examination, roentgenologic evidence of a duodenal ulcer, and "blood in the urine," attributed to "nephritis." Transfusions of blood were followed by moderate but only transitory relief of symptoms. In June 1955, weakness progressed in severity with dyspnea on exertion, palpitation and swelling of the ankles. There was no family history of anemia!

Physical examination disclosed good nutritional status, moderate pallor, but no jaundice,
a solitary retinal microaneurysm in the right eye, mild cardiomegaly and tachycardia, slight enlargement of the liver, a barely palpable spleen tip, and pitting pedal edema.

The initial laboratory observations included hemoglobin, 4.0 Gm. per cent; erythrocytes, 1,320,000 per cu. mm.; leukocytes, 3,300 per cu. mm. with a normal differential; platelets, 138,000 per cu. mm.; and reticulocytes, 9.0 per cent. The peripheral blood smear showed red cells normal in size, shape and degree of hemoglobinization. The sternal bone marrow smears and histologic sections revealed normoblastic hyperplasia, and the Prussian blue-stained sections disclosed only a few hemosiderin granules.

The fecal excretion of urobilinogen was 253 mg./24 hr., the serum bilirubin concentration, 0.3 mg. per cent direct and 0.5 mg. per cent total, and the apparent half-survival time of the patient’s radiochromium (Na$_2$Cr$_6$O$_7$) tagged red cells was 10 days, the normal range in our laboratory being 27 to 33 days. The following procedures for suspected hemolytic disorders gave negative results: Kahn serologic test for syphilis, sodium metabisulfite sickling preparation, erythrocyte osmotic fragility test, Coombs direct anti-human globulin test, L. E. cell preparation, Donath-Landsteiner cold-hemolysin test, qualitative examination for urinary porphyrins and Watson-Schwartz test for urinary porphobilinogen, hemoglobin paper electrophoretic pattern (adult hemoglobin), and the Ham presumptive test for PNH (vide infra). The benzidine test for urinary hemoglobin was negative, but hemosiderin in the urine was demonstrated repeatedly by Prussian blue staining of the sediment. The following examinations for PNH gave positive results: Ham complete acid hemolysis test (although with certain atypical results to be described below), Crosby thrombin-activation test, and the Dacie anti-A serum hemolysis test.

The urine was clear yellow, showed a trace of protein and reducing substance, but no ketone bodies; a moderate number of leukocytes, but no birefringent lipid in the centrifuged sediment; and, when cultured, yielded E. coli and alpha streptococcus. The stools exhibited no gross blood but gave positive tests with benzidine while the patient was on a meat-free diet. The blood urea nitrogen was 87 mg. per cent; serum phosphate, 6.1 mg. per cent; serum calcium, 8.2 mg. per cent; serum carbon dioxide, 12.5 mEq. per L. and serum pH 7.22. In the oral dextrose tolerance test, performed with 62 Gm. glucose (1 Gm. glucose per Kg. of body weight), the blood sugar concentrations were: fasting, 104 mg. per cent; half-hour, 138 mg. per cent; 1 hour, 160 mg. per cent; 2 hours, 176 mg. per cent; and 3 hours, 159 mg. per cent. Roentgenographic examination disclosed mild cardiac enlargement and a duodenal deformity without an ulcer crater. The electrocardiogram revealed atrioventricular nodal rhythm during a paroxysm of tachycardia.

The course of the patient’s illness from August 1955, when she was first seen at the University, until her death 11 months later is, in part, depicted in figure 1. The patient was given transfusions, initially of whole blood and subsequently of washed blood cells. With each rise in circulating hemoglobin there was amelioration of symptoms, but these periods of improvement were brief. A total of 19 transfusions was given with mild, febrile reactions noted on two occasions. The management of the acidosis is reported below. The infection of the urinary tract was treated with sulfisoxazole and chloramphenicol, but the urinary cultures continued to be positive. Antacid powders and a bland diet were given for the questionably active duodenal ulcer. There was relief of the paroxysmal tachycardia by quinidine. The diabetes mellitus remained mild, and no insulin or special dietary measures were prescribed.

In July 1956, the patient returned to the hospital with vomiting, headache, palpitation and ankle swelling. Laboratory examinations revealed a hemoglobin concentration of 2.0 Gm. per cent; white cells, 1,600 per cu. mm.; platelets, 12,000 per cu. mm.; reticulocytes 4.8 per cent; blood urea nitrogen, 145 mg. per cent; and serum carbon dioxide, 7.0 mEq. per L. Acute, severe abdominal pain with distention supervened. In spite of transfusions with washed blood cells, antimicrobial drug therapy and digitalization, she became stuporous and died 11 days following admission.

Special Studies

1. Ham presumptive test.$^5$ A sample of the patient’s sterile, defibrinated blood was incubated at 37.5 C. and observed for hemolysis at 6 and 24 hours.
While the spontaneous formation of lactic acid lowers the serum pH sufficiently to hemolyze PNH cells, the blood of our patient did not hemolyze.

2. Ham complete acid hemolysis test. In typical PNH, little or no hemolysis results when the patient's red cells are incubated for one hour with the patient's unacidified serum. When the serum is acidified, hemolysis occurs. A summary
of the results noted when our patient's blood was subjected to this test is shown in table 1. When the red cells were incubated with her serum, with or without acidification, there was no hemolysis. However, when the erythrocytes were incubated with acidified normal serum, hemolysis occurred. Red cells from another patient with established PNH and the same blood type, responded identically. That is, when incubated with our patient's acidified serum, known PNH cells did not undergo hemolysis, although these cells were readily hemolyzed in the normal acidified serum. Appropriate control tests using normal red cells were performed and no hemolysis was observed.

3. Nature of the serum peculiarity. The incapacity of our patient's acidified serum to hemolyze her own red cells or those from another patient with established PNH distinguished her serum from that of the normal and typical PNH sera. To determine the reason for this difference, the model of the Ham complete acid hemolysis test was followed (table 1). The addition of 7.5 units of purified human properdin* to our patient's acidified serum restored full hemolytic potency, while the addition of guinea pig serum, as a source of complement, resulted in only slight hemolysis, and magnesium chloride had no hemolytic effect. Assays of the patient's unaltered serum for properdin, by the zymosan method of Pillenwr,10 revealed a concentration of less than 2 units per ml. (normal range 4 to 8 units per ml.) and for total complement, a level of 50 units per ml. (normal control value of 40 units per ml.).

4. Crosby thrombin-activation test.1 Our patient's erythrocytes were incubated for 15 minutes with acidified normal serum, with and without 50 units of thrombin (Upjohn). A greater degree of hemolysis was noted in the tube containing thrombin. In a separate experiment, when the patient's red cells and her acidified serum were incubated with and without thrombin, hemolysis was noted only in the tube with the added thrombin.

5. Dacie Anti-A serum hemolysis test.3 When our patient's erythrocytes, which were blood type A, were incubated with anti-A serum, hemolysis was noted at a serum titer of 768, whereas normal (non-PNH) type A red cells were much less vulnerable, being lysed with a serum titer of 48. However, the titer of serum for agglutination was 768 for both our patient's and the normal cells.

6. Acidosis and PNH hemolytic activity in vivo. The effect of the acidosis associated with renal insufficiency on PNH hemolytic activity in vivo was studied by noting the response to the daily oral administration of 12.0 Gm. of sodium bicarbonate (fig. 1). The serum carbon dioxide rose from 12.5 to as high as 28.1 mEq. per L. and the serum pH, from 7.22 to 7.43. In spite of this apparent improvement in serum acid-base equilibrium, there was a progressive decline in circulating hemoglobin and a persistence of reticulocytosis at 5 per cent. The Na,Cr51O4 apparent half-survival time of the patient's

*Human properdin, purified by the method of Pillenwr,10 furnished through the courtesy of Dr. K. C. Robbins of Armour Laboratories, Chicago, Ill.
1Assays kindly performed by Dr. Carl Hinz, Jr. of Western Reserve University School of Medicine, Cleveland, Ohio.
red cells remained shortened, being 7½ days. Forty-seven days after starting alkali, because of the reduction of the circulating hemoglobin to 5.2 Gm. per cent, it was felt necessary to resume blood transfusions. The alkalinization program was abandoned after a total of 67 days.

Necropsy

At autopsy the proximate cause of death was found to be an acute generalized fibrinopurulent peritonitis, the pathogenesis of which was not apparent. Although gram-negative bacilli and gram-positive cocci were seen in the peritoneal exudate, there was no evidence of bowel perforation. The duodenum was deformed by scar, but there was no ulcer crater.

The bone marrow was hyperplastic especially in the erythroid series but contained only sparse amounts of stainable iron. In spite of heavy deposition of hemosiderin in the spleen (fig. 2), in the parenchymal as well as Kupffer cells of the liver (fig. 3), and the moderate quantities of iron in the mucosa of the stomach, the entire small intestine and the cecum, these tissues exhibited no significant pathologic reaction to the deposited pigment.

However, the kidneys, which were also sites of iron accumulation, were scarred and small, each weighing 80 Gm. The convoluted tubules showed the most intense siderosis, with separation of pigment-laden epithelial cells, plugging of the lumina, tubular dilatation and atrophy (fig. 4). Around the collapsed and atrophic nephrons were areas of fibrosis suggesting condensation of stroma (fig. 5). The interstitium demonstrated diffuse chronic inflammation, most pronounced in the deeper medullary portions, extending with less severity into the cortex with periglomerular fibrosis (fig. 6). Most of the glomeruli were severely hyalinized but were generally free of iron. No discrete hyaline nodulation of the glomeruli characteristic of diabetic glomerulosclerosis was discernible.

The pancreas displayed small aggregates of iron granules in the acinar cells, but no scarring, and the islets of Langerhans showed no morphologic abnormality. The heart was dilated and hypertrophied, but there was no siderosis of the myocardium. No evidence of vascular thrombosis was seen in any of the tissues.
Fig. 2.—Spleen showing heavy concentration of iron pigment without pathologic tissue reaction (Prussian blue, neutral red x 75).

Fig. 3.—Liver demonstrating pronounced deposition of iron in parenchymal as well as Kupffer cells without pathologic reaction to pigment (Prussian blue, neutral red x 160).

DISCUSSION

The demonstration of susceptibility of the patient's red cells to hemolysis in acidified serum and the exclusion of other conditions that occasionally may
account for a positive acid hemolysis test, appear sufficient to justify the diagnosis of paroxysmal nocturnal hemoglobinuria in this case. Support for this diagnosis was given by the positive results of the Crosby thrombin-activation and the Dacie anti-A serum hemolysis tests.

The Ham presumptive test was negative and thus the diagnosis of PNH was excluded initially. Nevertheless, with the discovery of hemosiderinuria, the complete acid hemolysis test was performed, demonstrating the abnormality of the red cell characteristic of PNH. The results of this test, however, were not entirely typical. It was shown that there was a peculiarity of the patient's serum as well, distinguishing it from typical PNH and normal sera, for it lacked PNH hemolytic activity in vitro. The factors in properly acidified serum necessary for demonstration of the PNH red cell fault have been found by Hinz et al. to be magnesium, complement and properdin. The special studies of our patient's serum demonstrated a deficiency of properdin, while magnesium and complement did not appear to be lacking. The unusually low concentration of properdin in the serum appears to provide at least a partial explanation for the negative presumptive test and the atypical results of the complete
acid hemolysis test, for addition of properdin restored in vitro PNH hemolytic activity to the serum. The factors accounting for the reduced serum properdin level are not evident. Hinz has noted low properdin values in other cases of PNH, but the serum has not been lacking in PNH hemolytic activity. Hypo-properdinemia has also been found in association with gram negative microorganism infections, so that one may speculate that the chronic renal infection due to E. coli in our patient may have contributed to the properdin deficiency.

Crosby, in 1950, reported that occasionally the serum of patients with PNH or even normal persons may lack PNH hemolytic activity in vitro. He found that the addition of thrombin activated the serum, and this was the basis for his proposed diagnostic test for PNH. Recent studies of Hinz and colleagues suggest that the degree of activation of the serum is proportional to the properdin content of the thrombin preparation used. Thus, it appears that the positive thrombin-activation test for our patient may be attributable, at least in part, to properdin furnished by the thrombin preparation to serum that was deficient in this material. This test may therefore have special diagnostic value.
in atypical cases of PNH in which there is a deficiency of serum properdin. Since the Ham presumptive test may be negative, the Crosby thrombin activation test is advisable as a screening procedure for PNH in any instance of chronic hemolytic anemia when the precise nature of the hemolytic defect is not apparent.

If properdin is required by the hemolytic system of PNH, and if our patient had a deficiency of properdin, the question arises as to why she exhibited this severe degree of hemolysis. At present there is no satisfactory explanation because the role of properdin in PNH hemolysis in vivo has not been clarified. Hinz and his associates have reported that although the serum properdin titer tends to parallel PNH hemolytic activity in vitro, the serum properdin level is commonly low rather than high during PNH hemolytic crises. Rapid utilization of properdin during in vivo hemolysis would explain the diminished serum value noted in our patient. However, the studies of Hinz and co-workers show neither utilization nor destruction of properdin during in vitro hemolysis of PNH cells. Hence, it may be that some other fac-
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

1083

tor, not necessary for the in vitro demonstration of the PNH red cell defect, operates in vivo accounting for this curious paradox.

Variations in plasma pH also have been thought to influence PNH hemolytic activity. The in vitro demonstration of the red cell abnormality requires acidification of the serum. The fall in plasma pH during sleep is considered responsible for the classical nocturnal augmentation of hemolysis. Acidification with orally administered ammonium chloride may increase hemoglobinuria in PNH, and alkalinization, by giving sodium bicarbonate, has been associated with reduced plasma hemoglobin values. With this evidence, it was believed that acidosis associated with renal failure in our patient might be enhancing the hemolytic process, and it was conceivable that, with correction of this disturbed acid-base state, a decreased rate of hemolysis would follow. Unfortunately, although there was a fairly reasonable alleviation of the acidosis, no measurable diminution in hemolytic activity in vivo occurred. It would appear that in this instance the renal acidosis was not a significant factor contributing to the hemolytic process.

The autopsy findings were in general characteristic of PNH with an impressive contrast between the paucity of iron in the marrow and the massive aggregation of this metal in the kidneys. The concentration of hemosiderin in the spleen, liver, and alimentary tract was probably in large part related to the blood transfusions given late in the course of the illness.

However, the severe alterations in the kidney suggest an etiologic relationship with the intimately associated pronounced siderosis of these organs. This view is contrary to the generally held belief that in spite of vast accumulation of iron in the renal tubules in PNH, tissue damage and functional impairment do not follow. This is not to assert that the iron alone was responsible for the renal lesions in our patient, for the presence of diabetes mellitus may have contributed to them, and the pyelonephritis may have occurred quite independently of the siderosis and the disturbed state of glucose metabolism. In spite of these complicating factors, whose contributory roles cannot be assessed accurately in this case, the necropsy observations suggest that the renal pathologic changes in PNH may be at least in part a consequence of the continuous and long-standing deposition of large amounts of iron.

SUMMARY AND CONCLUSIONS

1. A case of paroxysmal nocturnal hemoglobinuria (PNH) has been presented in which the Ham presumptive test for PNH was negative.

2. The basis for this false negative test and the atypical results of the Ham complete acid hemolysis test for PNH appeared to be due to a loss of the in vitro PNH hemolytic activity of the serum associated with a deficiency of properdin.

3. The acidosis accompanying the renal failure in this patient did not appear to accelerate the hemolytic process, for there was no apparent reduction of hemolytic activity in vivo when the acidosis was corrected by the administration of sodium bicarbonate.

4. The autopsy observations suggest that the protracted deposition of mas-
sive quantities of iron in the kidneys may play a role in the not uncommon occurrence of renal disease in PNH.

SUMMARIO IN INTERLINGUA

1. Es presentate un caso de nocturne hemoglobinuria paroxysmal (NHP) in que le "test per presumption" de Ham esscva negative.

2. Le base del false negativitate de ille test e del resultatos atypic obtenite in le complete test a hemolyse acide como indice de NHP esscva vidite in un perdita in vitro del activitate hemolytic de NHP in le sero, associate con un carentia de properdina.

3. Le acidose que accompaniava le insufficientia renal in iste patiente non pareva accelerar le processo hemolytic, proque nulbe apparente reduction del activitate hemolytic occurreva in vivo quando le acidose eseva corrigite per le administration de bicarbonato de natrium.

4. Le observationes necroptic suggere qtie le prolongate deposition de massive quantitates de ferro in le renes ha possibilemente un rolo in le occurrentia non incommun de morbo renal in casos de NHP.

REFERENCES


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