THE RH FACTOR*

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THE Rh factor is a property of human blood, the latest of a series of previously discovered blood factors. In order to understand the Rh factor, it will be necessary to review the other known properties of blood.

Since the discovery by Landsteiner of the first two blood factors, A and B, in 1901,¹ several additional factors have been discovered. Some are little known; others are known better but have assumed no importance in the practice of clinical medicine and surgery, though they are applied in the practice of legal medicine. This discussion will be concerned mainly with blood factors of interest to clinicians, especially in connection with blood transfusions.

HEMAGGLUTINATION AND HEMOLYSIS

The frequently tragic results of early attempts at blood transfusion were due mainly to lack of knowledge of two reactions that may occur when the blood of two individuals of different species, and often when blood of two individuals of the same species, is mixed. One of these reactions is clumping or agglutination of the red blood cells of one individual by serum or plasma of the other. This special form of agglutination is called hemagglutination to differentiate it from other forms like bacterial agglutination. The second reaction is laking or lysis of the red cells by serum or plasma and is called hemolysis. The latter phenomenon, though of greater importance in blood transfusion reactions, is more complex than hemagglutination and more difficult to demonstrate. As hemolysis does not occur in the absence of hemagglutination, this discussion will be limited

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Two elements are essential for hemagglutination: the red cells which are clumped, the agglutinogen, and a substance in the serum or plasma which brings about the clumping, the agglutinin. The injection of red cells (the antigen) of one animal species into another leads to the production of agglutinating antibodies or hemagglutinins. They clump specifically the blood cells of the injected species.

In addition to these agglutinating antibodies produced by immunization, or immune agglutinins, another type of hemagglutinins, not due to previous immunization, has been known as normal hemagglutinins. The agglutination of red cells of one species by the serum of another was not surprising in view of other differences between species. Very startling was the discovery by Landsteiner of agglutination of red cells of one person by the serum of another, both of them normal and healthy. Here for the first time individual differences were observed in the blood of members of the same species, isoagglutination, as contrasted with heteroagglutination or agglutination in mixtures of blood of different species. Even after Landsteiner’s discovery there were reports in the literature referring to isoagglutination as a pathologic phenomenon, a manifestation of disease, and various authors claimed it to be a specific diagnostic test for a variety of diseases. Later studies confirmed Landsteiner’s concept of isoagglutination as a normal physiologic phenomenon.

BLOOD GROUPS

In experiments which led to the discovery of blood groups, Landsteiner studied the behavior of mixtures of serum and of red cells of various persons. He found from the behavior of these mixtures that people could be divided in three groups: the largest group consisted of persons whose red cells were not clumped by serum of any other person; the red cells of a slightly smaller second group, and of a considerably smaller third group were clumped by certain serums. Landsteiner assumed the existence of two different agglutinable substances in the two last mentioned groups, factor A in the larger and factor B in the smaller group. He explained the failure of the red cells in the first mentioned, largest group to be clumped by the absence in
them of both of these factors and expressed it by the numeral "0." Later it became customary to refer to the "0" as a letter instead of a numeral.

Corresponding to the factors in red cells there are agglutinins in the serum: anti-A (also called "a" or α) reacts with factor A, and anti-B (also called "b" or β) reacts with factor B.

Factor A and anti-A cannot be present in the blood of the same person, nor can B and anti-B, as this would not be compatible with life. This is expressed in Landsteiner's law: Every person has in his serum agglutinins that react with the factor or factors absent in his red cells. Persons of group A have anti-B isoagglutinins, persons of group B have anti-A agglutinins, and persons of group O have anti-A and anti-B agglutinins.

To these three groups a fourth, very rare group was added when a small number of persons was found whose red cells were clumped by the serums of persons belonging to the other three groups. It was shown that the red cells of this group contain both factors, A and B, and it became known as group AB. The serum of these persons lacks both anti-A and anti-B and does not agglutinate cells of any blood group.

Distribution of blood groups varies in different races. In the white population in this country, about 45 per cent belong to group O, about 40 per cent to A, about 11 per cent to B, and about 4 per cent to AB.

APPLICATION OF THE DISCOVERY OF BLOOD GROUPS TO BLOOD TRANSFUSIONS

It would seem that Landsteiner's discovery of differences in the properties of the blood and of incompatibilities arising therefrom should have been followed immediately by the practical application of this knowledge to blood transfusion. This is indeed the prevalent opinion, even among those who are especially interested in blood transfusion. Actually, a study of the literature of the first decade of this century reveals a gap of several years during which one fails to find any clear evidence of a practical application of the discovery of blood groups.

Hektoen was the first to recommend in 19078 the selection of compatible donors in order to avoid transfusion reactions. His recommendation was that there be "selection of a donor whose corpuscles are not agglutinated by the serum of the recipient and whose serum does not agglutinate the corpuscles of
the latter [to avoid the danger of] erythrocytic agglutination within the vessels of the subject transfused.” He suggested at the same time a simple procedure for cross-matching tests claiming that the actual isoagglutinative relation of the donor and the recipient is readily determinable. Hektoen’s fundamental contribution to the safety of blood transfusions by the practical application of the knowledge of blood groups to the selection of compatible donors has been generally overlooked.

Landsteiner and Levine discovered in 1926 two additional blood factors, M and N. About 30 per cent of the population have factor M, about 20 per cent have N, and the rest have both M and N, thus forming three types M, N, and MN. The term “blood types” is used in contradistinction to the “blood groups” A, B, AB, and O. The factors M and N play no part in relation to blood transfusions because there are no agglutinins against them in our blood normally, nor is a person of type N able to produce immune agglutinins against M when transfused with blood of type M or vice versa.

**DISCOVERY OF THE RH FACTOR**

The Rh factor was discovered in 1940 by Landsteiner and Wiener. Rabbits were injected with the blood of the rhesus monkey (*Macacus rhesus*). Following a series of injections their serum clumped the red cells of the monkey, as was to be expected, but also the red cells of about 85 per cent of human beings. Landsteiner and Wiener accordingly assumed that there is a common antigenic factor in the blood of the rhesus monkey and of about 85 per cent of human beings. The factor was designated as Rh to indicate its relation to the animal species. The 85 per cent who have the Rh factor in their blood are called *Rh-positive*, the remainder are *Rh-negative*.

It was shown that the Rh factor is independent of the other blood factors. It occurs with the same frequency in persons of all groups and types, and is not related to sex. It differs from the factors A and B in several ways: There are no normal agglutinins against it; i.e., Rh-negative persons do not have in their serum agglutinins capable of clumping the blood of Rh-positive persons.

Diamond observed anti-Rh agglutinins in a child with nephrosis without a history of previous transfusions, but such instances must be extremely rare. Agglutinins capable of
clumping Rh-positive blood are due to immunization by the Rh factor and will be discussed later.

The reference to factors A and B as blood properties is not entirely correct, because they are present not only in the blood but in tissues and in secretions, for instance saliva, of most persons of groups A, B, and AB. The Rh factor is found only in red blood cells. In both respects, in the absence of agglutinins against it, normally, and in its exclusive presence in red blood cells, the Rh factor is similar to the blood type factors M and N.

The rapidity with which the Rh factor became widely known is due to the fact that it helps to make understandable certain, previously unexplainable transfusion reactions, and that it has become the basis for a plausible hypothesis of the genesis of erythroblastosis foetalis.

**COMMON ERRORS IN TYPING AND CROSSMATCHING**

In the course of the years rules have been evolved governing the selection of safe donors for transfusions. The profession learned to recognize causes of errors and to guard against them in the typing of blood and in crossmatching tests. Some of these errors are due to the method of preparing reagents (typing serums, cell suspensions), some are due to the technic of the tests (temperature, speed of performance, bacterial contamination, and so forth), and some are due to inherent properties of the reagents (such as rouleaux formation, titer of agglutinins and irregular agglutinins). It is known that as a rule it is preferable to use donors of the same group as the patients, that the use of donors of group O (Moss IV) or so-called universal donors is to be restricted by various considerations which cannot be discussed in detail on this occasion. It has also been learned that crossmatching tests must be done before each transfusion.

The technic of transfusion has also been greatly improved, thus eliminating other sources of danger. The result of these advances has been that when a suitable donor was selected and when the transfusion was done according to established rules, there was good reason to expect that there would be no serious reaction.

**THE RH FACTOR IN TRANSFUSION REACTIONS**

In spite of all precautions, and in spite of using donors of the same groups as patients, instances of severe and even fatal trans-
fusion reactions* have been known to occur. In some such cases a subsequent recheck of typing and of crossmatching failed to reveal any evidence of incompatibility. There was no explanation for these tragedies. They had to be dismissed as unexplainable accidents. When the donor and recipient were of the same group the cases are referred to as intragroup transfusion reactions.

Although there was no satisfactory explanation and no known means of avoiding such reactions, accumulated experience brought out two facts: (1) most of these reactions occurred in recipients who had received previously one or several transfusions without any reaction or with mild reactions and then the last transfusion was followed by a severe, usually hemolytic, and sometimes fatal reaction, and (2) a smaller number of instances in which the first transfusion was followed by a severe or even fatal hemolytic reaction. Here too an interesting phenomenon has been observed: The majority of these reactions following a first transfusion occurred in pregnant women, usually during delivery or soon after childbirth or abortion.

Levine and Stetson⁷ were the first to throw some light on the second type of these reactions when they reported in 1939 a hemolytic reaction in a woman who was given a transfusion following an abortion. The recipient and donor belonged to group O. Reexamination after the transfusion revealed the presence in the blood of the patient of an irregular agglutinin which clumped the blood of about 80 per cent of persons regardless of their blood group, even those of group O. Levine and Stetson suggested that the irregular isoagglutinin was the result of immunization of the patient in the course of pregnancy by an antigenic substance coming from the fetus. The substance was assumed to have been inherited by the fetus from the father and to have been transmitted from the fetus to the mother through the placenta. Later developments have shown the correctness of this ingenious hypothesis, although the discovery of the Rh factor was needed to furnish a complete explanation of the exact mechanism.

Shortly after the discovery of the Rh factor, Wiener and Peters⁸ studied three cases of hemolytic transfusion reactions in patients

* The most serious, so-called hemolytic reactions are characterized by hemoglobinemia, hemoglobinuria, icterus, oliguria with progressive azotemia, anuria, uremia and sometimes death.
who had received previously one or several transfusions without untoward manifestations. The recipients and donors were in the same group. An investigation of the serum of the recipients by means of an especially sensitive technic revealed the presence of an irregular agglutinin reacting with red cells of the donors and of about 80 per cent of persons, regardless of the blood group and including the usually inagglutinable group O. The irregular agglutinin in the patient was found to be similar to the agglutinin produced in rabbits by injection of rhesus blood. Both agglutinins clumped the cells of the same persons. The patients were shown to be Rh-negative, the donors whose blood was responsible for the reactions were Rh-positive and so were some of the donors whose blood was used in one or several of the previous transfusions.

Here was an obvious explanation of the transfusion reactions. The injection of Rh-positive blood into Rh-negative persons may be followed by development of anti-Rh agglutinins, just as it happened in the rabbits that were injected with rhesus blood. There will be, of course, individual variations. Some Rh-negative persons will develop agglutinins after only one transfusion. Some will require several transfusions, and there may be persons who will not develop any demonstrable antibodies. After sensitization has taken place, the recipient and the donor, though of the same group, are not compatible any more as they had been before the recipient developed antibodies against the Rh factor.

The argument was clinched when Rh-negative recipients reacting unfavorably to transfusion of Rh-positive blood stood transfusions of Rh-negative blood without an untoward reaction and with the usual evidences of beneficial effects of successful transfusions. Thus the majority of severe transfusion reactions in recipients with a history of previous uneventful transfusions was explained by Rh factor incompatibility.

In some cases of such reactions, agglutinins against Rh-positive blood could not be detected in the serum of the recipient. Three possible explanations were offered for their absence: (1) the tests were done too soon and the agglutinins were neutralized by the injected Rh-positive blood and a repetition of the tests after varying intervals of time revealed their presence in some instances; (2) the tests were done too late after the transfusion and the agglutinins have disappeared, as it is known to happen in other immunizations; (3) demonstrable agglutinins may not have been present in the circulating blood even before the transfusion—they may have been attached to the cells of the reticulo-endothelial system or their titer may have been too low to be detected by the available methods. The third explanation was substantiated by actual experience: Patients who developed hemolytic reactions fol-
lowing transfusion were found to be Rh-negative but did not show anti-Rh agglutinins on repeated examinations. The donors were Rh-positive. Transfusions with Rh-negative blood were free of reactions, while further transfusions with Rh-positive were followed by severe reactions.

The hypothesis of Wiener and Peters, confirmed by a long series of reports, can be accepted at the present time as offering an explanation for a majority of previously unexplained intra-group transfusion reactions and as furnishing means of avoiding many of them.

The second group of severe hemolytic reactions following first transfusions, as was mentioned, have been observed to occur mainly in pregnant women during or soon after delivery or after abortion. The application of sensitization by the Rh factor to the previously quoted case reported by Levine and Stetson7 gave the clue for an explanation.10 An Rh-positive husband of an Rh-negative wife may transmit the Rh factor to the fetus. Antigenic substances containing the Rh factor may pass from the fetus through the placenta to the mother and stimulate in her the development of anti-Rh agglutinins. If the mother needs a transfusion, the natural procedure would seem to be to use the husband as a donor if he belongs to the suitable blood group. It is obvious from our present knowledge, however, that the husband may be the least suitable donor, even if he is of the same group as his wife. If the foregoing situation regarding the Rh factor prevails and his blood is transfused, the result may be similar to that following a transfusion of incompatible blood: a severe or even fatal hemolytic reaction. Even if another donor is used instead of the husband, the chances are four out of five that the donor is Rh-positive and the result will be similarly disastrous.

A study of the blood of women who have suffered such transfusion reactions, of donors whose blood was responsible for them, and of babies (whenever that was possible) confirmed the existence of this immunologic state of affairs in a sufficiently large number of cases to permit the conclusion that this explanation is applicable to most instances of such reactions.

The questions may be asked at this point: Why is it that cross-matching tests prior to these transfusions done with the usual methods failed to show incompatibility? Why did repetition of
crossmatching tests, after the reactions had occurred, fail to reveal incompatibility? The answer is that testing for the Rh factor and for anti-Rh agglutinins requires a special technic, more sensitive and delicate than tests for the usual isoagglutinins. The isoagglutinins anti-A and anti-B, which are used to detect the four blood groups, A, B, AB and O, have as a rule a higher titer than the anti-Rh agglutinins. They react within a wide range of temperature, from icebox temperature where the titer is at its peak, declining slightly through room and body temperature, and remaining detectable in most instances even at temperatures close to 50° C. Anti-Rh agglutinins react, as a rule, best at body temperature, and lose their strength rapidly with changes of temperature in either direction, so that at room temperature, at which the usual grouping and crossmatching tests are done, Rh-agglutination is in most instances difficult to detect or is even absent.

Rare instances have been reported of anti-Rh agglutinins reacting better, or exclusively, at icebox temperature. The usual grouping and crossmatching tests are done on slides, Rh agglutination has to be carried in test tubes and requires prolonged sedimentation or centrifugation, which are known to favor all agglutination reactions. Thus it is apparent that the reason for the failure to discover Rh incompatibility prior to the introduction of the special sensitive technic was due to the usual methods of doing grouping and matching tests on slides and at room temperature, while Rh agglutination requires that the tests be done in test tubes at 37° C, and that the reaction be reinforced by fairly long incubation or by centrifugation.

THE RH FACTOR AND ERYTHROBLASTOSIS FOETALIS

After Levine had explained the transfusion reactions in pregnant women as due to sensitization of mothers by antigenic substances passing through the placenta from the fetuses, it was logical to consider the further consequences of this unique immunologic situation. The placenta is a two-way road. If antigenic substances pass through it from the fetus and cause the production of antibodies in the mother with the resulting potential danger to her, the passage of these antibodies from the mother through the placenta may have a damaging effect on the fetus. This line of thought had been taken up in the past by several investigators. Darrow came closest to what seems to be the solution of the problem when she incriminated chemical and antigenic differences in the hemoglobin of the fetus and mother as the responsible agents. She reasoned correctly that
the mother may be sensitized by the hemoglobin of the fetus, and that the antibodies thus produced may damage the child. The hypothesis was well conceived but lacked confirmation.

Levine's hypothesis substituted the Rh factor in the red cells of the fetus for the hemoglobin. Rh antibodies passing from the mother to the fetus damage its erythropoietic system and produce a hemolytic anemia and the other manifestations of the disease known as erythroblastosis foetalis.

Levine and his associates checked this hypothesis by the simple means of comparing the incidence of the Rh factor in mothers of babies with erythroblastosis with that in the general population. Levine found about 90 per cent of them Rh-negative, while the incidence of Rh-negatives in controls was about 15 per cent. The husbands of these women and the babies afflicted with the disease were found to be Rh-positive in almost 100 per cent of cases, while the incidence of this property in the general population is about 85 per cent. Statistical data published by others are in close agreement with Levine's.

It was found that many mothers of babies with erythroblastosis have anti-Rh agglutinins reacting with the blood of the baby. The serum of these mothers became the most important reagent for the detection of the Rh factor.

Two pertinent questions present themselves at this point. If erythroblastosis is based on the presence of the serologic condition of an Rh-positive father, an Rh-negative mother and an Rh-positive baby, how can this hypothesis be reconciled with the finding of about 10 per cent of Rh-positive mothers in Levine's own series and with similar findings by other authors? This can be answered by calling attention to the fact that fetal erythroblastosis is not a disease due to one specific cause, as for instance typhoid fever is due to the typhoid bacillus. It is in keeping with known facts about the disease that any agent capable of damaging the blood of the fetus and of producing a hemolytic anemia may start the chain of events that eventually produce the clinical syndrome known as erythroblastosis foetalis. Thus Rh antibodies are not the only agents capable of producing the disease, though apparently they are in most instances responsible. It is likely that various diseases of the mother may cause transmission of hemolytic substances to the fetus through the placenta and produce a result similar to or indistinguishable from fetal erythroblastosis. It is known that syphilis may do it. It is possible that other factors, thus far unknown, are operative.
The existence of antigenically differing fractions of the Rh factor has been demonstrated. It is theoretically possible, though still to be proved, that a mother and her child may both be Rh-positive but have different antigenic fractions. The mother may lack a fraction possessed by the child and thus become sensitized. It is therefore not necessary to construe the finding of Rh-positive mothers or of Rh-negative fathers or babies as in any way detracting from the validity of Levine's hypothesis. The fact is that the hypothesis is applicable in about 90 per cent of the cases, and this is as good a corroboration as any hypothesis may be expected to furnish.

The second question is: Why is erythroblastosis foetalis such a rare disease? The question is justified by the discrepancy between the relative frequency of the combination of Rh-positive husband and Rh-negative wife (calculated to occur in about 13 per cent of marriages), and the reported rarity of the disease (quoted in statistics to be from 0.1 to 0.2 per cent of births). This contradiction is more apparent than real and it can be explained by a consideration of various factors which are involved in the mechanism of the genesis of the disease.

In the first place, an Rh-positive father does not have to transmit the Rh factor to his child. The Rh factor of a person, his phenotype, is the result of inheritance from his parents. He may inherit the Rh-positive property from both parents. Then the formula of his blood is Rh Rh and he is homozygous; or he may inherit from one parent the Rh-positive property and from the other parent the Rh-negative property (designated as rh), and then his formula is Rh rh and he is heterozygous. There is no test available for distinction of homozygous persons from heterozygous. The child of a homozygous Rh-positive father will always be Rh-positive, while the child of a heterozygous father may be either Rh-positive or Rh-negative. This mechanism cuts considerably the incidence of Rh-positiveness in children. Kariher reported recently erythroblastosis in one of twins who was Rh-positive, while the Rh-negative twin remained well.

The next point has to do with the production of Rh antibodies in the Rh-negative mother of an Rh-positive child. It does not follow that in each case of such a combination the mother must produce Rh-antibodies. It is possible that passage from the fetus to the mother must be facilitated by an abnormally increased permeability of the placental barrier in order to permit the entrance of sufficiently large amounts of antigenic substances. We know from experimental investigations in man and animals that active immunization depends on such quantitative factors. This consideration is supported strongly by clinical evidence. Erythroblastosis
foetalis is extremely rare in the first child. As a rule, one or more normal children precede the birth of a child with the disease. This may be interpreted as indicative of the need of several successive immunizations before the antibodies in the blood of the mother reach the level necessary to exert the destructive action in the child. In this connection it may be added that women may differ in ability to produce antibodies, as is known to occur in infectious diseases and in animal experiments.

The previously mentioned fact that the incidence of erythroblastosis increases with the number of pregnancies, suggests another factor contributing to the rarity of the disease: the tendency to limit the number of children in modern marriages. In Javert's series, multiparity was associated with erythroblastosis in 92 per cent of the mothers. The disease is rare in primiparous women. It is interesting that in a case reported by Diamond the mother of a first-born child with erythroblastosis had received eight transfusions prior to pregnancy. The last two transfusions were followed by hemolytic reactions.

After the antibodies have been produced in the mother in sufficient strength they have to pass to the baby. Here again the permeability of the placenta enters into the picture. It is possible that it has to be abnormally increased and the permeability in the direction from the mother to the baby may be of a different nature than in the opposite direction. Both may have to be increased to permit the working of the postulated mechanism, thus again limiting the frequency of the disease.

Finally, recent reports suggest that erythroblastosis may occur in mild form, (sometimes as a relatively mild hemolytic anemia), and that it may not infrequently escape recognition. This point will be taken up later in greater detail.

The factors just enumerated seem to me to be sufficient to account for the apparent discrepancy between the true incidence of the disease and the number of marriages between Rh-positive men and Rh-negative women.

Although Levine's hypothesis is strongly supported by statistical evidence, it is possible to conceive certain further corroborative observations:

1. An Rh-negative wife of an Rh-positive husband, mother of one or several children with erythroblastosis, may marry again, this time an Rh-negative man, and have with him healthy Rh-negative children.

2. An Rh-negative mother of Rh-positive children with erythroblastosis, wife of an Rh-positive man, may consent to arti-
ficial insemination from an Rh-negative donor and conceive normal Rh-negative children.

3. An Rh-negative wife of an Rh-positive man may give birth to twins, one Rh-negative free of disease, the other Rh-positive suffering from erythroblastosis. The husband has to be, in such a case, heterozygous. The previously mentioned report of Kariher\textsuperscript{19} is the first instance of this type.

The Development of Manifestations of Erythroblastosis Foetalis

If it is agreed that Rh antibodies may produce hemolytic anemia in the fetus, how is the development of the other manifestations of the syndrome known as erythroblastosis foetalis to be explained? This is a question worthy of consideration in view of the heterogeneous manifestations of the disease of which the four most important ones are hemolytic anemia, erythroblastemia, icterus gravis, and congenital hydrops.

Figure 36 shows how these manifestations follow each other, step by step, in logical order. The results of hemolytic anemia are threefold: (1) Anoxemia (anemic anoxia) occurs as a result of a reduction of the oxygen-carrying capacity of the blood. (2) Hemolytic icterus develops from the destruction of blood and as a result of the presence of excessive amounts of components needed for formation of bile. This is an exaggeration of the normal tendency to jaundice in the newborn (icterus neonatorum). It differs from it by being present at birth (icteric vernix caseosa) or by appearing during the first day of life, while icterus neonatorum does not, as a rule, become manifest before the end of the second day. (3) Erythroblastemia, an abnormally increased number of nucleated red blood cells in the circulation, is a response of the bone marrow to hemolysis. The bone marrow of the newborn and of the child is known to respond to stimuli more intensely, quantitatively as well as qualitatively, than the marrow of the adult. It is known that nucleated red cells have a lower oxygen-carrying capacity than mature cells. A large number of such cells in the circulation adds to the anoxemia.

Each of the three results of hemolytic anemia leads to further developments, and these in turn are followed by others. The changes are frequently interconnected with one another. I hope that the chart (Fig. 36) will help to clarify this seeming maze.

In response to the anemia, foci of erythropoiesis develop in different organs. For the purpose of this discussion the most important are the changes in the liver. One finds frequently large masses of immature erythroblasts in the sinusoids compressing the liver parenchyma. In addition liver cells are frequently gorged with
hemoglobin and finally damage to the endothelium of the bile capillaries by the anemic anoxia adds the third factor leading to interference with normal hepatic function. Damaged liver function manifests itself in three ways, as shown in Figure 36. One is hypoproteinemia, the formation of blood proteins being one of the main

**Figure 36.—Pathogenesis of erythroblastosis foetalis.**

functions of the liver. Hypoproteinemia, in turn, leads to edema which has the oldest history of all manifestations of erythroblastosis, having been known for centuries as congenital hydrops. The other result of hepatic damage is a hepato-cellular type of jaundice, which tends to intensify the previously mentioned hemolytic jaundice. Liver damage is known to be associated with macrocytosis, which
is present in erythroblastosis. Macrocyes are less efficient carriers of oxygen than normocytes and so contribute to the anoxemia, similar to erythroblasts.

The anoxemia, the development of which we have traced from at least three sources, hemolytic anemia, erythroblastemia, and macrocytosis, results in endothelial damage, which is responsible for three types of changes. It causes edema, thus acting synergistically with the hypoproteinemia. Endothelial damage explains the purpura, an occasional complication of the disease. Finally, the damage to the endothelial lining of biliary capillaries together with the stasis of bile due to hemolytic jaundice contribute to formation of bile plugs in the liver which lead to intrahepatic biliary obstruction and to obstructive jaundice. Thus we have now the third element in the formation of the severe jaundice of erythroblastosis, the icterus gravis.

The chart makes it clear, I hope, why edema is the most serious manifestation of erythroblastosis and the least frequently reversible. That is the reason why infants with generalized congenital hydrops recover so rarely. In Javert’s series the mortality was 100 per cent. Anemia and erythroblastemia are usually most pronounced in this form; jaundice may be masked by edema. Icterus gravis is next in seriousness, with a mortality of 54 per cent (Javert). It is likely that its seriousness and the chances of recovery may depend on the associated liver damage. It is justifiable to conclude from the chart and in keeping with clinical experience, that hemolytic anemia may be present as the only manifestation of the disease, with or without excessive erythroblastemia. If mild, it may be overlooked entirely or it may not be interpreted as a manifestation of erythroblastosis. The suggestion has been made that the disease would be better called hemolytic disease of the newborn. The suggestion has much in its favor, as hemolytic anemia is the basic change in the disease and erythroblastemia is a less significant finding.

There is a tendency in recent publications to consider every case of hemolytic anemia of the newborn, even when it is moderate or mild, and even without erythroblastemia or jaundice, a manifestation of, and synonymous with erythroblastosis, especially if the distribution of the Rh factor in the family is of the type characteristic for erythroblastosis, father and child positive, mother negative. This is an extreme view likely to lead to inclusion of cases which have nothing to do with erythroblastosis with resulting confusion. No student of the subject will deny that there are instances of hemolytic anemia of the newborn which are not erythroblastosis foetalis. Septic infections, neonatal and prenatal bronchopneumonia
may be associated with severe hemolytic anemia. It is sometimes extremely difficult and even impossible to diagnose the underlying infection clinically. Only an autopsy may reveal it.

The diagnosis of erythroblastosis is not always easy, especially in mild abortive cases, and an autopsy may be essential for a definite diagnosis. There are instances in which even autopsy findings may not be unequivocal, especially in premature infants and in stillbirths. In such cases a study of the Rh factors in the parents and child and of anti-Rh agglutinins in the mother may be of considerable help in arriving at a correct interpretation. It has already been stated that this cannot be relied on too implicitly and I have had one example of an Rh-positive husband of an Rh-negative mother of a perfectly healthy Rh-positive infant, with well developed anti-Rh agglutinins in the mother. Levine reported similar findings in two women.

Illustrative Cases

Two cases will be quoted to illustrate the role of tests for the Rh factor in obscure blood disease in infants.

Case I.—A five-month-old white girl, patient of Dr. E. Padnos, was admitted on July 12, 1943, suffering from a severe anemia, with a history of jaundice at birth lasting for over a month. The child did not thrive well and passed bloody stools on several occasions. She was very pale. The spleen and liver were palpably enlarged.

The accompanying tabulation summarizes some of the blood findings. The differential blood smears showed marked macrocytosis, moderate anisocytosis, poikilocytosis and hyperchromia, and slight polychromatophilia. The reticulocytes were 3.6 per cent, platelets 108,000, coagulation time nine minutes, bleeding time over ten minutes, icterus index 11 and 13. The fragility test showed normal values. Serologic tests for syphilis were negative.

In view of the history of jaundice at birth and of the severe anemia, hemolytic disease of the newborn was considered. The father and the child were B Rh-positive, the mother O Rh-negative. There were no demonstrable anti-Rh agglutinins in her serum. The father was used as donor in both transfusions. The use of an Rh-negative donor was not considered necessary in view of the age of the child. Any antibodies that the child may have had at birth would have been eliminated by this time.

The child left the hospital eighteen days after admission, very much improved, and has remained well since.
# Tabulation

**Blood Findings in a Young Infant with Severe Anemia, Enlarged Liver and Spleen and a History of Icterus Neonatorum**

<table>
<thead>
<tr>
<th>Date</th>
<th>R.B.C.</th>
<th>Hb.</th>
<th>W.B.C.</th>
<th>Neutrophils %</th>
<th>Eosinophils %</th>
<th>Lymphocytes %</th>
<th>Monocytes %</th>
<th>Normoblasts*</th>
</tr>
</thead>
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<tr>
<td>7/13</td>
<td>1,110,000</td>
<td>4.8</td>
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* Per 100 white blood cells.
Was this a case of erythroblastosis foetalis? The history of prolonged jaundice at birth, the clinical picture and hematologic findings would fit into that diagnosis. Purpura is not infrequently observed as a complication. The prompt response to transfusions is also more commonly seen in this disease than in other blood disorders. Finally, the Rh formula in the family is in favor of the diagnosis of late erythroblastosis foetalis with purpura.

Another diagnostic problem presented itself in Case II.

Case II.—A five-month-old girl, patient of Dr. B. M. Gasul, was admitted with a history of extreme restlessness with rigidity of the body and retraction of the head for the past two months. The weight at birth was 8 pounds and was the same on admission. The child was born at term, the delivery was spontaneous. The mother stated that the child became jaundiced at the age of four days and that jaundice persisted for two months.

The red blood count was 4,830,000 and 5,380,000, the hemoglobin was 15.4 gm. (92.4 per cent) and 14.9 gm. (89.4 per cent). The other findings including the differential blood count were not remarkable. Serologic tests for syphilis were negative.

In the course of blood grouping and crossmatching tests it was found that the serum of the mother clumped cells of groups A, B, O and AB, though she was of group AB. Serum of group AB should not clump cells of any group. It was established that she was Rh-negative and had strong anti-Rh agglutinins, that her husband was A Rh-positive, and that the child was AB Rh-positive. This was the third child in the family; the first, a girl five years old, was normal, the second a three-year-old boy, was jaundiced at birth but had been well since.

In the differential diagnosis between birth injury and a residual involvement of the central nervous system with Kernicterus due to icterus gravis, the serologic findings of an Rh formula typical for erythroblastosis foetalis and of anti-Rh agglutinins in the mother favored the last mentioned diagnosis.

Kernicterus with spastic manifestations and evidence of mental deficiency has long been considered a late complication of icterus gravis. This case shows the value of Rh tests in such cases. In view of the opinion that some forms of juvenile cirrhosis may be another residual condition after erythroblastosis, as a result of severe hepatic damage, studies of the Rh factor in such families may be of interest.
Testing for the Rh factor may be done either with animal (guinea pig) or with human immune serum. The serum of guinea pigs, immunized with rhesus blood, may show significantly higher titers for Rh-positive blood.

Human serum with anti-Rh agglutinins may be obtained, rarely, from Rh-negative patients who had received repeated transfusions of Rh-positive blood, and more frequently, though still rarely, from Rh-negative mothers of babies with erythroblastosis.

For technical details regarding preparation of animal serums and their uses, detection of anti-Rh agglutinins in human serums and their uses, elimination of interfering isoagglutinins, crossmatching tests for Rh incompatibility, which can be done without available anti-Rh serums, the interested reader is referred to the bibliography.\textsuperscript{8, 9, 16, 17, 22, 23}

**PRACTICAL APPLICATIONS**

**Repeated Transfusions**

Patients who are receiving repeated transfusions may benefit by having their blood tested for the Rh factor. If it is found negative and if Rh-negative blood is available it is advisable to use it for two reasons: reactions will be avoided if the patients have become sensitized previously, otherwise sensitization will be prevented. Experience has shown that severe hemolytic reactions in patients receiving repeated transfusions have, as a rule, been preceded by mild or moderate reactions which have been disregarded. The lesson from this is to test for the Rh factor the patient who is receiving repeated transfusions and in whom a reaction, especially with hemolytic features, has been observed.

It should be noted that the sensitive crossmatching technic for Rh incompatibility may give a patient in need of transfusion a measure of protection even if Rh tests are not done.

**Transfusions in Pregnancy**

Pregnant women, or those recently pregnant, should receive the benefit of the new knowledge. They are especially in danger if there is a so-called bad obstetrical history of previous births of babies with edema, jaundice, and of stillbirths. Rh-
negative donors are to be used, of the same group as the patients, or of group O. The latter when Rh-negative are better than Rh-positive ones, even of the same group as recipients. The husband should not be used, unless expert serologic tests have eliminated any evidences or even possibility of isoimmunization during pregnancy.

**Transfusion Therapy in Erythroblastosis Foetalis**

Transfusion is the therapy of choice for newborn children with any manifestations of erythroblastosis. It is important that blood be given as early as possible and that indications for further transfusions be controlled by the condition of the child. It is essential that Rh-negative blood be given, although the infants are Rh-positive. It is assumed that they may have some of the anti-Rh antibodies in their circulation or attached to the reticulo-endothelial system, ready to react with injected Rh-positive blood. If known Rh-negative blood is not available the next best course is to use the mother’s blood cells, after the plasma is removed and the red cells washed twice with sterile saline and resuspended in sufficient saline to make up for lost volume. This is always a safe procedure because an infant never has isoagglutinins in his serum directed against the cells of his mother.

It has been recommended that mothers be not permitted to nurse infants with erythroblastosis, because anti-Rh agglutinins have been found in breast milk. The results of prompt transfusion therapy in erythroblastosis have been extremely gratifying. Javert reported a drop in mortality from 73 per cent to 14 per cent since 1941 in his series. This success is especially significant in view of the fact that erythroblastosis has been listed as responsible for 3.2 per cent of fetal and infant mortality.

**BIBLIOGRAPHY**


