Section of Pathology

President Professor J V Dacie MD

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President's Address

Paroxysmal Nocturnal Hæmoglobinuria

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Paroxysmal nocturnal hæmoglobinuria (PNH) is a very remarkable and rather rare disorder. It is one of the three 'classical' types of hæmoglobinuria which were described in the nineteenth century. The other two were paroxysmal cold hæmoglobinuria and march hæmoglobinuria.

As emphasized by Crosby in 1951, Paul Strübing's remarkably complete description of PNH in 1882, in which he noted the association between sleep and hæmoglobinuria, and many of the other characteristic features of the disease, failed to impress his contemporaries and was in fact overlooked almost completely for more than half a century. Crosby suggested that Strübing made two tactical errors: he failed to give his 'new' disease a name – the title of his paper was simply 'Paroxysmale Hæmoglobinurie' – and by his prescient observations and descriptions he leaped far ahead of contemporary thought.

It was not until 1911 that the next accounts of PNH were published, from Italy by Marchiafava & Nazari and from Holland by Hijmans van den Bergh. Marchiafava & Nazari remarked on the presence of what they took to be granules of hæmoglobin in the urine. We now know that the granules contain easily demonstrable iron (so-called hæmosiderin), and Hijmans van den Bergh noticed that his patient's red cells underwent hæmolysis in the laboratory in an atmosphere of carbon dioxide although not in air. Biffis in Italy in 1915 noted that the hæmoglobinuria in his patient, whom he thought was suffering from the same disease as that of Marchiafava & Nazari, occurred only in the morning or at night, but it was not until 1928 that the term 'paroxysmal nocturnal hæmoglobinuria' was coined by Enneking, for what he otherwise described as a 'new form of intermittent hæmoglobinuria'. In 1928 two further papers appeared in Italy; by Marchiafava and by Micheli, respectively, in which both authors stressed the perpetual nature of the hæmosiderinuria.

Two years later the eponym 'Marchiafava-Micheli type' was added, I think unfortunately, by Donati (1930) to his already overlong title of 'Splenomegalic anæmia with hæmoglobinuria-hæmosiderinuria'. The eponym Marchiafava-Micheli became popular in the late 1930s and 1940s but recently it seems to have been quietly dropped in favour of paroxysmal nocturnal hæmoglobinuria, a title which is now almost universally used.

I shall refrain from saying anything more about the fascinating history of this disease – it is all set out in considerable detail in Crosby's (1951) paper, but the publications I have mentioned have all in their various ways been landmarks in the development of knowledge of the disease; in them may be found described the main clinical and pathological features and in Hijmans van den Bergh's paper a forerunner of the diagnostic test for the disease – the acidified serum test.

My own personal interest in PNH dates from 1937, when I saw and investigated my first case: since then my interest in the disease has been almost continuous and it is for this reason that I have chosen PNH as the subject for this Presidential Address. I shall review as succinctly as I can what I know is known about it; needless to say a great deal remains to be found out.

I shall be mainly concerned with the nature of the red cell abnormality and the pathogenesis of hæmolysis, and the identity of the plasma factors which bring about this hæmolysis, but before attempting to get to grips with these complex

problems I should like to sketch briefly some points in the clinical history of the disease and describe the blood picture and other main pathological findings.

Clinical Aspects

PNH affects both sexes in about equal proportions; it is a disease of adults, of all ages, and is very rare, although not unknown, in childhood; it is apparently acquired and is not genetically determined, and as far as I know more than one case in a family has never been described. It is a rare disease, but it is probably not as rare as was at one time thought. Crosby (1953*a*) suggested that its incidence may be about 1 in 500,000 of the population. This would mean that there should be about 100 cases of PNH in Great Britain at the present time. It would be interesting to know how close to the truth this figure is. We have in fact investigated 48 patients at the Postgraduate Medical School of London between the years 1946 and 1963. They comprise some local patients, i.e. Londoners, but the majority have been patients referred to me, or samples of their blood sent to me, by friends from other parts of England. The age and sex incidence of 43 of these patients are summarized in Fig 1.

PNH is a disease accompanied by very varied symptoms. Although classically associated with hæmoglobinuria at night-time and freedom from hæmoglobinuria during the day-time, this is by no means always so. In some patients hæmoglobinuria is very infrequent and in others it may never occur at all. Occasionally, too, the nocturnal rhythm is not obvious, the episodes of hæmoglobinuria, when they occur, lasting continuously over a period of days. In other patients anæmia is not the presenting or obvious sign; instead, nausea, vomiting or attacks of recurrent abdominal pain may lead the patient to the gastroenterologist rather than to the hæmatologist. Ellenhorn and his colleagues reported in 1951 the history of a patient who presented with abdominal symptoms and recounted how seven years elapsed before the correct diagnosis was reached. This patient was a boy who, when 10 years of age, complained of recurrent nausea and vomiting; he was found to be anæmic two years later; five years later PNH was diagnosed, and this is stated to be the 22nd diagnosis to be considered.

This case is only exceptional in the multitude of diseases considered as possible diagnoses rather than in the length of time which elapsed between the boy's first symptoms and the diagnosis being established.

PNH is seldom diagnosed without some delay, and this is by no means always the pathologist's or physician's fault. Sometimes, as in the case mentioned above, the first symptoms do not suggest a blood disease at all; in other patients anæmia is obvious but it appears to be hypochromic and of the iron-deficiency type rather than hæmolytic, and in still others the disease may be heralded by a period of marrow hypoplasia and pancytopenia and, as I have just mentioned, even if the patient's anæmia appears to be clearly hæmolytic in type, hæmoglobinuria may be inconspicuous or absent. Finally, PNH is often not established as a diagnosis simply because it is not thought of. This is a pity because the necessary diagnostic test are very easy to carry out and the results are or should almost always be absolutely clear cut. In Fig 2 is summarized the delay in making the diagnosis in 38 of the patients in the present series in whom the onset of their illness is known with fair certainty.

Prognosis

PNH is a life-long disease but, as will be stressed again later, it is also a disease of widely varied intensity. At one end of the scale in some patients hæmolysis is so intense that they have to be sustained and kept alive by repeated transfusions; at

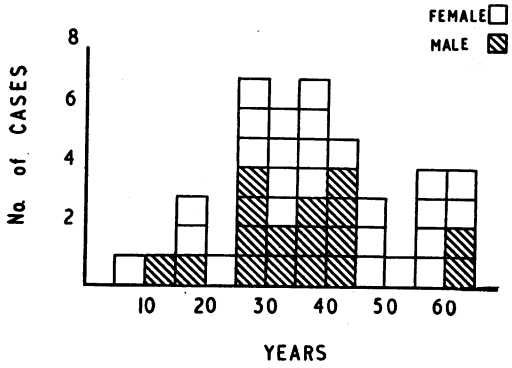

Fig 1 *Age at onset in 43 cases of paroxysmal nocturnal hæmoglobinuria*

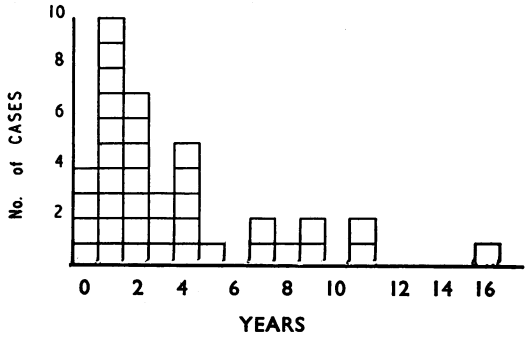

Fig 2 *De.ay in making diagnosis in 38 cases of paroxysmal nocturnal hæmoglobinuria*

the other end of the scale there are patients whose anæmia and symptoms are never serious. Rarely patients recover; their hæmolysis gradually diminishes and the hæmoglobin becomes normal. Usually, however, in such cases the laboratory signs of the disease remain positive.

A well-documented example of this is referred to by Wagley & Rumsfeld (1958) who describe how one of Hamburger & Bernstein's patients, reported on in 1936, was clinically well thirty-two years after the known onset of her disease. More rarely still, recovery is both clinical and hæmatological, and 2 of the patients in my series have recovered completely from their disease, in one of them after it had lasted for seventeen years. I must stress, however, that this happy outcome is exceptional. Most patients, unfortunately, die of their disease or of complications arising from attempts at treatment. The record for survival may be Rosenthal's (1931–32) patient who died following splenectomy thirty-three years after the onset of symptoms and Wagley & Rumsfeld's patient was, as I have mentioned, reported as being still alive, in fact clinically cured, after thirty-two years.

Venous thromboses are conspicuous amongst the causes of death, as has been stressed by many authors (e.g. Scott *et al.* 1938, Crosby 1953*a*) and of the 9 known deaths in my series venous thrombosis was the cause in 5. Infections, post-operative collapse following splenectomy, severe anæmia, and hæmorrhage are alternative but less frequent causes of death.

Blood Picture

Anæmia is usually moderate or severe; the red cells are normocytes or macrocytes and aniso-poikilocytosis is but slight or moderate in extent. Polychromasia will be present according to the reticulocyte count. Spherocytes are not seen and the MCV is usually in the 100–120 cu.μ range. The MCHC is normal or may be a little low. The red cell picture, in short, is not a dramatic or characteristic one; occasionally, the picture is that of an iron-deficiency hypochromic anæmia with signs of regeneration.

The leucocyte count is normal or low and the differential count usually normal. The leucocytes look normal but the neutrophil alkaline phosphatase is often very low or absent. The platelet count is normal or low; the platelets themselves look normal. On the whole, most patients show some degree of leucopenia or thrombocytopenia at some stage in their illness.

Dausset *et al.*, in a review published in 1956, found records of platelet counts in 41 cases; in 20 of these (49%) thrombocytopenia had been reported, i.e. the counts were less than 150,000. Leucocyte counts were recorded in 109 cases; in 32% the counts were less than 3,000/c.mm and in 10% they were less than 2,000/c.mm.

Our own data are even more striking: 44% of the patients were leucopenic and 65% thrombocytopenic at some stage of their illness (Table 1).

Table 1
Leucocyte and platelet counts in PNH

	Total No. of patients	*No. of patients with counts less than:*		
Leucocytes	34	*2,500/c.mm* 15 (44%)	*1,500/c.mm* 9	*1,000/c.mm* 2
Platelets	29	*100,000/c.mm* 19 (65%)	*50,000/c.mm* 12	*25,000/c.mm* 6

The reticulocyte count is typically raised and may exceed 30%; but not infrequently it is lower than might be expected taking into account the patient's anæmia.

A significant proportion of patients presents with a pancytopenic blood picture, i.e. with marked leucopenia and neutropenia and severe thrombocytopenia, and the reticulocyte count, despite severe anæmia, may be less than 5%. The blood picture may then be indistinguishable from that of aplastic anæmia. As will be mentioned later, I believe that these patients *are* in fact suffering from aplastic anæmia.

Some patients may be pancytopenic throughout the course of their illness, but it is more common for the pancytopenia to become less obvious as time passes and for this to be superseded by a clearly hæmolytic phase. In still other patients transient pancytopenic phases may develop during the course of their illness. Rather uncommonly, it is thrombocytopenia which is the dominant abnormality in the peripheral blood, and then the patients may appear clinically to be suffering from thrombocytopenic purpura.

The peripheral blood picture is a reflection of the patient's bone-marrow activity. Typically there is hyperplasia with erythropoiesis (normoblastic in type) predominating, but where there is pancytopenia, the marrow is indistinguishable from that of a moderate to severe grade of aplastic anæmia.

Intravascular Hæmolysis

The central feature of PNH is intravascular hæmolysis. This is continuous, but it is characteristic for the plasma-hæmoglobin level to rise considerably while a patient is asleep and to fall while he is awake – hence the nocturnal rhythm of hæmoglobinuria in typical cases. Whether the exacerbations in hæmolysis during sleep with con-

sequent rises in plasma-hæmoglobin levels always occur is uncertain. The fact that in some patients there seems to be no clear relationship between sleep and hæmoglobinuria suggests that the changes may sometimes be slight or that they may not always occur.

The reason for the exacerbations in hæmolysis which occur during sleep has not been satisfactorily established. Retention of carbon dioxide, with perhaps a consequent slight fall in plasma pH, has been suggested as a possible mechanism. However, Crosby (1953*a*) was able to show that when he placed a patient in a Drinker respirator and adjusted the amplitude of respiration so that this was even greater during sleep than when his patient was awake, and there was thus no question of CO_2 retention, the nocturnal sleep rhythm of hæmoglobinuria persisted. Other subtle changes presumably may occur during sleep which are capable of causing an increase in the plasma lytic activity, but the nature of these changes has not yet been clarified.

Hæmosiderinuria: The intravascular hæmolysis leads to depletion of serum haptoglobins and to the continuous presence of hæmoglobin in the glomerular filtrate in the kidney. Much of this hæmoglobin is adsorbed by the cells of the first convoluted tubules which become in consequence heavily loaded with iron and it is the subsequent excretion of this iron in the form of granules which gives rise to the characteristic perpetual hæmosiderinuria.

Hæmosiderinuria is a valuable sign of chronic intravascular hæmolysis, however this may be caused. In practice, its presence is a pointer to PNH as a possible diagnosis. In PNH the urine deposit typically contains large amounts of iron, and the excretion of iron may persist for years in the absence of overt hæmoglobinuria. Some patients in fact become seriously iron-depleted in this way; in others, the hæmosiderinuria acts as a safeguard against transfusion siderosis.

Diagnosis of PNH

Acidified-serum test: Diagnosis depends upon the demonstration of a positive acidified-serum test, i.e. the demonstration that the patient's red cells undergo hæmolysis *in vitro* in fresh normal human serum when the pH of the serum is suitably adjusted. The optimum pH for the reaction is between 6·5 and 7·0, hence the name acidified-serum test, also referred to as Ham's test. Using serum obtained by defibrination of blood a one-tenth part of 0·2 N HCl is about the optimum amount of acid to add. Over-acidification leads to inhibition. I think that this test, if carried out with suitable controls, is specific for PNH. The red cells in PNH have, however, some other interesting characteristics and these, too, can be used as a basis for diagnostic tests, but none, I believe, is as specific, or is as simple to carry out, as is the acidified-serum test.

The fact that the red cells undergo lysis in normal serum pinpoints the abnormality in PNH as a defect of the patient's red cells rather than of the environment in which the cells circulate. Needless to say, too, normal cells do *not* undergo lysis in a patient's serum *in vitro*, and the life span of normal red cells transfused to a PNH patient is essentially normal.

Two further points in relation to hæmolysis in acidified serum should be stressed. One is that sera vary in their ability to bring about lysis and the other is that lysis is not complete, even in the most potent sera and at optimum pH. Even if the serum is changed several times there appear to be always some cells which resist hæmolysis. From the practical point of view it is a good thing to test the sera of as many members of your laboratory staff as you can (group A, or AB if possible) so as to select the most potent serum. A fresh sample of this serum can then always be used in subsequent diagnostic work – it must of course be ABO compatible. It is preferable *not* to use the patient's own serum; not only may this contain free hæmoglobin to start with, but frequently the samples will be found to be relatively impotent.

Some of the central problems connected with PNH are: (1) The nature of the red-cell defect. (2) The nature of the factors in normal plasma which bring about hæmolysis of the PNH red cell. (3) Whether the patient's leucocytes and platelets are abnormal. (4) The relationship between PNH and thrombosis. (5) The ultimate problem – the ætiology of the disease and its relationship to marrow hypoplasia.

The Nature of the Red Cell Defect in PNH

Has the PNH red cell an abnormal morphology? I think no one will contest that when viewed with the light microscope in suspension in plasma or in stained dried films the cells show no particular abnormal features.

By electronmicroscopy, however, starting with the observations of Matthes *et al.* (1951), an unusually coarsely granular or irregularly pitted surface has been described; however, I should add that a minority of workers have felt that the appearances they obtained were normal or not specific for PNH. My colleague Dr S M Lewis, working in collaboration with Dr D Danon of Israel, has , however, obtained abnormal pictures (Fig 3) (Lewis, Danon & Marikovsky, unpublished).

The results of electronmicroscopy are certainly

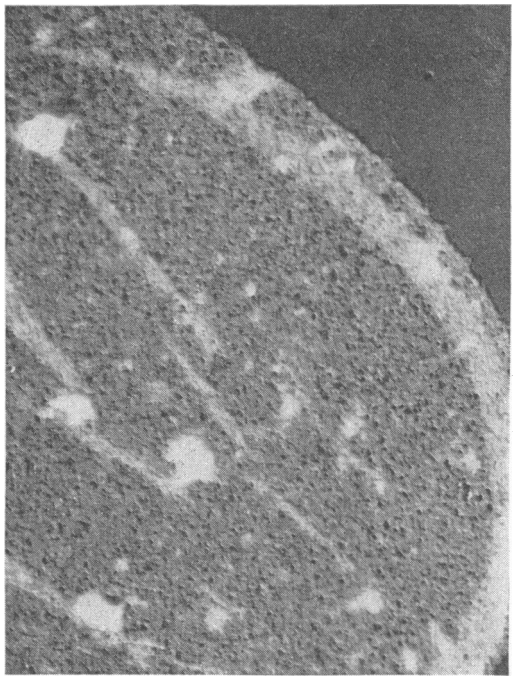

Fig 3 *Electronmicrograph of the membrane of a red cell from a patient with PNH. The presence of pits in the red cell surface is thought to be abnormal. (From a study by S M Lewis, D Danon and Y Marikovsky, unpublished observations).* ×*15,000*

interesting but perhaps not unexpected. The phenomenon of an abnormal tendency to lysis in normal plasma or serum suggests an abnormality at the cell surface and another interesting characteristic of the PNH cell points in the same direction. This is that the cells are remarkably sensitive to hæmolysis by iso-antibodies (or hetero-antibodies). Whereas human red cells are normally strongly agglutinated by, say, the antibodies anti-A or anti-B, and are but weakly lysed to a much lower titre, PNH cells are at least as sensitive to lysis as they are to agglutination. This seems to hold good for all antibodies which have any potential lytic activity, i.e. all complement-fixing antibodies. This interesting phenomenon was first demonstrated by Ham & Dingle (1939). The explanation is not obvious. It seems likely that antibodies bind on complement unusually easily to the PNH cell surface rather than that unusually small amounts of complement are capable of bringing about lysis. The exact mechanism is, however, an interesting problem that deserves full investigation.

This increased sensitivity to lysis by iso- or hetero-antibodies is not, however, as specific as is lysis in acidified normal serum from the practical point of view of diagnosing PNH, although we have in fact used the phenomenon as a confirmatory diagnostic test. Actually, normal cells vary somewhat from subject to subject in their sensitivity to lysis by iso-antibodies and red cells from patients suffering from blood diseases characterized by dyshæmopoiesis seem to be a little more sensitive than normal, at least to lysis by cold antibodies of the anti-I type (Dacie *et al.* 1960). There is in fact a small overlap in sensitivity between the mildest examples of PNH and some dyshæmopoietic blood-disease samples. However, it is only in PNH that the acidified-serum test using normal serum is positive. It is interesting to note, too, that in cases of PNH the sensitivity to acidified serum lysis and iso-antibody lysis does not run exactly parallel although the two phenomena are clearly positively correlated (Dacie *et al.* 1960).

In both types of test complete hæmolysis is never observed. This leads to the question as to whether in PNH one is dealing with a population of abnormal cells of widely differing degrees of abnormality or whether two populations of cells are always present – abnormal cells, more or less easily lysed *in vitro* and *in vivo* and a population of strictly normal cells. That two populations are present is quite clear both from the laboratory tests and also from the results of red cell survival studies carried out *in vivo*, using ^{51}Cr labelling. It is characteristic of PNH that the survival curve is at first steep and then much less steep and that the slope of the less steep part approximates to that of the survival curve of normal cells in health.

The apparent proportion of short-lived to long-lived cells varies widely in different patients. Needless to say, it is the patients who have a high proportion of cells which are easily lysed (both *in vitro* and *in vivo*) who are severely ill and it is the patients with a small proportion of abnormal cells who tend to have little or no anæmia or symptoms of any kind. In connection with the cells which cannot be lysed *in vitro* and which are long-lived *in vivo* it must be admitted that it is very difficult, if not impossible, to distinguish between cells which are so slightly abnormal as to be not appreciably affected by the hæmolytic mechanism to which they are subjected, so that they pass as normal, and cells which are completely normal. This is perhaps as much a matter of semantics as medicine.

The proportion of cells which appears to be resistant to hæmolysis *in vitro* and *in vivo* overestimates the proportion of insensitive to sensitive cells actually produced by the bone marrow. It is certainly reasonable to suppose that the insensitive cells will accumulate in the peripheral blood by virtue of their longer life-span. The point can be illustrated in another way. If blood is centrifuged and the upper reticulocyte-rich layer is tested for sensitivity to hæmolysis, more hæmolysis will be

observed than with a reticulocyte-poor blood sample.

Red Cell Acetylcholinesterase (AChE)

Another abnormality which appears to be characteristic of the PNH cell is a low (or perhaps absent) acetylcholinesterase activity. This was first reported from Italy by De Sandre *et al.* (1956) and it has been subsequently confirmed by other groups including ourselves. Not unexpectedly, however, normal AChE values occur in patients who are but mildly affected – where less than 20% of the red cells can be lysed in the acidified-serum test, and where even if the PNH cells had no activity at all this might well be masked by the enzyme activity of the normal or apparently normal cells present in great majority (which might reasonably be expected to have a normal AChE content).

Whether the acetylcholinesterase activity of the individual PNH cell is low in every case is not quite clear. Leaving aside mild cases, where only a small proportion of PNH cells is likely to be present, there is at least one report of normal activity in a patient said to be suffering from active disease, whose blood would be expected to contain many abnormal cells. This was a patient of Auditore *et al.* (1960) but no details are given.

As might have been anticipated, because reticulocyte-rich blood contains a higher proportion of cells sensitive to lysis in acidified serum, the AChE activity of reticulocyte-rich blood is less in PNH than that of reticulocyte-poor blood (Auditore & Hartman 1959, Auditore *et al.* 1960, Metz *et al.* 1960). This finding is the opposite to what is found with normal blood where reticulocytes have a greater enzyme activity than normal cells.

I have already suggested that the AChE activity of the red cells in PNH which are resistant to hæmolysis might be normal. Metz *et al.* (1960) have in fact made some measurements which tend to support this contention.

Other Possible Biochemical Abnormalities

Various chemical and metabolic estimations have been carried out, but the results fail to add up to any clearly-defined abnormal picture.

In summary, Rodbard (1950) in Holland seems to have been the first to suggest that the PNH red cell might have an abnormal lipoid surface structure. However, although some workers have claimed to have demonstrated abnormalities in the ratios of the various types of phospholipids present (Harris *et al.* 1957), their observations have not been confirmed by others (Formijne *et al.* 1957, Barry 1959). Similarly, it has been claimed that there are abnormalities in the ratios between the constituent fatty acids (Munn & Crosby 1957, Lovelock & Prankerd 1960) but again other workers have been unable to confirm these claims (Leibetseder & Ahrens 1959). On the metabolic side, in studies using ^{32}P-labelled phosphate, Hellem & Skang (1955) and Altman *et al.* (1958) observed subtle differences from the normal which they thought could be explained by changes in the structure of the surface of the cells. From a different point of view, Pondman & Mastenbroek (1954) observed an abnormally slow migration of a proportion of a PNH patient's red cells when subjected to microelectrophoresis.

A still further aspect of red cell behaviour was studied by Auditore *et al.* (1959) who, using ^{42}KCl, found red cell permeability, as measured by potassium flux, to be normal. Finally, from Italy, Vaccari & Baldini (1960) have reported a marked decrease in SH groups in the red cells of a PNH patient and concluded that this indicated a severe abnormality of protein structure. The significance of this finding is obscure and, as I mentioned above, no clearly defined biochemical abnormality, other than deficiency in AChE activity, has yet been demonstrated.

The Plasma Lytic System

PNH red cells are, I personally believe, uniquely abnormal, in that they lyse readily in normal plasma *in vivo* and in normal serum *in vitro*. The problem of the plasma components responsible has a long and complicated history of unravelment, stretching back to the extensive studies of Ham & Dingle, primarily concerned with the role of complement, published in 1939. Subsequently, the problem was taken up intensively by Crosby (Crosby & Dameshek 1950, Crosby 1953*b*), who concluded that clotting factors, in particular Ac-globulin and thrombin, were involved. Crosby (1953*b*) in fact postulated that four interacting plasma factors were required, two being heat-stable and two heat-labile, and two being inhibitors and two hæmolytic, and that calcium and magnesium were required also. Fortunately, and perhaps rather unexpectedly, this complex situation was resolved when it was demonstrated by Hinz, Jordan & Pillemer (1956) that it was the properdin complex – properdin, complement and magnesium – which was responsible for the hæmolysis. This work seems generally to have been widely accepted and the inhibiting effect of anticoagulants in *in vitro* tests – hence the necessity for using serum rather than plasma – is explainable by their anticomplementary action and on the removal of magnesium ions. Whether thrombin or clotting factors play any part in promoting hæmolysis still seems to lack proof. The *in vitro* effect of thrombin in increasing hæmolysis in the acidified-serum test (Crosby's test) has been widely used as a confirmation of the diagnosis of PNH, but I do not believe that Crosby's test is ever positive when the acidified-serum test without

thrombin is negative. How thrombin works has been the cause of the liveliest controversy and there is quite a large to-and-fro literature on the point.

I mentioned earlier that the patient's own serum might be ineffective in causing lysis in the acidified-serum test and that normal serum should always be used. One reason may be that PNH sera are often demonstrably low in properdin (Hinz, Weisman & Hurley 1956); presumably, this substance is utilized faster than it is synthesized and, perhaps fortunately for the patient, its low concentration may put a brake on hæmolysis. Complement activity in PNH sera, as measured with sensitized sheep cells, is, however, usually apparently normal.

Role of properdin: We are now getting a little closer to understanding why (if not how) the PNH cell undergoes premature hæmolysis. Its surface is abnormal and it is possible that this leads to the adsorption of the properdin complex, while normal cells by virtue of their normal surface fail to do this to a significant degree. The fixation of the complex to the cell surface then leads enzymatically to hæmolysis. Presumably it is the complement complex which actually brings about the lysis, with the properdin, by fixing the complement to the cell, playing a role analogous to that played by classical amboceptor. Isliker (1959) suggested that normal red cells are covered with a protective layer which prevents the fixation of properdin. He then suggests that the 'layer is lacking in PNH cells, thus enabling properdin and complement to exert their lytic action.' He also suggests that 'erythrocytes from patients with PNH are a typical example of a properdin-active substrate of non-bacterial endogenous origin'. These are rather exciting hypotheses.

Role of Acetylcholinesterase

Can lack of this enzyme, or at least lack of demonstrable activity, be fitted into the above concept? As yet its role normally is unexplained. However, it has variously been thought to play a part in the renewal of the phospholipids of the stroma (De Sandre & Ghiotto 1958), to take part in an enzyme buffer system, to play a part in controlling pH at the cell membrane, and to control membrane permeability (Auditore *et al.* 1959). Against it having any important role in protecting red cells from hæmolysis are the *in vivo* observations of Metz *et al.* (1961) who administered an inhibitor to normal subjects and reduced their red-cell AChE activities to the levels found in PNH, but without altering in any discernible way the rate of hæmolysis. It is obvious that the exact connexion between low AChE levels and the hæmolysis of the PNH cell has in no way yet been explained. Personally, however, I feel that the presence of these low enzyme activities and the correlation between enzyme activity and sensitivity to lysis must have some meaning in relation to the pathogenesis of the disease.

Possible Qualitative Defects of Leucocytes and Platelets

Is there such a thing as a PNH leucocyte or platelet in addition often to there being leucopenia and thrombocytopenia? Crosby (1953*a*) reported that the leucocytes and platelets of a PNH patient underwent unusually rapid autolysis *in vitro*. This observation remains unconfirmed as far as I am aware. On the other hand, Flexner *et al.* (1960) reported that platelets survived normally *in vitro*, as judged from clot retraction and platelet numbers, and repeated studies have not demonstrated any abnormality in platelet thromboplastic function (McKellar & Dacie 1958, Flexner *et al.* 1960, Newcomb & Gardner 1963). Cohen *et al.* (1961), too, have demonstrated normal *in vivo* survival of platelets tagged with ^{51}Cr in 2 patients with PNH. These studies do not therefore support the concept that the platelets are abnormal in PNH, but the reservation must be made that the presence of a small proportion of abnormal platelets would pass undetected.

On the other hand a definite abnormality of leucocytes has been demonstrated. Beck & Valentine (1951) reported a low leucocyte alkaline phosphatase and this has been confirmed by other workers (Hartmann & Auditore 1959) as well as by ourselves. In most cases of PNH the neutrophil leucocyte alkaline-phosphatase activity appears to be virtually absent. However, this is not so in all cases. In mild cases the results are generally normal. This is what would be expected if, as seems to be the case with the red cells, only a proportion of the leucocytes is abnormal.

The significance of the diminished neutrophil alkaline-phosphatase activity is obscure, but clearly it must have some meaning, and to those who believe that red cells, leucocytes and platelets arise from a common stem cell the occurrence of abnormalities affecting more than one type of blood cell will hardly be the occasion for surprise.

Mechanism of Thrombosis in PNH

Venous thrombosis is one of the commonest causes of death. Crosby (1953*a*), in particular, has stressed the importance of platelets and thrombin in this respect, but as I have just indicated there is little concrete evidence that the platelets in PNH are abnormal and are lysed in the circulation (Flexner *et al.* 1960). This, how-

ever, does not prove that a hypercoagulable state does not exist. Newcomb & Gardner (1963) have recently reported supporting laboratory evidence, and clinically there is every reason to believe in its existence. Recent work does in fact suggest that it is thromboplastic-like material derived from hæmolysed red cells or even from PNH red cells prior to hæmolysis that may be responsible for the thrombotic tendency (McKellar & Dacie 1958, Bradlow 1961). Bradlow (1961) demonstrated, too, that reticulocytes *in vitro* liberate thromboplastic material into plasma if kept at 37°C and this may be relevant to PNH thrombosis also.

Ætiology of PNH: Relationship between PNH and Marrow Hypoplasia

PNH is remarkable in being the consequence of an acquired disorder of the red cells. This is in striking contrast to the other well-known red cell disorders of intrinsic type, such as hereditary spherocytosis and sickle-cell disease, which are congenital diseases based on genetically-determined defects. The evidence that PNH is an acquired disease seems to be conclusive. As I have already mentioned, it is a disease of adult life, and it has never been described in infants and it is rare in children. No example of a familial incidence has I think yet been observed and the disease has occurred at least once in one member only of a pair of similar twins (Dameshek 1942). There is nothing to suggest that it is a recessive disease. Relatives, when tested, have been found to have normal blood pictures and to give negative acidified-serum tests, while Auditore & Hartmann (1959) and De Sandre & Ghiotto (1960) each found that the red cells of relatives of their patients had normal AChE activity.

If it is accepted that PNH is an acquired disease, what sort of disease is it? It appears to occur in all races and roughly equally in the two sexes. Its maximum incidence between the years 25 and 45 is unlike that of a degenerative or neoplastic disease. Can it be of nutritional, endocrine, infective or immunological origin? There seems to be no substantial evidence for any of these origins. Dameshek & Fudenberg (1957) pointed out that occasionally a positive antiglobulin test or other serological abnormality had been observed in patients with PNH and argued that PNH might have an immunological origin. The abnormal serological findings are, however, uncommon and the conclusion that PNH itself is based on an abnormal immunological mechanism is a hypothesis for which direct evidence is still lacking.

Somatic-mutation hypothesis: Is it possible that PNH occurs as the result of somatic mutation, that is to say as the result of a self-perpetuating change affecting the erythropoietic stem cells in the bone marrow? This is a more attractive hypothesis, as difficult to prove as to refute. It *could* be that the patient's bone marrow becomes occupied more or less completely, but possibly never entirely, by a race of hæmocytoblasts which give rise to defective cells, i.e. PNH cells, and that in the great majority of instances the hæmocytoblasts and their progeny continue to occupy the marrow until the patient dies of his or her disease. Presumably, on this hypothesis the abnormal cells must have some, as yet not understood, biological advantage – as is the case in leukæmia where an analogous process can be imagined to occur. The rare cures of PNH could on this hypothesis be the result of the gradual elimination of the clone of abnormal cells for one reason or another. It is legitimate to ask, if this hypothesis is considered, why the mutation giving rise to PNH cells should occur at all. Could it be fortuitous, the result of a summation of subtle changes occurring in a random manner with age? The age incidence with a peak in early adult life seems against this. Could the mutation be caused by a virus? This does not seem to be impossible but there is no evidence for the idea. There is a further possibility.

Significance of marrow hypoplasia: The remarkable association of PNH with marrow hypoplasia perhaps provides a clue. As has already been stressed, leucopenia and thrombocytopenia are frequently found but, more interestingly, there is an increasing literature on the occurrence of marrow hypoplasia (Dacie & Lewis 1961). This has been reported at the onset of the disease in most instances, before PNH was diagnosed or thought of, but occasionally, the hypoplasia has persisted throughout the patient's illness or has recurred after periods of partial or complete marrow recovery. In my own series of 48 cases, no less than 7 patients were at first confidently diagnosed as suffering from aplastic anæmia; that is to say they had a pancytopenia of severe degree with only slightly raised reticulocyte counts – as is not infrequent in aplastic anæmia – and hypoplastic bone marrows with severe reductions in the numbers of hæmopoietic cells. I believe that these patients were in fact suffering from aplastic anæmia and that PNH developed as a complication. The odds that the two rare diseases could occur in the same patient as the result of chance are astronomical – they must be causally related.

It seems to me to be possible that the PNH change, resulting perhaps from somatic mutation, may be particularly likely to occur in damaged marrows, perhaps where early or abortive attempts at regenerative hæmopoiesis are occurr-

ing. By damaged marrows I mean marrows which have become hypoplastic or aplastic. This does not mean that the PNH change could not occur spontaneously without preceding aplasia or hypoplasia; it apparently often does, although it is true that the more one looks for evidence of marrow hypoplasia the more frequently one finds it. Of particular interest, too, are cases clinically and hæmatologically of aplastic anæmia where only a small proportion of PNH cells is present – such patients have no obvious signs of hæmolysis but nevertheless they have hæmosiderin in their urine and give a positive acidified-serum test, with perhaps less than 10% of cells lysed.

It can of course be argued that the patients with PNH whose illness was ushered in by marrow hypoplasia or where the marrow hypoplasia continues throughout their illness have not got aplastic anæmia at all. I think the evidence is against this view-point. Of particular significance is, I feel, the fact that the sequence of marrow aplasia progressing to PNH has been seen in at least one patient with aplastic anæmia of familial origin (Dacie & Gilpin 1944), and in probable drug-induced marrow aplasia, too, as well as in aplastic anæmia of unknown origin.

Martin (1955) and Schubothe (1958) described a remarkable case. Their patient developed severe skin sensitivity to resorcin and a few weeks later developed marked pancytopenia and became critically ill with an extremely hypocellular marrow. The aplastic phase lasted six to nine months. At some time during this phase, perhaps about nine months after the start of the illness, tests for PNH became positive and hæmosiderin was found in the urine. Twelve months after the aplastic crisis the patient presented the complete and typical picture of PNH and the marrow showed erythroblastic hyperplasia. It seems extremely likely that this patient's aplastic anæmia was drug induced and based on sensitivity to resorcin. It may (or may not) be significant that there are several other reports in the literature where PNH has been observed in patients thought to have been exposed to chemicals known to have caused aplastic anæmia.

It must not be thought, though, that the acidified-serum test will *often* be positive in patients whose blood and marrow pictures indicate aplastic anæmia. Nevertheless, the proportion is appreciable. Dr S M Lewis and I have in fact tested 32 cases of apparent aplastic anæmia in recent years and obtained positive acidified-serum tests and found hæmosiderin in the urine in 4 of them.

I do not wish to suggest that I feel that this concept of the relationship between aplastic anæmia and PNH is proved, but I think it is a good working hypothesis. With this in mind I thought

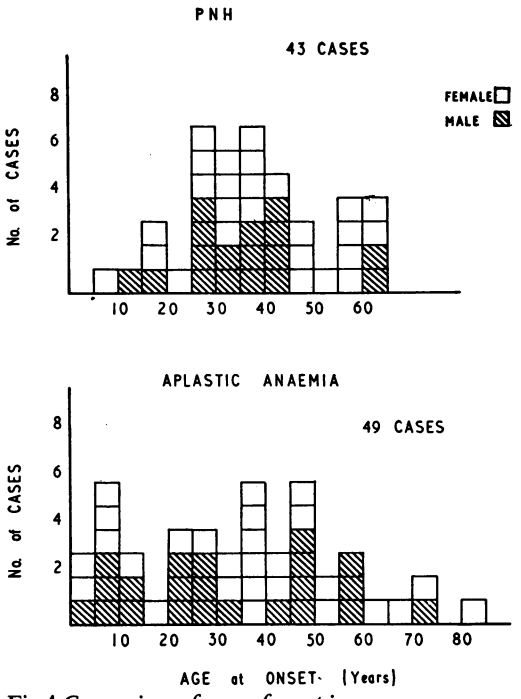

Fig 4 *Comparison of ages of onset in 43 cases of paroxysmal nocturnal hæmoglobinuria and 49 cases of aplastic anæmia*

it might be useful to compare the age incidences of the two diseases. Our own data are fairly similar, but children clearly suffer from aplastic anæmia and escape PNH disproportionately (Fig 4).

It is pertinent to ask, too, if PNH really does develop as a complication of aplastic anæmia, whether the disorder ever develops as a complication of other blood diseases. The answer to this is that it may well do so, although rarely. Aside from possible cases of autoimmune hæmolytic anæmia, Dameshek & Fudenberg (1957) reported the occurrence of PNH in patients suffering from myelosclerosis and chronic lymphocytic leukæmia, respectively. However, it is clear that these occurrences are rare. This, also, needs an explanation if PNH is really based on marrow dyshæmopoiesis. But perhaps it is the peculiar dyshæmopoiesis of aplastic anæmia or the ætiological factors which lead to the aplasia which engender the PNH change rather than dyshæmopoiesis *per se*.

Finally, before I close on this speculative note I should like to thank sincerely the many friends, pathologists and physicians, who have referred patients suffering from PNH for me to study, and my colleagues and assistants who have helped so much in their investigation. I should like to mention particularly Dr S M Lewis, Dr J Metz, Mr D

Tills and Mr L H Wallett. As I mentioned earlier, I saw my first case of PNH over twenty-five years ago now, and I must confess I still look upon it as *the* blood disease, unique in its pathology and remarkable in its clinical diversity and hæmatological interrelationships.

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Meeting June 15 1963

The Summer Meeting was held at the Beecham Research Laboratories, Brockham Park, near Dorking. Demonstrations were given.