Studies in Rh Sensitization

III. Effect of Rh-Positive Pregnancies on Rh Antibody Titer

By ALEXANDER S. WIENER, M.D., F.A.C.P., RAFFAELE NAPPI, M.D. AND EVE B. GORDON

In the preceding paper1 our methods for performing Rh antibody titrations were described in detail, with special reference to the precautions taken to insure objective and reproducible results. Using these techniques, it was shown that pregnancies with Rh-negative fetuses and the birth of Rh-negative babies have no significant effect on the Rh antibody titer of sensitized Rh-negative mothers. This paper is a continuation of these studies on Rh sensitization and will present the results of observations on the effect of pregnancies with Rh-positive fetuses and birth of Rh-positive babies on the maternal Rh antibody titer. As will be pointed out, these findings have important implications with regard to the frequency with which pregnant Rh-negative women are apt to become isosensitized and give birth to erythroblastotic babies.

RESULTS

Observations were made on 65 sensitized Rh-negative women pregnant with Rh-positive babies. In the majority of the cases the babies were delivered prematurely, mostly by induction of labor and in several cases by cesarean section, in order to reduce the period of exposure of the fetus to the maternal Rh antibodies and to treat the baby by exchange transfusion. The data have the limitation that in many of the cases the antenatal antibody titrations were not repeated a sufficient number of times. Among 59 cases in which 2 or more antenatal titrations were carried out, (table 1) a significant rise in titer was observed 13 times, a questionable rise in titer 8 times, no significant change in titer in 30 cases and in 4 cases the Rh antibody titer fell.*

As pointed out in the previous paper, a definite rise in the antibody titer of a sensitized Rh-negative woman during pregnancy could only occur following a leakage of Rh-positive blood from the fetus into her circulation. Since observations on antenatal antibody titers are necessarily crude and incomplete, any estimate of the frequency with which such a leakage occurs† based on a rise in titer, must also be crude. For practical purposes,

* The criteria by which we ascertain whether or not the antibody titer has risen have been presented previously.1,2 The mean of all the titer values is first determined. Deviations from the mean of less than one dilution (100 per cent) are not considered significant because this could be due to the technic. A rise in titer of more than two dilutions is considered significant, while a rise of between one and two dilutions is considered of only doubtful significance. The objectivity of the conclusions can be judged from the fact that all three authors analyzed the data independently and arrived at essentially the same conclusion.

† As Levine3 has emphasized repeatedly, it is not necessary to postulate a gross placental lesion, since leakage of a minute volume of blood is sufficient to bring about sensitization.
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However, one may conclude from the observations presented in table 1 that even in the absence of manifest obstetric complications, a leakage of fetal blood into the maternal circulation is apt to occur antenatally in approximately 1 out of 3 or 4 pregnancies.

In 56 cases, the Rh antibody titer was determined one fortnight after the delivery as well as antenatally. In 15 of these cases there was a definite rise in antibody titer, in 5 cases there was a questionable rise in titer, in 29 cases there was no significant change in titer and in 1 case a questionable fall. These data have the limitation that in some cases the rise in titer observed after delivery merely represents a continuation of an antenatal rise in titer. On the other hand, where the rise in Rh antibody titer was small (less than 100 per cent) this would not be demonstrable serologically since the technic itself has an error of this magnitude; and in addition, in some cases a rise in titer may have not occurred because the patients were in a refractory state. Therefore, any estimate of the frequency with which fetal blood is apt to leak into the maternal circulation during delivery, based on the occurrence of a rise in antibody titer, could only be approximate.

### Table 1.—Effect of Pregnancy with and Delivery of Rh-Positive Babies on Maternal Rh Antibody Titer

<table>
<thead>
<tr>
<th>Nature of investigation</th>
<th>Number of cases studied</th>
<th>Number of cases showing</th>
<th>Definite rise in titer</th>
<th>Doubtful rise in titer</th>
<th>No significant change</th>
<th>Fall in titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Periodic antenatal Rh antibody titrations</td>
<td>59</td>
<td>13</td>
<td>8</td>
<td>30</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>II. Comparison of postpartum and antepartum Rh antibody titers</td>
<td>56</td>
<td>15</td>
<td>5</td>
<td>29</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

However, for practical purposes, one may estimate from the data presented here that a leakage of a significant amount of fetal blood into the maternal circulation is apt to occur in about one-third of all deliveries. This confirms our previous observations regarding the importance of parturition in bringing about Rh sensitization. It would appear that the chance of such leakage during a cesarean operation is about the same, though our data on this point are meager.

With information now available concerning the approximate frequency with which fetal blood is apt to leak into the maternal circulation during pregnancy and during delivery, one can estimate how often an Rh-negative expectant mother is likely to become isosensitized and give birth to an erythroblastotic baby. Two types of cases have been distinguished, namely, those in which the expectant mother has received a previous transfusion or injection of Rh-positive blood and those in which there is no history of such an injection. As has been pointed out previously, in order for Rh sensitization to result, an Rh-negative individual must have received Rh-positive blood by the parenteral route on at least two occasions. The first injection acts as a "primer," and then after a latent period of about three or four months the individual may be ready to produce antibodies in response to a second injection of Rh-positive blood. In experiments on male volunteers, it was found that fully 40 per cent of Rh-negative indi-
individuals become sensitized after two such injections, and if the injections were continued the incidence of Rh sensitization rose so that as many as 80 per cent become sensitized after six injections.

Where the expectant mother has received a previous injection of blood, the patient is already primed. The chance of her marrying an Rh-positive man is approximately $6:7$. About $2:5$ of such husbands are homozygous for the Rh factor in which case the baby will surely be Rh positive, while about $3:5$ of Rh-positive husbands are heterozygous in which case there is a 50 per cent chance that the first baby will be Rh positive. Therefore, the chance that the Rh-negative woman will wed an Rh-positive man and have an Rh-positive baby at her first pregnancy is $\frac{6}{7} \times \frac{2}{5} = \frac{12}{35}$. As has already been shown, the chance of fetal blood leaking into the maternal circulation during a normal pregnancy is approximately $1:3$, and since the chance of sensitization resulting after a second injection of Rh-positive blood is $2:5$, the chance that an expectant mother who has received a previous injection of Rh-positive blood will become sensitized during her first pregnancy is $\frac{3}{5} \times \frac{1}{3} \times \frac{2}{5} = \frac{2}{25}$ or about 1 in 12 or 13. If the blood originally injected intramuscularly or intravenously was not typed for the Rh factor, the chance that it would be Rh negative is $1:7$, in which case no harm will have resulted. But in $\frac{6}{7} \times \frac{2}{25} = \frac{12}{175}$ or roughly 1 case in 14 or 15, the injection of untyped blood into an Rh-negative female will cause her to have an erythroblastotic baby at her first pregnancy.

On the other hand, the chance of an erythroblastotic baby being born at the first pregnancy is quite small in women who have never received an injection of Rh-positive blood. The reason for this is that for isosensitization to occur during the first pregnancy, it would be necessary for fetal blood to leak into the maternal circulation on at least two occasions antenatally and at an interval of at least three or four months, for reasons just explained. This never occurred in our series of cases, and such instances must be very rare indeed. This accounts for the clinical observation that in the great majority of cases where a primipara gives birth to an erythroblastotic baby it is possible to elicit a history of a previous injection of blood.\textsuperscript{3} \textsuperscript{9} \textsuperscript{17}

Where a woman has never received an injection of blood but has a baby and is pregnant for the second time, the chance that the second baby will be erythroblastotic can be calculated as follows: Firstly, the husband and both children must be Rh-positive, and the chance that this will occur is approximately $\frac{6}{7} \times \frac{2}{5} \times \frac{3}{4} \times \frac{1}{4} = \frac{33}{70}$. During the first pregnancy there is approximately one chance in three that fetal blood will leak into the maternal circulation, and, similarly, there is one chance in three for a leakage to occur during the second pregnancy, and finally, one chance in three for a leakage to occur during the first delivery. Therefore, the chance that fetal blood will enter the maternal circulation on at least two of these three occasions is $\frac{1}{27} + 3 \times \frac{2}{27} = \frac{7}{27}$. Moreover, since approximately 40 per cent of Rh-negative individuals become sensitized after the
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second injection of Rh-positive blood, the chance that an Rh-negative woman will become isosensitized before the end of her second pregnancy becomes

\[
\frac{33}{70} \times \frac{7}{27} \times \frac{2}{5} = \frac{11}{225}
\]

This fits very well with the clinical observations that about 1:15 to 1:25 of each Rh-negative women who have never received a previous injection of Rh-positive blood will nevertheless have an erythroblastotic baby at the second delivery.

Recently Wiener and Hallum have reported the demonstration of Rh antibodies in follow-up tests on Rh-negative primiparae who had Rh-positive babies, and the studies described here provide an explanation for their observations. Since there is about one chance in three for a leakage of fetal blood into the maternal circulation antenatally, and again one chance in three at parturition, and in two-fifths of the cases where a leakage occurs both times sensitization is likely to occur, the chance of demonstrating Rh antibodies in follow-up tests on such primiparae is \(\frac{1}{3} \times \frac{1}{3} \times \frac{2}{5}\) or 2 times in 45 cases. Actually Wiener and Hallum demonstrated Rh antibodies in 2 out of 62 cases, which agrees satisfactorily with the theoretical expectations.

### TABLE 2.—Results of Rh Antibody Titrations in Case 1

<table>
<thead>
<tr>
<th>Date of test</th>
<th>Period of gestation (lunar month)</th>
<th>Rh antibody titer* (units) by method of</th>
<th>Enzyme-treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Agglutination</td>
<td>Blocking</td>
</tr>
<tr>
<td>7–10–1950</td>
<td>4th</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>9–16–1950</td>
<td>8th</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10–21–1950</td>
<td>9th</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>11–4–1950</td>
<td>10th</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>11–11–1950</td>
<td>10th</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Titrations by the antiglobulin method of Coombs et al. were not done. In our hands this method gives values intermediate between the albumin-plasma conglutination method and the test with enzyme-treated cells.

† Enzymes used were ficin and papain.

**Case Reports**

A few cases will be described in detail in order to illustrate the management of pregnancies in which the expectant mother is Rh-negative and sensitized to the Rh factor.

**Case 1.** This patient was referred during her second pregnancy because she was known to be Rh negative and gave a history of having received a blood transfusion after an appendectomy fifteen years previously. The first pregnancy had terminated with the spontaneous delivery of a full term, normal, female baby who showed no jaundice or anemia during the neonatal period and who is alive and well.

Grouping and Rh-Hr tests showed the father to be of type A, MN Rh,Rh, the mother of type O, MN rh, and the daughter type O, N Rh,rh. Since the husband belonged to type Rh,Rh, he was most likely homozygous for the Rh, factor, so that there was hardly any doubt that the patient was carrying an Rh-positive fetus.

Tests for Rh antibodies showed the patient to be sensitized to the Rh factor and the antibody titrations were repeated periodically with the results shown in table 2. It will
be seen that the Rh antibody titers showed no significant change between the fourth and ninth lunar months, the average titer by the albumin-plasma conglutination method during this period being 9 units. At the beginning of the tenth lunar month, however, there was about a fivefold or a sixfold increase in antibody titer up to 60 units by the albumin-plasma conglutination method. Since in our experience, antibodies of this magnitude result in stillbirths in almost half the cases, induction of labor was carried out and the baby treated immediately after birth by exchange transfusion, using 120 cc. of packed group O, type rh blood cells.*

Subsequent tests showed the baby to be group O, type M, type Rh,rh, confirming the prediction that the baby would be Rh positive. The icterus index of the cord serum was 20 units and tests for coating of the baby's cells by Rh antibodies were positive both by the conglutination and antiglobulin methods. The baby's hemoglobin concentration at birth was 15.9 Gm. per cent before the transfusion, and was found to be 19.6 Gm. per cent the next morning. Only a mild evanescent jaundice appeared and the baby was discharged from the hospital with his mother. When seen at the office two weeks later the baby appeared entirely normal, the hemoglobin concentration at that time being 14.3 Gm. per cent. The subsequent course was uneventful.

The symptoms in the case just described, after the exchange transfusion, were so mild that the uninitiated may wonder whether the baby was ever really ill and whether treatment was actually necessary. That such doubts can arise is a tribute to the value of antenatal Rh antibody tests which can enable one to predict before birth which babies will be erythroblastotic, and to treat them promptly by exchange transfusion and thus prevent the development of serious disease. It is only after one has seen several cases where babies apparently normal at birth become ill and die on the third or fourth day of life that one appreciates the importance of treating such babies before instead of after they have become obviously sick.

Since erythroblastosis fetalis occurs in only 1 out of 200 babies, those not specializing in the field will rarely see a case, and it was therefore thought worthwhile for purpose of contrast to quote a case which did not have the benefit of antenatal antibody tests.

Case 2. A couple consulted us because of the following history. The wife had been pregnant three times, and had never received any blood or plasma injections. The first pregnancy yielded a premature female infant who weighed 54 pounds. Aside from evanescent cyanosis this baby had no neonatal illness and is alive and well. The second pregnancy, seven years later, yielded a full term male infant with a birth weight of 6 pounds, 5 ounces. This baby appeared normal at birth but on the third day became jaundiced and died shortly thereafter. The third baby was another full term infant with a birth weight of 6 pounds, 14 ounces. Because of what had transpired after the second baby was born, the patient was promised that a specialist would be called to see her new baby. However, when the baby was born the obstetrician stated that no specialist was necessary because the baby was entirely normal. This baby nevertheless became jaundiced on the third day and died shortly thereafter. We were consulted by the parents one month later in order to determine the cause of the deaths of their two infants.

Grouping and Rh-Hr tests on the family showed the father to belong to type A, MN Rh,Rh2, the mother to type A2 M rh, and the daughter to type A, MN Rh2rh. Since the father belonged to type Rh,Rh2 he was most likely homozygous for the Rh0 factor (most

* In this procedure, 120 cc. of packed type rh cells are introduced through the saphenous vein while 120 cc. of the baby's blood are withdrawn from the radial artery. The method is not to be confused with simple transfusions of concentrated red cells as advocated by Pennell and previously by others.
likely genotype R:type), and there was hardly any doubt that the two babies who died were Rh positive. Moreover, tests for Rh antibodies showed the mother to be sensitized to the Rh factor; the antibody titer by the agglutination method was 18 units, and by the albumin-plasma method 28 units. These results indicated that the two babies described had died of erythroblastosis.

In the case just described the serologic findings and the clinical story are comparable to those of Case 1. Since the babies did not become ill until the third day of life, it seems reasonable to conclude that the outcome would have been different if the babies had been treated by exchange transfusion directly after birth. One might argue, however, that the Rh antibodies detected by us may not have been present until after the third pregnancy, and that the babies died from some condition other than erythroblastosis. Since we did not have the opportunity to perform antenatal Rh antibody tests in Case 2, it is necessary to report still another case in order to refute this argument.

Case 3. A couple gave the following history. The woman had been pregnant three times. The first two pregnancies had been artificially aborted. During the third pregnancy, the patient was found to be Rh negative and strongly sensitized to the Rh factor. The baby showed early jaundice but the blood count and physical examination repeated twice a day were normal. On the fifth day the jaundice deepened, the baby became apathetic, and there was a precipitous drop in the red blood cell count to 3 M. per cu. mm., with a hemoglobin concentration of 11 Gm. per cent. A transfusion was started, but the baby died before it could be completed.

We repeated the Rh typings and found the husband to be group A2 MN Rh2Rh while the wife's blood was group A, MN rh. Tests for Rh antibodies showed the wife to be sensitized to the Rh factor, the titer being 5 units by the agglutination method, 9 units by the albumin-plasma conglutination method and 70 units for enzyme-treated cells. These results agreed with the antenatal findings and confirmed the diagnosis of erythroblastosis as the cause of the baby's death. Since the baby survived five days without treatment, it seems reasonable to assume that his life might have been saved had he been treated immediately after birth by exchange transfusion.

Not every erythroblastotic baby requires treatment. Where the maternal Rh antibody titer is low* and there have been no previous erythroblastotic children, the baby even though Rh positive, may be so mildly affected that no treatment will be necessary. However, if the antenatal Rh antibody titer is low at first but rises to a high level before term, the baby may be severely affected and a stillbirth may even result, because a relatively brief exposure to a potent antibody can cause serious damage. Therefore, when the expectant mother is known to be sensitized, the antenatal Rh antibody titrations should be repeated at frequent intervals, ideally once weekly, so that a rise in titer can be detected as soon after it occurs as possible. If the titer rises to a dangerously high level, labor should be induced without delay, provided that the fetus is sufficiently mature. If this is done promptly where the elevation in titer has not been of long standing, the manifestations in the baby may be so mild that treatment is unnecessary as in the following case.

* In general, if sensitization is so mild that Rh antibodies are demonstrable in the maternal serum only with enzyme-treated cells or by the antiglobulin method, but not by the albumin-plasma conglutination method, the baby, even though Rh positive, will hardly ever exhibit manifest signs of disease.
Case 4. This couple was referred for Rh studies because the expectant mother was known to be Rh negative and was pregnant for the second time. The first pregnancy had resulted in a full term normal baby who showed no jaundice or anemia and was alive and well. Grouping and Rh-Hr tests showed the father to be of type A, N Rh, Rh₂, the mother type A, N rh, and the daughter type A, N Rh₂rh. Since the father belonged to type Rh, Rh, it was reasonable to conclude that the expected baby would probably be Rh positive (either type Rh, or type Rh₂). Rh antibody tests were carried out periodically and the results are summarized in table 3. As shown in the table, aside from one occasion when low titered Rh antibodies were detected in tests with enzyme-treated cells, no evidence of Rh sensitization was exhibited by the patient until the last month of pregnancy. Then, two weeks before term, when the Rh antibody titer rose to a significant level (albumin-plasma method 5 units), labor was induced and a normal appearing male infant weighing 8½ pounds was delivered. This baby belonged to type Rh,rh, so it was Rh positive as predicted. Moreover, the icterus index of the cord serum was 24 units and weak positive reactions were obtained by the conglutination and antiglobulin tests for coating of the baby's cells. However, the blood count was within normal limits and the baby remained clinically well except for mild jaundice which appeared on the second day and disappeared a few days later. The baby was discharged from the hospital with his mother, and when seen at the office two weeks later was well except for a mild anemia (hemoglobin concentration 70 per cent, and red blood count 4.05 M. per cu. mm.). The mother's serum was retested at that time, and as shown in table 6, the titer was found to have risen still further to 22 units by the albumin-plasma conglutination method. The baby is now four months old and entirely well.

There seems hardly any doubt that if this pregnancy had been allowed to go to term a severely affected erythroblastotic baby would have resulted. With regard to future pregnancies, since the father is homozygous Rh positive (the first child belongs to type Rh₂ and the second to type Rh₁, so that the father's genotype must be RR²), and since the mother is strongly sensitized to the Rh factor, there is hardly any doubt that every future baby would be severely erythroblastotic, and there would be a strong possibility of a stillbirth. Therefore, had this baby been lost due to improper management, this couple would have been deprived of their last chance to have a normal baby.

Unfortunately, not every case turns out so happily, even with the best of care. The next case is representative of a problem for which there is no satisfactory solution in the present state of our knowledge.

Case 5. This patient was referred to us during her sixth pregnancy, and gave the following obstetric history. Her first pregnancy, in 1937, yielded a full term, normal, female in-
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fant, delivered by low forceps, who had a birth weight of 7 pounds and 11 ounces. This baby had no neonatal jaundice or anemia and is alive and well. The second pregnancy, in 1939, also yielded a full term, normal, female infant delivered by low forceps, with a birth weight of 6 pounds and 14 ounces. This baby also showed no jaundice or anemia, and is alive and well. The third pregnancy, in 1947, yielded a male infant; there was a protracted labor lasting 18 hours because of malposition (L. O. P.). At birth this baby exhibited evidence of intracranial hemorrhage and erythroblastosis, and was given two blood transfusions, and is alive and well. The fourth pregnancy in 1949 terminated in an early complete abortion. The fifth pregnancy in the same year terminated with the birth of a 5 pound macerated stillborn fetus, whose death was ascribed to erythroblastosis.

Grouping and Rh-Hr tests done on the family gave the results shown in table 4. As expected the mother was Rh negative while the father and all 3 children were Rh positive. Moreover, the father belonged to type Rh,Rh, so that he was most likely homozygous for the Rh, factor, and there was hardly any doubt that the mother again was carrying an

<table>
<thead>
<tr>
<th>Blood of</th>
<th>Group</th>
<th>M-N type</th>
<th>Rh-Hr type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>O</td>
<td>MN</td>
<td>Rh,Rh₁</td>
</tr>
<tr>
<td>Mother</td>
<td>O</td>
<td>M</td>
<td>rh</td>
</tr>
<tr>
<td>1st child</td>
<td>O</td>
<td>MN</td>
<td>Rh,rh</td>
</tr>
<tr>
<td>2nd child</td>
<td>O</td>
<td>MN</td>
<td>Rh,rh</td>
</tr>
<tr>
<td>3rd child</td>
<td>O</td>
<td>M</td>
<td>Rh,rh</td>
</tr>
</tbody>
</table>

**TABLE 4.—Blood Groups and Rh-Hr Types in Case 5**

Summary and Conclusions

The results of repeated Rh antibody titrations on 65 sensitized Rh-negative pregnant woman carrying Rh-positive fetuses are presented and analyzed. Considering that a rise in antibody titer antenatally could result only from a leakage of fetal blood into the maternal circulation, the findings indicate that such a leakage occurs antenatally in about 1 out of every 3 or 4 normal pregnancies. Similarly, evidence is presented indicating that fetal blood leaks into the maternal
circulation in about 1 out of 3 deliveries. Since, previously, it has been shown that approximately 40 per cent of Rh-negative individuals can be sensitized by two injections of Rh-positive blood spaced four months apart, one can calculate the chance of sensitization resulting from pregnancy with an Rh-positive fetus. The calculations show that in Rh-negative women who have received a previous injection of Rh-positive blood, there is about one chance in 12 or 13 that the first baby will be erythroblastotic (cf. Levine and Waller). In women who have never received any injection of blood which might sensitize them, the chance that the first baby will be erythroblastotic is negligible, because for this to occur it would be necessary for blood to leak into the maternal circulation antenatally on at least two occasions spaced four months apart, once to prime the patient and the second time to stimulate the appearance of antibodies. This never occurred in the present series of cases. It was also calculated that in Rh-negative primiparae who have never received an injection of Rh-positive blood, the chance of Rh antibodies appearing after the birth of an Rh-positive baby is approximately 2 out of 45, and, as a matter of fact, this has been found to occur by other workers in 2 out of 65 cases. It was further calculated that in Rh-negative women who had never received an injection of Rh-positive blood, the chance of their second baby being erythroblastotic is about 1 in 20, which agrees well with the actual observations. By extending the calculations, one could also estimate the chance of an erythroblastotic baby at the third, fourth, and later pregnancies.

Five cases are described in detail in order to illustrate the value of periodic antenatal Rh antibody titrations in the management of sensitized Rh-negative women.

REFERENCES

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