Hemolytic Disease of the Newborn Infant Caused by Maternal Sensitization to the Blood Factor hr' (c)

Report of Two Cases with Special Reference to the Etiologic Significance of Multiple Blood Transfusions in Rh Positive Women Prior to Gestation

By JOSEPH GRUNDORFER, M.D.

HEMOLYTIC DISEASE of the newborn infant due to maternal sensitization to the Hr blood factor is no longer an unusual event. Case reports on this subject, however, are infrequent and in some instances lack sufficient information to elucidate the role of this factor in the pathogenesis of this disease. Though some evidence on the occurrence of hemolytic transfusion reactions due to Hr sensitization had been presented by some investigators prior to the report of case 2 in a previous communication by the present author, no well described case of an Rh-positive woman sensitized by multiple blood transfusions prior to pregnancy had as yet been reported.

The following report presents two Rh-positive women who, prior to gestation, had been transfused repeatedly and subsequently gave birth to infants afflicted with hemolytic disease. The management of such cases demonstrates the application of our knowledge of the Rh-Hr blood group system. With complete serologic and hematologic data on hand, this presentation may be regarded as another contribution towards a better understanding of the role of this blood factor in the mechanism of maternal isoensitization.

Case Reports

Case 1

Mrs. V. M., 34 years old, white, a Para I, Gravida III, with a history of an uneventful first full term pregnancy in 1946. In February 1951, she became pregnant again and a routine prenatal Rh test was performed on her. The results were as follows: Group AB, type Rh. These findings appeared to be of no further significance. A spontaneous miscarriage at ten weeks of gestation terminated this pregnancy. Because of profuse hemorrhage she had to be transfused, and two pints of citrated blood were given to her. Shortly after, she became pregnant for the third time. This pregnancy she carried through to full term. On April 7, 1952, she was delivered of a female infant weighing 6 pounds 6 ounces. Labor had lasted two hours and fifteen minutes.

The infant appeared extremely pale and markedly icteric. Sclerae and mucous membranes were yellow. Petechial hemorrhages could be noticed on the face, abdomen, and both legs. Liver and spleen appeared considerably enlarged. The free liver edge was palpable at the level of the umbilicus; the lower pole of the spleen three fingers below the left costal arch. The cry was faint. The general condition appeared very critical.

The laboratory findings were as follows: red blood cells 1,740,000; white blood cells (corrected) 31,000; hemoglobin 50 per cent; total nucleated cells 288,000; differential count: polymorphonuclear leukocytes 54 per cent, stab cells 12 per cent, eosinophils 2 per cent,
HEMOLYTIC DISEASE OF THE NEWBORN

basophils 1 per cent, myelocytes 11 per cent, myeloblasts 6 per cent, lymphocytes 7 per cent, monocytes 7 per cent; 816 normoblasts, 79 erythroblasts, and 36 megaloblasts were counted per 100 white blood cells. The infant was group A,B, type Rh1Rh2 (table 1). The direct anti human globulin test (Coombs' test) was strongly positive (4 plus). The serum showed a bilirubin content of 8.4 mg. per cent.

The diagnosis of icterus gravis neonatorum had to be made though the mother appeared Rh positive and hemolytic disease due to major group incompatibility could be ruled out, since mother and infant both belonged to group AB (table 1). In spite of the critical con-

<table>
<thead>
<tr>
<th>Blood of</th>
<th>Group and subgroup</th>
<th>M-N type*</th>
<th>Rh-Hr type</th>
<th>Possible genotypes†</th>
<th>Reactions with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti-hr' (c)</td>
</tr>
<tr>
<td>Father</td>
<td>A2</td>
<td>MN</td>
<td>Rh1Rh2</td>
<td>RPR2 (cDE/cDE) or RPR' (cDE/cdE)</td>
<td>Positive</td>
</tr>
<tr>
<td>Mother</td>
<td>A1B</td>
<td>N</td>
<td>Rh1Rh1</td>
<td>RPR1 (CDe/CDe) or RPR' (CDe/Cde)</td>
<td>Negative</td>
</tr>
<tr>
<td>1st child (male, age 6 years)</td>
<td>A1</td>
<td>N</td>
<td>Rh1Rh2</td>
<td>RPR2 (CDe/cDe) or RPR* (CDe/cDe)</td>
<td>Positive</td>
</tr>
<tr>
<td>2nd child—patient (female)†</td>
<td>A2B</td>
<td>N</td>
<td>Rh1Rh2</td>
<td>As in 1st child</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* The M-N types are not important clinically.
† The second child is the result of a third term pregnancy. One year ago, a second pregnancy had been terminated by a spontaneous miscarriage at twelve weeks of gestation. At that time, the mother received two pints of citrated blood belonging to group A1B, Rh1rh and AB, rh respectively.
‡ Listed in order of their frequency.

dition of the infant, no transfusion was attempted until the mother's blood was carefully re-examined. The result was identical with that previously established. However, additional tests revealed her as hr' negative, her serum containing a potent antibody against the hr' factor (table 2).

The conclusion was drawn that blood not being agglutinated by this antibody would be most suitable for transfusions of the infant. From this viewpoint, donors whose red cells did not react with the maternal serum and also appeared negative for the hr' factor by other testing fluids were selected. Applying the indirect anti human globulin technic, such blood was also crossmatched with the infant's serum, and the first transfusion started six hours after birth. This transfusion consisted of the administration of 360 cc. of citrated whole blood—group B, Rh1Rh1 (CDe/CDe)—through the right antecubital vein and the withdrawal of 270 cc. of venous blood from the anterior fontanel. This procedure took four hours and no difficulties were encountered in removing blood from the fontanel. During the following night the temperature rose to 102 F.

On the next day, the red cell count was 3,655,000, the white cell count (corrected) 14,200, the hemoglobin 80 per cent, the nucleated cell count 61,800; the blood smear showed 414...
<table>
<thead>
<tr>
<th>Technic of Titration</th>
<th>Test Cells</th>
<th>Dilution of the maternal serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Undil.</td>
</tr>
<tr>
<td>Agglutination (saline-)</td>
<td>Rh,rh (CDe/cde)</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Rh,rh (cDE/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>rh rh (cde/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Rh,Rh (CDe/CDe)</td>
<td>-</td>
</tr>
<tr>
<td>Albumin-plasma conglutination</td>
<td>Rh,rh (CDe/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Rh,rh (cDE/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>rh rh (cde/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Rh,Rh (CDe/CDe)</td>
<td>-</td>
</tr>
<tr>
<td>Anti human-globulin (Coombs' test)</td>
<td>Rh,rh (CDe/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Rh,rh (cDE/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>rh rh (cde/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Rh,Rh (CDe/CDe)</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme-treated cells (trypsin)</td>
<td>Rh,rh (CDe/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Rh,rh (cDE/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>rh rh (cde/cde)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Rh,Rh (CDe/CDe)</td>
<td>-</td>
</tr>
</tbody>
</table>
HEMOLYTIC DISEASE OF THE NEWBORN

normoblasts, 13 erythroblasts, and 10 megaloblasts per 100 white blood cells. Another replacement transfusion was performed; 350 cc. of cells—group O, Rh1Rh1 (CDe/CDe)—resuspended in normal saline solution were given through a malleolar vein of the right foot while 195 cc. were again removed from the fontanel. This transfusion lasted four and a half hours. During the first twenty-four hours of the infant’s life, a total volume of 710 cc. of blood was given while 465 cc. of blood were withdrawn. To protect the infant from tetany which is sometimes a direct result of the toxic effect of the citrate administered with the donor’s blood, 1 cc. of a 10 per cent calcium gluconate solution was given for each 100 cc. of transfused blood. On the following day, the jaundice appeared to be markedly deepened; however, the infant seemed generally improved. The cry was vigorous, the temperature normal. The red cell count rose to 5,765,000, the hemoglobin to 104 per cent.

In the meantime, complete blood group studies were carried out on the infant and the other members of the family, the results of which are summarized in table 1. Although the entire family was found Rh positive, it could be observed that the mother belonged to type Rh1Rh1 (CDe/CDe, or CDe/Cde) while the father showed Rh2Rh2 (cDE/cDE, or cDE/cdE) and both infants consequently Rh1Rh2 (CDe/cDE, or CDE/cDe, or CDe/cdE, etc.). The latter possessed the factor hr' which the mother was lacking. As already indicated major group incompatibility could be ruled out. The transfusion history also appeared to be of considerable significance, and revealed that this hr'-negative mother had received two pints of blood, both positive for hr', (one pint was group AB, type rh, the other group A, type Rh1rh) at the time of the miscarriage in 1951. It was evident that, in addition to pregnancy, the mother had become sensitized to hr' by these blood transfusions.

The antibody was studied on serum obtained from the mother six hours and five and twenty-three days postpartum. In addition to tests performed in saline and albumin-plasma media, the indirect anti human globulin method and the technic with enzyme-treated red cells were applied in all further investigations. The serum strongly agglutinated all cells negative for rh' and presented a selective reactivity towards cells containing rh'; type Rh1Rh1 cells were not affected. The serum was tested against one hundred and eighty-five random blood specimens. One hundred and forty-nine (80 per cent) were agglutinated by this serum while thirty-six (20 per cent) showed no reactions at all. Among ninety-five 1)1001)100 specimens belonging to type Rh1 or rh', 62.1 per cent were found of subtype Rh1rh (CDe/eDe) or rh'rh (Cde/eDe)—heterozygous, and 37.9 per cent of subtype Rh1Rh1 (CDe/CDe)—homozygous (table 3). Titer studies of this antibody disclosed a marked activity in saline (titer: 32 units) and the reactions multiplied in strength when the other methods were used (table 2).

In order to rule out the presence of an antibody for the blood factor rh'', absorption experiments were carried out. For that purpose, the serum was absorbed with type rh blood and lost its capacity to agglutinate cells of type Rh1rh, rh, and Rh0. (Suggestive for the presence of an antibody for rh'' would have been an unaffected reaction with type Rh0 blood, and no reaction at all in control studies after absorption with type Rh0 blood. Though the presence of an hr antibody appeared unlikely in this case, the serum was also explored in this direction. It was calculated that blood containing the factor hr' (c) and lacking the factor Hr (d) would best be suitable for this absorption experiment. This requirement could be fulfilled by obtaining group O, Rh3Rh3 (cDE/cDE) blood from an individual, the homozygosity of which for Rh0 (−D−/−D−) could be proven by blood group studies of its family. The serum was, therefore, absorbed with such blood; the final outcome of all investigations resulted, however, in negative findings. The conclusion could finally be drawn that this mother had exclusively become sensitized against the factor hr'.

Additional titration experiments to determine the quantity of maternal Hr antibodies in the infant's serum were carried out. They revealed the absence of any activity of this serum in saline and an appreciable quantity of an antibody demonstrable by the albumin-plasma method (titer: 16 units) and the anti human globulin technic (titer: 128 units). The cells used in the experiment were of type Rh1rh, Rh0, and rh; type Rh1Rh1 was used as a control.
Though the clinical condition of the infant remained satisfactory, no regression of the deep icteric discoloration of skin and sclerae could be noticed while the red cell count and hemoglobin kept on dropping slowly. On the fifth day of life, the red cell count was 4,250,000, the hemoglobin 85 per cent. The blood smear showed 5 normoblasts per 100 white blood cells. The infant received intravenously 70 cc. of saline suspended cells (group O, Rh1Rh1) and a prompt improvement of the blood picture could be recorded (red cells:

\[
\begin{array}{c|c|c|c|c|c|c|c|c|c}
\text{Number} & \text{Reactions} & \text{Most frequent genotype} & \text{Incidence of positive reactions} \\
\text{of tested} & \text{with Rh antisera} & \text{in this group of reactions} & & \text{Antic} & \text{Antie} & \text{Maternal serum} \\
\text{cells} & & & & & & & & \\
\hline
2 & + + - - + & r'r' Cde/cde & 2 & 2 & 2 & heterozygous \\
37 & + + + - + & R'r CDe/cde \ R'r R' & 57 & 57 & 57 & (62.1\%) \\
36 & + - - - + & R'r' C'cDe/cDe \ R'r R' & 0 & 36 & 0 & homozygous \ (37.9\%) \\
24 & - + - - + & r'r cde/cde & 24 & 24 & 24 \\
2 & - - + + + & r'r cde/cde & 2 & 2 & 2 \\
19 & - + + + + & R'r R' cDE/cDE \ R'r' & 19 & 19 & 19 \\
4 & - + + + - & R'r R' cDE/cDE \ R'r' & 4 & 0 & 4 \\
17 & - + - - + & R'r cDe/cde & 17 & 17 & 17 \\
24 & + + + + + & R'r R' cDe/cDE \ R'r' & 24 & 24 & 24 \\
185 & & & & 149 & 181 & 149 & (Total) \\
\end{array}
\]

Anti-C . . . anti-rh'; anti-D . . . anti-Rh0; anti-E . . . anti-rh*; anti-e . . . anti-hr'; anti-e . . . anti-hr*.

The incidence of reactions with known anti-hr' (e) and anti-hr* (e) sera is compared with the incidence of reactions with the maternal serum.

5,360,000; hemoglobin: 104 per cent). Two days later the infant had a profuse hemorrhage from the base of the necrotic stump of the umbilical cord and lost approximately 40 cc. of blood. The infant had to be taken to the operating room where an active bleeding vessel had to be ligated. Afterwards the infant appeared relatively well though a considerable drop in the red cell count (4,230,000) and hemoglobin (76 per cent) had taken place again. Another transfusion of 80 cc. of saline suspended cells (group O, Rh1Rh1) was given whereupon the infant rapidly improved. Three weeks after birth, the jaundice had almost disap-
HEMOLYTIC DISEASE OF THE NEWBORN

peared and the skin presented a normal color. On April 21, the red cell count was 4,520,000, the hemoglobin 102 per cent. The infant was kept under observation and was discharged on May 18, 1952. Since then the baby has repeatedly been seen and has never exhibited any neurologic sequelae.

Case 2

Mrs. E. H., 36 years old, white, a Para 0, Gravida I, had received several blood transfusions for the treatment of gastric hemorrhage in 1947. Early in 1951, when she became pregnant, a prenatal Rh test was performed on her and her husband. The results were as follows: Mrs. E. H.: Group A, type Rh,Rh (CDe/CDe), type MN; Mr. H. H.: Group O, type Rh, Rh (CDe/cde), type N. The incompatibility of hr' (c) was apparent but antibody studies yielded negative findings.

Her physician requested a repetition of the test because she had been found "Rh negative" in the hospital where she was transfused. The results of the second test were the same. No further serologic investigations were attempted and her pregnancy followed an entirely uneventful course.

On October 11, 1951, she was delivered of a full term, female infant. Labor had lasted for seven and a half hours and a breech presentation did not complicate delivery. At birth, the baby appeared well developed and weighed 5 pounds and 10 ounces. The general condition was good; no anomalies were noted. Liver and spleen were not palpable. On the following day, physical examination revealed no unusual findings except for a moderate jaundice of skin and sclerae and some pallor of the mucous membranes. The infant appeared alert with intact reflexes.

The hematologic findings were as follows: red blood cells 3,620,000; white blood cells (corrected) 27,100; hemoglobin 88 per cent; total nucleated cells 35,700; differential count: polymorphonuclear leukocytes 33 per cent, stab cells 19 per cent, eosinophils 2 per cent, lymphocytes 15 per cent, monocytes 4 per cent, myelocytes 7 per cent, 132 normoblasts per 100 white blood cells (276 normoblasts per 100 white blood cells were found in one blood smear taken immediately after birth). The serum showed a bilirubin content of 7.3 mg. per cent. Serologic studies were carried out on venous blood obtained from the fontanel. The baby was group O, type Rh,Rh (CDe/cde), type MN. The direct anti human globulin test (Coombs' test) was strongly positive (4 plus). The serum did not show any agglutination in saline, but a specific activity against group O, Rh negative cells could easily be noted in albumin-plasma media (titer: 4 units); an indirect anti human globulin test also showed 4 units, while a titration against group O, Rh negative cells treated with trypsin yielded 32 units. A homozygous Rh cell was used as a control.

On the basis of these findings, the diagnosis of hemolytic disease of the newborn infant due to maternal hr' sensitization was made, and the infant received, through the antecubital vein, 60 cc. of citrated blood, group O, Rh,Rh (CDe/CDe).

For the next three consecutive days, the hematologic findings were as follows:

<table>
<thead>
<tr>
<th></th>
<th>October 14</th>
<th>October 15</th>
<th>October 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>5,620,000</td>
<td>4,340,000</td>
<td>4,370,000</td>
</tr>
<tr>
<td>White blood cells</td>
<td>21,800</td>
<td>15,100</td>
<td>9,900</td>
</tr>
<tr>
<td>Hemoglobin (in per cent)</td>
<td>118</td>
<td>106</td>
<td>108</td>
</tr>
<tr>
<td>Differential count:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphonuclear leukocytes</td>
<td>72</td>
<td>71</td>
<td>61</td>
</tr>
<tr>
<td>Stab cells</td>
<td>17</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>4</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>6</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Monocytes</td>
<td>—</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Normoblasts (per 100 white blood cells)</td>
<td>78</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>
Along with the improved hematologic picture, the clinical condition of the infant appeared satisfactory. The formula was taken well and the baby made good gains in weight. The jaundice gradually disappeared and, on October 19, the baby was discharged with the following hematologic findings: red blood cells 5,140,000, hemoglobin 104 per cent, absence of normoblasts. The anti human globulin test, however, was still positive (3 plus).

Additional information was obtained from the hospital where the mother stayed in 1947. According to the records she had received 500 cc. of citrated blood on admission without any ill effects. Another transfusion, started forty-eight hours later, caused chills and an elevated temperature of 104 F. and had to be discontinued. The Rh factor had been determined and undoubtedly she was erroneously typed as Rh negative. (The report from the hospital stated that, at that time, an Rh factor determination along with a blood typing was not a routine procedure.) She later received Rh-negative blood. In summary, six transfusions, each consisting of 500 cc., and four transfusions, each consisting of 250 cc., of citrated blood had been administered to her.

An extensive study of the serum of this mother was now carried out. All tests were performed in saline and albumin-plasma media; the slide test as well as the indirect anti human globulin technic (Coombs’ test) was also employed and proved to be extremely valuable in all tests found negative to saline agglutination. Thus findings, especially on type Rh1 bloods, established with the albumin-plasma technic could easily be checked. Potent anti-hr’ sera from two different sources* were used to verify all the results.

One hundred and seventy-eight blood specimens were examined; one hundred and thirty-eight (77.5 per cent) were agglutinated by this serum while forty (22.5 per cent) showed no reactions at all. Among one hundred and four blood specimens belonging to type Rh1 or rh’, 61.5 per cent were found to be heterozygous and 38.5 per cent homozygous. Titer studies which also included the technic with enzyme-treated red cells revealed that this serum possessed a selective reactivity in saline towards all Rh-negative bloods and to a lesser degree towards bloods belonging to type Rh1, Rh0, and RhRhRh respectively. Heterozygous bloods (R1r’ or r’r) showed no agglutination in saline, but a strong reaction in albumin-plasma media; these findings corresponded to titer readings established by the anti human globulin technic as well as against enzyme-treated red cells; in no instance could any abnormal reaction be obtained against bloods which had been found homozygous (R’R’ or r’r’) with the available known anti-hr’ sera. The pronounced higher titer findings in the infant’s blood could only be explained by the altered condition of its red cells, which had been found to be already considerably coated by the maternal antibody when the direct anti human globulin test was first performed.

The conclusion could finally be reached that this serum contained an antibody of the specificity anti-hr’ (anti-c). It showed an agglutinating (saline) titer of 2 units in strength against all bloods negative for rh’ and some bloods belonging to type RhRhRh exhibited a specific conglutination (albumin-plasma) titer of 32 units in strength against all heterozygous Rh1 bloods. The coating property of this antibody became particularly evident from the tests performed with the anti human globulin reagent. Attempted blocking tests always yielded negative results.

**DISCUSSION**

The purpose of this presentation is to offer additional evidence of two atypical cases of hemolytic disease of the newborn infant, the cause of which could only be explained by a thorough study of all so-called Rh-Hr blood types. The rarity of this unusual pattern of sensitization is best illustrated by reviewing some data established during the first five year period of this laboratory.

Between April 1, 1947, and March 31, 1952, a total of 12,226 pregnant women had been examined. Sixteen hundred and twenty-eight (13.98 per cent) had been

* Certified Blood Donor Service, Jamaica, N. Y.; Ortho Pharmaceutical Corporation, Raritan, N. J.
found Rh negative by routine tests while five hundred and eighty women known as Rh negative had specifically been referred for further studies. One hundred and thirty-nine women showed evidence of sensitization against the rhesus factor. Fifteen cases of isosensitization in Rh-positive women due to the ABO blood group system had been seen while only two cases due to the hr' blood factor could be encountered within the same time period.

Many investigators agree that Hr sensitization may produce a disease identical with that caused by Rh sensitization. The incidence of sensitization to this blood factor remains, however, extremely small. From this viewpoint the aforementioned findings closely resembling those made by other workers were established. At the Chicago Lying-in Hospital, Potter found, among 7000 women tested for the Rh factor, only one who during her second pregnancy became sensitized to the factor hr'. The infant afflicted with severe hemolytic disease died at the age of three days. Her third pregnancy terminated in the delivery of an infant with apparent hydrops who died at birth.

In the early investigations, Levine and Javert reported one case of Hr sensitization in an Rh-positive mother. In this case, the second pregnancy was terminated by the delivery of an erythroblastotic infant who died on the second day of life. Her serum contained an antibody which had the property of agglutinating all Rh-negative bloods and all those Rh-positive bloods which did not react with anti-rh' serum. In 1943, McCall, Race, and Taylor reported the first case of Hr sensitization in England. A case of hemolytic disease in a newborn infant was described where the father and all three infants were Rh1 heterozygous while the mother was Rh1 homozygous. Her serum contained an antibody which reacted with all Rh-negative bloods. However, it differed from the serum of Levine's case in the higher percentage of positive reactions (80 per cent instead of 30 to 50 per cent). This antibody was named St and later was shown by Wiener to be identical with Hr. Wiener also pointed out that the difference in the number of positive reactions was due to the lower titer of Levine's serum which failed to react with heterozygous blood. Another similar case was subsequently reported by McCall and Holdsworth in 1945.

Levine was the first to describe the Hr factor. Wiener offered the explanation that the Hr factor can only be responsible for hemolytic disease in the offspring if the mother is Hr negative while the father and infant are Hr positive. The conclusion that an infant with hemolytic disease due to maternal Hr sensitization may be Rh negative has to be considered as incorrect. The infant cannot possibly be Rh negative, because it must inherit either gene R' or gene r' from its mother. The hr' factor can only be involved in cases of hemolytic disease of the newborn where the mother belongs to type Rh1 (or, very rarely, to type rh') and the infant does not belong to type Rh2, Rh0, rh", or rh. It is quite plausible that there need not be any limitations as to the Rh type of the father because Hr-positive individuals occur in all Rh types.

This conclusion is in accordance with the findings in the two cases reported by the British workers and in other investigations and as well as with those established in our two cases. In each instance, the entire family was also found Rh positive (mother—homozygous, father and infant—heterozygous). Both mothers had been transfused with Rh negative and heterozygous Rh
positive blood (Rh₁rh) prior to a first and third pregnancy respectively. The repeated administration of these bloods had a decisive influence on the mechanism of sensitization of the Hr-negative mother with respect to her Hr-positive offspring. Had the latter been homozygous, the transfusion history would never have risen to any appreciable degree of importance and thus the opportunity would have been missed to realize all the implications of giving such transfusions to an Rh-positive woman prior to or during her child bearing age. Because of its early occurrence during pregnancy, a miscarriage, recorded in the first case, could definitely be dismissed as an etiologic factor in this immune process.

Hemolytic disease in the newborn as a result of previous maternal Hr sensitization due to transfusions is a rare event and no clearly described case has as yet been reported in the literature. Boorman and associates²⁶ reported one case of an Hr-negative mother who had been transfused prior to her second pregnancy and showed an Hr antibody in her serum after delivery. However, the presence of that antibody was not conclusive evidence that the mother had been sensitized by the transfusion she received because Rh typing of the transfused blood was never carried out.

That Hr sensitization may eventually play an important role as a cause of hemolytic transfusion reactions in Rh-positive individuals, has been demonstrated by Wiener¹ in a series of thirty-two Rh-positive patients who experienced various reactions after the administration of blood. Of twenty-two individuals of this group, who exhibited febrile reactions and evidence of hemolysis after transfusions, seventeen were reported as Hr negative; on the other hand, febrile reactions with absence of post-transfusion hemolysis were reported in ten patients, none of whom were Hr negative. This report is of value as it fosters a better understanding of the relationship of this blood factor to clinical medicine, especially in regard to the mechanism of isosensitization in any case like these two presented in this paper.

The Hr blood factor is generally considered as a poor antigen. Evidence of Hr sensitization manifested by antibody formation is very infrequent, and according to reports on this subject, Hr sensitization, when it occurs, appears to be usually mild in degree.¹,⁹ Nevertheless, the danger which potentially may arise from transfusions of Rh-negative blood to an Rh-positive woman especially in regard to her future offspring should be emphasized. It is well known that when several transfusions of Rh-positive blood are given to an Rh-negative individual at intervals of only a few days, antibodies are rarely formed in sufficient quantities to cause a reaction following any of these transfusions. Transfusion reactions seem to occur most commonly in patients who have had two or more transfusions within a few days or weeks and who have not subsequently received blood for several months or years. According to Wiener,¹,¹⁷,¹⁸ the wide spacing of transfusions is probably the most important factor in the development of isosensitization. Furthermore, pregnancy seems to contribute a more effective agent in producing sensitization than does one or even several transfusions.

The second case reported in this paper demonstrates especially well the time lag in the process of immunization. The mother had received a series of transfusions supposedly within a week or two. Four years later when she became pregnant no evidence of sensitization could be found and apparently the entire
pregnancy was necessary to provide a sufficient stimulus for the formation of the antibody. Without previous transfusions she probably would never have formed this antibody against a blood factor of such poor antigenicity. This conclusion could definitely be drawn with regard to the numerous case histories of first pregnancies with a true Rh incompatibility but without preceding transfusions. From these observations we know well that during the course of such pregnancies the chance of developing a concentration of antibodies sufficient to produce hemolytic disease in the newborn infant is very slight. On the other hand, the important role of blood transfusions in female infants and adults prior to their child bearing period with the consequently higher incidence of fatal forms of hemolytic disease in the first born is well known and described in the literature, and needs no further emphasis.

In our two cases the severity of the resulting disease in each infant varied greatly. In case 1, the infant presenting the typical clinical picture of icterus gravis neonatorum appeared critically ill. Immunohematologic findings additionally gave ample evidence of the severity of the disease. The favorable outcome could be ascribed to the prompt recognition of this unusual immune process and proper treatment of the infant by exchange transfusions of carefully prepared blood. The resulting disease in the infant of the second case was mild. The symptoms appeared identical with those usually encountered in cases of icterus praecox while the laboratory findings gave more accurate information as to the extent of the actual hemolytic process. In this case, the favorable outcome could be directly correlated with the late occurrence of the maternal antibody during pregnancy as well as with its moderately elevated albumin-plasma titer.

Transfusions in women with possible previous sensitization can be properly controlled today by checking their Rh-Hr status. Nevertheless, pregnancy in such women will always present a certain problem with respect to the welfare of their future offspring. The medical profession should, therefore, be aware of the possible danger in using Rh negative blood as a universal safe donor in either ungrouped or erroneously grouped Rh-positive cases. Routine pretransfusion testing for the Hr blood factor as well as Rh subgroups may not appear very practicable; however, in the light of our present knowledge and with the ever increasing popularity of transfusions as a therapeutic measure the routine performance of such tests appears highly advisable especially when a female must be transfused prior to her child bearing period.

**SUMMARY AND CONCLUSIONS**

1. Intragroup Rh incompatibility between an Rh-positive mother and an Rh-positive infant presents an unusual pattern of maternal isosensitization.
2. The antibody found in the sera of two Rh-positive mothers appeared to be of anti-hr' (anti-c) specificity.
3. Evidence of hemolytic disease in both infants was found and the findings directly correlated with this maternal antibody.
4. The welfare of the infant greatly depends on prompt recognition of this unusual pattern of maternal isosensitization and proper treatment of its disease.
5. An attempt has been made to evaluate the role of multiple transfusions of
Rh-negative blood which had been given to an Rh-positive mother four years prior to gestation.

6. The wide spacing of several transfusions or transfusion and pregnancy respectively appears to be probably the most important factor in the development of isosensitization.

7. The possible danger in using Rh-negative blood as a universal safe donor is briefly discussed.

REFERENCES

20. Dunne, C. J. and Innella, F. P.: Dual sensitization to the rh" (E) and hr' (e) factors detected antenatally. A case report. Blood 7: 526, 1952.
Hemolytic Disease of the Newborn Infant Caused by Maternal Sensitization to the Blood Factor hr' (c): Report of Two Cases with Special Reference to the Etiologic Significance of Multiple Blood Transfusions in Rh Positive Women Prior to Gestation

JOSEPH GRUNDORFER