Paroxysmal Nocturnal Hemoglobinuria—New Thoughts*

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Introduction

The illness I am going to discuss bears some relationship to what we have been discussing previously —that is, basically the problem is one of hemolysis brought about by a defect in the red cell (Dacie, 1967). However, it differs in several major respects from the other hemolytic anemias under discussion. First, the defect is not congenital but rather acquired. Also, there may be 100 million to 200 million people with a glucose-6-phosphate dehydrogenase deficiency, but mercifully paroxysmal nocturnal hemoglobinuria (PNH) is rarer. The incidence is probably not as low as has been suspected, but increases as one looks for the disease. In our own clinic we have, within the last three years, found 16 patients with PNH, some by referral to be sure. It is important to identify the patients with PNH because of the implications that are inherent in treating these patients.

Clinical Presentations of PNH

PNH is probably one of those diseases that is badly misnamed. With its present name, people tend to think of PNH only when the patient comes to the physician complaining of passing dark urine in the morning which clears by noon. Under this definition, hardly twenty percent of those patients with PNH would be ordinarily identified.

It has become apparent that there is a great deal more wrong with the hematopoiesis of patients with PNH than was originally thought. This defective hematopoiesis has two characteristics which may be manifest in different proportions in different patients. On the one side, the predominant problem may be marrow hypoplasia, not simply of the red cells but also of the white cells and of the platelets (Lewis and Dacie, 1967). The other hematopoietic problem is that the cells that are made are defective. The manifestation easiest to investigate is, of course, the defect in the red cell which renders it sensitive to the lytic action of complement. This is traditionally demonstrated by the acidified serum test (Ham and Dingle, 1939). This defect in the red cells leads to hemolysis, which then leads to hemoglobinuria. It has become apparent in recent years, though, that the white cells and platelets are likewise defective (Aster and Enright, 1969).

The classic clinical presentation of the patient with PNH is, of course, hemoglobinuria (Table I); when it is paroxysmal and nocturnal, it is a striking finding. But, as I have said, if we relied upon this symptom we would detect, I suspect, not more than twenty percent of the patients with the disease. When the symptom is present, the morning urine may be very dark indeed and the evening urine may not be clear. The amount of hemoglobinuria may vary greatly from day to day. One of our patients who was also a wine connoisseur, reported his urine in terms of the wine it most nearly resembled. If the color resembled the color of Chablis, he was pleased but when it looked like Port, he became apprehensive.

The more usual presentation of PNH is chronic hemolysis without, at least to the patient's eye, hemoglobinuria. In any patient with chronic hemolysis, one ought to look for the presence of PNH.

As we mentioned, the bone marrow hypoplasia may be a major part of the illness, and PNH may grow out of patients with bone marrow hypoplasia as a primary event. The bone marrow hypoplasia may be due to drugs, as it has become apparent that some patients taking chloramphenicol may, as they recover, show the manifestations of PNH (Quagliana, Cartwright and Wintrobe, 1964). The cytopenia in PNH patients may be simply leukopenia, and/or thrombocytopenia, without much anemia. But when the patient is carefully observed, and blood is carefully examined, the defect in the red cells may be demonstrated.

Iron deficiency is a common presentation of patients with PNH. Usually the reticulocytosis is higher than one would expect for a similar degree of iron deficiency in patients not hemolyzing. This is one of

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the few causes of hemolysis in which the loss of iron is a major problem, and, therefore, iron deficiency is not uncommon.

Venous thrombotic phenomena are not uncommon in patients with PNH, possibly because of the release of thromboplastic substances during intravascular hemolysis. These thromboses are not simply the usual arteriosclerotic variety, such as coronary thrombosis or cerebral thrombosis. In PNH the thrombi are usually venous and result in unusual syndromes. Adbominal venous thrombosis may occur in the portal vein, the splenic vein, or most characteristically the hepatic veins, leading to the Budd-Chiari syndrome. The presentation of the hepatic vein thrombosis may be very insidious in its onset, but many patients that die of PNH, have evidences of hepatic vein thrombosis. Cerebral vein thrombosis can lead to very bizarre neurological syndromes due to cerebral venous or sinus thromboses.

Finally, a most interesting complication of PNH has been the occurence of acute leukemia. Dr. Dameshek predicted many years ago that acute leukemia might well occur in these patients, and indeed it has been reported in three patients (Dameshek, 1967). In all three, the leukemia is acute myelogenous and the PNH defect in the red cells disappeared with the onset of leukemia.

TABLE I

Clinical Presentation and Complications of Paroxysmal Nocturnal Hemoglobinuria

1. Nocturnal hemoglobinuria.
2. Chronic hemolytic anemia.
3. Pancytopenia - aplastic anemia.
   leukopenia
   thrombocytopenia
4. Iron deficiency.
5. Acute myelogenous leukemia.

Diagnosis: Demonstration of the Red Cell Defect

How do we make the diagnosis of PNH? We now define the disease in terms of the red cell defect which is manifest as an abnormal sensitivity of the red cells to lysis by complement. It is present in patients with PNH, by definition, and leads to the problems of the hemolysis on which we will now base most of our remaining discussion.

The sensitivity to complement lysis may be detected in several ways. In Fig 1, I have illustrated the way we do this (Rosse and Dacie, 1966). To generate this graph, cells are sensitized with an antibody, anti-I cold agglutinin in this case, and are lysed with different amounts of normal human serum as a source of complement. In the ordinary complement titration using sheep cells, differences in the amount of lysis reflect not differences in the red cells, since sheep cells are assumed to be always the same, but rather differences in the amount of complement present. In the complement sensitivity lysis test illustrated here, the amount of complement present at a given dilution of serum is the same since normal human serum is used, so that differences in lysis will therefore reflect not differences in the amount of complement, but differences in the sensitivity of the cells to lysis by complement in the presence of antibody.

The cells from a normal patient form a single straight line in this test when one plots the amount of complement present on a logarithmic scale against

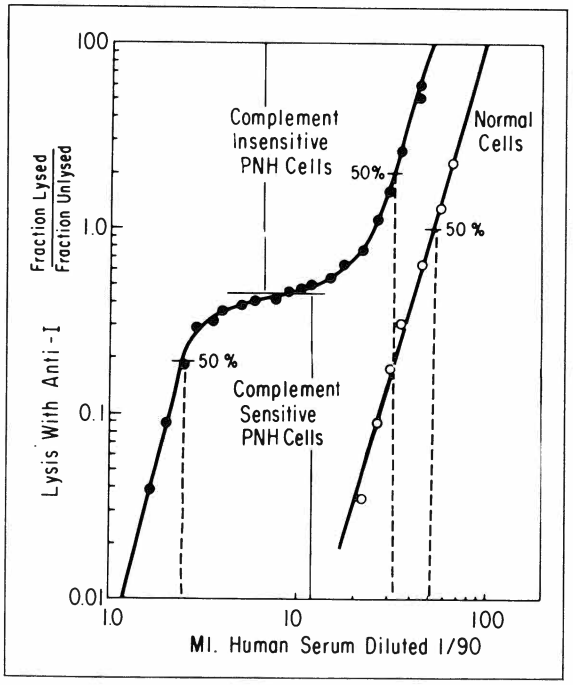

Fig 1—The lysis of normal and PNH cells by anti-I and normal human serum. The single population of normal cells and the two populations of PNH cells are seen.

the logarithm of the fraction lysed over the fraction unlysed (please forgive that way of expressing the data; it has been a tradition since 1916, at least, for plotting this sort of thing in order to obtain a straight line). The straight line with normal cells indicates that there is a single population of cells with respect to complement sensitivity. The graph also illustrates that it requires very large amounts of complement in order to lyse these cells. When PNH cells are examined in the same system, a double curve is obtained. One population of cells is nearly normal, although almost never entirely normal with respect to their complement lysis sensitivity. Another population of cells, called the complement-sensitive cells, are extremely sensitive to lysis by complement; that is, they require very small amounts of complement to lyse the cells. The proportion of cells in each population will vary from patient to patient.

It does not really make any difference what antibody one uses in order to demonstrate this phenomenon. In Fig 2 the same thing is demonstrated using a rabbit antihuman antibody. Here again, the normal is a single straight line, and both sensitive and insensitive populations in PNH cells are demonstrated. In this instance, the difference between the complement sensitive and the complement insensitive populations is not quite so striking as with the anti-I, but is

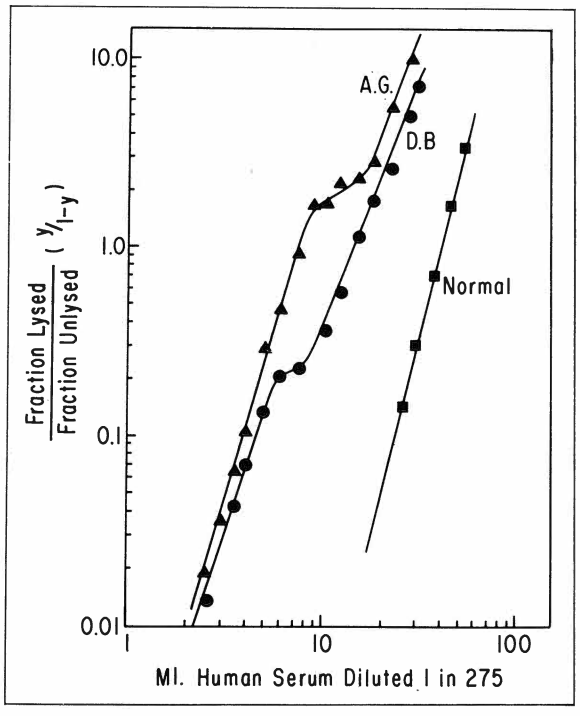

Fig 2—The lysis of normal and PNH cells by rabbit antihuman red cell antibody and human serum.

nevertheless apparent. From this and other data (Rosse and Dacie, 1966), we have concluded that the increase in immune lysis under these circumstances is not an effect of antibody but rather is due to the fact that the cells have increased sensitivity to the effect of complement.

What do we mean by increased sensitivity to the effect of complement? We mean simply that the completion of the complement sequence is more efficient. In the normal human red cell, the complement sequence appears to be very inefficient; ie, for every complement sequence that is begun, few go through to completion—probably less than 1 in 500 on the normal cell. In the PNH cell, the chances of a complement sequence, once begun, going through to completion are very much greater although there is still a considerable amount of inefficiency in the system. The nature of the defect which leads to this complement sensitivity is, at the present time, totally unknown. There is a very interesting set of compounds which can render cells which are normal to appear somewhat similar to PNH cells. These are the compounds that contain the reactive thiol groups such as glutathione, cysteine, and so on. The concentrations of these compounds that are required are often very high, however, and the exact relationship of the effect of these compounds to the defect in a PNH cell is not at all certain at the present time.

What is the relationship of this complement sensitivity to the phenomena with which we are more familiar, ie, the diagnosis of PNH from the *in vitro* tests that we use, or the clinical syndrome that we observe in the patient? The diagnosis of PNH is not, under ordinary circumstances, made by generation of the complex curves which I have shown. Rather, at least historically, this diagnosis has been made by the Ham test, or the acidified-serum lysis test. In the Ham test, one simply acidifies normal serum and incubates the cells of the patient to observe whether hemolysis takes place. It was early observed, by Ham himself, that anything that destroyed complement (that is, heating the serum, adding agents which would chelate calcium, and so on) would destroy the ability of that serum to hemolyze the cells of patients with PNH. Further evidence that there is a relationship between the complement lysis and lysis observed in the Ham test is obtained from electron microscope pictures. When the cells of patients with PNH are lysed in acidified serum and examined on the electron microscope, small, round, 100 Å defects with the heaped-up edge which are characteristic of destruction of the membrane by complement are observed (Rosse, Dourmashkin and Humphrey, 1966).

What initiates the lysis in the Ham test is not at all certain. There is no demonstrable antibody present in the normal serum. It is thought that fluid-phase activation of complement may be sufficient to bring

about the lysis of the cell. In the early days, it was proposed that another immune system related to properdin was responsible for the initiation of complement lysis, and this may still have a certain amount of validity although the exact nature of this system is not at all certain (Hinz, 1966). Nevertheless, the difference between the normal cells and PNH cells is probably not a difference in the amount of complement that is added to the cell, but in the efficiency with which the complement sequences are able to be completed in order to bring about lysis of the cell.

It was early observed by Dacie and others that the amount of lysis in the Ham test was highly variable from patient to patient. When we examined the relationship between the amount of lysis in the Ham test and the proportion of complement-sensitive cells present in patients with PNH, we found that, in general, the percentage lysis in the Ham test is less than the proportion of complement-sensitive cells. In addition, different specimens of normal serum may tend to lyse different numbers of cells. In most instances the complement-sensitive population is not entirely lysed on the first exposure to normal acidified serum. However, on exposure a second or third time, the entire complement-sensitive population may be lysed. If the complement-sensitive population is very small, the lysis may not be detected in the Ham test; this has occurred in three patients in our experience. In general the Ham test is a reasonably good screen, although it is rather cumbersome when done with all controls. It does detect most patients with PNH, but does not quantitate very well the proportion of the complement-sensitive population.

In addition to the false negative test, there is a "false-positive" acidified serum lysis test. This occurs in a group of patients, first described in detail by the Crookstons in Toronto (Crookston, et al, 1969) and by Vervilghin in Belgium, who have a congenital defect in erythropoiesis, characterized by a dyserythropoietic anemia, multi-nucleated red cell precursors, and a membrane defect. The membrane defect is manifest in three ways. First, it makes the cell more complement-sensitive than normal, but the complement sensitivity is distributed in the single population, and the complement sensitivity increase is five to six times normal rather than 30 times normal as seen in PNH. Second, an antigen is exposed on the red cell surface which is not present on normal cells, and to which normal people have isoantibodies. The reaction of a naturally occurring antibody present in most normal serum, together with a modest increase in complement sensitivity, renders the acidified lysis test positive with these cells. However, the serum of these patients does not contain the antibody, and therefore their own cells will not lyse in their own serum, despite acidification.

More recently, Drs. Hartmann and Jenkins at Nashville have introduced the sucrose lysis test, which likewise appears to depend upon complement lysis (Hartmann and Jenkins, 1966). The reduction in ionic strength increases the efficiency of several of the steps in the complement sequence. Therefore, by incubating the cells in the presence of normal serum, the complement-sensitive cells in PNH will lyse. With this test there is a much better correspondence between the percentage of cells in the complement-sensitive population, and the amount of lysis that one obtains, although the degree of variability is somewhat greater than in the acidified serum test. To date, we have not found any false negatives in patients with PNH having small complement-sensitive populations which do not lyse in the sucrose lysis test. There are, on the other hand, instances of false-positives of which one must be aware. The cells of patients with immune hemolytic anemia in which the antibody can fix complement may lyse, if the cells are incubated in their own serum and sucrose. However, if the cells are incubated in someone else's type compatible serum, lysis will usually not occur.

We have related the *in vitro* phenomena that we commonly associate with PNH to the complement-sensitive population. In order to relate these to *in vivo* phenomena, we have investigated the life span of the red cells in PNH. If the complement-sensitivity has anything to do with the hemolysis that one observes, then there should be a difference in the life span of the complement-sensitive cells, compared to the complement-insensitive cells.

If the life span of the red cells is determined using glycine-2-C_{14} as a cohort label, the complement-sensitive cells are seen to be destroyed extremely quickly in a random fashion. The half-time of this random destruction is in the order of four to six days, indicating that the life span of an individual complement-sensitive cell may be extremely short. The complement-insensitive cells are also lysed in a random fashion to a far greater extent than normal. Ordinarily, less than five percent of normal cells are lysed before reaching senescence. In PNH, probably 25 percent of the complement-insensitive cells are lysed. Those cells that miss the random destruction process are destroyed at approximately 110 to 120 days. The complement-sensitive cells do not become complement insensitive, since as the complement-sensitive population is lysed and falls, the amount of label in the whole population falls, indicating that the cells in the complement-sensitive population are born bad and die young.

We have also studied the red cell life span of both populations of cells with DFP^{32}. Again, the life span of the complement-sensitive cells was much shorter than that of the complement-insensitive cells, reaffirming that the complement-sensitive cells are de-

stroyed more rapidly than are the complement-insensitive cells. However, the life span of the complement-insensitive cells is shorter than normal, again demonstrating that these cells are not normal. So, we can see then that the lysis of the complement-sensitive cells is an important *in vivo* correlation to an *in vitro* demonstration of complement-sensitivity.

Determinants of the Rate of Hemolysis

One might ask, on what does the daily rate of hemolysis depend? In Table II, we have listed some of the things which appear to determine the amount of hemolysis in patients with PNH. In the first place, probably the most important parameter is the proportion of complement-sensitive cells. Among most patients with PNH, the sensitivity to complement of this population is nearly the same. In general, those patients with a high proportion of complement-sensitive cells have a higher rate of hemolysis. On the other hand, an occasional patient does have a complement-sensitive population which, instead of being 15–25 times more sensitive than normal, is 5–7 times more sensitive. These patients have less hemolysis and less difficulty with the disease. Thus, on rare occasions, the decreased sensitivity of the complement-sensitive population may spare the patient some of the trial and tribulations of the disease.

On occasion, the complement-insensitive cells may also be markedly abnormal. If this population is more sensitive to complement than usual, then the rate of hemolysis may in part depend upon destruction of cells in the population.

Another important determinant of the amount of hemolysis is the rate of erythropoiesis. The proportion of precursors of cells in the complement-sensitive population is far greater than the proportion of these cells in the peripheral blood. Therefore, each day a large number of complement-sensitive cells are delivered to the circulation. If the ability to make red cells is increased, the number of complement-sensitive red cells that are delivered to the circulation, and thus the number that are susceptible to hemolysis is increased. Thus patients who are iron deficient with PNH may have a bout of hemoglobinuria when given iron because of the increase in erythropoiesis.

Fig 3 illustrates a patient with iron deficiency who, after initial studies, was given intravenous iron. The reticulocyte count did not increase until four to five days later. It was only when the reticulocyte count began to increase that there was an increase in urine hemoglobin. As the reticulocyte count rose, the amount of urine hemoglobin rose precipitously. During this time, the hematocrit rose and the percentage of complement-sensitive cells rose somewhat. At this point, the patient began to have abdominal pains, a fairly frequent complication of a hemolytic episode in patients with PNH, probably due to minor thromboses in the mesentery and the gut wall. The patient was transfused, and the reticulocyte count and the urine hemoglobin fell. He was next given iron dextran and did not have a hemolytic response because erythropoiesis had been suppressed by elevation of the hematocrit by transfusion. Thus we were able to demonstrate that the hemoglobinuria which follows iron

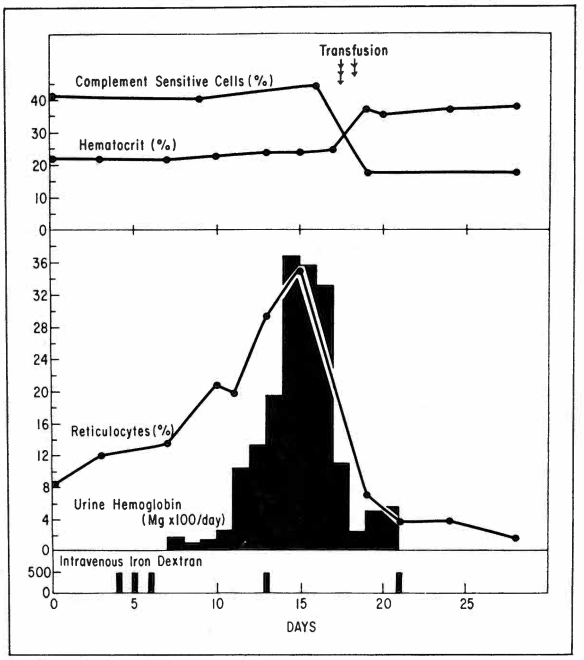

Fig 3—The effect of iron therapy on the rate of hemolysis in a patient with PNH.

TABLE II

Determinants of Hemolysis in Patients with Paroxysmal Nocturnal Hemoglobinuria

1. *Cellular Abnormality.*
   a. Proportion of complement-sensitive cells.
   b. Sensitivity of complement-sensitive cells.
   c. Sensitivity of complement-insensitive cells.
2. *Rate of Erythropoiesis.*
   a. Response to iron therapy.
   b. Response to androgen therapy.
3. *Initiating Mechanisms.*
   a. Immunologic phenomena.
      i. infections—especially viral
      ii. vaccinations
   b. Drugs—? heparin.
   c. Unknown.
      i. nocturnal-acting.
      ii. others.

therapy is not due to a toxic effect of the iron, but rather due to a sudden increase in erythropoiesis. We have seen the same effect in patients given androgens, but the hemolytic episode is not so abrupt or so dramatic as in iron therapy.

Other determinants initiating hemolysis are not well known. Some are immunologically related, since the red cells are susceptible to immunologic lysis. One would expect that if immunologic reactions were going on in the patient, hemolysis might be increased. This is, in fact, true. Certain virus infections, especially the Hong Kong flu, vaccinations, especially typhoid vaccinations, and some bacterial infections have been incriminated as precipitating crises in hemolytic episodes.

The cause of the nocturnal variation is totally unknown. It was originally thought that maybe there was an accumulation of acid during sleep which would then mimic the acidified serum lysis test. This is probably not true. It has also been suggested that the diurnal variation might be related to diurnal variations in erythropoiesis. This is difficult to demonstrate, but it may be true. Most of the time, we do not know why patients with PNH have a paroxysm of hemoglobinuria.

Summary

I have tried to summarize some of the facts we know, and some of the questions we need to ask in a disease which, although it is not common, probably is not as rare as we once thought. Once the diagnosis is made, one must be very careful in tending to the patients, since there are instances in which they react much differently than would normal people—either by the hemolytic episode or with other complications. This is especially true with regard to surgery, which may be extremely dangerous in these patients. The post-operative course may be complicated by thrombosis, infections, and other forms of morbidity. Identification of these patients is important, simple, and relatively helpful in their care.

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