ERYTHROBLASTOSIS FETALIS IN THE FIRST-BORN

Prevention of Its Most Severe Forms

By Philip Levine, M.D., and R. K. Waller, M.D.

A result of our knowledge of the pathogenesis of erythroblastosis fetalis, a new diagnostic procedure, testing for the Rh factor, has been made available.1, 2 Another outcome of the early work was the recommendation3 that human anti-Rh agglutinins derived from mothers of erythroblastotic infants are far preferable to experimental anti-Rh blood immune serum, which, by and large, is now no longer used.* Tests with potent diagnostic anti-Rh serums were recommended for all women with a history of fetal or neonatal morbidity, in order to prevent intragroup transfusion accidents, and for the selection of Rh negative blood for the affected infants of Rh negative mothers. The findings presented below indicate that future emphasis should be placed on the prevention of iso-immunization by transfusions in the group of Rh negative female patients at any time prior to possible pregnancies.

Iso-immunization by the Rh factor occurs in two groups of cases, namely, (1) Rh negative individuals after repeated transfusions of Rh positive blood, (2) Rh negative women immunized by Rh positive fetal blood. A third group may be mentioned in which both factors, transfusions and pregnancy, are operative.

This paper deals mainly with selected cases of the third group in which iso-immunization was initiated by transfusions and, after a variable interval, became intensified through pregnancies. Evidence is presented which indicates that the combined action of both factors in the order given served to increase the number of infants with erythroblastosis fetalis as well as the severity of the condition in the infant.†

It is now generally accepted that 92 per cent of all cases of erythroblastosis fetalis result from (1) immunization of the Rh negative mother by Rh positive fetal blood, and (2) subsequent action of maternal anti-Rh antibodies (agglutinins and blocking antibodies) on the susceptible Rh positive fetal blood. The obstetric histories of mothers of erythroblastotic infants reveal that this condition occurs in the first-born only very rarely. Obviously, one or more pregnancies with Rh positive infants are required to induce a sufficient degree of iso-immunization. Once an Rh negative woman is immunized, each successive pregnancy with an Rh positive fetus results in increasingly severe forms of erythroblastosis fetalis.‡

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* In the statistical studies on the pathogenesis of erythroblastosis fetalis, human anti-Rh serums were used exclusively.
† For a preliminary report, cf. Levine.4
‡ In this paper the terms Rh positive and Rh negative refer to reactions with the diagnostic serum of choice, i.e., anti-Rho, which detects about 92 per cent of all instances of iso-immunization by the Rh factor. It is not advisable for the clinician to commit to memory several terminologies based on the action
The observation in 1944 of a young Rh negative woman, aged 20 (case 1, table 2), whose first pregnancy resulted in the most severe form of erythroblastosis fetalis—fetal hydrops—suggested the possibility that the patient was already immunized at the time of her first pregnancy. On being questioned, she stated that at the age of 6 (fourteen years before) she had been transfused several times with her father's blood. Although immune antibodies (anti-Rh agglutinins or blocking antibodies), in contrast to normal iso-antibodies, have a comparatively short duration in the blood plasma—i.e., from several weeks to one or more years after pregnancy—the reticulo-endothelial cells responsible for antibody production probably retain the sensitized state for a long time, if not permanently. Under such conditions, the response to readministration of the same antigen even many years later, in the form of either fetal blood or transfusion, is more rapid and more intense. These cases serve as excellent examples of the phenomenon known to immunologists as the 'anamnestic reaction.'

The anamnestic reaction represents "the production in response to an antigenic stimulus of an antibody that has been produced in the tissues on some previous occasion." In the past, most workers were interested mainly in nonspecific response, but the term is used here to indicate a response to restimulation many years later by the same antigen, i.e., the Rh factor. In 3 cases published by Levine, the immunization in Rh negative patients was initiated by pregnancy, and many years later transfusion of Rh positive blood resulted most unexpectedly in severe reactions with varying degrees of oliguria. Cases of a similar nature were also observed by Weiss.

In one of the cases cited, an Rh negative woman born in 1885, had one daughter (Rh positive) in 1904. There were no other pregnancies. In 1937, the patient had one uneventful blood transfusion from her Rh positive daughter, given because of mastoiditis. This additional stimulus apparently immunized the patient, so that in May 1941, four years later, when a diagnosis of leukemia was established, another transfusion of her daughter's blood was followed by a delayed reaction.* A further transfusion from another Rh positive donor three months later resulted in a severe reaction and oliguria. Anti-Rh agglutinins of moderate activity were found.

Accordingly, the possibility suggested itself that erythroblastosis fetalis in the first-born might occur far more frequently if the Rh negative patient had been transfused at any time prior to the pregnancy, even in her childhood or girlhood. This view could be put to the test in a statistical analysis of the large material tested in the years from 1940 to 1945.

In this study, limited to approximately 700 Rh negative women, only twenty-eight instances were found in which the first Rh positive infant had erythroblastosis fetalis.† Of these, nineteen mothers gave histories of one or more transfusions of other varieties of anti-Rh serums that are either important in very few cases or not available for distribution. It is, however, necessary for the clinician to be guided by consulting with those serologists who conduct active research in this field. For a description of a similar case with a delayed reaction to a transfusion, cf. Levine et al.† The incidence of erythroblastosis fetalis in the first-born cannot be derived from this study, since complete obstetric histories were not always available.
at various times prior to the first full term or almost full term pregnancy, and no such history could be elicited in a control group of nine mothers. By itself, the ratio of 2:1 was disappointing, because it was felt that the effects of previous iso-immunization by transfusion would be far more striking. Further reference to the possible role of other immunizing stimuli in the control group will be made below. In any event, the true state of affairs was revealed by taking into account the severity of the condition in the infant. These findings are summarized in table 1.

These findings seemed to justify the conclusion that previous transfusions initiate iso-immunization, which is intensified by the first pregnancy.* In nine of eleven subsequent pregnancies, the end result was fetal death, while two women are still to be delivered. The most severe forms, ending in intra-uterine fetal death, are twelve times as frequent as mild cases.† Assuming that all severe cases can be successfully treated with transfusions of Rh negative blood and by other measures, only 7 of the 19 cases could have been saved, in contrast to 8 of 9 cases in the control group.

<table>
<thead>
<tr>
<th>Table 1. — Erythroblastosis Fetalis in the First Rh+ Infant in Rh− Women</th>
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<tbody>
<tr>
<td>Transfusion History</td>
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<tr>
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<tr>
<td>No. cases</td>
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<tr>
<td>Severity of disease</td>
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<tr>
<td>mild</td>
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<tr>
<td>severe</td>
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<tr>
<td>fetal death</td>
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</table>

The single death in the control group may possibly be attributed to causes other than erythroblastosis fetalis. The blood of this patient, Mrs. Mar (case 15, table 4), group O, Rh negative, was submitted by Dr. Lowell Erf. The patient was 24 years old, and her first pregnancy was to terminate in the middle of November 1944. However, she failed to develop spontaneous labor, on Dec. 4 fetal heart sounds were no longer heard, and a medical induction was started. After much difficulty, a yellow, macerated fetus was expelled: "microscopic sections revealed that the bone marrow was hyperplastic and that there was some extra-medullary hematopoiesis in the spleen and liver." The mother developed septicemia subsequent to a pelvic peritonitis and expired Dec. 12. Tests of the mother's blood three days prior to her death were negative for agglutinins and blocking antibodies.

The essential details in the cases of the nineteen Rh negative mothers previously immunized by transfusions are set forth in table 2.

Of the nineteen Rh negative women, three (cases 10, 16, 19) had early pregnancies prior to the first full term or almost full term pregnancy with an Rh positive infant. Two of these had early abortions, while the third had a ruptured ectopic gestation. These cases can be included in the series, since iso-immunization cannot be initiated by fetal blood unless the pregnancy is so well advanced that the placenta presents a sufficiently large surface area of fetal villi to the maternal

* On a theoretic basis, this possibility has already been mentioned by Katzin and Mollinson.
† A high incidence of fetal death was also reported by Diamond in a similar group of cases.
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Patient (Mother)</th>
<th>Physician</th>
<th>Interval between transfusion and pregnancy (yr.)</th>
<th>No. transfusions</th>
<th>Indication for transfusion</th>
<th>Condition of First Rh+ Baby</th>
<th>Subsequent Pregnancies</th>
<th>Rh of Father</th>
<th>Anti-Rh Antibodies in Mother</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dw</td>
<td>F. Auerbach Irvington, N. J.</td>
<td>14</td>
<td>4</td>
<td>measles, scarlet fever, ovarian cyst; pelvic abscess</td>
<td>fetal hydrops (1944)</td>
<td>severe, 4 transfusions, death (1942)</td>
<td>Rh-</td>
<td>negative</td>
<td>Rh-Hr negative</td>
</tr>
<tr>
<td>2</td>
<td>Gw</td>
<td>J. Pinkston Nashville, Tenn.</td>
<td>1</td>
<td>9</td>
<td>bleeding after tonsillectomy</td>
<td>fetal death (1942)</td>
<td>1944 stillbirth</td>
<td>Rh-</td>
<td>Rh-Hr weak</td>
<td>weak (1944)</td>
</tr>
<tr>
<td>3</td>
<td>Mc</td>
<td>C. A. Dille Dayton, O.</td>
<td>6</td>
<td>1</td>
<td>bleeding after tonsillectomy</td>
<td>fetal death (1942)</td>
<td>1944 stillbirth</td>
<td>Rh-</td>
<td>Rh-Hr weak</td>
<td>weak (1944)</td>
</tr>
<tr>
<td>4</td>
<td>T</td>
<td>W. Tirman Brooklyn</td>
<td>10</td>
<td>2</td>
<td>mastoidectomy</td>
<td>fetal death at full term (1943)</td>
<td>now pregnant</td>
<td>Rh- negative</td>
<td>Rh-Hr weak</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ri</td>
<td>C. Kingsley Staten Island, N. Y.</td>
<td>5</td>
<td>1</td>
<td>menorrhagia</td>
<td>fetal death at full term (1939)</td>
<td>1941 fetal death at 30 wk.</td>
<td>Rh-Hr weak</td>
<td>weak (1944)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ko</td>
<td>L. R. Pyle Brooklyn</td>
<td>2</td>
<td>1</td>
<td>bleeding after tonsillectomy</td>
<td>fetal death at 6 mo. (1942)</td>
<td>1943 fetal death at 30 wk. 1945 hydrops at 34 wk.</td>
<td>Rh-Hr strong</td>
<td>strong</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Do</td>
<td>H. H. Ware Richmond, Va.</td>
<td>11</td>
<td>several</td>
<td>pseudo-hemophilia</td>
<td>severe; several transfusions, recovery (1944)</td>
<td>mild, not transfused (1942)</td>
<td>Rh-Hr weak (homozygous)</td>
<td>strong (1945)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Pl</td>
<td>H. H. Ware Richmond, Va.</td>
<td>4</td>
<td>several</td>
<td>injuries</td>
<td>macerated fetus at full term (1942)</td>
<td>1944 fetal death at 31 wk.</td>
<td>Rh-r (heterozygous)</td>
<td>strong</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Wat</td>
<td>R. Johnston Houston, Tex.</td>
<td>2</td>
<td>3</td>
<td>pyelitis, anemia</td>
<td>macerated fetus at full term (1942)</td>
<td>1945 fetal death at 100 wk. 1945 hydrops at 34 wk.</td>
<td>Rh-</td>
<td>negative</td>
<td>Rh-Hr weak</td>
</tr>
<tr>
<td>10</td>
<td>Li</td>
<td>R. Johnston Houston, Tex.</td>
<td>8</td>
<td>1</td>
<td>fractured pelvis</td>
<td>fetal hydrops, 7 mo. (1944)</td>
<td>1945 fetal death at 30 wk.</td>
<td>Rh-Hr weak</td>
<td>weak (1945)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Ha</td>
<td>J. Davenport New Orleans</td>
<td>9</td>
<td>1</td>
<td>septicemia</td>
<td>severe, death (1944)</td>
<td>1945 fetal death at 2 mo.</td>
<td>Rh-Hr weak</td>
<td>weak (1945)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>DeB</td>
<td>J. Davenport New Orleans</td>
<td>2</td>
<td>8</td>
<td>ruptured appendix, peritonitis</td>
<td>macerated fetus (1945)</td>
<td>now pregnant</td>
<td>Rh-</td>
<td>weak (1945)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Haf</td>
<td>G. V. Herrman Kansas City, Mo.</td>
<td>12</td>
<td>2</td>
<td>orthopedic operation</td>
<td>severe: recovery after transfusion of Rh- blood (1945)</td>
<td>1945 fetal death at 30 wk.</td>
<td>Rh-Hr strong</td>
<td>strong</td>
<td>baby's blood behaved like Rh-, owing to action of blocking antibody</td>
</tr>
<tr>
<td>No.</td>
<td>First Name</td>
<td>Last Name</td>
<td>City, State</td>
<td>Age</td>
<td>Sex</td>
<td>Diagnosis</td>
<td>Outcome</td>
<td>Rh Type</td>
<td>Reaction</td>
<td></td>
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</tr>
<tr>
<td>14</td>
<td>Haff</td>
<td>P. Liebling</td>
<td>New York</td>
<td>2</td>
<td>1</td>
<td>Empyema, thrombocytopenia</td>
<td>Severe: death (1945)</td>
<td>Rh Rh</td>
<td>Strong</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ham</td>
<td>W. L. Mitchell Jr.</td>
<td>Newark, N. J.</td>
<td>1</td>
<td>1</td>
<td>Ectopic pregnancy (6 wk.)</td>
<td>Severe: death at 33 wk. (1945)</td>
<td>Rho</td>
<td>Weak</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Sm</td>
<td>G. W. Schelm</td>
<td>Battle Creek, Mich.</td>
<td>2</td>
<td>1</td>
<td>Duodenal ulcer</td>
<td>Fetal death at 33 wk. (1945)</td>
<td>Rh Rh—</td>
<td>Strong</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Ne</td>
<td>A. E. Rakoff</td>
<td>Philadelphia</td>
<td>1</td>
<td>1</td>
<td>Middle ear infection</td>
<td>Severe: several transfusions, recovery (1944)</td>
<td>Rho</td>
<td>Strong</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Ga</td>
<td>R. H. Stewart</td>
<td>Seattle</td>
<td>1</td>
<td>1</td>
<td>Bleeding after abortion</td>
<td>Fetal death (1944)</td>
<td>Rh Rh—</td>
<td>Distinct</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Wa</td>
<td>M. Wachtstein</td>
<td>Middletown, N. Y.</td>
<td>1</td>
<td>1</td>
<td>Macerated fetus</td>
<td>Fetal death (1945)</td>
<td>Rh Rh</td>
<td>Strong</td>
<td></td>
</tr>
</tbody>
</table>

* Transfused once more following delivery, with severe reaction.
* Not immunized by early pregnancy; 3 transfusions at operation uneventful advised to terminate pregnancy but refused.
* Not immunized by early pregnancy; transfusion of husband's Rh+ blood uneventful.
ERYTHROBLASTOSIS FETALIS IN THE FIRST-BORN

Furthermore, as the pregnancy progresses, the blood vessels in the villi become large and approach nearer to the maternal sinuses. That distinct iso-immunization was not induced in the early pregnancies was also indicated by the absence of untoward reactions in 2 of the 3 patients who required immediate blood transfusion.

It is significant that the intensity of the hemolytic process in the first Rh positive fetus was not influenced by the number of transfusions preceding the pregnancy (table 3).

A few of the cases listed above are selected for additional comment.

Patient Gw (case 2) was transfused nine times uneventfully in 1940 for ovarian cyst. In 1943, in her first pregnancy, she was delivered of an infant with severe anemia and jaundice. The infant was transfused four times, but expired. There were no anti-Rh agglutinins in the mother's serum tested one month after the delivery, but it may be assumed that blocking antibodies had been present.

Patient Mc (case 3), group O, Rh negative, was transfused once because of excessive bleeding following a tonsillectomy in 1936. Her first pregnancy, in 1942, ended in a full term stillbirth, fetal heart sounds having ceased in the latter part of the ninth month. In 1944 she was again pregnant and during the course of this pregnancy received a series of subcutaneous injections of her husband's blood "for the purpose of desensitization." The end result was another stillbirth. When tested in May 1944, the patient was found to be immunized (anti-Rh agglutinins). Her husband was in group O, Rh positive (type Rh\textsubscript{1}, Rh\textsubscript{2}). It is quite evident that 'desensitization' cannot be accomplished by injection of small amounts of Rh positive blood. It is more likely that these injections could only have the opposite effect of increasing the degree of iso-immunization.

Patient Wat (case 9), group O, Rh negative, was transfused uneventfully twice during her first pregnancy and once post partum. This case can be included in this series because the first child, born in 1940, was Rh negative and normal. The husband (group O, Rh\textsubscript{1}, Hr\textsubscript{2} positive) was used as donor twice. These transfusions immunized the patient, so that two years later her second pregnancy—the first with an Rh positive fetus—resulted in intra-uterine fetal death. A third pregnancy resulted in intra-uterine fetal death at thirty-one weeks. Tests eleven months after the last pregnancy revealed the presence of strong blocking antibodies.

Patient Ha (case 11), 28 years old, group O, Rh negative, was transfused only once, from her Rh positive uncle (Rh\textsubscript{1}, Rh\textsubscript{2}), in 1935. Her first pregnancy terminated in August 1944, and the infant had a severe form of erythroblastosis fetalis. This case is significant because only one transfusion, of 9 years before, was sufficient to initiate the iso-immunization. Tests of the patient's serum seven months after the delivery revealed the presence of weak anti-Rh agglutinins.

Patient Sm (case 16) was transfused three times uneventfully in 1939 for an early ectopic pregnancy. In 1945, the first full term pregnancy was terminated by cesarean section at thirty-three weeks and a stillborn infant was delivered. The patient was immunized, as indicated by the presence of strong blocking antibodies. As already mentioned, this case can be included in the series because iso-immunization is hardly likely to occur in the presence of an early ectopic pregnancy.

<table>
<thead>
<tr>
<th>Severity of Disease</th>
<th>Immunized by</th>
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<tr>
<td></td>
<td>1 transfusion</td>
</tr>
<tr>
<td>Mild</td>
<td>0</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Fetal death</td>
<td>6</td>
</tr>
<tr>
<td>Totals</td>
<td>9</td>
</tr>
</tbody>
</table>
Two patients, P1 and Ham (cases 8, 15) who were transfused during the state of active iso-immunization, suffered transfusion reactions, and one of them (P1) had complete anuria, from which she finally recovered. This patient subsequently had several uneventful transfusions of Rh negative blood.

It was not always possible to test the blood of the affected infant, but evidence of iso-immunization could be obtained in all but three instances, and these were studied prior to the description of the tests for blocking antibodies. Judging from the high incidence of blocking antibodies in the remaining cases, it is safe to assume their existence in at least two of the three mothers who were tested soon after their deliveries.

In two instances, the affected infant's blood tested at birth failed to agglutinate with anti-Rh₀ serums, but their mothers (cases 7, 13) had strong blocking antibodies that rendered their blood inagglutinable with anti-Rh₀ serums. In case 7 the infant's blood reacted with anti-Rh' serums, so that the blood behaved as though it belonged to subtype Rh'.

Although histologic evidence to support a diagnosis of erythroblastosis fetalis was frequently not available, the selection of the cases can now be accepted on the basis of serologic findings in Rh negative mothers who have been immunized, as indicated by the presence of agglutinins or blocking antibodies. These serologic tests were of particular value in cases in which autopsies were not performed or in which the tissues were too much macerated to permit of histologic examination.

Of the sixteen fathers tested, three were homozygous for the Rh₁ factor, as indicated by a negative reaction with anti-Hr serums. Four fathers were Rh positive, of the subtype Rh₁Rh₂. Bloods of this type are heterozygous from a genetic viewpoint, but in matings of these men with Rh negative women all the offspring must be Rh positive, 50 per cent of the type Rh₀ and 50 per cent of the type Rh₁. To all intents and purposes, these seven mothers had been deprived of an opportunity of having normal offspring as a result of previous transfusion.

Of the remaining nine fathers, only one (case 9) is definitely of the heterozygous genotype, Rh₁Rh, since the first child was Rh negative. The blood of four fathers was not tested with anti-Rh' serum, so that the outlook for future pregnancies in these four matings and in the four matings in which the father is of the type Rh₁Hr positive could not be determined.

The incidence of an additional incompatibility involving the blood factors A and B in this group of cases is 37.5 per cent, or almost identical with that found in the much larger group of Rh negative mothers of erythroblastotic infants (35 per cent).

THE CONTROL GROUP (NOT PREVIOUSLY TRANSFUSED)

As indicated above, it was most surprising to find as many as nine instances of erythroblastosis fetalis in the first full term presumably Rh positive infants in the group of mothers not previously immunized by transfusions. The pertinent data in these cases are presented in outline form in table 4. A few of these cases are selected for brief comment.

Case 21. Mrs. Rei had four pregnancies in the years from 1941 to 1945. Her blood, tested at irregular intervals during the last three years, always contained anti-Rh agglutinins. Her first baby had mild
erythroblastosis fetalis that required no treatment. Her second child (1942) had severe hemolytic symptoms and in addition exhibited spasticity, probably indicative of kernicterus. Her third child (1943) died after a few days of severe anemia and jaundice. At this time, Mrs. Rei was advised not to become pregnant again until her agglutinins had disappeared. Unfortunately, the patient soon became pregnant while moderately active antibodies were still present. Since her husband was homozygous for the Rh factor, it was suggested that the circumstances were such as to justify a termination of the pregnancy, which was carried out. It is quite probable that the intensity of the iso-immunization in cases of this sort can be diminished by longer intervals between pregnancies. At any rate, in carefully selected cases, characterized by (2) history of several instances of fetal or neonatal morbidity, (2) active iso-immunization, and (3) a homozygous (clinically or genetically) husband, further pregnancies should not be encouraged. In the event that such patients accidentally become pregnant, there is already sufficient clinical evidence to justify termination of the pregnancy.

**Case 26.** Mrs. No had an early pregnancy that terminated in an abortion. In her first full term pregnancy, the infant had severe hemolytic disease as well as the syndrome known as Mongolian idiocy.
This was followed by a miscarriage in 1944 and delivery of a full term normal Rh negative infant. Accordingly, the husband is heterozygous for the Rh factor, and there is good outlook for future normal Rh negative infants.

Case 25. Mrs. Mar's history is given above and referred to again because the fetal death in this case, the only one in this group, could be attributed probably to other circumstances, particularly since there was not much pathologic evidence of blood destruction.

Case 27. Mrs. Ma's first baby had severe erythroblastosis and died Apr. 25, 1943, in spite of four transfusions of presumably random (Rh positive) blood. During her puerperium, Mrs. Ma received one transfusion, which was followed by a severe chill and fever. In December 1944 the patient became pregnant again, and tests during her fourth, sixth, and eighth months of her pregnancy gave no evidence of iso-immunization. A specimen drawn one month before delivery contained moderately active blocking antibodies. Accordingly, labor was induced, and the infant was normal at birth (Aug. 6, 1945) but became slightly jaundiced in a few hours. The jaundice deepened gradually for thirty-six hours and then receded slowly, probably because the baby was transfused with Rh negative blood within from two to three hours after delivery. In his letter, Dr. Herrman writes: "I am certain we prevented a more severe case, but it can't be proven."

Of the seven husbands tested, five are Hr negative, so that the fetus in each succeeding pregnancy must be Rh positive. Of the remaining three, one (case 26) must be heterozygous. One of the patients (case 27) had a severe transfusion reaction following her first pregnancy.

Apparently this control group as well as the transfused group selects those Rh negative women who produce antibodies with great ease. The existence of individual differences in response to iso-immunization, probably determined by genetic factors, was postulated by Levine in order to explain the general low incidence of erythroblastosis fetalis, in spite of a high incidence of random incompatible matings.3, 17

It is now believed that erythroblastosis fetalis occurs in about one of 150 to 200 random full term deliveries.3, 12, 19 This figure, based on Rh tests to detect all instances of iso-immunization, is to be contrasted with a value of 1 case in 438 deliveries based on clinical grounds only.20 If all Rh negative women responded readily to iso-immunization, one should expect erythroblastosis fetalis to occur in almost all of the 13 per cent of matings in which the father is Rh positive and the mother Rh negative. At the same time, erythroblastosis fetalis in the first full term Rh positive infant should occur very frequently.

Granting that these women were not transfused, one cannot exclude the possibility that the iso-immunization in at least some of them may have been initiated either many years previously or at any time prior to or during a pregnancy by the common practice of administering small amounts of blood intramuscularly.4 In the days preceding the use of vitamin K, blood was routinely given to the newborn infant, or to the pregnant woman in repeated doses. Intramuscular injection of blood is still being used as a form of nonspecific therapy.

* It is of interest that 3 cases of this group were referred by Dr. Herrman, who assured the writer that these three mothers were not transfused prior to their pregnancies. In reply to a query regarding the possibility of intramuscular injection of blood, Dr. Herrman writes: "I'm afraid you will have a hard time getting correct information concerning intramuscular blood in infancy. It has been almost routine with some doctors in the Middle West to give intramuscular blood at the time of delivery especially if a difficult labor was anticipated. Many of the patients know nothing about it unless their husbands..."
The objection may be raised that the small quantities of blood used for intra-muscular injection may be insufficient to induce iso-immunization. There is, however, considerable support from immunologic literature to indicate that immunization may be stimulated by injection of unbelievably minute amounts of blood or other antigens. So far as iso-immunization by the fetal blood across the placenta is concerned, Levine calculated that the passage of as little as 0.13 cc. of fetal blood is required to immunize the Rh negative mother. Indeed, pregnancy and the intramuscular injection of blood provide conditions held to be favorable for iso-immunization, i.e., the continual presence of minute quantities of blood acting over a long period.

By and large, it is not possible to obtain a history of intramuscular administration of blood, but in view of its routine and indiscriminate use in the past, this procedure must be considered as a possible source of the immunization of Rh negative individuals even in infancy or the neonatal period. While infants do not produce antibodies as readily as adults, these intramuscular injections may at least serve to initiate the process. In short, it is advisable to avoid this form of therapy in Rh negative girls or women unless Rh negative blood is used.

Recently, Wiener recommended the so-called biologic test to detect Rh incompatibility.* This consists of preliminary intravenous injection of 0.5 cc. of Rh positive blood, and 'comparison with the naked eye of the color of the patient's original citrated plasma with that of a comparable specimen taken one and one-half hours after the injection.' Aside from the fact that the method is cumbersome and not always reliable, it should also be kept in mind that the injection of quantities of much less than 50 cc. of Rh positive blood may cause severe hemolytic reactions accompanied by oliguria. In the absence of severe reactions, the injection of small amounts of Rh positive blood may initiate iso-immunization or intensify this process if already induced. If the test is to be used to detect incompatibility due to finer variations of the Rh factor or other blood factors, these considerations are to be kept in mind. The biologic test has very limited value, since the tests in vitro (agglutinins, blocking antibodies, slide test of Diamond) that will detect almost all instances of iso-immunization are less cumbersome, more reliable, and do not inconvenience the patient.

GENERAL CONSIDERATIONS

In the material presented above, two highly contrasting groups of cases were selected in order to determine the role of transfusions prior to pregnancy. However, supporting evidence can be derived from still another group of cases, briefly referred to below.

It is not generally appreciated that the degree of iso-immunization required to induce symptoms of the hemolytic disease in the infant is far more intense than that required to produce a severe hemolytic reaction in the mother following

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* This test was used previously as a further check on the grouping and compatibility tests.
transfusion of Rh positive blood. Now that the pathogenesis of erythroblastosis fetalis is established, it is obvious that the hemolytic disease is the result of prolonged intra-uterine action of maternal anti-Rh agglutinins on the susceptible fetal blood. Accordingly, one may expect to find instances of Rh negative women with anti-Rh antibodies who nevertheless have normal Rh positive children, while in the following pregnancy the degree of iso-immunization is sufficiently severe to produce the hemolytic disease.

In one of the earliest cases studied with Burnham, the blood of an Rh negative mother who had just been delivered of a normal Rh positive infant was found to be incompatible with her husband’s group-compatible blood. Because the patient’s condition was not serious, the transfusion was not carried out. In 1944, the patient was delivered of an infant that had a mild to moderate form of icterus gravis, with complete recovery. One may speculate that had this patient received and survived the transfusion of Rh positive blood, the pregnancy of 1944 would have ended in fetal death. This indeed was the outcome in two Rh negative women who were transfused with Rh positive blood following delivery of normal Rh positive infants.

**Case 28.** Patient McC, 30 years old, blood group B, Rh negative, had been married eight years. In 1939, she had a presumably normal child whose blood was of group B, Rh positive, type Rh2. After delivery, the mother was transfused with group-compatible blood. Soon after the transfusion was over, the patient had a violent reaction. No other details are available. In 1943, the patient was delivered of a full term fetus that had died twenty-four hours before birth. In 1944, when she was tested for the first time, in the fifth month of her third pregnancy, strong blocking antibodies were present and, as was to be anticipated, the end result was fetal death.

**Case 30.** Mrs. So is in group O, Rh negative. Her first pregnancy was in 1936, and she was delivered of a normal Rh positive infant. (Although not tested, the infant was undoubtedly Rh positive, because the father’s blood failed to react with anti-Hr sera. The genotype of the father is most likely Rh1Rh1.) Shortly after the delivery, the mother received two uneventful transfusions, and it is therefore safe to assume that she was not immunized as a result of the first pregnancy. Each of four subsequent pregnancies (1938, 1939, 1943, 1945) resulted in fetal death. Anti-Rh agglutinins were demonstrable several weeks after the last delivery.

These 2 cases cannot be included in the series because it was the second pregnancy with an Rh positive fetus that presumably resulted in erythroblastosis fetalis. In the first case, the first pregnancy probably immunized the mother, but the antibody production, insufficient to cause the disease in the fetus, was intense enough to induce a severe transfusion reaction. In both cases, the additional antigenic stimulus of transfusions of Rh positive blood probably induced a degree of iso-immunization in the following pregnancies sufficient to induce fetal death. One may well speculate that if these patients had received Rh negative blood, the infant in the second pregnancy might have had a milder form of erythroblastosis fetalis.

**DISCUSSION**

Soon after the clinical importance of the Rh factor was established, emphasis was placed on the prevention of intragroup hemolytic transfusion reactions by the use of Rh negative donors for all Rh negative patients already immunized either
by previous transfusions or by pregnancies. For an index of iso-immunization, the obstetrician was advised to be guided mainly by the history of fetal and neonatal morbidity. An additional preventive measure that now becomes imperative is the exclusive use of Rh negative blood for all Rh negative patients to be transfused. In other words, the emphasis should now be placed on the prevention of iso-immunization. This holds true particularly with regard to the female population of all ages, but the present study was limited to the influence of previous transfusions on the incidence of erythroblastosis fetalis.

The deliberate iso-immunization of the Rh negative female population, even as infants, by transfusion or perhaps even by intramuscular injection of Rh positive blood, can now be prevented. This simple measure should, by itself, reduce the incidence of erythroblastosis fetalis, especially in its more severe forms. By and large, this is the responsibility to be shared by pediatricians and those hematologists who perform compatibility tests and transfusions.

The fundamental fact is that once an Rh negative individual is immunized, he or she must be considered as remaining potentially immunized for the remainder of his or her natural lifetime. So far as any of our female population is concerned, the outcome of pregnancies, even many years after transfusion, can be influenced by the type of blood used in transfusing Rh negative female infants or girls. Similarly, it is important to determine the Rh factor of an adult female patient before transfusions are carried out. Furthermore, one should not be completely guided by the presence or absence of a history of fetal or neonatal morbidity, particularly if more than one transfusion is contemplated. To be more specific, all Rh negative mothers of normal Rh positive children are liable to serious intragroup transfusion reactions if, even many years later, they receive more than one transfusion.

Although the role of previous intramuscular injection of blood in initiating iso-immunization is not well established, it must be considered as probably significant, since only minute quantities of blood are required to induce iso-immunization. Certainly, with the introduction of vitamin K for the treatment of hemorrhagic disease of the newborn, this factor will become less important.

The more widespread use of Rh tests cannot be recommended without taking into account the matter of a supply of potent anti-Rh serums. It can be assumed that at present there is not yet available an experimental serum of a potency equal to that of human serums. Certainly efforts should be directed in the future toward improvement of the quality of experimental Rh serums.

Undoubtedly there will be sufficient human anti-Rh serums if all women having potent anti-Rh serums are bled periodically. The yield from these women can be increased to a considerable degree by the use of several measures. In the first place, the amount withdrawn should always be replaced by a transfusion of compatible Rh negative blood. Second, the method of Hill and Haberman, of maintaining a high degree of iso-immunization in selected cases on a voluntary basis, should be adopted. These workers inject such women intravenously with minute amounts of Rh positive blood after the agglutinin titer of their blood drops below a level sufficient for routine use.

The program, initiated several years ago, of concentrating weak anti-Rh serums,
thus rendering them useful as diagnostic reagents, should be encouraged. Furthermore, Levine and Waller have shown recently that anti-Rh serums containing both potent agglutinins and blocking antibodies can be absorbed so that the latter antibodies are removed without diminishing to any great extent the agglutinin titer.

The difficulties in obtaining sufficient quantities of potent anti-Rh serums from patients immunized by repeated transfusions are obvious, but occasionally some of the measures suggested above may be applicable.

SUMMARY

The incidence of fatal forms of erythroblastosis fetalis in the first-born can be diminished by the simple measure of transfusing all Rh negative female patients, even as infants, with Rh negative blood. Once a female patient is found to be Rh negative, all subsequent transfusions must be carried out with Rh negative blood. The indications are that sufficient human anti-Rh serums will become available for the more extensive Rh tests required for the prevention of iso-immunization by transfusion.

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ERYTHROBLASTOSIS FETALIS IN THE FIRST-BORN: PREVENTION OF ITS MOST SEVERE FORMS

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