

THE Rh FACTOR *

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THE first step in the discovery of a new agglutinable substance in human blood was made by Landsteiner and Wiener in 1937 (1), when they observed that agglutinins would form in certain anti-rhesus immune sera which were specific for the human agglutinogen. Following this they immunized rabbits with red blood cells of a rhesus monkey and obtained an immune serum which contained agglutinins specific for a human agglutinogen different from A, B, M, N and P (2). To show that rhesus blood was used in its production, the factor was given the name Rh. It was then found to be present in 85 per cent (designated Rh positive), and absent in 15 per cent (designated Rh negative) (2, 3) of the white population tested. Later in 1940, Wiener and Peters (3) reported cases in which repeated transfusions of properly grouped blood gave rise to severe hemolytic reactions and two deaths. The reacting substance in the serum of each patient corresponded to the agglutinin of the immune rabbit serum prepared by Landsteiner and Wiener by the injection of rhesus blood.

The red blood cells of Rh positive persons contain the Rh agglutinogen (antigen). In Rh negative persons this factor is lacking, but on the introduction of Rh positive red cells in their circulation, agglutinins (antibodies), which are specific for the Rh agglutinogen, can develop in their serum. This is contrary to the normal presence in the blood of specific agglutinins for the agglutinogens A and B.

The basis, then, for a reaction would be, first, the transfusion of Rh positive blood into an Rh negative recipient, thus offering an antigen for immunization, and, secondly, a subsequent transfusion of Rh positive blood after anti-Rh agglutinins have developed in the recipient's serum.† In the latter, the combination of agglutinogen and agglutinin produces agglutination or clumping (fig. 1).

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† In answer to a recent inquiry regarding the length of time for the normal processes of immunization to come into play after Rh positive blood is transfused into an Rh negative individual, Dr. A. S. Wiener said, "Almost all Rh negative individuals do not sensitize against the Rh factor when Rh positive blood is injected into them, or when they bear an Rh positive

The Rh factor usually can be disregarded in the selection of donors for primary transfusions because anti-Rh agglutinins do not develop naturally in Rh negative persons, although some evidence has been presented to show this may occur (4). In a person receiving repeated transfusions, the possibility of such a reaction becomes of increasing importance since 85 per cent of donors' bloods contain the Rh antigen for iso-immunization if the recipient is Rh negative. Evidence of incompatibility with the donor may be lacking by the usual crossmatching technic, and even the most delicate methods often fail to detect agglutination (5). The determination of the presence or absence of the Rh factor in the recipient's blood will determine whether or not a reaction can occur. If the recipient is Rh negative, he, of course, should receive blood only from an Rh negative donor.

MECHANISM OF Rh+ TRANSFUSION REACTION

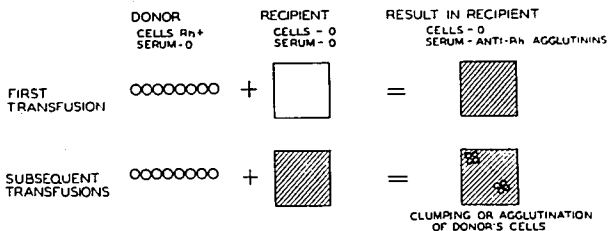


FIGURE 1.

The following case, which was previously reported by Norcross (6) illustrates a transfusion reaction which was caused by the Rh factor.

Case 1.—A 41-year-old man entered the Clinic complaining of weakness of three months' duration. He had received frequent injections of nearsphenamin between 1934 and 1936 and had had intermittent treatment since that time. Two months before admission bleeding of the gums developed and he received four transfusions over a ten-day period.

Examination revealed marked pallor, a questionably enlarged spleen, hemorrhagic retinitis and profound anemia with a hemoglobin of 2.8 Gm. (19 per cent). A specimen of bone marrow obtained for biopsy showed hypoplasia of all blood elements.

In view of the findings a diagnosis of aplastic (more correctly, hypoplastic) anemia was made. On the day of admission, during transfusion, lumbar pain developed, with a severe chill followed by fever after only 200 cc. of blood had been given. The blood was Rh negative and contained anti-Rh agglutinins.

fetus. Only about 1 in 50 become sensitized, and these vary greatly in the ease with which they were sensitized. Some become sensitized after a single transfusion or at the very first pregnancy, while others require up to ten or more transfusions or pregnancies before becoming sensitized."

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The donor was Rh positive. Following this the patient was given only Rh negative blood, and in the course of the next nine months he received a total of twenty-two transfusions without reaction.

An important exception to disregarding this factor in the selection of donors for primary transfusion is intrapartum and postpartum patients. Wiener and Peters (3), in a review of the literature, pointed out that apparently every recorded instance in which a first transfusion produced a hemolytic reaction with properly grouped blood had occurred in intrapartum and postpartum patients. Levine and Stetson (7), in 1939, reported a case of intragroup transfusion reaction following delivery of a dead fetus, and demonstrated agglutinins in the patient's serum for her husband's cells and those of 80 per cent of donor tested. They postulated the occurrence of an immunizing property in the blood or tissues of the fetus which must have been inherited from the father. Iso-agglutinins resulting from iso-immunization in pregnancy and causing transfusion reactions were reported by Levine and Katzin (8) with one serum corresponding in specificity to the anti-Rh of Landsteiner and Wiener (2). Subsequently the responsible agglutinin in most of these reactions was found to correspond to the anti-Rh agglutinin.

Here the setting for a reaction is an Rh negative mother and an Rh positive fetus, the latter having inherited the Rh factor from the father. During pregnancy some of the fetal red blood cells containing the Rh agglutinogen find their way into the maternal circulation, presumably through some placental defect, and immunize the sensitive mother to produce anti-Rh agglutinins. Should a transfusion then be necessary the chances are that the husband or another Rh positive donor (85 to 15) will be selected and a reaction, which is often fatal, may follow. An Rh determination on the blood of the mother would give cause to anticipate such a reaction with the indiscriminate use of donors and the reaction could be avoided. Compatible blood would be found only in Rh negative donors.

The following case, previously reported by Burnham (9), shows the difficulty in pregnancy that may arise from the Rh factor.

Case 2.—A woman, aged 32 years, had had two previous pregnancies. The first infant was delivered at term and died two hours later. A diagnosis of erythroblastosis foetalis subsequently was made. The second pregnancy was terminated at eight months by the delivery of a macerated hydrocephalic fetus. In the third pregnancy an elective cesarean section was performed before term in the hope of obtaining a living child. The infant showed anemia and jaundice and died in twelve hours in spite of transfusion, given six hours after birth, 50 cc. of blood from the father.

The diagnosis at autopsy was erythroblastosis foetalis.

Because of considerable loss of blood, the mother was given a 500 cc. blood transfusion, using the husband as donor, following the operation. There was no obvious evidence of a reaction, but the patient continued to bleed more than

usual. Using the husband again as donor, a second transfusion of 500 cc. was given on the first postoperative day. One hour later there was severe pain in the abdomen and a shaking chill occurred which lasted thirty minutes. Oliguria was followed by retention of nitrogenous end products. The danger of further transfusion was appreciated, but the need seemed so great that a transfusion of 250 cc. was given on the fourth postoperative day. This was followed in one hour by a severe chill. Plasma and fluids intravenously in large amounts were started. An Rh determination showed the mother to be Rh negative. The baby and husband were Rh positive and the blood used for the first three transfusions was Rh positive. Anti-Rh agglutinins were demonstrated in the patient's serum on the eighth day. On the twelfth day, 500 cc. of blood from an Rh negative donor was given without a reaction of any kind. Administration of slightly incompatible blood on the fifteenth day was followed by a chill. Three transfusions of Rh negative blood were given on the eighteenth, twentieth and twenty-third days, respectively, without reaction. The patient began to recover and was discharged on the thirty-sixth day.

Light was thrown on an enigma of the past when Levine and his associates (10, 11, 12) showed the importance of the Rh factor in the

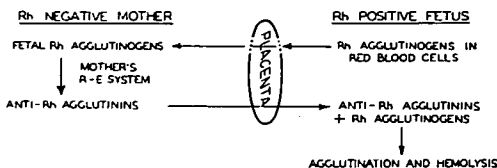


FIGURE 2.

pathogenesis of erythroblastosis foetalis. An Rh positive fetus which has inherited the factor from the father iso-immunizes a sensitive Rh negative mother. Anti-Rh agglutinins produced in the maternal circulation cross the placenta into the fetal circulation and destroy the fetal red blood cells, producing jaundice, edema with effusions into the serous cavities and compensatory hematopoiesis. The mechanism causing the disease is illustrated in figure 2 (6).

Over 90 per cent of the mothers of these infants with erythroblastosis foetalis are Rh negative, and in 50 per cent of the mothers the anti-Rh agglutinins are demonstrable in vitro two months after delivery. With lapse of pregnancy they gradually disappear, but in 2 cases they have been found present two years following delivery. In the remaining mothers who are Rh positive and produce infants with erythroblastosis foetalis, there is evidence that the Rh factor may also be related to the production of the disease (13). The correct selection of donor in these infants is often lifesaving. Anti-Rh agglutinins derived from the maternal circulation may be stored in the tissue cells of the infant and be released slowly into the infant's blood stream unabsorbed follow-

ing birth. Introduction of Rh positive blood would only present more antigens for agglutination. The donor of choice is an Rh negative person but not the mother since her serum contains anti-Rh agglutinin. The mother's breast milk has been shown to transmit the agglutinin (14), and the forbidding of breast feedings may be a valuable adjunct in the treatment of babies who are suffering from erythroblastosis foetalis.

The following case, reported by Wiener and Wexler (15), illustrates the value of the careful selection of donors for these infants.

Case 3.—A female infant, 25 days old, was admitted to the hospital with diagnosis of acute hemolytic anemia of the newborn. The hemoglobin was 15 per cent. She had been delivered at another hospital where, it was stated, she was observed to have become extremely pale shortly after birth. When she was 7 days old the hemoglobin was 27 per cent, and she was transfused with blood from an apparently compatible donor, without taking the Rh factor into account. Following the transfusion, the hemoglobin rose to 36 per cent, and on the eleventh and fourteenth days, after additional transfusions, the hemoglobin was 45 and 52 per cent, respectively. The exact amount of transfused blood was unknown. Because of the poor response to treatment, further studies were carried out, and the mother's blood was found to be Rh negative. This, then, was evidently a case of erythroblastosis.

The patient was given transfusions of Rh negative blood totaling 160 cc., and the hemoglobin rose from 35 per cent on admission (age 25 days) to 80 per cent on discharge, eight days later.

The patient was readmitted at 7 weeks of age with a hemoglobin of 53 per cent, a red cell count of 2,900,000 and reticulocytes of 1.5 per cent. She was given 100 cc. of Rh negative blood, following which the hemoglobin rose to 70 per cent, and, as far as could be ascertained five months later, there has been no recurrence of the anemia.

The first child of parents producing infants with erythroblastosis foetalis often escapes the disease. The explanation is thought to be that a single pregnancy may not afford the necessary stimulus for the production of adequate amounts of anti-Rh agglutinins. Other children in these families may all be born with erythroblastosis foetalis or some may be normal. Explanation of this difference is found in the genetic make-up of the father. Landsteiner and Wiener (16) concluded that the factor is inherited as a simple mendelian dominant which is not sex-linked and is apparently not linked to the red blood cell agglutinogens A and B or subtypes M, N and P. It is transmitted by a pair of genes designated Rh (dominant) and rh (recessive). The dominant gene Rh determines the presence of the factor in the individual. Three combinations or genotypes therefore would exist and are Rh Rh, Rh rh, and rh rh. The first two containing dominant genes would correspond to the phenotype Rh positive, and the third containing only recessive genes would correspond to the phenotype, Rh negative. The possible combinations of genotypes in a marriage are shown in table 1.

TABLE 1
HEREDITY OF THE Rh FACTOR

Possible Combinations of Genotypes	Genotypes Producing Erythroblastosis Foetalis			
	Father	Mother	Offspring	
			Erythroblastosis Foetalis	Normal
Rh Rh - Rh Rh	Rh Rh	rh rh	Rh rh	rh rh
Rh Rh - Rh rh				
Rh rh - Rh rh	Rh rh	rh rh	Rh rh	rh rh
Rh Rh - rh rh				
Rh rh - rh rh				
rh rh - rh rh				

Rh negative (Rh -) persons are homozygous, genotype rh rh.

Rh positive (Rh +) persons are homozygous, genotype Rh Rh, or heterozygous, genotype Rh rh.

All pregnancies resulting from a homozygous father (Rh Rh) and an Rh negative mother (rh rh) could be expected to result in erythroblastosis since each infant inherits the Rh factor. When the father is heterozygous (Rh rh), only half of the pregnancies would be expected to result in the disease since the factor is not inherited by those with recessive genes (rh rh).

Three main varieties of anti-Rh serum have been identified in the white race and are classified by the characteristic percentages of positive reactions they give with the agglutinin. These are called anti-Rh₁, anti-Rh₂ (11), and anti-Rh₃ and anti-Rh₄ (5, 16). The percentages of positive reactions they give are 87, 85, and 73, respectively. The antiserum used as standard is Rh₁. Recently a new anti-Rh agglutinin has been described which reacts with about 35 per cent of white persons tested. With these different antisera the Rh agglutinin has been subdivided into different varieties (17).

The statistics for table 2 were gathered by Levine (18) and show the distribution of the Rh factor as reported in different races, using anti-

TABLE 2
TESTS WITH ANTI-Rh SERA

Race	Anti-Rh ₁			Anti-Rh ₂		
	Number Tested	Percentages		Number Tested	Percentages	
		+	-		+	-
White (19)	334	85	15	334	73	27
Colored (18)	264	95.5	4.5	118	46	54
Colored (20)	113	92	8			
American Indians (21)	120	99.2	0.8	69	58	42
Chinese (22)	150	99.3	0.7	150	93	7

serum Rh, and antiserum Rh. From this a low incidence of erythroblastosis can be expected in the colored race, and the disease should be rare among the Chinese.

Satisfactory anti-Rh serum for testing usually is produced in guinea pigs since more uniform results are given. Human anti-Rh serum, as a rule, is satisfactory, but it is difficult to obtain, gradually decreases in titer of antibodies and results obtained vary, depending on the variety of anti-Rh serum used. Agglutination produced by anti-Rh serum is much weaker than agglutinative reactions produced by anti-A and anti-B sera. A very sensitive technic, therefore, is a necessity in most cases where the Rh agglutination is to be demonstrated. Directions for preparing anti-Rh sera in guinea pigs and the sedimentative technic employed in determining the presence of the Rh factor are adequately described elsewhere (16, 23).

CONCLUSIONS

From this discussion, it is suggested that an Rh determination be done on the blood of any patient who is to receive repeated transfusions or who has received transfusions in the past.

When it is seen that conditions are present for a reaction, but the presence or absence of the Rh factor is not known, two courses are open, either an Rh determination on the recipient's blood or the use of an Rh negative donor. Of the two, the former is recommended as this settles the doubt and may conserve the supply of Rh negative donors. An Rh determination should be done on the blood of all pregnant women. All expectant mothers who are Rh negative should be hospitalized where Rh negative blood can be obtained both for the mother and child if need for transfusion arises following delivery. An adequate list of Rh negative donors in the four major blood groups should be made available for emergency use in any institution equipped to do transfusions.

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The Section on Anesthesiology of the American Medical Association will be held during the week of the Scientific Session in Chicago, June 12-16, 1944. The scientific sessions and the technical exhibits will be held in the Stevens Hotel and the scientific exhibits in the Palmer House.