CASE REPORT

Possible Paroxysmal Nocturnal Hemoglobinuria with Pronounced Pancytopenia, Reticulocytopenia, and without Hemoglobinuria Simulating Aplastic Anemia

By Henning Letman, M.D.

Paroxysmal nocturnal hemoglobinuria (PNH) was originally established as a clinical concept characterized by the peculiar nocturnal hemoglobinuria. Subsequent investigators have shown that in this disease there is a fundamental anomaly in the red blood corpuscles causing them to become hemolyzed in the blood stream under the influence of a normal thermo-labile plasma component. There are constantly varying amounts of free hemoglobin in the plasma of these patients. When the amount of plasma hemoglobin exceeds the renal threshold (30 to 50 mg. per cent), hemoglobinuria results. The hemoglobinemia increases if the pH of the plasma is reduced, e.g., by the administration of ammonium chloride. This can presumably explain why the hemoglobinemia and hemoglobinuria increase at night, since diminished respiration during sleep may cause an accumulation of carbon dioxide thus resulting in increased acidity of the blood.

In vitro, too, slight acidification of the blood causes increased hemolysis. This fact has been used as a diagnostic test, as described by Ham. However, the test is not entirely specific, as hemolysis may also occur after admixture of acid to the blood of patients with hemolytic anemia caused by hemolysin.

In 1950 it was shown by Crosby and Dameshek that the factor in the plasma which produces the hemolysis is either identical with or closely related to serum accelerator globulin (Owen's factor 6). This substance is present in an inactive state in plasma (plasma accelerator globulin or factor 5) and is activated by thrombin. On this basis, Crosby described a modification of Ham's test for PNH, which he stated was specific for this disease. An admixture of thrombin to PNH blood corpuscles suspended in normal serum will cause the inert hemolytic factor to become converted to the active hemolytic factor resulting in hemolysis of the erythrocytes. The present report is concerned with a case of severe anemia with pancytopenia, in which many features suggesting the diagnosis of PNH were present. Examination of the spleen following splenectomy revealed granulomatous lesions resembling those of Beoc's sarcoid.

METHODS

Crosby's modification of Ham's test is made as follows: (1) The patient's blood is cautiously defibrinated and the red blood corpuscles are washed three times with a 0.9 per cent solution of sodium chloride. At the last centrifugation all sodium chloride is poured off.
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(2) Two ml. of normal serum from a person of the same blood group as the patient and 0.1 ml. of N/3 hydrochloric acid are run out of a pipet into a test tube. An admixture of 0.2 ml. of the washed red blood corpuscles is then made. (3) A quantity of 50 units of thrombin is put into another test tube. An admixture is then made of 1 ml. of the cell suspension in the first test tube. Both test tubes are closed and left for 15 minutes at 37 C. after which centrifugation is made. If the erythrocytes originate from a patient with PNH, hemolysis will have occurred in both test tubes, but to the highest degree in the one containing thrombin.

In order to arrive at a measure of the number of defective erythrocytes, the test was made with accurately measured quantities of blood and reagents, and the proportion of hemoglobin liberated was determined by means of Wu’s method. The results are stated in mg. of hemoglobin per 1 ml. of serum.

Plasma hemoglobin was determined according to Bing and Baker’s modification of Wu’s benzidine method in citrated plasma, centrifuged and pipeted off immediately after the blood sampling. In control tests with normal plasma, values of about 2 to 3 mg. per cent were found.

CASE RECORD

The patient was a 24 year old married woman, admitted to the Medical Department of the Odense County and City Hospital on May 28, 1951. There were no known cases of diseases of the blood in the family. The patient was always in completely good health previously. Menstruation was normal. She had one normal parturition four years prior to her admission to the Department.

Present disease: since this parturition, the patient had been feeling increasingly fatigued. For three to four months before admission the fatigue had been so pronounced that she could hardly manage her daily household chores. She became breathless even on slight exertion, had a ringing in her ears and everything went dark for periods.

For three to four months she had noticed, in addition, a tendency to bleeding of the gums but otherwise there had been no tendency to hemorrhage.

She had never observed yellow discoloration of the skin or the sclerae nor dark or bloody urine at any time.

Four weeks before her admission to the hospital the patient’s doctor had ascertained anemia (Hgb. 43 per cent), which he had treated with an iron and liver preparation without result.

The general physical examination showed pronounced anemia of the skin and mucous membranes, no jaundice, no glandular swelling and no palpable enlargement of the spleen or liver.

The ordinary hematologic examination gave the following result (for details see table 1): hemoglobin 31 per cent, erythrocytes 1.30 ml./mm.³ Color index 1.11. Leukocytes 1,400/mm.³. Differential count: polymorphs. 55 per cent, lymphocytes 40 per cent, monocytes 5 per cent, thrombocytes 27,800/mm.³. Bleeding time 14 minutes. Clotting time 4 minutes, 10 seconds. Prothrombin time normal. Capillary fragility test (Bexelius) 16 petechiae. Blood group O, Rh negative. Reticulocytes 0.8 to 6.2 per cent, rather high values immediately after admission, later falling to normal and subnormal values. Serum bilirubin 0.8 mg. per cent; serum iron 0.13 mg. per cent. The bone marrow showed some parts which were moderately hyperplastic, and some with a normal distribution between fat and active marrow. The cell types found were all normal and the erythropoiesis was normocytic. Later, the bone marrow became increasingly hypoplastic.

At the roentgen examination, it was considered that a slightly enlarged spleen could be demonstrated. All bones were normal.

A few examinations with the benzidine test for blood in urine and feces were negative.

Because of the perhaps slightly hyperplastic bone marrow and the rather high reticuloocyte figures during the first time of observation a suspicion of hemolytic anemia was aroused. But in spite of severe anemia the content of bilirubin in the serum was normal in repeated examinations.

Moreover, the Coombs antiglobulin test and the Donath-Lansteiner test were negative. The osmotic resistance was normal and there was no spherocytosis.
Both the Ham test and Crosby’s modification of Ham’s test for PNH were then made. The latter test was made with both the patient’s and with normal red blood corpuscles. There was a vigorous hemolysis with the patient’s blood corpuscles most pronounced in the test tube containing thrombin. No hemolysis occurred with large numbers of normal red blood corpuscles. In addition, the test was made with the patient’s serum and with normal serum heated for half an hour to 56 C. In the former case there was vigorous hemolysis; in the latter there was none.

The urine was examined for hemoglobin with benzidine regularly for several weeks. Weakly positive reactions were often found, but as there were also a few erythrocytes in the urine on many occasions, presumably because of the thrombocytopenia, it was impossible to decide whether there was actually any hemoglobinuria. It must at any rate have been very slight. On a few occasions traces of hemosiderin were found in the urine.

Immediately before the splenectomy (see below) the plasma hemoglobin was 30 mg. per cent.

The hemolytic process must be considered very moderate, as the serum bilirubin was normal (on a few occasions doubtfully increased), the plasma hemoglobin was relatively low and urobilin in the urine was found in the dilution 1:40 at the utmost. Unfortunately, the bile pigment output in the feces was not determined.

There were certain discrepancies. Thus, the bone marrow was only slightly if at all hyperplastic and the reticulocyte figures were normal or slightly increased. This, in connection with the pronounced leukopenia and thrombocytopenia, showed that there must be a relative inhibition of the bone marrow. A normal bone marrow would have responded to the hemolytic process with a marked hyperplasia and would thus have been able to some extent to make up for the increased destruction of erythrocytes, so that the anemia would only have become a moderate one. This relative inhibition of the marrow might be of splenogenic origin. If so,

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<th>Date</th>
<th>R.B.C. millions per mm.¹</th>
<th>W.B.C. per mm.²</th>
<th>Granulocytes</th>
<th>Platelets per mm.³</th>
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<th>Hgb in plasma mg. %</th>
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Fig. 1.—Spleen. Low power photomicrograph of section (X50) showing extensively large malpighian nodules, some of them coalescing to form a granuloma.

Fig. 2.—Spleen. Photomicrograph (X200) of section showing development of granuloma from large, pale-staining cells of the reticulum series.

A material improvement might be expected after splenectomy. After repeated transfusions, splenectomy was therefore performed on August 31. The spleen was of normal size. On the surface and on cut surfaces nodules to the size of peas were seen.

The histologic preparations were examined by Dr. G. Teilum and reported as follows:
Microscopic examination of the spleen (figs. 1 and 2) showed a fairly dense structure with abundant accumulation of blood pigment in the red pulp. The macroscopically observed nodules were localized to the Malpighian bodies. Some of these had coalesced to form well defined granulomas, composed of large, pale, epithelioid cells, interspersed with giant cells, some suggesting Langhans giant cells, others suggesting foreign body giant cells. Around the granulomas was seen a zone of lymphocytes. There was no necrosis. The arteries showed no pathologic changes. The histologic findings bore a certain resemblance to Boeck's sarcoid.

After splenectomy the leukocyte figures became normal for three to four weeks; then there was increasing neutropenia with a strikingly great number of cells with rod-shaped nuclei. The thrombocytopenia and the number of reticulocytes remained quite uninfluenced. A bone marrow puncture twenty-two days after operation showed that the marrow was poor in cells. On the other hand, the hemolytic process appeared to be favorably influenced. Both the plasma hemoglobin and the serum bilirubin seemed to be at lower values after the splenectomy than before. Unfortunately, only one determination of the plasma hemoglobin had been made prior to the operation, but it was higher than all the other determinations.

Crosby's hemolysis test remained quite constant, and was uninfluenced by the splenectomy.

In order to attempt to stimulate the function of the bone marrow, a short course of ACTH therapy was given but without any effect whatever.

DISCUSSION

The case reported was one of chronic severe anemia which remained uninfluenced by iron and liver therapy and showed pronounced pancytopenia and a slightly hyperplastic, but otherwise normal bone marrow. The serum bilirubin was normal although on one occasion slightly increased. The reticulocyte values were at first slightly increased, later normal or subnormal. Crosby's modification of Ham's test, which has been stated to be specific of paroxysmal nocturnal hemoglobinuria, was positive.

Further examinations showed that the patient had most of the essential symptoms of the latter disease: constantly free hemoglobin in the plasma, inconstant hemosiderin in the urine, chronic anemia despite fairly high reticulocyte figures, and normal or increased function of the bone marrow. Hemoglobinuria was never observed with certainty. This was presumably due to the hemoglobinemia being so slight that it never exceeded the renal threshold for hemoglobin.

The following findings were peculiar in this case: (1) the absence of hemoglobinuria, (2) the extremely slight signs of a hemolytic process, despite the severity of the anemia, (3) the pronounced neutropenia and thrombocytopenia with increased tendency to bleeding and (4) the presence of Boeck-like granulomas in the spleen.

Concerning (1), the previously published cases of PNH have all had hemoglobinuria, in most of the cases most pronounced during sleep. Ham mentions, however, that the hemoglobinuria should only be considered an immaterial symptom, which may be lacking for considerable periods. Our case shows that this symptom need not occur at all.

Concerning (2) and (3), in 1948 it was shown by Owen in 6 cases of congenital hemolytic anemia that during a crisis the serum bilirubin and the reticulocyte figures fell, while at the same time the anemia was intensified. At the same time a reduction of the erythroblastic tissue in the bone marrow could be demon-
strated. He concluded from this that in congenital hemolytic anemia the crisis is not hemolytic but aplastic.

Furthermore, it was shown by Owren that the crisis was accompanied by neutropenia and thrombocytopenia. Correspondingly there was an inhibition of the ripening in the bone marrow, manifesting itself by a left shift. When the crisis was almost over, the number of neutrophils rose first, then the number of thrombocytes, and finally the reticulocyte crisis occurred.

Dameshek and Bloom, on the other hand, maintained in a publication of 1948 that the crisis in congenital hemolytic anemia could be both aregenerative and hemolytic, for during the crisis they found an increased urobilinogen excretion and marked spherocytosis, indicative of increased hemolysis, as well as reticulocytopenia and apparent inhibition of bone marrow activity. Of their 7 published cases, some showed slight thrombocytopenia and others slight neutropenia. They considered that both the increased hemolysis and the inhibition of the marrow were due to a hyperactive spleen, as both symptoms subsided after splenectomy.

In 1942 Wiseman and Doan in their publication on “Primary Splenic Neutropenia,” stressed the close relation between neutropenia, thrombocytopenia, and hemolytic anemia. In addition to neutropenia all their cases showed a more or less pronounced thrombocytopenia and signs of hemolytic anemia. All symptoms subsided after splenectomy.

In the so-called hypersplenic hemolytic anemia the neutropenia or the thrombocytopenia are occasionally seen. Among Evans and Duane's 11 cases there were 5 with accompanying thrombocytopenia which improved or subsided after splenectomy, and in 2 cases there was leukopenia, which subsided after splenectomy.

The writer has previously observed a case of subacute hemolytic anemia in a 10 year old girl associated with aplasia of the bone marrow, severe pancytopenia and hemorrhagic diathesis causing the patient's death.

In PNH moderate neutropenia and thrombocytopenia are usually present. The lowest leukocyte figure I have seen is 1,300, the lowest thrombocyte figure being 95,000. These low values were transitory.

In the present case the severe anemia was in no reasonable proportion to the hemolytic process which as a matter of fact could only be demonstrated by means of special technics. This, in connection with the leukopenia and thrombocytopenia showed that there was probably an inhibition of bone marrow activity. The supposition suggested itself that the latter was of splenogenic origin although the spleen was not definitely enlarged. The negative effect of the splenectomy apart from a transient rise in the number of neutrophils showed that this concept was incorrect. In PNH there may also be an increased destruction of leukocytes and thrombocytes and it may be that the factor which renders the erythrocytes defective may also influence the marrow.

Concerning (4), similar changes of the spleen have never been described in PNH. In this disease the spleen is usually slightly enlarged, displaying a normal histologic picture. However, Carling et al. mention a case of hemoglobinuria of a type not defined in detail (it may have been a case of PNH) with nodes in the
spleen measuring up to 2 x 2 cm. and consisting of large, pale cells and a number of giant cells. It is impossible from the very brief description to decide whether the lesions presented the same microscopic picture observed in our case, but this is highly probable.

**Summary and Conclusions**

1. This paper describes a case of chronic severe anemia with very pronounced neutropenia and thrombocytopenia and a varying degree of reticulocytopenia. Crosby’s modification of the Ham test for paroxysmal nocturnal hemoglobinuria proved to be strongly positive. Despite a prolonged period of observation no unquestionable hemoglobinuria was found, but slight hemoglobinemia was found. As the serum bilirubin was normal and there was no reticulocytosis an ordinary hematologic examination could not have revealed the hemolytic nature of the anemia.

   Because of the pronounced pancytopenia and the lack of response to all therapy, the case would presumably have been classified as one of aplastic or “refractory” anemia.

2. The supposition suggests itself that other cases of aplastic or “refractory” anemia and of hemolytic anemia without hemoglobinuria are actually atypical forms of paroxysmal nocturnal hemoglobinuria. The hemoglobinuria in this disease is an immaterial symptom. It should be possible to reveal such atypical cases by means of Ham’s and Crosby’s tests.

3. Pancytopenia is seen not only in the so-called hypersplenic hemolytic anemia, in which the bone marrow is hyperplastic but in congenital hemolytic anemia during crisis and in certain cases of auto-immune acquired hemolytic anemia. It is therefore possible that other forms of hemolytic anemia may be cloaked by a picture of “aplastic anemia.”

4. From the standpoint of therapy, it is naturally important to settle the question as to the true nature of the anemia. In congenital and in hypersplenic hemolytic anemia, splenectomy exerts a curative effect; in acquired hemolytic anemia caused by antibodies, treatment with ACTH and cortisone will be effective in many cases; and, finally, in PNH no therapy other than transfusions is presently available.

**References**

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