A SURVEY OF THE SIGNIFICANCE OF THE Rh FACTOR

By Philip Levine, M.D.

Following Landsteiner's brilliant description in 1900-1901 of the four blood groups, transfusions gradually became on the whole a safe and routine procedure. However, intragroup transfusion reactions were still observed in two groups of cases: (1) patients who had previously received a series of uneventful transfusions, and (2) women at the first transfusion associated with pregnancy. It was for a long time suspected that differences other than the 4 blood groups were responsible for isoimmunization and the production of new antibodies, but the actual demonstration of immune isoagglutinins was very seldom observed. In the course of studying this material, an entirely new field of clinical importance was opened by the discovery of the role of fetal blood in isoimmunization, a subject hitherto of theoretical interest only.

HISTORICAL DEVELOPMENTS

In 1900 two other discoveries pertinent to isoimmunization and erythroblastosis fetalis were made. The first was the description by Ehrlich and Morgenroth of the phenomenon of isoimmunization in goats and later extended by other workers to many species of animals. In general, the procedure used was cross-transfusion within any particular species which resulted in the appearance of immune isoagglutinins or isohemolysins. This work led to the concept of the individuality of animal blood by virtue of combination and permutation of a number of antigenic and hereditary substances in the red blood cells. In 1900, the Mendelian laws of heredity, published 35 years previously, were independently rediscovered by Correns, Tchermak and de Vries. With the description of the human factors M, N, and P, in 1928, the concept of the individuality of blood was extended to include human blood also, although in these discoveries Landsteiner and Levine used heteroimmune sera.

In 1939, Levine and Stetson offered an explanation for the origin of an atypical agglutinin held to be responsible for a severe transfusion reaction in a recently delivered woman at her first transfusion. The serum of this patient, who had just delivered a macerated fetus, agglutinated the cells of 80 per cent of group O individuals. The intragroup agglutinin was at least as active at 37°C. as at room temperature, and in this respect it differed widely from the normal atypical isoagglutinins (anti-A, anti-O, and anti-P), studied extensively by Landsteiner and Levine.

Levine and Stetson assumed that the fetus inherited a dominant agglutinable factor from the father, but not present in the mother's blood. Isoimmunization could then result from the transplacental transfer of minute quantities of fetal red blood cells and/or tissue cells into the maternal circulation.

The authors were led to suggest transplacental isoimmunization because of paral-
The observations made by Irvin, Gorer, and Lumsden demonstrated mutual isoimmunization in back-cross hybrids of two species of doves joined by parabiosis. Gorer described the appearance of immune isoagglutinins in certain strains of mice following transplantation of a mouse sarcoma and an identical finding was reported by Lumsden in rats. The latter worker noted necrosis of the transplants in certain animals, which was attributed to the development in the host of immune isoagglutinins directed against antigenic factors in the red cells of the donor transplants.

It was this concept of placental isoimmunization which paved the way for the subsequent findings on the pathogenesis of erythroblastosis. At the same time Levine and Stetson described a new blood factor which was independent of the other hitherto known blood properties such as A, B, M, N, and P. However, no name was assigned to this new blood factor which was antigenic in the same species (isoimmunization), but not antigenic or, as we know at present, poorly antigenic in animals (heteroimmunization).

At about the same time (1937), Landsteiner and Wiener were investigating a factor in the red blood cell of the rhesus monkey, related to but not identical with the human M factor. In the course of their studies of the antibodies in sera produced in animals injected with rhesus blood, another human blood factor was discovered which they called Rh. As in the case of the M factor it indicated a property in rhesus blood related to but not identical with the factor in human blood.

At first these workers did not suspect that their heteroimmune agglutinin derived from animals was identical in specificity with the isoimmune antibody found in the patient studied by Levine and Stetson. Because the serum was derived from the experimental animal, Landsteiner and Wiener had no way of knowing that their factor was important clinically. Later, in 1940, however, Wiener and Peters observed that the Rh factor was antigenic in Rh-individuals who had been transfused several times with Rh+ blood.* These workers now suspected that the human Rh factor was probably identical with the unnamed factor previously described by Levine and Stetson.

Later in 1940 Levine and Katz in studied several cases similar to the one described with Stetson. In each case there was a severe or fatal transfusion reaction at the very first transfusion in a woman who had recently delivered. As in the remarkable case of Zacho, these agglutinins were more active at 37°C. than at lower temperatures. Accordingly, Levine, Burnham and Katz referred to them as "warm agglutinins." The obstetrical histories of this group of women were striking because of the high incidence of fetal and neonatal morbidity, and it was suggested that the phenomenon of immunization with fetal blood, responsible for intra-group transfusion reactions, was directly correlated with the fetal and neonatal morbidity. When the histories revealed that these infants suffered from one or another form of erythroblastosis fetalis it was suggested that the intra-uterine

* It is of interest that all their anti-Rh sera were of the "cold" variety and the authors suggested a compatibility test with incubation at icebox temperature. In the light of our present knowledge, it is probable that these sera contained blocking antibodies.
blood destruction was brought about by the action of the maternal antibodies which found their way into the fetal circulation to react with and destroy the infants' Rh+ blood.18

**STATISTICAL PROOF**

The proof of the concept presented is indicated in table 1 which shows the striking statistical differences in a series of mothers of erythroblastotic infants as compared with a random white population.

Further proof was supplied in a demonstration that the incidence of the disease in any given population depends upon the incidence of Rh— individuals in the test with anti-Rh0 (anti-D) agglutinin.

**Table 1.*—Statistical Proof**

<table>
<thead>
<tr>
<th></th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rh+</td>
</tr>
<tr>
<td><strong>Random population, male or female</strong></td>
<td></td>
</tr>
<tr>
<td>350 mothers of erythroblastotic infants</td>
<td>85</td>
</tr>
<tr>
<td>204 husbands of Rh— mothers</td>
<td>10</td>
</tr>
<tr>
<td>139 affected infants of Rh— mothers</td>
<td>100</td>
</tr>
</tbody>
</table>

* After Levine20.

**Table 2.*

<table>
<thead>
<tr>
<th>Race</th>
<th>Number tested</th>
<th>+ (%)</th>
<th>— (%)</th>
<th>Incidence of Erythroblastosis fetais</th>
</tr>
</thead>
<tbody>
<tr>
<td>White19</td>
<td>334</td>
<td>85.0</td>
<td>15.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Negro20</td>
<td>264</td>
<td>95.5</td>
<td>4.5</td>
<td>1.7</td>
</tr>
<tr>
<td>American Indian21</td>
<td>110</td>
<td>99.2</td>
<td>0.8</td>
<td>?</td>
</tr>
<tr>
<td>Chinese22</td>
<td>150</td>
<td>99.3</td>
<td>0.7</td>
<td>very rare</td>
</tr>
<tr>
<td>Japanese23</td>
<td>150</td>
<td>98.0</td>
<td>2.0</td>
<td>very rare</td>
</tr>
</tbody>
</table>

* After Levine21.

In 1941–1943 Levine and his coworkers established the following additional facts:

1. In the 8 per cent Rh+ mothers of erythroblastotic infants, the isoimmunization was attributed to finer differences of the Rh factor (anti-C),* to a new blood factor called Hr (genetically related to Rh), and to the factors A and B.19–26

2. The Rh factor was presumably limited to red blood cells.27 Certainly in the affected infants the Rh factor could not be demonstrated in the body fluids.

3. Anti-Rh agglutinins could be demonstrated in less than 50 per cent of the Rh— mothers of erythroblastotic infants.19 It was assumed that in the remaining cases the hemolytic process was induced by the action of an antibody of another variety

* For a discussion of the terminology see page 7.
not detectable by methods hitherto employed.\(^{28}\) (In 1944 and 1945, Race,\(^ {29}\) Wiener\(^ {30}\) and Diamond\(^ {31}\) independently described the blocking antibody.)

4. Intra-group transfusion accidents could be prevented by the administration of Rh- blood to Rh- patients.\(^ {19}\)

5. Anti-Rh sera differ in specificity, 2 of them now called anti-D (anti-Rha) and anti-C (anti-Rh') giving 4 types of reactions.\(^ {32}\) The anti-Hr serum (anti-c) and anti-Rh' (anti-C) gave only 3 types of reactions, since a blood failing to react with both sera was not found.

6. The final statistical study revealed the far greater importance of the anti-D serum, presumably because the D (Rha) factor was more antigenic.\(^ {22}\)

7. Human anti-Rh sera were superior to the experimental serum produced in animals by injections of either rhesus\(^ {13}\) or human blood.\(^ {33}\) Accordingly the experimental serum was largely abandoned at a very early date.

8. The hemolytic process in the affected infant could be treated more effectively by transfusion of Rh- blood since the transfused Rh+ blood as well as the infant's own Rh+ blood were still subject to hemolysis in the neonatal period.\(^ {19}\)

9. Several factors of safety were listed which were responsible for the comparatively low incidence of erythroblastosis fetalis in spite of a high frequency of incompatible mating \((85 \times 15 = 12.75\) per cent\): (a) the current tendency to small families, (b) a high incidence of heterozygous fathers and (c) the failure of many Rh- women to produce anti-Rh antibodies. It was suggested that the capacity to produce antibodies is dependent upon one or more genetic factors.\(^ {19}\) \(^ {38}\)

In the 6 year interval since the description of the pathogenesis of erythroblastosis fetalis, some notable contributions were made by the British workers, Fisher, Race and their colleagues, Taylor, Coombs and Mourant, and by the American workers, Wiener, Diamond, Witebsky, Chown, Hill and Haberman. These dealt mainly with finer methods for detection of immunization, theories on the genetics of Rh-Hr system, attempts to enlarge the supply of human anti-Rh sera for diagnostic purposes, and replacement transfusion of the affected infant.

**GENETICS OF THE RH-HR SYSTEM**

Reference has already been made to the 4 types of reactions given by two anti-Rh sera (anti-D and anti-C) in striking contrast to 3 types of reactions observed on testing several hundred bloods with anti-C and anti-c. Because of the historical significance of these facts and their bearing on the linkage theory of Fisher, table 3, showing the relationship of these 3 sera, is reproduced.

Two of the 3 sera, anti-D and anti-C were produced by Rh- mothers of erythroblastotic infants, while anti-c (anti-Hr') was observed in an immunized Rh+ mother.\(^ {19}\) \(^ {49}\) In the latter case the husband was Rh- but the mother's serum satisfied the criteria of isoimmunization; i.e., a factor in the blood of the husband and affected infant, not present in the mother's blood, and causing the production of specific antibodies by the mother.

Because anti-C and anti-c gave only 3 types of reactions, it was suspected that the genetic relationship of factors C and c (Rh' and Hr') was analogous to that observed by Landsteiner and Levine for M and N., i.e., 2 allelomorphic genes at a
It is for this reason that the letters "Rh" were reversed to yield the term "Hr" for the new blood factor. Accordingly, the 3 serologic types correspond to the 3 genotypes: (1) CC, homozygous, (2) Cc heterozygous and (3) cc, homozygous. The sum of the first 2 gives the incidence of positive reactions with anti-C (about 70 per cent) and the sum of the second and third gives the number of positive reactions with anti-c or anti-Hr' (about 80 per cent).

It is true that Levine's original anti-Hr' serum (anti-c) was of weak activity, but the incidence of the factor could have been calculated by taking into account the incidence of positive and negative reactions with anti-C which was maximally active. Levine, however, was unable to explain the genetics of the factor D, determined by reactions with anti-Rh₀ (anti-D) or the factor E discovered independently by Race and Wiener.

TABLE 3.*—The Cross-Roads Experiment

<table>
<thead>
<tr>
<th>Terminology of Wiener and Landsteiner</th>
<th>Mrs. M.F. anti-Rh₀</th>
<th>Mrs. M.S. anti-Rh'</th>
<th>Mrs. K.F. anti-Hr</th>
<th>Incidence of type (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh₁</td>
<td>+</td>
<td>+</td>
<td>o or ±</td>
<td>71</td>
</tr>
<tr>
<td>Rh₂</td>
<td>+</td>
<td>o</td>
<td>+</td>
<td>71</td>
</tr>
<tr>
<td>Rh'</td>
<td>o</td>
<td>+</td>
<td>o or ±</td>
<td>1</td>
</tr>
<tr>
<td>Rh₀</td>
<td>o</td>
<td>o</td>
<td>+</td>
<td>13</td>
</tr>
</tbody>
</table>

* After Levine.†

† At the request of Dr. Wiener, the scheme of the reactions indicated was made available to him for inclusion in the third edition of his book, "Blood Groups and Transfusion." pp. 253-254 (C.C. Thomas).

Without taking into account the Hr factor, Wiener suggested that the 4 types of reactions could be explained on the basis of multiple alleles, at first 3 genes and after the description of the E factor, 6 genes.*

Subsequently, Fisher and Race suggested the alternative theory of linkage at 3 different loci on a particular chromosome. The two contrasting theories are illustrated below:

\[
\begin{array}{c|c|c|c}
\text{Multiple Alleles} & \text{Linkage} \\
\hline
R^1R^+ & D & · & d \\
R^1 R^+ & R'R'' & C & · & c \\
\text{Wiener} & E & · & e \\
Fisher & Race & \\
\end{array}
\]

Fisher produced some genetic evidence to indicate that the gene C is located in a position intermediate between D and E.

* For a discussion of this theory see Wiener.
Accordingly, Fisher extended the concept of the MN type of relationship to include genes for the 2 remaining Rh factors D and E. In doing so he had to postulate the existence of 2 additional Hr genes d and e allelomorphic respectively with genes D and E. One of these, e, was subsequently discovered by Mourant when he described a new human antibody which gave 96 per cent positive and 4 per cent negative reactions. As will be shown below, these figures correspond almost exactly to the theoretical values derived from a calculation of gene frequencies.

The existence of the third variety of anti-Hr antibodies, anti-d, has not yet been definitely established. This, however, does not destroy the validity of the theory since with rare exceptions, all anti-Hr sera can be produced by the much smaller group of 8 per cent Rh+ mothers of affected infants or Rh+ individuals immunized by transfusion of Hr+ blood. In contrast to the 92 per cent Rh— mothers who produce anti-Rh antibodies, only those homozygous for D(DD) can produce anti-d.

Apparently the varying incidence of the several antibodies observed is an index of the degree of antigenicity of the corresponding factors, since the number of incompatible matings cannot differ for factors determined by allelomorphic genes. These considerations are illustrated below:

<table>
<thead>
<tr>
<th>Rh of Mother (anti-D)</th>
<th>Husband X Wife</th>
<th>Incidence of Types (%)</th>
<th>Incidence Mat-</th>
<th>Antibodies Produced</th>
<th>Incidence of Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D × d</td>
<td>85 × 15</td>
<td>13</td>
<td>anti-D</td>
<td>frequent</td>
</tr>
<tr>
<td></td>
<td>C × c</td>
<td>73 × 27</td>
<td>10</td>
<td>anti-C</td>
<td>rare</td>
</tr>
<tr>
<td></td>
<td>E × e</td>
<td>30 × 70</td>
<td>21</td>
<td>anti-E</td>
<td>occasional</td>
</tr>
<tr>
<td></td>
<td>d × D</td>
<td>63 × 37</td>
<td>23</td>
<td>anti-d</td>
<td>rare</td>
</tr>
<tr>
<td></td>
<td>c × C</td>
<td>80 × 10</td>
<td>16</td>
<td>anti-c</td>
<td>very rare</td>
</tr>
<tr>
<td></td>
<td>e × E</td>
<td>97 × 3</td>
<td>3</td>
<td>anti-e</td>
<td></td>
</tr>
</tbody>
</table>

Wiener's view expressed in numerous papers that the genetics of the Rh factor was based on a series of multiple alleles at 1 locus does not take into account the existence of the Hr factors. Furthermore, it is difficult to incorporate into the multiple allelic theory such new Rh genes as those described by Stratton (D*) and Race (C*). The complex antigenic structure of the Rh factor can be more readily explained in terms of linkage at several loci along the length of the chromo-
It must be stated, however, that geneticists always find it difficult to differentiate multiple alleles from closely linked genes.

Because genetic usage requires variations of a symbol for allelic genes at a given locus, the linkage theory necessitates a departure from the term "Rh" as a gene. Accordingly, Fisher arbitrarily selected the letters C, D, E for the Rh genes and c, d, e for the corresponding Hr genes. There is no reason for discarding the terms Rh and Hr as blood factors.

In a sense the choice of D for the factor described by anti-Rh0 serum is most fortunate. The student in this field can readily orient himself since the anti-D serum, already referred to in the literature as the "diagnostic serum" is clinically the most important one because of the greater antigenicity of the D factor. In terms of the linkage theory, an Rh+ individual is one whose blood contains the factor D as indicated by a positive reaction with anti-D, the diagnostic serum (anti-Rh0). Such individuals may also possess the factor C, i.e., DcE or Rh1, or the factor E, i.e., either DcE or Rh2 or all 3, i.e., DCE or RhRh.

An Rh0 individual is represented as Dcc since his blood will react with only anti-Rh0 serum and with 2 anti-Hr sera. By the same token an Rh— individual is any one whose blood fails to react with anti-D. The several possibilities are given below:

<table>
<thead>
<tr>
<th>Rh—</th>
<th>dCc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh'</td>
<td>dCe</td>
</tr>
<tr>
<td>Rh*</td>
<td>dcE</td>
</tr>
<tr>
<td>Rh Rh*</td>
<td>dCE</td>
</tr>
<tr>
<td>Rh negative</td>
<td>dce</td>
</tr>
</tbody>
</table>

In this paper the term Rh— indicates the composite group while "Rh negative" refers to the absence of all Rh factors and by the same token the presence of all 3 Hr factors.

These considerations are important for the clinician since they aid in the differentiation of Rh+ husbands as homozygous (DD) or heterozygous (Dd). Obviously the prognosis in pregnancies is far better for matings in which the husband is heterozygous since 50 per cent of the offspring will be Rh— (dd) and, therefore, incapable of immunizing the Rh— mother (Dd x dd). With 2 genes at a particular locus serologic tests for the corresponding blood factors reveal directly the 3 phenotypes (serologic types) corresponding to the 3 genotypes as shown in Table 4.

* For an excellent summary of the Fisher theory, see Race.48
The Johnson formula previously used by Landsteiner and Levine for the genetics of the MN factors, is generally applicable for all genetic systems in which there are two allelic genes and three types corresponding to three genotypes. This useful formula is based on the application of the binomial theorem to the gene frequencies. The frequency of any one gene is equal to the square root of the percentage of non-reactors who must be homozygous.

The brilliance of Fisher's contribution is seen from an application of Johannsen's formula to Mourant's recent discovery of the e factor. Anti-E gives 30 per cent positive reactions and 70 per cent negative reactions in both English and American populations. The 30 per cent containing the E factor consist of a certain percentage of homozygous EE and the remainder are heterozygous or Ee. Application of the formula gives the following.

\[
\begin{align*}
\text{Frequency of gene } e &= \sqrt{0.30} = 0.55 \\
\text{Frequency of gene } E &= 1 - 0.30 = 0.69 \\
\text{Frequency of } EE &= \frac{1}{2} \times 0.55 \times 0.69 = 0.21 \\
\text{Frequency of } Ee &= \frac{1}{2} \times 0.55 \times 0.69 = 0.21 \\
\text{Frequency of } ee &= (0.55)^2 = 0.30.
\end{align*}
\]

Remarkably enough these derived values are in almost perfect agreement with the data obtained by Mourant for anti-c, i.e., 96 per cent. These findings constitute very significant evidence in support of Fisher's linkage theory.

| Table 5. Determination of Gene Frequencies for the Rh-Hr Systems (C-c, D-d, and E-e) |
|:---------------------|:------------------|:-----------------|
| X = Incidence one dominant gene | X = Incidence of the other dominant gene |
| X + x = 10 | X^2 + 2X + x^2 = 100 |
| 0 + 10 | 0 0 100 |
| 1 + 9 | 1 19 81 |
| 2 + 8 | 4 32 64 |
| 3 + 7 | 9 49 49 |
| 4 + 6 | 16 48 36 |
| 5 + 5 | 25 50 25 |
| 6 + 4 | 36 48 16 |
| 7 + 3 | 49 42 9 |
| 8 + 2 | 64 32 4 |
| 9 + 1 | 81 18 1 |
| 10 + 0 | 100 0 0 |

In dealing with immune isoagglutinins of human origin which occur very rarely, it is not surprising that a particular antibody, perhaps the only one available at the time, will not give maximally potent reactions. Although this occurred with the first anti-Hr serum (anti-c) nevertheless its genetic relationship with the factor C was obvious, since anti-C gave maximally potent reactions. Because of these considerations, the writer hesitated to publish at length on the Hr factor until a more potent serum became available.

Historically it was of interest that anti-D and the rare anti-c sera were available to the British workers (Race and Taylor) for their first studies. Somewhat later...
an anti-E agglutinin was found and curiously enough anti-C serum was first used late in 1943 and published in 1944. Fortunately, Levine had available large quantities of potent specimens of anti-D, anti-C and a weaker anti-c in his first studies in 1941.

Until anti-d sera become available there is no choice but to use anti-c for differentiation of homozygous and heterozygous Rh+ husbands of Rh− mothers. This is possible because there is a high degree of correlation of genotypes DD and CC for the 71 per cent of Rh+ individuals of type DCE and DCE (Rh1 and Rh2Rh3). As first pointed out by Levine5, anti-c cannot be used for the 14 per cent of Rh+ individuals of types DcE (Rh1) or Dcc (Rh0). For this group an anti-d serum is essential.

Through the use of the hemolytic effect of anti-Rh and anti-Hr serums in the presence of whole blood and complement, Hill and Haberman6, 97 have been able to show a difference in the hemolysis of homozygous and heterozygous bloods. In the presence of the specific antiserum (anti C, D or c) homozygous cells showed approximately twice the hemolysis observed in heterozygous bloods.

SOME PRACTICAL CONSIDERATIONS

With the aid of anti-D the diagnosis of erythroblastosis fetalis is established in 92 per cent of all cases if the mother’s blood is negative (Rh−) and if her serum contains anti-Rh antibodies (agglutinins or blocking antibodies). In the smaller group of 8 per cent Rh+ mothers, the blood should be submitted to special workers in the field for testing with the other anti-Rh and anti-Hr sera.

The mother who has already delivered an affected infant should not become pregnant until an interval of several years elapses, long enough for residual antibodies to disappear and for a “test” period for the antibody producing cells. In the event of another Rh+ fetus in the next pregnancy, it is possible that the degree of isoimmunization will not be so intense. Should periodic tests show an increasing antibody production, many authorities believe that labor should be induced in order to shorten the period of intrauterine blood destruction.

Since antibodies have been shown to persist for a number of years, further pregnancies are to all intents and purposes excluded especially for the older Rh− woman whose husband is homozygous. With another pregnancy already in progress when antibodies residual from the preceding pregnancy have not yet disappeared, it will be very difficult—if not impossible—to differentiate newly formed from residual antibodies, particularly if the husband is heterozygous. Reference will be made later to the value of the anti-human globulin test on the cord blood but this test can be applied only after delivery (see page 13).

Until our knowledge is extended, the number of pregnancies of Rh− women already immunized by transfusions and/or previous pregnancies, should be limited. In practice this is equally applicable to Rh+ women immunized by other factors. Even though some of these infants will recover with replacement transfusions, there is always the increasing danger of later complications because of kernicterus.

In the case of Rh− women in general the number of pregnancies to be recom-
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mended will depend upon the ease with which antibodies are produced. With routine testing for antibody production with each pregnancy, the much smaller group of Rh− women who are readily immunized can be selected. As already mentioned the capacity for antibody production is determined by one or more genetic factors. In any event, the incidence of erythroblastosis fetalis can be lowered by prevention of isoimmunization in all of our Rh− female population who may be candidates for transfusion or intramuscular injection of blood (see page 17).

In view of the high fetal morbidity in intensively immunized Rh− women whose husbands are homozygous, termination of accidental pregnancies by therapeutic abortion seems justified. In the several cases in which this recommendation was made, the Rh− patient had potent antibodies residual from the preceding pregnancies. Except for those immunized by previous transfusions, these women already had one or more normal Rh+ children and had lost one or more affected infants. For this group of cases either artificial insemination or adoption may be recommended.

AGGLUTININS AND BLOCKING ANTIBODIES

In the initial study on the pathogenesis of erythroblastosis fetalis anti-Rh agglutinins were observed in less than 50 per cent of the Rh− mothers.* It was obvious that the remaining Rh− mothers were immunized because their infants had hemolytic symptoms and these mothers were equally subject to severe transfusion reactions. The view was expressed that “antibodies capable of reacting in vivo cannot be demonstrated because of limitations in the sensivity of the technic employed.”

It is of interest that time-honored methods for testing were used, i.e., Rh+ cells suspended in saline after previous washing to remove serum elements. Curiously enough, the logical step of testing the mother’s serum under the conditions existing in vivo, i.e., Rh+ cells suspended in plasma, was not taken.

In 1944, Race, Wiener, and Diamond independently described “incomplete,” “blocking” or “inhibiting” antibodies produced by Rh− mothers whose serum failed to agglutinate saline suspensions of Rh+ cells. The indirect method was employed, so that 3 reagents were required for their demonstration. The mother’s serum presumably coated the Rh+ cells suspended in saline and such treated cells now became resistant to the action of anti-D agglutinins.

In a number of sera, titration with saline suspended cells revealed the presence of a prozone in the higher concentrations and increasingly strong reactions on further dilution. In the light of present knowledge these sera contained a weak blocking antibody and a stronger agglutinin. It was shown that under certain conditions, by absorption of undiluted sera the blocking antibody could be specifically removed and the anti-Rh agglutinin could be recovered almost quantitatively.

The indirect test, however, was tedious and time-consuming because 2 incubation periods were required so that it is no longer employed as a routine procedure.

* Undoubtedly, this figure is probably too high since in several cases weak reactions were elicited on the addition of an excess of serum. Under these conditions the effect could be attributed to the action of the blocking antibody.
A direct reaction given by blocking sera was discovered by Diamond and Abelson, when they recommended the slide test and concentrated cell suspensions. Subsequently, it developed that serum or plasma was essential as a suspending medium for the test cells and that the test could be carried out in test tubes, thus making it possible to carry out quantitative studies. A notable contribution was made by Diamond and Denton when they demonstrated that bovine albumin was suitable for suspending the test blood.

Wiener’s application of the term ‘conglutination’ appears unfortunate, particularly since there is no evidence indicating that many proteins, serum, albumin, globulin and fibrinogen in the form so-called X protein are essential for the direct reactions. In this connection it may be cited that certain concentrations of acacia, Le Page’s glue No. 7 (by virtue of its acacia content), polyvinyl alcohol, pectin and numerous other nonprotein material may be used to elicit the reaction. However, these are not as satisfactory as bovine albumin because of their tendency to form rouleaux formation. With the addition of minute amounts of saline, the rouleaux disappear and the specific reaction still remains (Levine and Wigod).

Another notable contribution was made by Coombs, Mourant and Race when they showed that after prolonged washing with saline, Rh+ cells coated with blocking antibodies are specifically agglutinated by precipitins for human serum. Presumably a layer of antibody is fixed to the surface of the red blood cells and on addition of the anti-human globulin serum, the specific union with the coated red cells results in varying degrees of agglutination depending upon the intensity of the coating. The British workers and Haberman and Hill found this test to be most useful in determining whether or not the infant’s cells at delivery had been sensitized with mother’s blocking antibodies. In a number of instances the author successfully applied this test to differentiate Rh+ cord blood from genetically Rh— blood exposed to but not damaged by maternal antibodies residual from the preceding pregnancy. The degree of coating can be determined by quantitative studies to determine the greatest dilution of anti-human globulin which will still agglutinate the washed blood. Of the several procedures to detect coated Rh+ cells, the anti-human globulin test is by far the most sensitive and it should become a routine procedure for testing the red cells obtained from cord blood. This reaction could safely be used as a guide for therapy and in the event of intense agglutination, replacement transfusion should be carried out.

Attempts were made by Wiener to correlate the clinical picture in the affected infant with the presence of agglutinins or blocking antibodies. Hemolytic symptoms were associated with blocking antibodies, while agglutinins were held to be responsible for severe jaundice, general toxicity and kernicterus. The claim was made that kernicterus was the end result of thrombi (consisting of specifically

* In titration of blocking antibodies, the author uses pooled male serum as a diluent and albumin suspended Rh+ cells. Under these conditions much higher titers are obtained than by Wiener’s original method (plasma both as a diluent and suspending medium). The results are at least as good as those obtained in the modified test in which Wiener employs for the first time albumin solution (1 part of 25% human albumin and 4 parts oxalated human plasma).
agglutinated cells) in the arterioles of the liver causing severe icterus which supplied the characteristic coloring to certain areas of brain tissue whose arterioles were likewise plugged with agglutination thrombi. Although these thrombi were found in numerous organs, harmful effects were assumed to be specifically localized in the liver and brain. The consensus is that the agglutination thrombi observed by Wiener and Brody represent not the specific lesion but rather postmortem changes.

Many exceptions to these claims have occurred, i.e. the severe anemia associated with strong anti-Rh agglutinins and no symptoms suggestive of kernicterus. The latter condition has now been observed in a number of cases in which blocking antibodies alone were found.

It is of course, established that maternal blocking antibodies in contrast to anti-Rh agglutinins pass into the fetal circulation and specifically unite with fetal blood. Remarkably enough, these cells remain unagglutinated in the fetal or infant’s circulation although they are in continuous contact with antibodies in a medium of plasma and, in a number of instances the antibody concentration in the infant’s circulation is sufficiently great to indicate a state of equilibrium on both sides of the placental barrier. In any event there is no justification for the use of the terms “univalent” and “bivalent” for blocking and agglutinating antibodies respectively. Since both sorts of antibodies exert their harmful effects in vivo, the difference seen in vitro would seem to become less significant. The fact is that in vivo both sorts of antibodies are associated with the identical clinical entity of intense blood destruction. The fact that blocking antibodies are frequently demonstrated in the infant’s serum, does not necessarily indicate that they are of smaller molecular size than agglutinins.

It was further claimed by Wiener that agglutinins exert their harmful effect mainly at delivery and not during the latter part of the pregnancy. It is difficult to accept the view that a concentration of maternal agglutinins sufficient to induce severe symptoms of blood destruction or icterus gravis could be attained from the process of parturition and delivery. Certainly it can not be expected in the case of infants delivered by Cesarean section. Furthermore, the clinical picture is almost identical in many anemic infants of mothers with either agglutinins or blocking antibodies.

In a number of instances qualitative tests for blocking antibodies with albumin suspended cells will be entirely negative, but titration in normal human serum as a diluent will reveal a prozone, i.e., gradually increasing reactions on further dilution. This obviously is a serious source of error which can readily be detected with the aid of the anti-human globulin test. As in the earlier reports on prozone due to a mixture of agglutinins and blocking antibodies, these findings indicate the presence of two varieties of blocking antibodies. More recently this view was confirmed by the results of specific absorption experiments by Levine and Wigod.62 On treatment with Rh + but not with Rh − blood the prozone is specifically removed and titration of the absorbed serum now reveals gradually decreasing reactions. Accordingly one may assume that the antibodies in the course of their pro-
duction by the immunized mother represent an ever changing configuration of the immune globulin which still retains its characteristic specificity.

On the basis of discrepancies in the behavior of blocking antibody and the anti-human globulin test for coated cells, Hill and Haberman assumed the existence of a third order of antibodies.

These authors classified the antibodies on the basis of saline agglutinins, blocking antibodies (those that saturate the Rh antigen without causing agglutination), and the ‘cryptagglutinoids’ (those antibodies that do not agglutinate in saline, do not block, but will be demonstrable by the anti-human globulin test or in albumin and serum). They applied the term ‘developing test’ to the use of the anti-human globulin serum on fetal erythrocytes.

In conclusion, serologic tests are now available for a somewhat more accurate correlation of symptoms in the infant and antibody content of the mother’s serum and infant’s serum and red blood cells. In the final analysis the intensity of the disease process and the therapy will be determined by serologic study of the cord blood, particularly on the red blood cells. However, more intensive studies on the serological and physico-chemical properties of purified preparations of the several varieties of antibodies are required for a fuller understanding of the subject.

SPECIFIC THERAPY OF THE AFFECTED INFANT

As first suggested by Levine, Burnham, Katzin and Vogel the affected infant of an Rh- mother should be vigorously transfused with Rh- blood which is not subject to the action of stored maternal antibodies. It is essential to maintain a hemoglobin level above 65–70 per cent. In some cases it is necessary to transfuse repeatedly until the infant is temporarily Rh- by virtue of the normally surviving donors’ Rh- blood.

In any event the infant’s red cell after exposure to maternal antibodies is an injured one and is not apt to survive long in the infant’s circulation. One cannot underestimate the degree of blood destruction resulting from the action of passively transferred maternal antibodies stored presumably in the infant’s tissue spaces. In one severely affected infant reported elsewhere, who was transfused several times, maternal blocking antibodies were still demonstrable on the twenty-fifth day of life. At this time the infant’s blood was Rh- by virtue of the surviving donors’ Rh- blood.

More recently several workers (Wallerstein, Wiener, Diamond) have been carrying out replacement transfusions by washing out the infant’s circulation with large quantities of Rh- blood. Wallerstein, Wiener and Vogel have been withdrawing the infant’s blood either from the fontanelle or the radial artery and administering the donor’s blood into one of the superficial veins. Units of 20 cc. of infant’s blood are withdrawn and replaced with 20 cc. Rh- blood and the proc-

* The method of choice seems to be the use of cord veins which can be cannulized with the aid of a special plastic catheter, as suggested by Diamond.
ess is continued until the infant’s circulation is washed out with 1 liter of Rh—blood. An additional quantity of Rh—blood corresponding to 10 cc. per pound should then be administered, preferably at the beginning of the transfusion.

In less severely affected infants, as indicated by the serological tests on the cord red blood cells, smaller volumes of Rh—blood may be used. Past experience reveals that many affected infants recover after 1 or 2 single transfusions. The radical replacement should be reserved for those infants who have been exposed to prolonged intrauterine blood destruction.

From the point of view of prognosis the most significant information will be derived from a study of the cord blood carried out soon after delivery. The presence of maternal antibodies in the cord serum is not as significant as the presence of maternal antibodies fixed to the infant’s red blood cells. In several cases the increase of antibody content in the mother proved to be misleading since the infant was shown to be Rh—. The mechanism of such nonspecific antibody production is still to be investigated. As indicated above, genetically Rh—blood can be differentiated from specifically coated Rh+ cells with the aid of the reaction of Coombs, Mourant and Race.

Assuming the infant to be Rh+ the ratio of blocking antibody in the maternal and cord blood is of some prognostic significance. With more intensive isoimmunization of long duration a state of equilibrium can be established on both sides of the placental barrier, in which case the prognosis is not favorable. By earlier induction of labor a more favorable ratio may be obtained. There is reason to believe that the same conditions which favor passage of fetal elements into the maternal circulation, i.e., gradual thinning of the placental barrier in the last third of pregnancy, are at the same time more favorable for passive transfer of maternal antibodies into the fetal tissues.

The author has recently studied a number of cases in which the infant, delivered by early induction of labor and given an immediate replacement transfusion, recovered completely from the anemia, although the infant in the preceding pregnancy had died of erythroblastosis fetalis. The prognosis, however, so far as freedom from symptoms due to kernicterus is concerned, must always be guarded since the damage to the brain may not become manifest for many months or even several years. In at least one such instance the infant, delivered 4 weeks early, did not require more than one transfusion at birth and another in the fourth day of life. Although completely recovered from the anemia, this infant is now developing symptoms indicative of kernicterus. However, the damage to the brain, whatever the cause may be, is determined by intrauterine action rather than by stored antibodies acting during the neonatal period.

The recent criticism by Darrow68 and others of the use of Rh—blood does not seem valid, at least not for the severely affected infant. It is highly probable that the mildly affected infant will recover either without benefit of transfusions or despite the transfusion of Rh+ blood. One is not justified in assuming that the burden placed on the mechanism for the disposal of large quantities of destroyed blood does not exert a deleterious effect on an anemic and jaundiced infant. To a lesser degree perhaps the same objections are applicable also for the replacement
transfusion with Rh+ blood. Undoubtedly the replacement is never complete and because of the residual antibodies in the tissue spaces, the Rh+ blood will not survive as long as Rh− blood.

It is advisable to take the erythroblastotic infant off breast milk because anti-Rh antibodies are frequently present in the mother's milk (Witebsky94). This precaution should certainly be taken for the more acutely ill infants even though it is not definitely established that maternal antibodies are absorbed from the intestinal mucosa, at least in appreciable amounts.

In the series of affected infants delivered by immunized Rh+ mothers, the infant should be transfused with group compatible Rh+ blood of the same Rh-Hr subtype as the mother. If the isoimmunization is induced by factors A or B (e.g., mother O, infant A or B), the infant should be transfused with group O blood along with the soluble group A and B substances of Witebsky.96

**PREVENTION OF ISOIMMUNIZATION OF RH− INDIVIDUALS**

All Rh− individuals requiring blood transfusions should receive Rh− blood only. In this way isoimmunization will be prevented and if present the clinician will be spared the trouble of treating the patient for transfusion anuria, perhaps unsuccessfully.

As pointed out by Levine,38, 43 once a patient is immunized, the individual remains potentially immunized for the remainder of his or her natural life time. This is most important in the case of young girls, even as infants. If Rh− girls are transfused indiscriminately as they have been in the past, their chances many years later for having 1 or 2 normal Rh+ children are considerably diminished. These women are thus deprived of the several factors of safety which tend to reduce the incidence of erythroblastosis fetalis so that even their first Rh+ infant may be lost as a macerated fetus, stillbirth, or the infant may have the more severe forms of erythroblastosis fetalis.83

There is reason to believe that those women who were not transfused, may in several instances have received intramuscular injections of blood, a routine procedure in the days preceding the use of vitamin K, or for prophylaxis against measles. In a larger series of cases of erythroblastosis fetalis in the first born, soon to be published by Levine and Rosenfield,69 histories of intramuscular injection were elicited in several instances.

Regardless of the influence of previous transfusions, the occurrence of erythroblastosis fetalis in the first born has some bearing on the mechanism of transplacental isoimmunization. Wiener 70 had assumed that fetal blood entered the maternal circulation only during labor and delivery. Although there may be another antigenic stimulus at delivery, the transfer of minute quantities of fetal blood in the latter half or third of pregnancy must be the determining factor even in those Rh− women who may have been transfused many years previously. With intervals of 9−14 years between the antigenic stimulus of a transfusion and the first pregnancy, one may well assume that the damage to the fetus or infant is not caused by residual antibodies which are evanescent in character, but rather by renewed immunization. On renewed contact with this antigen, many years later the
antibody producing cells of the reticulo-endothelial system respond more rapidly (anamnestic reaction).

It is obvious that the biologic test, as recommended by Wiener,\textsuperscript{7} i.e. the administration of small quantities of Rh+ blood, will also serve to immunize. More sensitive tests are now available for detection of immune antibodies specific for differences within the Rh− and Rh+ group, or new blood factors other than Rh. Incompatibility can be excluded if the patient's serum does not agglutinate the donor's cells suspended in his own serum, plasma or bovine albumin.

A woman who has delivered an erythroblastotic infant, if transfused many years later may tolerate one transfusion of Rh+ blood.\textsuperscript{*} In any event this transfusion will restimulate antibody production so that the following transfusion may result in a severe reaction perhaps with anuria. This is another example of the so-called anamnestic reaction.

\textbf{Table 6.—Erythroblastosis Fetalis in the First Rh+ Infant in Rh− Women}

<table>
<thead>
<tr>
<th>Transfusion History</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cases</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Severity of disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>severe</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>fetal death</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

Since erythroblastosis fetalis is the result of prolonged intrauterine blood destruction the number of immunized Rh− women exceeds the number of affected infants. In other words, an Rh− woman may have anti-Rh antibodies and yet her Rh+ infant may be entirely normal, because the antibody production may have started too late in the course of the pregnancy to allow for much if any intrauterine blood destruction. However, the next Rh+ infant will certainly be affected probably with an unexpectedly severe form of the disease. In any event these Rh− women are always subject to severe transfusion reactions should they require blood even many years after their last pregnancy.

Intra-group transfusion reactions in Rh− male patients can be readily prevented because, as a rule, after a series of uneventful transfusions, the patient eventually will have a slight chill or mild jaundice following a particular transfusion. This should serve as a warning to carry out Rh tests and if found to be Rh−, only Rh− blood should be used for all future transfusions. Unless these precautionary measures are taken the following transfusion will result in a severe, if not fatal, hemolytic reaction.\textsuperscript{72, 73} On the whole the transfusion risks are far greater in women than in men.

For transfusion purposes it is preferable to think in terms of 8 different types rather than the 4 blood groups, with the provision that Rh+ individuals may

\* In one instance there was a delayed reaction probably attributable to traces of residual antibodies produced by a transfusion 4 years previously.\textsuperscript{38}
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receive either Rh+ or Rh− blood. Although certain Rh+ patients may be immunized by the subtypes of the Rh factors (C, E) or by the Hr factors (d, c, e), this occurs very rarely. In any event, it is not feasible to take into account all possible antigenic differences, at least for transfusion requirements.* However, Rh+ individuals should not receive Rh− blood for the very practical consideration that Rh− blood should be reserved for Rh− patients.

A PUBLIC HEALTH PROGRAM

In 1941, immediately after the role of the Rh factor in the pathogenesis of erythroblastosis fetalis was established, the writer drafted 2 rules which were discussed by the Board of Medical Control of the Blood Transfusion Association and public health authorities. These follow:

1. In all individuals receiving repeated transfusions in whom untoward (intra-group) transfusion reactions have been noted, tests for the Rh factor shall be performed before any subsequent transfusion. No subsequent transfusion shall be given to any such recipient found to be Rh negative except from an Rh negative donor whose red blood cells are shown to be compatible with the recipient’s serum at 37°C.

2. No woman with an obstetrical history characterized by habitual abortion, stillbirth, macerated fetus or erythroblastosis fetalis, shall receive a transfusion unless tests for the Rh factor have been made; and then, if her blood shall prove to be Rh negative, such transfusions shall be made only from an Rh negative donor whose red blood cells shall have been shown to be compatible with the recipient’s serum at 37°C.

Although these preventive measures were generally approved, no official action could be taken because no assurance could be given at the time that sufficient quantities of potent anti-Rh serum would be available.

In the light of our present knowledge regarding the influence of indiscriminate intravenous or intramuscular injection of blood, it now becomes necessary to modify the second proposal so that it may perhaps read as follows:

“‘No transfusions in a female of any age, from infancy on, may be carried out unless she has been tested for the Rh factor. If found to be Rh negative in tests with potent standard diagnostic anti-Rh (or anti-D) serums, she must receive only Rh negative blood, whether it be given intravenously, subcutaneously or by intramuscular injection.’”

The general adoption of these rules will result in a reduction of the incidence of erythroblastosis fetalis and a striking reduction of serious and fatal intra-group transfusion reactions.

The writer has been encouraging public health authorities to adopt a comprehensive program of Rh testing and in several states the measure is now under consideration. For example, there is the successful experience reported by Lee, Van Saun and Brown,74 in Passaic County, New Jersey. Since many states already have compulsory premarital and prenatal tests, no statutory authority is required, except perhaps to obtain financial support for the program.

All workers in this field are agreed that the Rh test should be done routinely

* It remains to be seen whether or not identity of antigenic factors in red blood cells of donor and host for skin or tissue transplants will give more satisfactory results. Theoretically at least, given a sufficiently large number of antigenic differences, the success of the transplant will depend upon proper selection of the donor.
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on the prenatal rather than the premarital specimen. Unfortunately the lay public has been unduly alarmed regarding Rh incompatibilities. There is almost never any indication for a couple to break their engagement solely because the young woman is Rh—. The only exception applies to those Rh— women who had already been immunized by transfusions of Rh+ blood.

In addition to routine screening tests for the selection of Rh+ and Rh— mothers, tests for the presence of active isoimmunization should be carried out. While these tests should also be performed in a state-wide program, all hospital laboratories, especially those with large obstetrical services, should be prepared to carry out these comparatively simple procedures. Unfortunately there are altogether too few workers with sufficient experience or background in this new and clinically important field. Certainly each of the larger hospitals should have at least one or more of their workers specially trained in all aspects of this field. It is further suggested that smaller hospitals pool their interests in organizing a properly equipped laboratory to serve the interests of their local community. Such a program can conveniently be organized in conjunction with a central blood bank.

In recommending a public health program it is pertinent to mention that the morbid effects incidental to isoimmunization, i.e. erythroblastosis fetalis and intra-group transfusion accidents, are observed far more frequently than those resulting from syphilis. Obviously the element of contagion is not present in the case of isoimmunization. The potential danger of Rh incompatibility varies in different races and is directly proportional to the incidence of Rh— individuals in any given population.

A SUPPLY OF POTENT ANTI-RH SERA

If Rh tests are to be done on a broad basis in all pregnant women and all patients prior to transfusion requirements it is important to maintain a continuous supply of potent human anti-Rh sera. Unfortunately the experimental serum produced in animals injected with rhesus or human blood is not sufficiently potent and its use was largely abandoned soon after the description of the phenomenon of transplacental isoimmunization. Even if an animal serum is produced in the future it will still be necessary to collect and store large quantities of human sera containing the other Rh and Hr antibodies, and any other of the very rare sera containing antibodies of unusual specificities.

In the past the outlook for large quantities of human anti-Rh sera was not favorable. More recently, however, a number of steps have been taken which should solve the problem of supply. With routine testing a greater number of immunized Rh— women will become available. Physicians should encourage these women to submit to periodic bleedings, followed by replacement transfusion of Rh— blood. In selected women who do not plan further pregnancies and in isoimmunized men, the potency of the antibody can be maintained by the intravenous injection of minute quantities of Rh+ blood, insufficient to induce unpleasant reactions (Hill and Haberman). Both Wiener and Diamond, and others, report the immunization of Rh— donors on a voluntary basis, by repeated administration of Rh+ blood. When antibodies begin to appear the dosage is appreciably
reduced so that there is no danger of untoward reactions from subsequent injections. Undoubtedly all these procedures will also be carried out for the production of other varieties of antibodies (anti-C, anti-E and their corresponding Hr antibodies).

Two additional sources of potent anti-Rh sera may be derived from the recent program of concentrating weak anti-Rh sera, and by certain treatment of so-called prozone sera. The latter sera contain both blocking antibodies and agglutinins, and were therefore considered not suitable for diagnosis. However, the blocking antibodies in such sera may be specifically absorbed without affecting to any considerable degree the activity of the recovered agglutinin (Levine and Waller).

Rarely, some of the measures mentioned above may be applicable also to patients immunized by repeated transfusions. In this connection it is appropriate to mention that anti-Rh sera may be used more economically by employing the capillary tube method of Chown instead of test tubes. For this procedure small quantities of sera may be used, and the tests carried out by Dr. Chown in my laboratory show that his method is almost as sensitive as the test tube method.

Recently the National Institute of Health has ruled that no anti-Rh serum be released for distribution unless it has a titration value of at least 1:32. In other words, a serum which has a titre of 1:320 may be diluted 10 times, but the diluent must be such as not to diminish the protein content below 25 per cent of the normal content. Accordingly, normal human serum of a group AB or bovine albumin may be used as the diluent (6 per cent for agglutinins and 30 per cent for blocking antibodies). The reagent of choice is the agglutinin which is active on blood cells suspended in saline.

More recently blocking antibodies have been recommended, (slide test) but the red cells to be tested must be suspended either in their own plasma, serum or bovine albumin. For large scale work it is preferable to use test tubes rather than the slide test. The main advantage in the use of blocking antibodies is their greater availability since most Rh—mothers of erythroblastotic infants produce blocking antibodies.

MECHANISM OF TRANSPLACENTAL ISOIMMUNIZATION

In 1939 Levine and Stetson suggested that products of the fetus (red blood cells or tissue cells) containing a dominant hereditary property not present in the mother's blood could find their way into the maternal circulation and thus stimulate the mother to produce specific antibodies. When the pathogenesis of erythroblastosis fetalis was described, experiments were carried out to determine whether or not the Rh factor was present in a water-soluble form. Using saliva as an index, it was established that the Rh factor unlike the secretor types of groups A and B, was limited to the red blood cells. Accordingly the question arose as to the mechanism which permitted formed elements, the size of a red blood cell, to penetrate

* The Minimum Requirements for Blood Grouping Serum and Anti-Rh Typing Serum were released on December 16, 1946 from the National Institute of Health, Washington, D. C.
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the placental barrier in sufficient quantity to stimulate antibody production on the part of the mother.

If the Rh factor were present also in a water soluble form, the material could readily enter the maternal circulation in sufficient quantity to immunize. In that event the maternal antibodies, after passage into the fetal circulation, would be completely inactivated by the excess of Rh soluble material, thus preventing the antibody from uniting with the Rh factor in the red blood cells. Accordingly, the hemolytic nature of erythroblastosis fetalis substantiates the finding of Levine and Katzin that the Rh factor is present in the red blood cells, presumably in a water-insoluble form. Possibly the Rh factor may also be present in tissue cells, but this observation of Boorman and Dodd is still to be confirmed.

Witebsky showed that Rh soluble material may be present in very small quantities in the amniotic fluid of some, but not all, Rh+ fetuses. However, this observation can have no bearing on the pathogenesis of erythroblastosis since each of the affected infants belonged to the so-called non-secretor type.

It does not seem necessary to assume the presence of gross lesions in the placenta, which would have to recur and become operative in each succeeding pregnancy with an Rh+ fetus, but not with an Rh− fetus. It is significant that in the vast majority of the cases the course of the pregnancy and the delivery of these mothers is entirely normal. Although there is no direct proof that the fetal red blood cells (a large, formed element) find their way into the maternal circulation, nevertheless, the statistical data on the pathogenesis of erythroblastosis fetalis permit of no other conclusion.

If one assumes that minute quantities of fetal blood, either as intact red blood cells or as stroma, pass the placental barrier, this must occur in every normal pregnancy. Isoimmunization may occur only if the fetus is Rh+ and the Rh− mother is genetically capable of producing antibodies. Accordingly, it becomes superfluous to assume the existence of genes determining placental permeability to formed elements.*

It is well known to the immunologist that remarkably minute amounts of antigenic material (soluble proteins, suspensions of bacteria or red blood cells) suffice to induce immunization. In recent experiments in rabbits, distinct increases in agglutinin titre were observed, following 14 daily injections of 2 cc. of a 1:5000 suspension of human blood, the total volume of which was 0.0056 cc. whole blood. The corresponding value for a woman weighing 120 pounds is only 0.13 cc.

It will be recalled that, in the latter part of the pregnancy, when isoimmunization is believed to begin, the blood vessels in the fetal villi are adjacent to the maternal sinus, and separated from it by a single layer of cells. It has been calculated by Dodds and Dees-Mattingly that the total area of fetal villi of the human term placenta exposed to maternal sinuses is 70-120 sq. ft. and total length of these villi, if laid end to end, would measure 11.4 miles. One-fourth or more of the fetal blood is outside the fetus and in the placenta.

In this connection, it is significant that the pathological effects of isoimmunization by the Rh factor are observed exclusively in the fully developed, or almost

*Cf. Haldane.
fully developed, fetus. More recent data do not support the view that isoimmunization by the Rh factor per se plays any role in early fetal death. If there is a higher than normal incidence of miscarriages in mothers of erythroblastotic infants, this may possibly result from the effects of isoimmunization from the preceding pregnancies. At any rate, this subject merits further investigation.

Erythrocytes can be observed in the yolk sac in the 4 weeks old fetus, and agglutinable properties have been demonstrated in the blood of the fetus, between the second and third months. There is reason to suspect that the more fundamental property of antigenicity and the capacity to unite with antibodies may be inherent, even in the forerunners of the red cells. Nevertheless, isoimmunization by the Rh factor probably is not initiated until the latter half of the pregnancy, when the blood vessels in the villi gradually approach the maternal sinuses, and are in intimate contact over an ever-increasing surface area. Incidentally, pregnancy offers certain conditions which are peculiarly favorable to isoimmunization, i.e., slow administration of the antigen, over a long period.

This concept of the mechanism of isoimmunization is compatible with the clinical observation that once an Rh- mother is immunized, the condition is likely to recur in all succeeding pregnancies in which the fetus is Rh+. Apparently, the isoimmunization is renewed even if an interval of several years elapses between pregnancies. Furthermore, the erythroblastosis is likely to be increasingly severe in successive pregnancies.

It has been observed on clinical evidence alone that erythroblastosis fetalis occurs about once in every 438 deliveries. If Rh tests are done in all cases of fetal and neonatal morbidity, one can assume an incidence of about 1:150 to 1:200 deliveries. Even this value is out of proportion to the number of pregnancies in the 13 per cent of susceptible matings; i.e. Rh+ father x Rh- mother. Accordingly, it is necessary to assume several factors of safety: (1) the current tendency to small families; (2) inability of many Rh- women to produce antibodies; and (3) the high incidence of heterozygous fathers. Reference already has been made to a recommendation which should decrease the incidence of erythroblastosis fetalis in the first born, particularly in its more fatal forms.

At present there is no specific measure which will prevent either the transfer of fetal blood across the placenta, or the formation of antibodies on the part of the mother. Possibly the injection of serologically active haptenes extracted from Rh+ blood may neutralize the action of antibodies as they are formed. Such haptenes lack the property of stimulating antibody formation, but are capable of specifically neutralizing antibodies. However, the extraction of such haptenes from Rh+ material presents many technical difficulties.

The suggestion has been made recently that the injection of typhoid vaccine in the course of pregnancy may prevent or delay antibody formation on the part of the Rh- mother. However, the experimental animal when injected with a mixture of numerous antigens, responds with the production of a corresponding multiplicity of antibodies. It is also conceivable that injection of nonspecific material may serve to stimulate rather than to depress antibody formation. If this suggestion is to be given a fair trial it is preferable to inject the Rh- women with
such antigens as tetanus and diphtheria toxoid and pertussis vaccine so that the mothers are at the same time producing antibodies which will be beneficial for their infants.

Erythroblastosis fetalis assumes importance altogether out of proportion to its low incidence because it is the first example in any species of a new cause of fetal and neonatal morbidity; i.e., genetic differences involving a particular blood factor which has a normal incidence in any racial group. Undoubtedly, many examples will be found in veterinary medicine, at least in those species which are characterized by a placenta which does not differ radically from that in man.

The essential feature of this form of fetal or neonatal morbidity is its selective effect on Rh+ offspring. Thus, in the case of twins only one of whom is affected, the normal member is always Rh−. Undoubtedly, the mechanism of isoimmunization may be operative in cases of selective fetal death in many animal species. As examples may be cited the observations of Corner99 and Robinson95 on the cause of selective intrauterine death in pigs and ferrets. These workers described multiple births in uteri which are normal in all respects, and yet they harbor dead and normal fetuses lying side by side. On the basis of the findings in man one is tempted to speculate that the dead fetuses have a particular blood property derived from the male parent which is absent from the blood of the normal fetuses and their mother.

It is significant that the method employed to supply the evidence for the pathogenesis of erythroblastosis fetalis is mainly statistical. Accordingly the same procedure may be used to determine whether or not isoimmunization by fetal blood (Rh or any blood factor) may or may not play a role in complications of pregnancy or other conditions of the fetal and neonatal period.91 Some preliminary findings on the role of A and B factors in causing stillbirths other than that due to erythroblastosis fetalis have already been published.86 In this connection mention may also be made of the recent findings of Yannet92 and Snyder93 on the high incidence of Rh negative mothers of infants and children affected with the so-called mental insufficiencies of the undifferentiated group. The possible relationship of these cases to the after-effects of kernicterus is still to be determined.

ADDENDUM

In this contribution the author made reference to several publications which appeared after this paper was delivered at Dallas (November, 1946).

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