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THE Rh FACTOR AND ITS CLINICAL APPLICATIONS

PHILIP LEVINE
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The author discusses the so-called incomplete or blocking antibody of Race and Wiener. With this reaction and a more recently described slide test of Diamond and its modification by Wiener, it is possible to provide proof of active iso-immunization in almost all instances.

With regard to the complexity of the RhHr system, the author believes that the clinician should think in terms of Rh positive and Rh negative as indicated by the most important anti-Rho serum, the only one available for distribution. This serum will aid in detecting 92 per cent of all those potentially immunized by the Rh factor. In order to detect iso-immunization in the remaining Rh positive individuals, the blood should be referred to specialists acquainted with the finer subdivision of the Rh and Hr factors and their heredity.

Finally the author stresses the importance of transfusing all Rh negative individuals, especially all of the female population even as infants, with Rh negative blood. This measure by itself will reduce the number of erythroblastotic infants and the severity of the condition.—P. L.

The author presents evidence that the Hr factor is an integral part of the Rh system of variants and its exclusion from any genetic theory is not warranted. Anti-Hr sera and anti-Rh' sera describe three varieties of reactions much like anti-M and anti-N. A type of blood failing to react with both antibodies does not exist. The view that anti-Hr sera are weak because of genetic reasons cannot be accepted. Anti-Hr sera are generally weak because of the simple statistical considerations that 92 per cent of Rh negative women produce anti-Rh agglutinins while only 1 or 3 per cent Hr negative women can produce anti-Hr agglutinins.

Anti-Hr sera are of value clinically because they select the homozygous Rh,Rh, where bloods lack the Hr factor.

(The British workers always took into account the Hr factor in their analysis of the genetics of the Rh factor. On a theoretical basis they assumed the existence of three varieties of anti-Hr sera to correspond with three varieties of anti-Rh sera to give 8 genes [Race: Nature 123: 774, 1944].) —P. L.

The author reports two of six cases of iso-immunization and erythroblastosis fetalis in which the mother was in group O and the affected infant in group A. That iso-immunization against the A factor occurred in individuals by a specific increase in the anti-A titer. It is implied that the fetus in these cases must be of the nonsecretor type. Iso-immunization by the A factor as well as the finer differences within the Rh-Hr complex explains the origin of erythroblastosis fetalis in the 8 per cent of Rh positive mothers.—P. L.

The author produced evidence that the slide test of Diamond depends upon the presence of serum rather than heavy suspensions of blood. The test could be carried out in test tubes with washed blood suspended in normal human serum to make about 2 per cent suspension.
The use of test tubes makes it possible to study the specificity of the blocking antibody which gives agglutination reactions if normal serum is used instead of saline both for suspension of the cells and in titrating the activity of the blocking antibody. At the same time, the supply of testing serum is increased.

(Wiener uses the term "conglutination," but the use of this term is criticized by Coombs, Mourant, and Race on the ground that it does not in any way resemble the phenomenon described by Border [Brit. J. Exper. Path., 1945]. There is still no evidence that this reaction is the basis for Chown's capillary tube test of saline suspended cells.)—P. L.

Prevention of Unintentional Isoimmunization of the Rh Negative Female Population. Philip Levine:

The author studied the incidence and severity of erythroblastosis fetalis in the first-born as influenced by previous immunization with Rh positive blood. He found about twice as many cases in the transfused series, and 60 per cent of these ended in fetal death. In the nontransfused group there was no fetal death and the symptoms were much milder. The possible role of intramuscular injection of Rh positive blood is discussed.

Levine recommends that the entire Rh negative female population requiring transfusion even as infants receive Rh negative blood only. This simple measure should reduce the incidence of erythroblastosis fetalis, especially in its most severe forms.

The biologic test of Wiener is not recommended on the ground that it may serve to immunize the Rh negative individual. (The one fetal death in the nontransfused group could be excluded from the series since the death could be attributed to other factors.)—P. L.


The principle of the test is the use of an anti-human globulin serum which will react directly with Rh positive blood previously coated with the incomplete antibody or with weakly agglutinating serum. The test is useful for the detection of fine degrees of sensitization not readily demonstrable by other methods (agglutinins or blocking antibodies).—P. L.


This general review of the subject contains a few noteworthy observations. Davidsohn points out that Rh negative women may be in the negative phase of iso-immunization for a short period following the delivery of an erythroblastic infant. Accordingly, the chances for detecting anti-Rh antibodies are best about eight to twenty days following delivery. Presumably at delivery fetal Rh positive blood must be released into the maternal circulation in sufficient quantities to neutralize circulating antibodies.

The observation of Witebsky that anti-Rh antibodies are found also in milk is confirmed. The persistence of anti-Rh agglutinins following delivery and its influence on the outcome of the next pregnancy are discussed. The observation of Levine that most anti-Rh agglutinins belong to the class known as warm agglutinin is confirmed. Although Davidsohn discusses the several varieties of anti-Rh agglutinins, he fails to emphasize that the anti-RhA serum is clinically the most important variety, which will detect more than 90 per cent of instances of iso-immunization by transfusion and pregnancy.—P. L.


The authors make the significant observation that the blood of each of fifteen chimpanzees possessed the Hr factor, but not the Rh factor. Accordingly, they behave like human Rh negative bloods. It is suggested that this uniformity is the result of selective effect of iso-immunization in pregnancy.—P. L.


The authors injected small amounts (7-10 cc.) of Rh positive blood into carefully selected Rh negative patients in order to maintain high levels of anti-Rh titer. It is highly probable that much smaller amounts of Rh positive blood will suffice. If this method, carried out on a voluntary basis, were entirely practicable, it would solve the problem of a sufficient supply of human anti-Rh diagnostic sera.
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By selecting the variety of Rh positive blood to be injected into these patients, it may be possible to produce anti-Rh agglutinins of the several varieties.—P. L.


The author concludes that iso-immunization by the Rh factor does not play an important role in early fetal death. It is only during the latter half of the pregnancy that the iso-immunization is initiated, and a certain interval of one or more months of intra-uterine hemolytic action on fetal Rh positive blood is required before the fetus is affected. Endocrine deficiencies are probably of more importance in habitual abortion.

(The author does not refer to the possible role of iso-immunization by the factors A and B in habitual abortion as shown by Levine in the J. Hered. 35: 71-80, 1945.)—P. L.


This brief report is significant because it represents the first organized effort to carry out prenatal tests as a public health measure on a country-wide basis. The list of Rh negative men and women was made available for the formation of a group of Rh negative donors whose blood was used for the treatment of erythroblastotic infants.—P.L.


Diamond and Abelson describe a procedure which facilitates the detection of the highest incidence of iso-immunization in Rh negative women immunized either by fetal blood or by repeated transfusions. The method consists of use of slides instead of test tubes and heavy suspensions of blood instead of 2 per cent cell suspensions. More specifically, they recommend the mixture on a slide of 0.1 cc. of the serum to be tested and 0.2 cc. of fresh oxalated blood or a 40-50 per cent washed blood suspension (presumably in saline).

The authors believe that "inhibiting," "blocking," or "incomplete" antibody, as it is variously termed, and agglutinins exist independently in most sera. Of 90 sera of mothers of erythroblastotic infants, only 37 had anti-Rh agglutinins tested by the incubation technic but the slide test was positive in all. In all but a few, the indirect test for blocking antibodies was positive in the 53 sera which were negative by the incubation technic.

In the presence of an excess of cells, 8 to 10 parts of cells and 1 part of serum, hemolysis was observed.—P.L.


The authors found that many Rh negative mothers of erythroblastotic infants contain their so-called inhibiting antibodies which are identical with the incomplete antibody of Race and the blocking antibody of Wiener. These antibodies unite with Rh positive blood, but the second stage of the reaction, i.e., the visible effect of agglutination, does not occur under the usual conditions of testing. These antibodies correspond in specificity to those of anti-Rh, anti-Rh', or anti-Rh" agglutinins.

Prolonged heating at 56° inactivates the agglutinin, but not the blocking antibody. Of 50 sera of Rh negative mothers recently immunized, 30 had strong agglutinins, 10 had strong "inhibiting" antibodies, and the remaining 10 had both sorts of antibodies.

Diamond and Abelson report an interesting experiment carried out by injecting an Rh1 individual with a serum containing "inhibiting" antibodies. The patient's cells were now 16 times less sensitive to agglutination by anti-Rh sera and, significantly, the patient's sera now contained anti-Rh agglutinins. The result can be understood if one assumes that serum used for the injection also had anti-Rh agglutinins which were masked by the action of the inhibiting antibodies. This was confirmed by the authors by in vitro absorption experiments.—P.L.


Ninety-eight Mexican Indians were tested for the several agglutinable properties. The results showed
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a high incidence of group O (91 per cent), 61 per cent M, 3.1 per cent N, and 78.9 per cent P. All were Rh positive with anti-Rh_2 serum, and the incidence of the subtypes was Rh_1 48 per cent, Rh_3 41.8 per cent, and Rh_0 1 per cent. The Hr factor was present in 55.8 per cent. Three individuals of type Rh_1 Rh_2, who were Hr negative contained the gene Rh_0, which is so rare in white individuals.—P.L.


Witebsky and Mohn present indisputable evidence that, contrary to the views previously held, the Rh factor occurs in a water-soluble form. In their studies using amniotic fluid, the authors found considerably less Rh active material than A and B substances in the amniotic fluid of secretors. As with the A and B substances, there are both secretors and nonsecretors, in about the same proportion as with A and B, i.e., about 80 per cent and 20 per cent respectively. There is, however, no correlation whatever of A and B and Rh secretors or nonsecretors.

In three cases of Rh negative mothers of erythroblastotic infants, it is significant that the three Rh positive affected infants proved to be nonsecretors.—P.L.


This comprehensive review is based on a large experience with material tested at the Blood Grouping Laboratory, Children's Hospital, Boston. The author stresses the severity of the hemolytic disease in infants if the Rh negative mother was previously immunized by transfusions. The incidence of erythroblastosis fetalis in the author's series is one in 150 full term deliveries. Diamond refers to experimental iso-immunization in ten Rh negative men, six of whom developed satisfactory titers.

(The author does not sufficiently differentiate the varying importance of the several varieties of anti-Rh sera, one of which, it was shown, will detect 92 per cent of all instances of iso-immunization. Also there is no evidence to indicate "that the placental barrier must be faulty" to permit transplacental iso-immunization.)—P.L.


The authors now believe that their slide agglutination test cannot be elicited with the use of washed blood. The presence of serum rather than a heavy cell suspension is the important factor. The various tests for detection of iso-immunization against the Rh factor are discussed and evaluated. The open slide test is most useful as a rapid screening test for selection of sera containing blocking antibodies.

The highest degree of accuracy is obtained by the several tests (agglutination, blocking, and the use of the serum suspended cells).

(The biological test of Wiener is described, but there should never be any need for this measure, which may serve only to increase the degree of iso-immunization.)—P.L.


The production of specific anti-Rh agglutinins in guinea pigs injected with human blood is described. Each of the six guinea pigs developed anti-Rh agglutinins which after absorption with Rh negative blood gave distinct differentiation of Rh positive and Rh negative blood. The agglutinins corresponded in specificity to the human anti-Rh_0.—P.L.


This antibody, produced by a patient immunized by repeated transfusions, agglutinated 96 per cent of all group O individuals. The 4 per cent nonreactors are mainly Rh_3 or Rh_4. The author believes that this agglutinin corresponds to the antibody predicted by Fisher, which should fail to react with the cells whose genotype is composed exclusively of Rh_3, Rh_4, Rh_0, and Rh_0.—P.L.


On purely theoretical grounds Fisher predicted three varieties of anti-Hr sera, called γ, δ, and η. The
Pathogenesis of Congenital Hemolytic Disease (Erythroblastosis Fetalis). I. Theoretical Considerations.


Some of the puzzles in the pathogenesis of congenital hemolytic disease (and of intragroup transfusion incompatibility) are: (1) Why only 1 in 25 to 50 Rh negative individuals exposed to Rh positive blood becomes sensitized to the Rh factor; (2) the lack of correlation between the titer of the anti-Rh agglutinins in the patient's serum and the intensity of sensitization; (3) why certain infants apparently normal at birth suddenly develop jaundice and anemia after several hours or days, often severe enough to cause death; (4) the role of the A and B factors in congenital hemolytic disease; (5) why the first-born is almost invariably spared, unless the mother has been sensitized by a previous injection of Rh positive blood; and (6) the occurrence of congenital hemolytic disease when the mother is Rh positive.

1. The author postulates the existence of a pair of allelic genes, K and k, where K confers the capacity to become sensitized readily, while k is the contrasting normal gene. Almost all (about 97 per cent) of individuals belong to genotype Kk. Such individuals, if Rh negative, are not apt to become sensitized to the Rh factor after a transfusion of Rh positive blood or after bearing an Rh positive fetus. About 3 per cent of individuals are believed to belong to genotype Kk, and these are the patients who are likely to have erythroblastotic infants or intragroup transfusion hemolysis. The rare individuals of genotype KK should be extremely easy to sensitize, and probably account for the rare cases of erythroblastosis in the first pregnancy, or instances of multiple sensitization to Rh and M, etc.

2. Individuals sensitized to the Rh factor may form other varieties of Rh antibodies besides agglutinins. Agglutinins are assumed to be bivalent (having two combining groups) or multivalent, and agglutination occurs as the result of the formation of a latticework (Marrack hypothesis) when the agglutinins combine with Rh positive red cells. Univalent Rh antibodies can be detected only by the use of special techniques: blocking tests or conglutination tests. Univalent antibodies are presumably composed of smaller molecules than agglutinins, and therefore should be capable of traversing the placental barrier into the fetus more readily.

3. The difference between agglutination and conglutination can be summarized as follows:

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\begin{align*}
\text{Rh positive red cells} & \quad + \quad \text{Rh negative red cells} \\
\text{Bivalent Rh antibodies} & \quad \rightarrow \quad \text{Agglutination} \\
\text{Univalent Rh antibodies} & \quad \rightarrow \quad \text{Conglutination}
\end{align*}
\]

4. Conglutinin (or X protein) appears to be a complex of serum albumin, serum globulin, and phospholipid, which is absorbed by antigen after it has been sensitized by its specific antibody. Because of its colloidal properties, X protein causes the sensitized red cells to stick together, and in vivo, probably in conjunction with complement, it brings about slow hemolysis. According to the author's theory, only the simple precursors of X protein are present in the fetus, but the physiologic changes occurring after birth cause these to aggregate into the larger molecules of conglutinin, thus accounting for the delayed onset of congenital hemolytic disease.

5. The natural A and B agglutinins are believed to be composed of large molecules. Only when individuals of genotype Kk (or KK) become sensitized are univalent A and/or B antibodies formed, which can traverse the placenta more readily. Congenital hemolytic disease occurring in this way may be indistinguishable clinically from cases caused by Rh sensitization; usually, however, the A-B-O cases are milder.

6. During pregnancy villi may become detached, but the fetal red cells contained in them will usually...
be too few in numbers to sensitize any but the rare patients of genotype KK. During labor and delivery, owing to the disturbances at the placental site, presumably larger numbers of fetal red cells could gain access to the maternal circulation, sufficient also to sensitize patients of genotype KK.

6. These cases are explained by sensitization to the A-B factors as indicated under (4), and by sensitization of mothers of one Rh blood type to blood of a different Rh type, or sensitization to the Hr factor, etc. Inasmuch as factors Rb', Rh", and Hr are much less antigenic than Rh\textsubscript{s}, these cases are rare.—A.S.W.


Eleven cases are presented which illustrate the following points:

1. The Hr test is a valuable aid in determining the homozygosity or heterozygosity of type Rh individuals. When the Hr test is negative, the individual is homozygous (either genotype Rb\textsubscript{1}Rb\textsubscript{1} or Rb\textsubscript{1}Rh\textsubscript{1}), while if the Hr test is positive, he is almost surely heterozygous. If a man is homozygous for the Rh factor and his wife's serum contains Rh blocking antibodies the prognosis for future pregnancies is virtually hopeless.  

2. In the presence of Rh sensitization, plasma transfusions should be given instead of blood transfusions unless Rh negative donors are available.

3. Even a small intramuscular injection of Rh positive blood may be sufficient to sensitize an Rh negative woman and thus prevent her from having viable infants.

4. The injection of a potent antigen may prevent an individual from becoming sensitized to a weaker antigen to which he or she is exposed simultaneously. For example, the injection of typhoid or pertussis vaccine during pregnancy may be worth while in the case of Rh negative patients with sisters who have had erythroblastotic infants. Once sensitization is developed, however, counterimmunization does not appear to affect the degree of sensitization.

5. That univalent antibodies (blockers) are more important than bivalent antibodies (agglutinins) in the pathogenesis of congenital hemolytic disease is proved by the ease with which the former can be demonstrated in the serum of erythroblastotic infants, in the absence of agglutinins.

6. In many cases, early cesarean section is ineffectual in the prevention of congenital hemolytic disease, because the disease often is initiated by the birth of the infant. According to the author's theory the profound physiological changes occurring after birth cause the formation of X protein in the infant's plasma and thus favor the onset of hemolysis. Besides, cesarean section subjects the mother to the additional hazards of an operation and the infant to the hazards of prematurity.

7. The presence of high hemoglobin concentration in an infant with hemolytic disease may merely mean that the disease process is mild, but frequently such cases have Kernicterus or hemorrhagic manifestations and die. In any event, there is nothing to be gained by prophylactic transfusions before the hemoglobin drops below 80 per cent, because the only effect of transfusion therapy is to correct the anemia, and the only infants benefited are those who would otherwise die from anemia. In any case, transfusion of too much blood is to be avoided, because this is dangerous.

8. Women differ in the ease with which they can be sensitized to the Rh factor, so that while most require only one or two pregnancies or transfusions, occasionally sensitization does not develop until there have been as many as seven to ten or more pregnancies. Once sensitization has developed, it appears to be permanent, though patients differ in the degree of sensitization. Thus some patients may have repeated stillbirths while others have viable infants who recover after transfusion therapy.

9. A woman developed high titered anti-Rh\textsubscript{s} and anti-Rh\textsubscript{a} agglutinins after giving birth to a type Rh\textsubscript{s} infant who died of hemolytic disease. The titer of the antibodies showed hardly any change after thirteen exchange transfusions with normal Rh negative male donors. The husband was shown to be homozygous for the Rh factor, so when the patient became pregnant a therapeutic abortion was performed.

10. For transfusing erythroblastotic infants, fresh blood is preferable to bank blood when available. Improperly stored blood breaks down quickly in the infant's circulation.

11. Mothers with latent diabetes may have an obstetrical history clinically indistinguishable from erythroblastosis fetalis. In one such case the difficulty of diagnosis was increased by the fact that the patient was Rh negative and the husband Rh positive. In such instances, testing for Rh antibodies on the maternal serum by the conglutination method is a reliable aid in the differential diagnosis.—A.S.W.