Studies in Rh Sensitization
I. Methods. II. Effect of Rh-Negative Pregnancies on Rh Antibody Titer

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

The role of iso sensitization in pregnancy in the pathogenesis of erythroblastosis fetalis raises a number of important questions. For iso sensitization to occur, it is necessary for fetal blood to gain access to the maternal circulation, but the normal placental barrier is believed to be impervious to erythrocytes. To be sure, it is easy to conceive that during parturition when the placenta separates, a sufficient number of fetal red blood cells might enter the maternal circulation to give rise to iso sensitization. However, no data are available giving the percentage of cases in which this actually occurs. It is also easy to conceive that during an abnormal pregnancy, such as an ectopic gestation, premature separation of the placenta, ruptured uterus, abortion followed by dilatation and curettage, or cesarean section, fetal red cells might gain access to the maternal circulation. Moreover, even during a normal pregnancy, judging from the high frequency with which infarcts are found in the placenta, occasional breaks may occur in the placental barrier, thus permitting fetal red cells to pass through. It is of interest to inquire how often this is apt to occur in sufficient degree to sensitize the mother. Furthermore, while it is generally conceded that once an Rh-negative individual has been sensitized to the Rh factor he remains so for life, there is dearth of data in support of this belief. Finally, the question has been raised whether pregnancy with an Rh-negative fetus and the birth of an Rh-negative baby can stimulate a rise in Rh antibody titer.

The present investigation was undertaken in an attempt to answer these questions, and the results obtained will be presented in a series of papers. For simplicity, the discussion will be limited almost exclusively to cases of Rh sensitization. Clinical cases of severe erythroblastosis due to A-B-O and Hr sensitization are relatively infrequent, but the principles involved are the same.

I. Methods

Manifestly, there is no practicable method of determining when a break in the placental barrier occurs during an apparently normal pregnancy. Such a break may be inferred if

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* Martland and Martland recently reported 2 cases in which pregnant women died from carbon monoxide asphyxiation; in both cases the maternal blood contained high concentrations of carboxyhemoglobin while none was demonstrable in the fetal blood. They consider that this observation disproves the idea that fetal red cells can gain access to the maternal circulation and sensitize her. As a matter of fact, the observation has no bearing on the question because it does not refute the possibility that during a fraction of a second, in the course of an entire pregnancy, a small amount of red cells might break through the placental
rise in antibody titer occurs during the course of pregnancy. Once a person has been sensitized to an antigen, the injection of a relatively small amount of the same antigen will stimulate a rise in the antibody titer, and this applies to the Rh factor as well as other antigens. The only exception is during a refractory period, namely, when the antibody titer is at a peak and incapable of rising further. Therefore, by following the Rh antibody titer during pregnancy in a large series of cases in which sensitized Rh-negative women are carrying Rh-positive fetuses, one may be able to estimate the frequency with which fetal blood enters the maternal circulation during a normal pregnancy. Similarly, by comparing the postpartum antibody titer with the titer before delivery, one can estimate how often fetal blood gains access to the maternal circulation during parturition.

The cases which will be analyzed in the present study have been accumulated during a period of seven years. While many of the cases did not come to our attention until the babies were born and exhibited signs and symptoms of erythroblastosis, other patients were studied antenatally and periodic antenatal Rh antibody tests were done, so that erythroblastosis in the baby could be anticipated. In such cases, the babies were often delivered prematurely by induction of labor, though early in the study some of the babies were delivered by cesarean section. In all cases where the baby survived, it was possible to follow up the maternal Rh antibody titer when the mother returned with the baby for periodic examinations. In this way, the antibody titers could be followed for periods up to four years or longer following the birth of the erythroblastotic baby.

There is considerable confusion since the technic of performing Rh antibody tests and the interpretation of Rh antibody titers often vary; a brief outline will therefore be given of the methods used in our studies. To test for Rh antibodies one must have on hand standard Rh-positive and Rh-negative red blood cells of group O. The exact subtype does not matter, but several Rh-positive and at least one or two Rh-negative bloods are needed for the tests. The tests are not simple, and for accurate results careful attention to details is essential.

The antibodies, when present, may be of the bivalent variety (agglutinins), or of the univalent variety (glutinins or blockers), or both. Tests in saline media will detect bivalent antibodies; there are several methods of demonstrating univalent antibodies, namely, the blocking test, the conglutination test, the anti-globulin test and the test with enzyme-treated red cells. The latter methods all determine the same antibody, but are of different sensitivities and may therefore give different titer values. Thus, corresponding to a titer of 1 unit by the blocking method, a titer of about 20 to 40 units by the albumin-plasma conglutination method may be expected, about 50 units by the antiglobulin technic and about 100 units using enzyme-treated red cells. Because of its low sensitivity, the “blocking” test is rarely used in clinical work. The antiglobulin test is a laborious procedure, though when carried out carefully by an expert it gives excellent results. For practical work, however, the conglutination test and the test with enzyme-treated red cells are to be preferred.

Since our study extended over a period of years during which more and more sensitive methods were being developed, the titer values obtained at different times were not comparable. For the sake of uniformity in this series of papers, unless otherwise stated, the titer values given are by the albumin-plasma conglutination method. However, early in the study, before this method was developed, the conglutination test was carried out on oxalated plasma. To convert titers obtained by the plasma conglutination method to equivalent titers by the albumin-plasma conglutination method, one should multiply by the factor 4.

Agglutination Test

A series of small test tubes (inside diameter 7 mm.) were set up, each containing a drop of a different group O blood suspension, some Rh-positive and some Rh-negative. (The
blood used for the tests was always fresh; the suspensions were washed once with saline solution and resuspended in saline to produce a 2 per cent concentration of red cells.) To each tube was added one drop of the serum being tested. The tubes were shaken and placed in the incubator or water bath at body temperature for one hour. The reactions were read in the usual manner by inspecting the pattern of the sediment with the naked eye; then the tubes were gently shaken to dislodge the sediment which was examined for clumping with the naked eye and under the low power of the microscope. For reliable results readings were taken blind; that is, one of us (E. B. G.) set up the tubes while another (A. S. W.) made the readings. Only if all the Rh-positive and Rh-negative bloods were correctly identified did we consider that the presence of Rh antibodies had been established.

When Rh antibodies were found they were titrated as follows: a series of progressively doubled dilutions of the serum was prepared with saline solution, using the same pipet for counting the drops throughout to insure accuracy, but avoiding "carrying over" by rinsing the pipet thoroughly with fresh saline solution between dilutions. One drop of each serum dilution (undiluted, 1:2, 1:4, 1:8, etc.) was transferred to a corresponding empty tube of a series, and to each tube was added one drop of a 2 per cent suspension of fresh Rh-positive cells in saline solution. The mixtures were allowed to stand for at least sixty minutes at 37 C. and the reactions were then read. The titer was taken as equal to the reciprocal of the highest dilution giving a distinct (one plus) reaction.

**Albumin-plasma Conglutination Method**

This test was carried out in two stages. The first stage was identical with the procedure described above for the agglutination test. After the cells had sedimented, the supernatant fluid was removed from each tube with a fine capillary pipet as completely as possible, and to each tube was then added a large drop of albumin-plasma mixture. The latter was prepared by pooling plasma obtained from fresh oxalated Rh-positive blood, and mixing 4 parts of the pooled oxalated plasma with 1 part of 30 per cent bovine albumin (Armour). The sedimented red cells were resuspended in the albumin-plasma mixture, and incubated at 37 C. for a second hour. The tubes were then shaken, somewhat more strongly than in the agglutination method, and examined for clumping with the naked eye and under the lower power of the microscope. Ability to identify the standard blood correctly blind was considered proof of sensitization to the Rh factor.

To titrate Rh antibodies by the albumin-plasma technic, again the first stage of the test was carried out as for the agglutination technic. After the red cells had sedimented, the supernatant fluid was removed and a large drop of the albumin-plasma mixture was added. The final readings were taken after an additional sixty to ninety minutes of incubation.

**Test with Enzyme-treated Cells**

This procedure was carried out exactly the same way as the agglutination technic, except that enzyme-treated cells were substituted for the untreated blood suspensions. To prepare the enzyme-treated cells a solution of trypsin, papain or other suitable proteolytic enzyme is necessary. The Difco solution of Bacto trypsin was used by us at times; we also prepared a one per cent solution of the powdered enzyme (trypsin or papain), allowed undissolved material to sediment, and used the supernatant solution for treating the red cells. The red cells to be trypsinated (or papainated) were washed 3 times, each time with about 10 volumes of saline solution, in order to remove enzyme inhibitors present in normal plasma. Nine parts of packed washed cells were mixed with 1 part of the enzyme solution, and the mixture incubated at 37 C. for one hour. A small amount of the enzyme-treated cells was then suspended in saline solution, washed twice in saline solution to stop the action of the enzyme, and a 2 per cent suspension prepared. These suspensions were used for the actual tests.

**Interpretation of Rh Antibody Titer**

Serologic titrations are relatively crude compared with chemical titrations, and wide discrepancies occur even in the hands of expert workers using the same serum and test cells. Therefore, all our titrations were done at least in duplicate using two different Rh-positive
cell suspensions and the average of the results taken. The titer values given in the tables, therefore, represent an average of 2 or more titrations and this accounts for the fact that they are not expressed merely as powers of 2. Despite careful work and repetition of tests, variations of one tube can hardly be avoided; for example, when the titer value obtained was 4 units, the actual titer might lie anywhere between 2 and 8 units. By the same token, the fact that on one occasion a titer of 2 units was obtained, while on another occasion the titer was found to be 8 units, does not necessarily prove a rise in titer because this seeming change might merely reflect the limitations of the test. Manifestly, the difference between 32 units and 128 units is of no greater significance than the difference between 2 and 8 units. Some of the misconceptions which prevail are due to the practice of presenting antibody titers graphically on an arithmetic scale which makes it appear as if the variation between 64 and 128 units is just as significant as between 0 and 64 units, while actually this is comparable only to the difference between 1 and 2 units.

The titrations as carried out in this study had an internal control, in that the antibodies were titrated by more than one method. For example, when it was found that the titer by the albumin-plasma method was 10 units, while with trypsinated cells the titer was 50 units, the findings were consistent. On the other hand, should the titer with enzyme-treated cells be lower than by the conglutination method, it would immediately be apparent that there had been some gross error in technic. Such errors occurred rarely in the present study, and when they did, they were readily detected and corrected. As a further control on the titer values, the antibodies were often titrated by the blocking method.

Whenever a rise in titer was observed in the course of a pregnancy, the patient was immediately recalled for another test. In this way, we could always determine whether we were dealing with a laboratory variation or with a true rise in titer. Antibodies of very low titer, that is, only of 1 or 2 units are easily overlooked due to the variations in the sensitivity of the tests from day to day. When patients returned for repeat tests, therefore, such weak antibodies might be picked up on some occasions and not on others. Observations of this type should not be misinterpreted as evidence of alternate appearance and disappearance of an antibody from a patient’s serum.

II. Effect of Rh-Negative Pregnancies on Rh Antibody Titer

We determined the antibody titers during the pregnancies of 11 sensitized Rh-negative women who subsequently gave birth to Rh-negative babies. We also studied 1 case (Case 10) of a type Rh₁ woman who was sensitized to the rh₂ factor and subsequently gave birth to a type Rh₁ baby. The findings obtained are given in table 1. As is shown in the table, in no case was there any tendency for the antibody titer to rise during the pregnancy. On the contrary, in Cases 10 and 11, the antibody titers exhibited a tendency to fall gradually as the pregnancy progressed, while in the remaining 10 cases the titer level did not exhibit any significant change during the entire pregnancy. In 7 cases, the antibody titers were determined again about a fortnight after the birth of the baby and in none of these cases was any significant change in titer observed. Thus, in our experience, pregnancy with a Rh-negative fetus and the birth of a Rh-negative baby has no effect upon the Rh antibody titer of an Rh-negative woman sensitized to the Rh factor.

The term “anamnestic reaction” has had two meanings applied to it and this has been a source of considerable confusion. It is applied firstly to describe the accelerated and exaggerated antibody reaction exhibited by immunized individuals, who after a long period of time are injected again with small amounts of the specific antigen. For example, when the Rh antibody titer has fallen after a lapse of years, if a small amount of Rh-positive blood is injected there will be a prompt rise in the Rh antibody titer. This description of an anamnestic reac-
tion is generally accepted, and in fact the phenomenon has been applied for
the production of Rh typing serum.\textsuperscript{12-13} According to the second meaning which
has been applied to the term, the injection of a totally unrelated antigen, or an
injection with an immunologically unrelated micro-organism, has been said to
produce a nonspecific rise in antibody titer. Most of the claims concerning such
nonspecific anamnestic reactions were published during the early days of im-
munology when the titration methods were even cruder than they are today,
and the observations interpreted too literally. With regard to the Rh factor, no
one would attempt to produce Rh typing serum or increase their titer by in-
jecting Rh-negative blood. Moreover, when sensitized Rh-negative patients are
given transfusions of Rh-negative blood the transfusions are well tolerated and
there is no noticeable effect on the Rh antibody titer. In view of these considera-
tions and the results shown in table 1, the claims\textsuperscript{14} of nonspecific anamnestic

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|}
\hline
Case Number & Antenatal Titers by Albumin-Plasma Conglutination Method During Lunar Month & Postpartum Titer \\
\hline
 & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\
\hline
1 & 32 & 28 & 57 & 32 & 32 & 32 & 32 & 32 & 32 & 32 \\
2 & 6 & 3 & 6 & 3 & 6 & 3 & 6 & 3 & 6 & 3 \\
3 & 56 & 52 & 22 & 72 & 36 & 28 & 48 & 28 & 48 & 28 \\
4 & 250 & 330 & 320 & 320 & 250 & 300 & 280 & 280 & 280 & 280 \\
5 & 9 & 8 & 9 & 16 & 9 & 16 & 9 & 16 & 9 & 16 \\
6 & 24 & 5 & 24 & 5 & 24 & 5 & 24 & 5 & 24 & 5 \\
7 & 5 & 5 & 5 & 5 & 5 & 5 & 5 & 5 & 5 & 5 \\
8 & 16 & 2 & 2 & 2 & 2 & 2 & 2 & 2 & 2 & 2 \\
9 & 0 & 6 & 0 & 0 & 2 & 2 & 2 & 2 & 2 & 2 \\
10* & 10 & 15 & 4 & 5 & 6 & 5 & 10 & 10 & 10 & 10 \\
11 & 10 & 10 & 10 & 10 & 10 & 10 & 10 & 10 & 10 & 10 \\
12 & 10 & 10 & 10 & 10 & 10 & 10 & 10 & 10 & 10 & 10 \\
\hline
\end{tabular}
\caption{Antenatal and Postpartum Titers in Sensitized Rh-Negative Mothers Bearing
Rh-Negative Babies}
\end{table}

reactions to pregnancy with Rh-negative babies must be attributed to faulty
technic or to the too literal interpretation of the results of antibody titrations.

Two of the cases will be presented in detail for the sake of illustration.

\textbf{Case 1}. This patient was seen for the first time in June, 1948 during her seventh preg-
nancy, with an L. M. P. of November 5, 1947, and an E. D. C. of August 12, 1948. The pa-
tient denied ever having received an injection of blood or plasma and gave the following
obstetric history.

First pregnancy yielded a full term, normal, female infant, born on April 11, 1940, who
was alive and well.

Second pregnancy terminated with a six week miscarriage.

Third pregnancy yielded a full term, normal, female infant, born May 3, 1942, who was
alive and well.

Fourth pregnancy terminated with a six week miscarriage.

Fifth pregnancy yielded a full term, male infant, born September 5, 1945, who became
jaundiced and developed severe anemia on the third day of life, with an RBC of only one
million per cu. mm. He was given a transfusion of 120 cc. of whole blood, and a second
transfusion was given when the baby was one week old. The baby recovered completely and exhibited no sequelae.

Sixth pregnancy terminated with a six week miscarriage.

During the seventh pregnancy, Rh antibody tests had been done after the sixth month and were said to show the presence of weak Rh antibodies (2 to 4 units). Our own grouping and Rh-Hr tests gave the result in table 2.

As expected, the tests showed the mother to be Rh negative while the father was Rh positive and belonged to the phenotype Rh2rh, so that his genotype was either R'r, R'R'
, or R'r". Genotype R'r" could immediately be excluded, because all 3 children belonged to type Rh2 indicating that the father was carrying the R2 gene. If the father belonged to the genotype R'R'
 he would be clinically homozygous for the Rh factor and every subsequent child would have to be Rh positive and therefore erythroblastotic. However, genotype R'r is much more common than genotype R2R'
, so that the odds were in favor of the father's being heterozygous.

### Table 2.—Results of Grouping and Rh-Hr Tests in Case 1

<table>
<thead>
<tr>
<th>Blood of:</th>
<th>Group</th>
<th>Rh-Hr Type</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expectant father</td>
<td>O</td>
<td>Rh2rh</td>
<td>R'r or R'R'</td>
</tr>
<tr>
<td>Expectant mother</td>
<td>B</td>
<td>rh</td>
<td>rr</td>
</tr>
<tr>
<td>1st child</td>
<td>O</td>
<td>Rh2rh</td>
<td>R'r</td>
</tr>
<tr>
<td>2nd child</td>
<td>O</td>
<td>Rh2rh</td>
<td>R'r</td>
</tr>
<tr>
<td>3rd child</td>
<td>B</td>
<td>Rh2rh</td>
<td>R'r</td>
</tr>
</tbody>
</table>

### Table 3.—Results of Rh Antibody Tests in Case 1

| Date of Tests       | Period of Gestation (Lunar Month) | Titer (Units) of Rh Antibody by Method of: | | | |
|---------------------|----------------------------------|------------------------------------------|---|---------|
|                     |                                  | Agglutination (Saline Media) | Blocking | Albumin-Plasma Conglutination |
| June 15, 1948       | Seventh month                    | 0 | ½ | 6 |
| June 20, 1948       | Eighth month                     | 0 | ½ | 4 |
| July 15, 1948       | Eighth month                     | 0 | ½ | 5 |
| July 26, 1948       | Ninth month                      | 0 | ½ | 10 |

* Not done.

Repeated Rh antibody titrations were done on the mother with the results shown in table 3.

It can be seen that the expectant mother's serum contained univalent Rh antibodies of moderate titer, and that none of the titer values differed significantly from the mean. These findings encouraged us in our hope that the patient was carrying an Rh-negative baby.

When the baby was born on July 30, 1948, he exhibited no evidence of erythroblastosis and immediate tests on the cord blood proved the baby to be Rh negative. The icterus index of the cord serum was within the normal limits, and free Rh antibodies were found in the cord serum of the same titer (6 units) as had been found in the mother's serum in the antenatal tests. The baby at no time showed any jaundice or anemia, and was entirely well when sent home from the hospital with his mother.

Case 2. This case was first studied in March, 1946, because of the following history: the mother had just completed her first pregnancy on March 15, 1946 at which time a female infant was born who quickly developed deep jaundice and anemia, with a hemoglobin concentration of 92 per cent. The baby was seen by us shortly before it died at the age of 3 days.
STUDIES IN Rh SENSITIZATION

Grouping and Rh-Hr tests done on the baby and its parents gave the following results shown in table 4.

Tests for Rh antibodies on the mother's serum at that time gave the following results: agglutination method = negative; albumin-plasma conglutination method = positive, 32 units; blocking test = positive 1½ units.

Another antibody test was carried out before the mother left the hospital which showed there had been a rise in titer, the blocking titer then being 4 units and the titer by the albumin-plasma conglutination method 160 units.

This case was unusual because the erythroblastotic baby was a firstborn. A history was then elicited that the baby's mother had received an intramuscular injection of blood from her own mother fifteen years previously, in order to prevent measles. Since the latter belonged to type A,Rh,rh, she was evidently the original source of the Rh sensitization.

The antibody tests were repeated in December, 1946 at which time the mother was pregnant for the second time, and the following results were obtained: agglutination method = negative; blocking test = positive, 6 units; albumin-plasma conglutination method = positive, 250 units.

| TABLE 4.—Results of Grouping and Rh-Hr Tests in Case 2 |
| Blood of: | Group and Subgroup | Rh-Hr Type |
| Father | A,B | Rh,rh |
| Mother | A, rh | rh |
| Erythroblastotic baby | A | Rh,rh |

<p>| TABLE 5.—Rh Antibody Titers During Third Pregnancy, Case 2 |</p>
<table>
<thead>
<tr>
<th>Date of Tests</th>
<th>Period of Gestation (Lunar Month)</th>
<th>Agglutination Method</th>
<th>Blocking Method</th>
<th>Albumin-Plasma Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 3, 1948</td>
<td>Third month</td>
<td>0</td>
<td>6½</td>
<td>250</td>
</tr>
<tr>
<td>October 2, 1948</td>
<td>Fourth month</td>
<td>0</td>
<td>20</td>
<td>320</td>
</tr>
<tr>
<td>October 6, 1948</td>
<td>Fourth month</td>
<td>0</td>
<td>11</td>
<td>320</td>
</tr>
<tr>
<td>October 27, 1948</td>
<td>Fifth month</td>
<td>0</td>
<td>8</td>
<td>320</td>
</tr>
<tr>
<td>November 12, 1948</td>
<td>Sixth month</td>
<td>0</td>
<td>5</td>
<td>320</td>
</tr>
<tr>
<td>November 19, 1948</td>
<td>Sixth month</td>
<td>0</td>
<td>18</td>
<td>250</td>
</tr>
<tr>
<td>January 8, 1949</td>
<td>Eighth month</td>
<td>0</td>
<td>9</td>
<td>300</td>
</tr>
<tr>
<td>February 8, 1949</td>
<td>Ninth month</td>
<td>0</td>
<td>15</td>
<td>280</td>
</tr>
<tr>
<td>March 17, 1949</td>
<td>Tenth month</td>
<td>0</td>
<td>6½</td>
<td>250</td>
</tr>
</tbody>
</table>

It was pointed out that the prognosis for this pregnancy depended entirely on the Rh type of the fetus. If the fetus was Rh negative it would not be harmed by the maternal Rh antibodies, while if it was Rh positive it would most likely die in utero and probably too early in the pregnancy (sixth or seventh month) to be saved by transfusion. In fact, the pregnancy did terminate on December 20, 1946 with the birth of a stillborn male fetus.

The couple then adopted a baby.

The patient returned for further studies in September, 1948 when she was pregnant for the third time with an L. M. P. of June 15, 1948 and an E. D. C. of March 22, 1949. The bloods of the husband's parents were typed in order to determine more exactly whether or not the husband was heterozygous for the Rh factor, but the results were not conclusive. The paternal grandfather belonged to type B,Rh,rh while the paternal grandmother belonged to type A,Rh,Rh, which left open the question whether the husband was heterozygous or homozygous for the Rh factor.

During the third pregnancy, the expectant mother was given five intramuscular injections of the so-called "Rh hapten" provided by Mrs. B. Carter, between October 2, 1948 and November 12, 1948, but there had been no effect on the antibody titer. As has been
pointed out elsewhere, it is now known that this preparation has no specific activity either in vivo or in vitro. The results of antibody titrations carried out during the third pregnancy are shown in table 5.

It is evident that there was no significant change in Rh antibody titer during the entire pregnancy; nor, in fact, had there been any appreciable change in titer for the past two and one-half years.

Since the fetus was still alive, we felt safe in predicting it was probably Rh negative. If the fetus had been Rh positive, it would most likely have died in utero by this time, as had happened during the second pregnancy. In fact, when the baby was born on March 21, 1949 it proved to be entirely normal. Grouping and Rh tests on the baby showed it to belong to group AB, type rh. The icterus index of the cord serum was only 12 units, and the conglutination and antiglobulin tests for coating of the baby's cells were negative, confirming the prediction that the baby would not be erythroblastotic. The baby's cord serum was titrated in order to determine its content of Rh antibodies and the following are the results:

<table>
<thead>
<tr>
<th>Agglutination Method</th>
<th>Blocking Method</th>
<th>Albumin-Plasma Conglutination Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother 0</td>
<td>9</td>
<td>300</td>
</tr>
<tr>
<td>Baby 0</td>
<td>6</td>
<td>250</td>
</tr>
</tbody>
</table>

It should be mentioned that since the mother belonged to group A and the father to group AB, the possibility had been considered that the mother might be sensitized to the B agglutinogen as well as the Rh factor. Therefore, during the pregnancy anti-A and anti-B titrations were carried out, but these were always within normal limits, and as expected the baby did not exhibit either jaundice or anemia even though he belonged to group AB.

TABLE 6.—Persistence of Passively Acquired Rh Antibodies in Serum of an Rh-Negative Baby

<table>
<thead>
<tr>
<th>Date of Test</th>
<th>Rh Antibody Titer (Albumin-Plasma) Method in Serum of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mother</td>
</tr>
<tr>
<td>March 21, 1949 (at birth)</td>
<td>300</td>
</tr>
<tr>
<td>June 21, 1949</td>
<td>280</td>
</tr>
<tr>
<td>September 12, 1949</td>
<td>180</td>
</tr>
</tbody>
</table>

Following the birth of the baby, the opportunity was taken to determine how long the passively acquired Rh antibodies persisted in the baby's circulation. The results are shown in table 6.

It will be seen that the Rh antibody titer of the mother showed only a relatively small drop during the six months following delivery, while by this time almost all the Rh antibodies had disappeared from the baby's circulation, in conformity with our previous observations. Since the Rh antibodies are serum globulins, their life can be taken as an index of the duration of life of serum globulin molecules in general. It is evident from the titer values obtained on the baby, that since one may assume that no new antibodies are being formed in the baby's body, the proportion of the passively acquired antibodies persisting at any given time is given by the approximate formula $e^{-0.0231d}$, where $d$ represents the age of the baby in days. Therefore, the half-life of the antibody molecule (and presumably any globulin molecule)
is approximately thirty days. It will be recalled that the Rh antibody titer of the mother's serum had been maintained with relatively small reduction in titer for more than three years, in the absence of any fresh antigenic stimulus. That means that during this period an amount of Rh antibody equivalent to the total amount required to maintain a titer of about 200 to 300 units had been produced by her body about every 35 days. This serves to underline the futility of attempts to reduce an actively sensitized individual's antibody titer by means of exchange transfusion.

**Summary and Conclusions**

The Rh antibody titer was studied during pregnancy and after delivery in 11 cases in which previously sensitized Rh-negative mothers had Rh-negative babies. In addition, 1 case was studied in which a type Rh1 mother, previously sensitized to the rh" factor, gave birth to a type Rh1 baby. In all cases the babies were normal at birth and the baby’s serum contained univalent Rh antibodies equal in titer to those present in the maternal serum. In no case was there a rise in the antibody titer in the maternal serum either during the pregnancy or after the baby was born. Thus, it has not been possible to confirm claims concerning a “nonspecific anamnestic rise” in Rh antibody titer said to be caused by an Rh-negative baby.

Two illustrative cases are described in detail. In one of the cases described, there was no appreciable change in Rh antibody titer during the pregnancy despite the use of so-called Rh hapten; nor had there been an appreciable change in titer for as long as two and one-half years previously. The Rh antibody titer of the baby's blood dropped about 98 per cent by the end of the sixth month, while during the same period of time the maternal antibody titer fell only approximately 35 per cent. Based on the course of the Rh antibody titer of the baby's serum, the half-life of the antibody globulin molecule is estimated to be about thirty days.

**References**

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