Hemolytic Anemias

Recent Advances in Diagnosis and Treatment

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SUMMARY

The hemolytic anemias of unknown cause can be separated into two main groups: (1) those produced by a defect in cell structure, which is usually hereditary, and (2) those due to a hemolysin of immune-body type.

The hemolytic anemias associated with hypersensitivity to drugs and disease processes such as leukemia are less well understood and need further investigation.

Splenectomy is the only effective treatment in congenital hemolytic jaundice and in acquired hemolytic anemia; the operation should be carried out promptly in most cases. Transfusion may be used in all varieties of hemolytic disease and is the only effective form of therapy in sickle-cell anemia and paroxysmal nocturnal hemoglobinuria.

ACCELERATED destruction of red blood cells, regardless of causation, is characterized by clinical and laboratory findings which can be listed as follows:

1. The erythrocyte count, hemoglobin and hematocrit fall until the bone marrow, working under increasing stimulus, produces new cells fast enough to keep pace with the accelerated destruction. A balance in the opposing processes may not be reached at a level of hemoglobin high enough to support life.

2. Increased blood formation as evidenced by polychromatophilia, reticulocytosis, and the presence of nucleated red blood cells is the characteristic finding in the peripheral blood.

3. There is an increased formation and excretion of hemoglobin breakdown products in the stool in the form of urobilin and urobilinogen. Some degree of hyperbilirubinemia always results from the additional bilirubin formed, but clinical jaundice is by no means a constant finding in hemolytic anemia. Bile does not appear in the urine, although the amount of urobilinogen in the urine is almost always increased.

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Presented as part of a Symposium on Hematology before a joint meeting of the Sections on General Medicine and Public Health at the 77th Annual Meeting of the California Medical Association, San Francisco, April 11-14, 1948.

CLASSIFICATION OF THE HEMOLYTIC ANEMIAS

Observations of the longevity of transfused cells and recently developed methods for detecting the presence of hemolytic antibodies now permit the division of the hemolytic anemias into three main groups:

A. Hemolytic anemia due to abnormality of the cell.
   1. Congenital hemolytic jaundice.
   2. Sickle cell anemia.
   3. Paroxysmal nocturnal hemoglobinuria (hemoglobinemia).
   4. Mediterranean anemia.

B. Anemias due to an immune body type of hemolysin.
   1. Acquired hemolytic anemia.
   2. Hemolytic disease of the newborn.
   3. Paroxysmal hemoglobinuria.

C. Hemolytic anemia associated with extrinsic factors or disease states.
   1. Parasitic infections of the red blood cells (malaria, aroya fever).
   2. Action of lytic substances (B. welchii toxin, phenylhydrazine).
   3. Hypersensitivity to drugs (sulfanilamide) or to other substances (favism) or to infectious processes (blackwater fever).
   4. Association with disease states but cause undetermined (lymphoma, leukemia, metastatic cancer).

CONGENITAL HEMOLYTIC JAUNDICE

In most cases the diagnosis of congenital hemolytic jaundice is clear-cut. In addition to the usual signs of accelerated blood destruction, the patient has a past history of subnormal energy and endurance since early childhood, and jaundice and anemia with periodic exacerbations may have been noted. There may be overt signs of hemolytic anemia in other members of the family, or examination of the blood of relatives may show the tendency toward spherocytosis of the erythrocytes and the increase in osmotic fragility that is characteristic of the disease.

The structural defect in the red cell which produces the tendency toward spherocytosis is not known, but Emerson and co-workers[12] have recently demonstrated that the erythrocytes from patients with this disease are particularly susceptible to stasis both in test tube experiments and in the spleen. Stasis produced a prompt increase in spherocytosis and a consequent increase in the osmotic and mechanical fragility which were not observed in

normal cells. Following splenectomy there is a disappearance of the most fragile cells and a return toward normal values for osmotic fragility. Normal cells, given to a patient with congenital hemolytic jaundice, did not share in the abnormal hemolysis and survived a normal length of time in the patient's circulation, as first shown by Dacie and Mollison.[6] Normal cells also showed no increase in osmotic and mechanical fragility after exposure to the patient's circulation.

The mechanism of the crisis in this hemolytic disease is still obscure. It has always been assumed that the abdominal pain, fever and increase in the anemia were due to a sudden acceleration of blood destruction. Owren[25] has recently thrown doubt on the hemolytic explanation of the crisis by demonstrating an aplasia of the marrow and a low reticulocyte count for a period of 10 to 14 days after the crisis. He believes that the rapid development of anemia is due to sudden decrease in blood formation. Others have observed the lack of rapid blood regeneration following the crisis but have attributed the symptoms and rapid increase in anemia to acceleration of the abnormal destruction. It is possible to be misled by the temporary absence of reticulocytes in patients who otherwise appear to have had a crisis of congenital hemolytic jaundice. However, we have observed a hemolytic crisis in a male, age 25, following transfusion, which was not associated with marrow hypoplasia, since the reticulocyte count remained between 10 and 17 per cent. Chart 1 shows that the transfused normal cells did not become involved in the abnormal hemolysis even during the crisis.

There is general agreement that splenectomy is highly effective in the treatment of congenital hemolytic anemia. During the last three years we have studied six individuals with this disorder who have had splenectomy. There has been a prompt and complete clinical remission in all cases and a tendency for a shift toward normal in the curve of hypotonic fragility. It seems clear that the spleen exerts its hemolytic effect by providing the principal site for stasis of the red cells and the development of fragile spherocytes. The time when splenectomy should be performed is largely optional, since, typically, the course is mild and even during and following a crisis the anemia does not threaten the patient's life. On the other hand, there is no point in permitting the patient to remain chronically ill for years when splenectomy would result in cure of anemia.

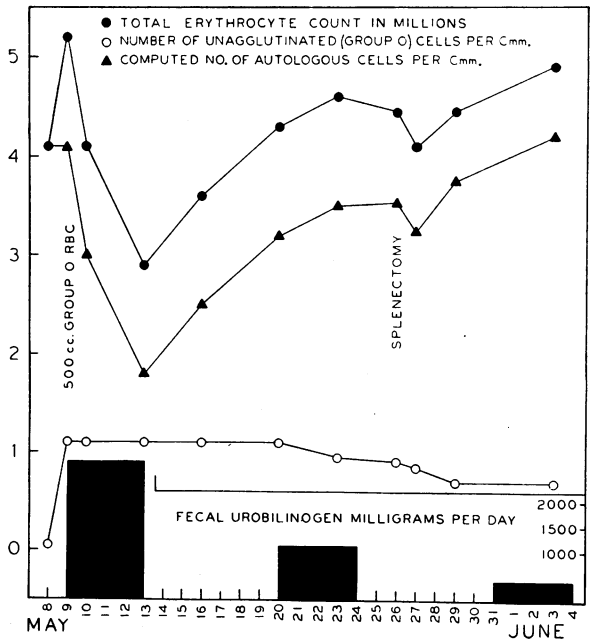

Chart 1.—Congenital hemolytic jaundice. Hemolytic crisis beginning 24 hours after transfusion of 500 cc. of Group O cells. The transfused erythrocytes did not share in the hemolytic process that reduced the erythrocyte count by over two million in three days. Studies of hypotonic fragility before and after the transfusion indicated that the transfused normal cells retained their normal morphologic structure and normal hypotonic fragility.

SICKLE-CELL ANEMIA

Since sickle-cell anemia is said to afflict about 1 per cent of all members of the Negro race, the disease should be suspected whenever anemia is observed in a Negro patient. Diagnosis is important and sometimes difficult, since the symptoms of sickle-cell disease may closely simulate those of rheumatic fever or intra-abdominal emergencies. On the other hand, not all anemias in Negro patients should be assumed to be due to sickle-cell disease, even if sickling is demonstrated, since the incidence of the sickle-cell trait is about 10 per cent.[10] The patient should show the cardinal signs of hemolytic anemia and sickling of the erythrocytes should occur readily in high percentage when exposed to decreased O_2 tension.

The chronic hemolytic anemia and the sudden hemolytic crisis are due to the sickling of erythrocytes in capillaries and venules which produces obstruction to the flow of blood and even thrombosis.[23] Fragmentation of the sickled red cells occurs largely as a result of stasis and increased mechanical fragility which results from the abnormal shape. In demonstrating the sickling the best results are obtained by first producing lowered O_2 tension in capillary circulation by placing a tourniquet on the finger for five minutes. A drop of whole blood is then sealed under a cover slip or a hanging drop preparation. Sickling of 90 to 100 per cent of the cells should take place rapidly under these conditions.

At present little can be offered to the patient with sickle cell disease in the way of sustained therapy for either the chronic forms or the hemolytic crisis. Much can be accomplished with transfusions if the necessary blood, particularly concentrated red cells, is available. Transfused normal cells do not become involved in the hemolytic process and therefore exhibit a normal survival time.[1] Building up the patient's blood count to a normal level with multiple transfusions has the effect of slowing down the rapid blood regeneration and replacing the patient's abnormal cells with normal red cells. When this is accomplished, the signs of rapid hemolysis may diminish abruptly as shown in Chart 2.

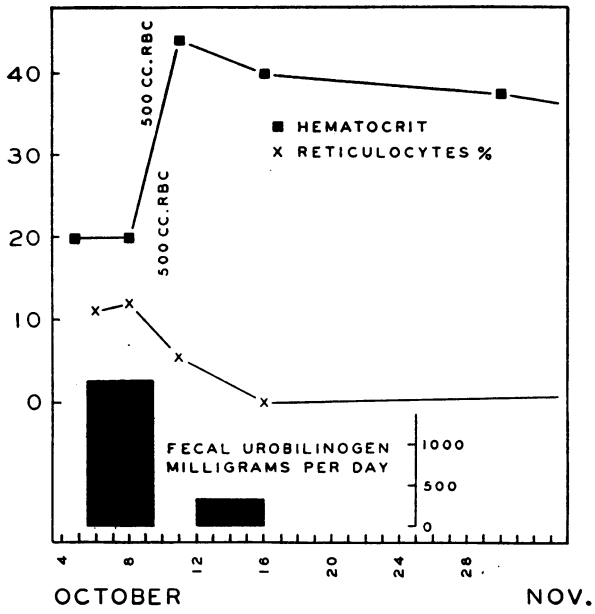

Chart 2.—Sickle cell anemia; demonstrating the marked reduction in rate of hemolysis following multiple red cell transfusions which temporarily eliminated the anemia and reduced the reticulocyte count to normal. The cells susceptible to sickling were 95 per cent before transfusion and 10 per cent during the remission in hemolysis following transfusion.

CASE REPORT

The patient from whose record Chart 2 was drawn was a 16-year-old Negro girl who was known to have had sickle-cell anemia for six years. She had also had migratory pains in the arms and legs, and because of cardiac enlargement and a rough systolic murmur the diagnosis of rheumatic fever had also been entertained. Signs of hemolytic anemia were exhibited and the liver was enlarged but the spleen was not palpable. Hematocrit reading showed packed red cells 21 per cent of the whole blood, the hemoglobin 7.5 gm. per 100 cc., and the red cells 2.15 million per cmm. with 10 per cent reticulocytes. In a sealed preparation, obtained after stasis, 95 per cent of the cells showed sickling. Following the administration of multiple red cell transfusions, the hemoglobin was 14.5 gm. per 100 cc. and red cells were 43 per cent of the whole blood. The rate of blood formation returned to normal, and the fecal urobilinogen fell to 315 mg. per day from 2,000 mg. per day. The patient was no longer icteric and appeared entirely well. Sealed preparations of blood showed only 10 per cent sickling. Transfusions of 500 cc. of red cells every three or four weeks were sufficient to keep circulating hemoglobin close to a normal level.

It is admittedly difficult and expensive to give the amount of blood necessary to alleviate this disease over a long period. However, multiple transfusions sufficient to eliminate anemia appear to be an excellent way for preparing a patient with sickle-cell anemia for operation or delivery. Reduction of the number of cells susceptible to sickling to 10 per cent, as was observed in our patient after transfusion, probably eliminates the possibility of a hemolytic crisis during a surgical procedure or an acute infection.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

In this rare variety of chronic anemia the erythrocytes have acquired a susceptibility to destruction by a hemolytic system which occurs in normal serum. Ham[17] demonstrated that the hemolysin is activated *in vitro* by lowering of the pH of plasma or serum, within physiological limits, by equilibration with CO_2 or acidification with mineral acids. In the patient hemolysis occurs most actively at night in the peripheral circulation so that hemoglobinuria occurs and hemoglobin usually appears in the morning urine. The explanation offered for the nocturnal peak of hemolysis is the lowering of the pH of the blood due to the accumulation of CO_2 during sleep, particularly in certain parts of the circulation.

A patient with this disease shows the cardinal features of chronic hemolytic anemia and in addition usually is alarmed by the passage of red urine, particularly in the morning. There is an absence of spherocytosis and the osmotic fragility of the red cells is normal. A simple, presumptive test for acid hemolysis is described by Hegglin and Maier[20] which consists in incubating a sealed tube of clotted blood at 37° C. A positive reaction results from hemolysis of susceptible cells as CO_2 accumulates during incubation. Normal blood and blood from patients with other varieties of hemolytic anemia do not show hemolysis under similar conditions unless, as we have observed, an extreme degree of spherocytosis exists. If the result of the presumptive test is positive, the more elaborate techniques described by Ham[17] should be carried out.

Active treatment of the disease is considered futile, since the patients do not respond to splenectomy. Administration of alkali, such as sodium bicarbonate, only temporarily delays hemolysis. However, as in sickle-cell anemia, transfusions are of considerable benefit, since the transfused cells do not become involved in the hemolytic process and exhibit a normal longevity.[7] For one patient with this disease who has been under observation for a number of years, regularly administered transfusions have been very beneficial:

CASE REPORT

The patient, a 53-year-old woman of Portuguese extraction, was first admitted to hospital in August, 1945, with severe anemia of undetermined cause. She had been found to be anemic about one year previously but there was no response to iron or liver therapy. Jaundice and hematuria had not been noted. The patient was obese and pale, but icterus of sclerae was not observed. The spleen was not felt. Packed red cells made up only 16 per cent of the whole blood, hemoglobin was 4.8 gm. per 100 cc., and erythrocytes numbered 1.7 million with 9 per cent reticulocytes. Leukocyte counts averaged 3,000 with a normal differential. The icteric index was 10 units. There was no spherocytosis or increase in hypotonic fragility, and sickling was absent.

Incubation of a sealed tube of the patient's blood at 37° C. overnight turned the serum surrounding the clot dark red with hemolysis. Red cells and serum were prepared from normal blood and the patient's blood by defibrination with glass beads. Ten per cent suspensions of cells in serum were mixed and equilibrated with air or CO_2 and then incubated for one-half hour at 37° C. Following centrifugation the supernatant serum was inspected for hemolysis.

	Cells	Serum	CO_2 or Air	Hemolysis
1	Patient's	Patient's	CO_2	+++
2	Patient's	Normal	CO_2	+++
3	Patient's	Patient's	Air	0
4	Patient's	Normal	Air	0
5	Patient's	Inactivated	CO_2	+
6	Normal	Patient's	CO_2	0
7	Normal	Normal	CO_2	0

These observations have been repeated on many occasions with closely similar results. Samples of blood collected at 2 a.m. have shown free hemoglobin in the plasma and serum although hemoglobinuria did not follow in the morning. Hemosiderin has been present in the urine on all examinations.

Studies of the excretion of fecal urobilinogen during 20 days of observation showed a fairly constant rate of 600 to 800 mg. per day. The patient was then given a transfusion of 500 cc. of concentrated red cells which did not produce immediate reaction, but two days later the temperature rose to 39.1° and hemoglobin appeared in the urine for the first and only time. In the succeeding two days two more transfusions were given, one of 500 and the other of 700 cc. of red cells, without reaction or recurrence of hemoglobinuria.

Following the third transfusion red cells were 45 per cent of the blood volume, and the red cell count and hemoglobin were normal. The reticulocytes fell to 1 per cent and the rate of excretion of urobilinogen in the feces dropped to a range close to normal during the first three weeks. With pallor gone the patient appeared well and said she felt well.

At the end of 45 days the red cell volume had dropped to 31 per cent of the whole blood and some of the pallor had returned. However, transfusions of concentrated cells given in two days restored the blood to normal. The patient has continued to receive multiple transfusions at intervals of 35 to 70 days and has felt well and been ambulatory. During the past year there has been some indication of general improvement in that the rate of fall of the hematocrit is slower now following transfusion than during the first year of treatment. Following the last transfusion it has been demonstrated by technique of differential agglutination that the transfused erythrocytes survived a normal length of time in the patient's circulation, as is shown by line A in Chart 3.

The frequency and severity of hemolytic episodes following transfusion has been noted by many observers. The severity of the reaction probably depends in large part on the number of susceptible cells in the patient's circulation at the time transfusion somehow sets off the increased hemolysis. It would appear that once a hemolytic episode has followed a transfusion, additional blood may be given with little danger of precipitating a similar reaction. The remission in hemolytic activity after transfusion has been noted previously by others. Once transfusions are started it may be possible to keep the hemolytic episodes under control by giving blood at regular intervals, thus preventing the building up of a high percentage of the patient's own cells in the circulation.

In the case reported, the absence of hemoglobinuria except in the one instance following transfusion does not throw doubt on the diagnosis since susceptibility of the patient's cells to acid hemolysis has been consistently demonstrated. Patients with this disease who show morning hemoglobinuria only occasionally have been described.[18] The absence of hemoglobinuria in the presence of hemoglobinemia

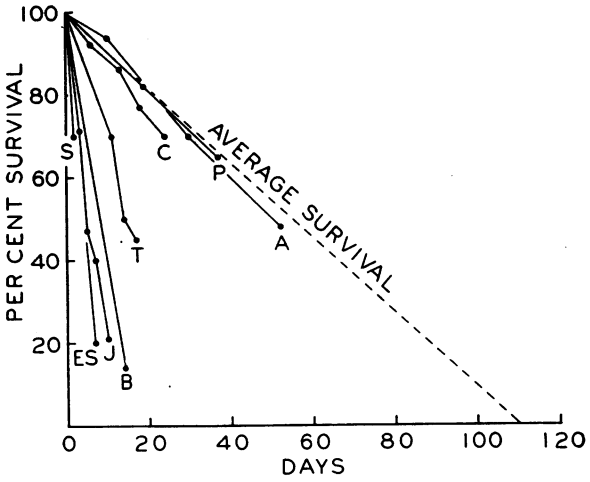

Chart 3.—Measurement of survival time of transfused erythrocytes in various types of hemolytic anemia. Lines labeled S, T, J, B, and ES are representative of acquired hemolytic anemia and show a sharp reduction in the survival time of transfused erythrocytes. Line C (congenital hemolytic jaundice), line P (Cooley's Mediterranean anemia), and line A (paroxysmal nocturnal hemoglobinuria), all show a survival time of transfused cells within the normal range during the period of observation.

simply means that the concentration of hemoglobin in the plasma does not reach a sufficient level to exceed the reabsorptive capacity of the renal tubules. Ham has suggested that the name "paroxysmal nocturnal hemoglobinemia" is preferable to "paroxysmal nocturnal hemoglobinuria." The case reported in preceding paragraphs represents a point in favor of the former name.

MEDITERRANEAN ANEMIA

It is now well recognized that Mediterranean anemia exists in varying degrees of severity and that the milder forms are compatible with adult life.[8] Many patients show evidence of an abnormal hemolytic process with hyperbilirubinemia, increased fecal urobilinogen, and reticulocytosis and nucleated red cells in the peripheral blood. However, other features of the disease may be present in the absence of much evidence of abnormal hemolysis. The most constant characteristics are hypochromia with a low mean corpuscular hemoglobin concentration which does not respond to iron, the decreased cell thickness with increased resistance to hemolysis in hypotonic saline, and the target cell or oval cell deformity of the erythrocytes in a stained smear. The disorder appears to be a hereditary defect in red cell formation, particularly as pertains to hemoglobin synthesis.

Since the disease in general appears to be due to a defect in the red cell, the abnormal hemolysis is probably on that basis. Transfused normal cells should exhibit a normal survival time in patients with this disease. We have observed this to be true in one case as shown in Chart 3. So far hemolytic antibodies have not been observed by any other method of study. Aside from repeated transfusions[21] there appears to be little available in the way of

therapy. Splenectomy may lessen the severity of the disease and diminish the frequency of transfusions in some cases.

ACQUIRED HEMOLYTIC ANEMIA

Acquired hemolytic anemia has been recognized as a separate entity on the basis of clinical observations since the first of the century. It is now possible to use laboratory methods as well as clinical evidence to distinguish between acquired hemolytic anemia and other types of hemolytic disorders, so that the syndrome may now be differentiated by the following characteristics:

1. No familial incidence has been noted in our cases nor in series reported by others.
2. The disease may begin in any period of life, and there is usually nothing in the history suggestive of previous hemolytic episodes.
3. The course is acute or subacute in contrast to the usual mild course of congenital hemolytic jaundice.
4. Spherocytosis and consequent increased osmotic fragility may be present, particularly in the severe cases, but it is not a constant feature of the disease.
5. Transfused normal cells become involved in the hemolytic process and are eliminated from the circulation at an accelerated rate.[3, 4, 6, 22]
6. Erythrocytes from patients with acquired hemolytic anemia consistently show evidence of sensitization by an immune body hemolysin,[2, 13, 27] by the Coombs technique.[5] Other observers report consistent demonstration of erythrocyte antibody in the serum.[24]
7. The response to splenectomy is so variable as to constitute a feature of the disease.

Twelve patients with acquired hemolytic anemia studied at Stanford University Hospital during the past six years have exhibited great variation in severity of the hemolytic process. In some cases the disease process has been violent with severe anemia, leukocytosis, fever, and prostration. In others the condition has been insidious in onset and the symptoms have come from the gradual development of moderately severe anemia. The patients have varied in age from 16 to 78 and only two of the 12 were men. In seven of the 12 patients there was no evidence of associated infection or neoplastic disease in the background and no history of drug ingestion or exposure to toxic substances. Of the remaining five, two patients, the youngest in the group, had a preceding or concomitant attack of virus pneumonitis. A third patient was receiving gold therapy for arthritis at the time of the onset of the hemolytic disease but there is no direct proof of etiological relationship. The oldest patient in the group, a man aged 78, was found to have hemolytic anemia in association with chronic lymphatic leukemia. One woman, aged 41, was in the third trimester of pregnancy at the time of onset of thrombocytopenia and hemolytic anemia. In her case the thrombocytopenia continued to overshadow the hemolytic disorder in severity.

Five of the patients did not show clinical jaundice when first seen. Consequently the diagnosis of hemolytic anemia was not entertained until blood studies and measurement of bile pigment excretion proved the hemolytic origin of the anemia. The excretion of urobilinogen in the feces was determined in seven patients, including those who did not exhibit clinical jaundice, and varied from 600 to 2,400 mg. per day. In all patients the icteric index and the plasma bilirubin were at least slightly above the normal level even though jaundice was not apparent. All the patients showed reticulocytosis which varied from 7 per cent to 30 per cent on the first examination. Nucleated red cells were found in the peripheral blood in every case, and sternal aspiration in 11 patients showed hyperplasia of the erythroid elements. The fragility of the erythrocytes in hypotonic saline was definitely increased in seven, but was within normal limits in five patients.

Leukocytosis with counts of 12,000 to 40,000 with polymorphonuclear leukocytosis was encountered in six patients, who in general had the most severe disease. On the other hand, leukopenia with counts varying from 1,700 to 5,000 was found in five patients. Thrombocytopenia was also common, and platelet counts of 60,000 or under were encountered in five patients, but only one patient exhibited a bleeding tendency.

Studies of the longevity of transfused cells by a modification of the Ashbey technique of differential agglutinations were made in five of the 12 patients. In all five the transfused normal cells were eliminated from the patients' circulation at an abnormally rapid rate, as is shown in Chart 3. In four other patients repeated multiple transfusions of red cells had a transitory effect so that it could be ascertained by simple calculation that the transfused red cells were involved in the hemolytic process. In one patient it was demonstrated that the transfused cells showed an increased hypotonic fragility 24 hours after transfusion.

Since the advent of the Coombs test, the nine patients in the group studied with this technique have all shown evidence of sensitization of the circulating red cells by an immune body. A technique for quantitating the amount of antibody on the cell has been devised and there is evidence that the activity of the hemolytic process before and after splenectomy is correlated with the amount of antibody on the cell surface.

The treatment of acquired hemolytic anemia is not as clear-cut as in the previous varieties discussed. In general the course of the disease is usually progressive and shows a tendency to increase in severity. Spontaneous cure of the hemolytic disease was observed in only one instance in a 16-year-old girl with associated pneumonitis. In her case one transfusion did not affect the course of the disease, and the hemoglobin remained at a level of 30 per cent for several days until the pneumonic process subsided. This was followed by a complete and apparently permanent remission in the hemo-

lytic process. A temporary partial remission was observed in one patient who a few weeks later relapsed and underwent splenectomy without improvement. One patient died without splenectomy because of the extreme severity of the disease, and one patient is now under observation and splenectomy has not been performed because of advanced age and complicating lymphatic leukemia.

Nine patients have undergone splenectomy with variable results typical of this type of hemolytic anemia. These patients may be divided into three groups according to their course since splenectomy:

1. Four have exhibited complete clinical remission for periods of from 18 to 24 months. One patient of this group died of metastatic carcinoma without recurrence of the hemolytic process. The patient exhibiting thrombocytopenia and hemolytic anemia during the sixth month of pregnancy showed little change following removal of the spleen although the bleeding tendency lessened. There was a gradual relief of anemia and thrombocytopenia following delivery.

2. One patient had a remission of 18 months following splenectomy but died in relapse.

3. In four, splenectomy was probably of some temporary or lasting value, but the hemolytic process has continued actively. One patient died of anemia and two died of infectious processes not connected with the hemolytic anemia. One patient is alive and ambulatory with chronic hemolytic anemia six years after splenectomy. In her case hemoglobin varies from 45 to 60 per cent.

Methods designed to quantitate the amount of anti-erythrocyte antibody on the surface of the red blood cell indicate that remission following splenectomy is associated with reduction of the quantity of antibody on the cell surface.[14] The reduction occurs immediately after splenectomy, indicating that the spleen is the major source of antibody. Relapse or continuation of the accelerated process is associated with amounts of antibody on the cell comparable to presplenectomy values. Evidently other tissues have assumed a larger role in the formation of the erythrocyte antibody in those cases in which there is either no improvement or relapse. Autopsy examination of two patients who died in relapse failed to reveal an accessory spleen.

The important practical question to be answered is the effect of early splenectomy, as opposed to delay, in the production of complete remission. Our series is small, but there is evidence that in general those patients in whom the disease was mild and who underwent splenectomy promptly after the diagnosis was made, did the best. On the other hand, patients on whom the operation was delayed because of uncertainty and because of the severity of the disease did less well after operation. It is our impression that the severity of the disease has more to do with remission than the time of splenectomy.

We have observed no general contraindications to transfusions in this group of patients and the majority received multiple red cell transfusions without incident during their hospital course. One patient is said to have had a hemolytic transfusion reaction before entering this hospital, which is probable, since hemoglobinemia was present on entry some 24 hours later. Subsequent multiple transfusions were well tolerated although the patient finally died of anemia and renal failure.

It should be kept in mind that these patients may exhibit atypical isoantibodies in addition to the hemolytic antibody causing the disease. They are likely to have cold agglutinins which add confusion to the determination of compatibility of blood. As a consequence it is recommended that in addition to the usual precautions of grouping and Rh typing, the cross-matching of blood be performed with a 2 per cent serum suspension of the donor's cells and a drop of the recipient's serum undiluted with saline. The mixture should be incubated at 37° C. for one-half hour and centrifuged in a warmed cup for one minute before a reading is made. This warm serum cross-match will serve to detect the presence of atypical isoantibodies and will eliminate false indications of incompatibilities due to cold agglutinins. In addition to these steps Hattersley[19] recommends centrifugation of the suspension immediately after mixing to detect the presence of atypical blocking antibodies which may not produce agglutination after incubation because of the zoning phenomenon. If the sedimented cells show no agglutination, incubation and recentrifugation are then carried out.

Multiple transfusions of red cell concentrate have proved to be of great value in preparing patients with acquired hemolytic anemia for splenectomy. In several cases the anemic state was temporarily eliminated in 24 to 36 hours by multiple transfusions. On the other hand, it should be kept in mind that transfusions have a very transitory effect because of the accelerated destruction of all red cells in the patient's circulation and that transfusions are not a substitute for splenectomy which as yet offers the only hope of a real remission in the majority of patients.

HEMOLYTIC DISEASE OF THE NEWBORN

A detailed discussion of hemolytic disease of the newborn is beyond the scope of this report, since it presents a special problem in the field. The infant is exposed to a hemolytic antibody of maternal origin *in utero* and the chief problem in therapy is the hemolytic anemia which continues after the removal of the infant from the source of antibody. Continued destruction of red cells for days or weeks following birth indicates that the antibody present is probably not neutralized or destroyed completely by hemolysis of the sensitized cells. It is possible that hemolysis may release the antibody to damage more erythrocytes and perhaps other tissues as well.

Recent advances in laboratory technique have made it possible to predict the occurrence of hemolytic disease prior to birth so that the necessary diagnostic and therapeutic measures can be arranged in advance of delivery. Experience to date with the replacement transfusion technique has been encouraging and so far parallels that reported by

Diamond.[9] The replacement transfusion provides the advantage of removing most of the destructive antibodies at the same time that the anemia is treated. In our experience only one replacement transfusion procedure has been needed in the treatment of severely anemic babies, whereas six to twelve separate procedures were required with the multiple small transfusion technique.

PAROXYSMAL (COLD) HEMOGLOBINURIA

The auto-antibody responsible for the hemolysis in paroxysmal (cold) hemoglobinuria requires a temperature below the average body range before it unites with erythrocytes. Therefore, although it can be demonstrated in the patient's serum between paroxysms of hemolysis, the titer tends to deminish or disappear following an attack. These well-known observations provide contrast with the findings in acquired hemolytic anemia where the immune body unites with erythrocytes at body temperature and the greatest part of the antibody is found on the surface of the erythrocyte at all times.

Paroxysmal hemoglobinuria should be suspected whenever the evidence of hemolysis, particularly hemoglobinuria, accompanies or follows chilling. The classical Donnath-Landsteiner technique of demonstrating the antibody in the patient's serum is too well-known to need description here.

HEMOLYTIC ANEMIAS ASSOCIATED WITH EXTRINSIC FACTOR

It is not the purpose of this report to discuss hemolytic phenomena produced by extrinsic agents. The cause of hemolytic anemias associated with parasitic infections and lytic substances that destroy the red cell is obvious and requires no further comment here. The mechanism of hemolysis in anemias which appear to be due to hypersensitivity is obscure, and further study with the transfusion and immunological techniques previously described is needed. The subject of the so-called symptomatic hemolytic anemias has recently been reviewed by Stats and co-workers;[26] but the underlying mechanism of hemolysis, whether cell defect, hemolysin or excessive stasis in the spleen and phagocytosis, has not been defined. We have studied one patient with hemolytic anemia associated with lymphatic leukemia who showed the usual evidence of hemolysin of an immune-body type and the rapid destruction of transfused cells. Whether or not an immune body will be a constant finding in this group of patients remains to be seen.

If treatment of symptomatic hemolytic anemia is required, therapy directed at the underlying disease should be attempted first. If the hemolytic process threatens the patient's life, splenectomy, if feasible, may produce relief. In connection with the association of hemolytic disease and malignancy, it is worth remembering that ovarian tumor has been found in the background in patients with hemolytic anemia and that the hemolytic process has subsided following removal of the tumor.

DISCUSSION

Although some light has been thrown on the subject of hemolytic anemias, it is obvious that many new problems have been created and many old questions remain to be answered. The classification presented is certainly tentative and is not complete even at this time. For instance, Haden[16] has recently described members of two families with familial hemolytic anemia without spherocytosis, which represents a new entity. On the other hand, Young and Lawrence[29] and Evans[15] have described single patients with hemolytic anemias that did not appear to be congenital or familial and could therefore be referred to as "acquired," in which hemolytic antibodies were evidently absent, since transfused cells were not involved in the hemolytic process. The defect therefore appeared to be in the red cell, yet the patients did not fit into any of the known categories of hemolytic anemia due to cell defect.

The structural defect which allows for spherocytosis, sickling, and susceptibility to acid hemolysis is as yet buried in the unknown regions of the physicochemical anatomy of the red cell. Watson[28] has recently reported extensive studies of sicklemia in the newborn of mothers with sicklemia, which show a low percentage of cells susceptible to sickling at birth as compared to four months after birth when the fetal red cells have been replaced by extra-uterine cells. The gradual increase in susceptibility to sickling during the first four months parallels the replacement of fetal hemoglobin by that formed following birth. Since qualitative differences such as resistance to alkaline denaturation have been demonstrated to exist between fetal and extra-uterine hemoglobin, the correlation with susceptibility to sickling is highly significant.

The demonstration of auto-antibodies in the great majority of patients with acquired hemolytic anemia provides considerable material for speculation. The antibody is really a tissue antibody since erythrocytes from other individuals are apparently destroyed as rapidly *in vivo* as the native cells. The concept of auto-antibodies is not new but the existence of this aberrant immunological activity has not been so clearly and consistently demonstrated in other diseases suspected of being due to similar cause. The acquired hemolytic anemias provide an excellent opportunity for observing the course of disease due to auto-antibodies, the effect of splenectomy, and other possible therapeutic measures. Observations made in this disease have a broad application in the field of medicine, particularly in those disease states of unknown cause, such as glomerulonephritis, rheumatic fever, rheumatoid arthritis, and other syndromes grouped under the general heading of the hypersensitive states.

The occurrence of idiopathic thrombocytopenia and hemolytic anemia of the acquired type in the same individual suggests that both diseases are due to the same process. Thrombocytopenia and leukopenia have been observed in almost 50 per cent of the cases of acquired hemolytic anemia observed by

the authors. Some of the patients described by Doan and Wright[11] under the heading of primary splenic panhematopenia have shown evidence of a hemolytic process. It seems unlikely that the hemolytic anemia in such cases is due to an erythrocyte antibody while the thrombocytopenia and leukopenia are due to suppression of marrow production by some hypothetical hormonal agent or to excessive phagocytosis in the spleen or reticuloendothelial tissue. The role of auto-antibodies in thrombocytopenia needs reinvestigation with improved immunological technique.

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