Rh ANTIBODIES; CORRELATION WITH CLINICAL FINDINGS

By I. DAVIDSOHN, M.D.

The discovery of so-called "incomplete" or "blocking" antibodies and of thermostable agglutinins reacting in serum or albumin but not in saline has stimulated discussion and speculation on their relation to each other and to the previously known Rh agglutinins which react in saline solution, and more recently on their relation to the various forms of fetal erythroblastosis.

The problem of the nature of the various forms of Rh antibody is one to be solved by immunologic and immuno-chemical methods. The second problem, of the respective role of the antibodies in the genesis of the different forms of the disease may be brought closer to solution by qualitative and quantitative study of antibodies during pregnancy and correlation with the condition of the newborn infant. This report aims to present the results of such a comparative investigation.

It is based on a study of the titres of Rh antibodies (saline agglutinins, serum-albumin agglutinins, and blocking antibodies) in the blood of 73 mothers of erythroblastotic infants or fetuses. In most instances repeated blood samples were obtained at intervals during the pregnancy and after childbirth. In some cases tests extended over several years. Ten women had only one sample examined, but the average number for the remaining 63 women was 4.5 samples.

The technic of the various tests was essentially the same as described by Wiener and by Diamond. It was presented in detail in a chapter on blood groups in the forthcoming new edition of Kracke and Parker's "Clinical Pathology." 1

Various opinions have been expressed regarding the nature of the 3 varieties of Rh antibodies. Agglutinins which react in physiologic saline solution are called bivalent antibodies by Wiener. The antibody which reacts best in serum or plasma or in a solution of albumin, human or bovine, is called by him glutinin (formerly conglutinin). Diamond refers to it as heat resistant antibody, an appropriate descriptive term. Diamond believes that blocking antibodies and antibodies reacting in serum-albumin are a manifestation of more intense immunization than agglutinins (hyperimmune antibodies).

It is not within the scope of this paper to take sides in the dispute on the identity or diversity of the various Rh antibodies. Factual evidence available does not yet justify definitive statements. Reactivity in one or another menstruum, thermal resistance or thermal range, may be manifestations of quantitative and not necessarily of qualitative difference.

The following terms will be used here: saline agglutinin for the thermodlabile antibody reacting in physiologic solution of sodium chloride; blocking antibody for the incomplete antibody of Race and Taylor; serum-albumin agglutinin for the...
Rh antibodies

so-called conglutinin or glutinin of Wiener; globulin antibody for the red cell coating globulin which reacts with the anti-human-globulin rabbit immune serum of the Coomb's test (Hill's developing test). The terms are admittedly cumbersome, but descriptive by inclusion of a prominent feature of the tests used for their detection. Some terms have been discarded because they are based on unproved hypothetical concepts. It is hoped that more fitting and less awkward terms will be substituted as soon as the true nature of the antibodies will become known. Table I presents the tests used for detection of the various forms of the Rh antibody.

Table I

<table>
<thead>
<tr>
<th>Serum tested</th>
<th>Diluent plus</th>
<th>Clumping</th>
<th>Forms of Rh antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known anti-Rh</td>
<td>Saline</td>
<td>Rh-+ red cells</td>
<td>Thermolabile Rh aggl. (saline aggl.)</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td></td>
<td>Incomplete blocking antibody (reacting in serum or albumin) (hyperimmune)</td>
</tr>
<tr>
<td>1. Unknown</td>
<td></td>
<td></td>
<td>Thermostable Rh aggl. (Coomb's test, Hill's developing test)</td>
</tr>
<tr>
<td>2. Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a. Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b. Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2c. Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d. Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thermolabile Rh agglutinin and blocking antibody are never present together.

Thermostable Rh agglutinin may be present together with the thermolabile Rh agglutinin or with the blocking antibody.

The material to be presented was divided into 2 main groups: (1) Cases with blocking antibodies and serum-albumin agglutinins, but without saline agglutinins. (2) Cases with saline agglutinins and serum-albumin agglutinins, but without blocking antibodies.

This division was suggested by the observation that in our material such combination was the one usually encountered. It is in accord with the fact that block-

* It is possible that it is not the presence or absence of sodium chloride which is responsible for the differences in reactivity of saline agglutinins and serum-albumin agglutinins but the water content of the diluent. In such a case the term hydrophilic may be the appropriate adjective for the saline agglutinin and hydrophobic for the serum-albumin agglutinin.
ing antibodies and agglutinins are mutually exclusive, at least within the range of certain dilutions. Table 2 is an example of the supposedly simultaneous presence of saline agglutinins and of blocking antibodies in the same serum. Many such records have been reported. In such serums there is agglutination in the undiluted serum, more or less distinct traces in one or two further tubes, perhaps up to dilutions of 1:10 or 1:20 with saline, fairly strong blocking antibodies (f.i. up to a dilution of 1:80) and still stronger agglutination (f.i. 1:2560) in dilutions of the serum with human serum or bovine albumin. Such tests are usually reported as: saline agglutinins 1:10, blocking antibodies 1:80, serum-albumin agglutinins 1:2560. Such interpretation and reading of the result is not justified, because what is interpreted as saline agglutinin in this and similar cases is not the usual saline agglutinin, but a serum-albumin agglutinin strong enough to react weakly in low dilutions of saline. In this report such serums are listed as lacking saline agglutinins.

As shown in table 3, in 28 cases (38 per cent) blocking antibodies were found. Twenty mothers gave birth to stillbirths or to infants with hydrops dying within a few hours after birth. Eighty-five per cent of these mothers had, and only 15 per cent lacked, blocking antibodies. In the remaining 27 deaths blocking antibodies were present only in 7 per cent of the mothers, while in the majority (93 per cent) of postpartum deaths not due to hydrops, blocking antibodies were absent. They were present in only 18 per cent of the 50 mothers who gave birth to babies with icterus gravis. In the mothers of 26 survivors blocking antibodies were present in 35 per cent.

The incidence of the 2 groups of antibodies was approximately equal in the total numbers of cases, deaths and survivals. Impressive differences are noticeable in mothers of stillbirths, of hydropic babies and in those receiving transfusions, where the blocking antibodies were higher, and in mothers of babies born alive and free of hydrops, and of babies with icterus gravis where the titre of blocking antibodies was low.

The relatively low figure for sera with agglutinins (62 per cent) is at variance
Rh Antibodies

with my previous report of an 82 per cent incidence. This is explained by the inclusion, previously, of serums with agglutinins, which are listed as free of saline agglutinins in this paper, and as containing blocking antibodies and serum-albumin agglutinins only. Tests for blocking antibodies and serum-albumin agglutinins had not been used in the study previously reported.

The high mortality in this series is due, to some extent, to the fact that some babies were sent in from other hospitals in critical condition, making this a select group of cases.

Table 3.—Distribution of Rh Antibodies

<table>
<thead>
<tr>
<th>Type of Antibody</th>
<th>No. of Families</th>
<th>Stillbirth &amp; hydrops</th>
<th>Icterus Gravis &amp; Nuclear Jaundice</th>
<th>Hemolytic Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% ±P.E.</td>
<td>No.</td>
<td>% ±P.E.</td>
</tr>
<tr>
<td>Blocking antibodies and serum-albumin agglutinins but no saline agglutinins.</td>
<td>18</td>
<td>60.7 ± 6.0</td>
<td>9</td>
<td>31.1 ± 5.7</td>
</tr>
<tr>
<td>Saline agglutinins and serum-albumin agglutinins but no blocking antibodies.</td>
<td>45</td>
<td>6.7 ± 1.6</td>
<td>41</td>
<td>91.1 ± 2.7</td>
</tr>
</tbody>
</table>

Twelve mothers had a history of transfusion prior to the pregnancy under consideration. The number is small but it may be significant that 11, or 92 per cent, had blocking antibodies; only 1 mother had none, and the baby of this mother was stillborn. Six of the transfused mothers (55 per cent) had stillborn babies.

In table 4 the antibodies are correlated with clinical manifestations in the newborn. Sixty per cent of mothers with blocking antibodies had stillborn or hydropic babies, 32 per cent had babies with icterus gravis and 7 per cent had babies in whom hemolytic anemia was the predominant finding and jaundice was not striking. On the other hand, only 6.7 per cent of mothers with saline agglu-
tinins and serum-albumin agglutinins (but no blocking antibodies) had stillborn babies, and 91 per cent had babies with icterus gravis. The incidence of hemolytic anemia as the presenting manifestation (without jaundice) was too small to permit evaluation. The small number of cases of fetal erythroblastosis with anemia as a predominant feature is probably also explained by the referral mostly of severely affected children as patients to the hospital, and of their mothers for serologic study in the laboratory.

Wiener suggested recently that agglutinins (saline agglutinins) are mainly responsible for icterus gravis, and the so-called conglutinins (serum-albumin agglutinins) and blockers (blocking antibodies) or, as he calls them, univalent antibodies, are mainly responsible for the severe damage leading to intrauterine death and hydrops. In his series agglutinins alone (saline agglutinins) were found in 64 per cent of mothers whose babies had icterus gravis, whereas blockers and conglutinins were present only in 14 per cent of such mothers. The mothers of stillborn babies were found to have agglutinins in only 8 per cent and conglutinins and blockers in 43.9 per cent of cases.

<table>
<thead>
<tr>
<th>Table 5.—Correlation of Saline Agglutinin/Serum-Albumin Agglutinin Ratio with Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of saline agglutinin-serum-albumin agglutinin titers</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Titre of saline agglutinins higher than or equal that of serum/albumin agglutinins</td>
</tr>
<tr>
<td>Titre of serum-albumin agglutinins significantly higher than that of saline agglutinins</td>
</tr>
</tbody>
</table>

The serious import of blocking antibodies is apparent from Wiener's and our results. On the other hand, agglutinins seem to have a less serious implication. In table 5 the 45 cases with serum-albumin agglutinins and saline agglutinins but without blocking antibodies were divided in 2 groups: one, in which the titre of saline agglutinins was higher than or equal to that of serum-albumin agglutinins (allowing one tube difference for technical factors); and the other group, in which the serum-albumin agglutinin titre was significantly higher than the saline agglutinin. The lower death rate in the first group is impressive though the sample may be too small to be significant.

Anamnestic Rh antibody reaction. It is a well known fact that nonspecific stimuli may cause a reappearance of antibodies that had in the past been produced by a specific antigen and had disappeared from the circulation in the course of time. This is the so-called anamnestic reaction, also known as the Hektoen phenomenon. The dictionary defines it as follows: "When antigens are introduced into the animal body in allergic states, there may exist an increased range of new antibody production which may include production of antibodies concerned in previous infections and immunizations."

We had an opportunity to observe what may be interpreted as anamnestic Rh
Rh antibodies

antibody reaction in case 6. The obstetrical history, the blood groups, the Rh factors of the family and the levels of Rh antibodies before and during pregnancy are recorded in figure 1. Anti-Rh agglutinins of a low titre (1:8) were found in 1943, 5 days after delivery of a stillbirth, with typical findings of fetal erythroblastosis (hydrops). Tests for serum-albumin agglutinins and blocking antibodies were not done. After artificial insemination (donor A, Rh negative MN) the woman became pregnant in November, 1945. No Rh antibodies of any kind were found at approximately the end of the third month of pregnancy, January 31, 1946. Seven specimens were examined at intervals varying from 2 weeks to 2 months. At the beginning of the fourth month saline agglutinins could not be found, but traces of serum-albumin agglutinins and blocking antibodies were observed for the first time. Serum-albumin agglutinins were very weak, detectable only in undiluted serum until the day of delivery, when the titre rose to 1:10. Blocking antibodies reached their peak (1:20) in the seventh month, and remained at that level except for a brief drop 2 weeks prior to delivery. Both antibodies were still present one week after delivery.

The appearance of the antibodies early in the pregnancy caused concern in view of the past obstetrical history. It was thought possible that the pregnancy resulted
not from the artificial insemination, though the patient and her husband asserted that they had adhered strictly to instructions and that the husband could not possibly be the father. The delivery of a normal B Rh-negative child showed that the antibody response could not have been due to a specific stimulus by the Rh factor. It is known that in heterospecific pregnancy the factors A and B may act not only as specific but also as nonspecific antigenic stimuli. For instance, in women of group O, a rise of anti-A agglutinins was observed in the course of pregnancy with a baby of group A. In the following pregnancy with a baby of group B there was not only a rise of anti-B but also of anti-A iso-agglutinins. A rise of both anti-A and anti-B iso-agglutinins was also observed in pregnancies where the infant was found to be of group O or of the same group as the mother. In case 6 the presence of factor A in the donor of the semen and in the mother made him an unlikely source of antigenic stimulation, especially since the baby did not inherit the A factor. The mother was not tested for factors M and N. If she lacked one of them, then it may possibly be incriminated, though factors M and N are extremely rarely antigenic in man. However, there is little doubt that our knowledge of individual antigenic differences is still too limited to exclude the presence of a hitherto unknown antigen in the fetus and its absence in the mother. According to present knowledge such an antigen would be nonspecific with regard to the Rh factor, thus justifying the reference to the observed phenomenon as anamnestic Rh antibody reaction.

The persistence of antibodies. The persistence of Rh antibodies in the circulation varies from case to case, depending on the intensity and duration of immunization, the potency of the antigenic stimulus, the individual response, and, in the case of immunization during pregnancy, on the number of pregnancies and on the permeability of the placenta. The role of transfusions prior to pregnancy has already been discussed. Case 10 is an example of persistence of Rh antibodies for at least 36 months and probably for over 4 years. Twenty-seven samples of blood were examined at regular intervals during the last 3 years. The titres of saline agglutinins and serum-albumin agglutinins are recorded in figure 2. Blocking antibodies have been absent consistently.

Value of repeated tests. Repeated examination of the blood of pregnant women for antibodies offers a unique opportunity for the study of responses to antigenic stimuli. The emphasis must be placed on the words "repeated examination," because a single examination may be misleading, partly due to variations in the agglutinability of red cells, even if the cells of the same person are used for all tests. Another reason is the possibility of persistence of antibodies from previous pregnancies. The examination should begin preferably not later than the third month of pregnancy. Repeated tests (at from 1 to 2 month intervals, and at biweekly intervals after the seventh month) make it possible to recognize a trend in antibody production which may permit clinical interpretation:

Case 34. Woman, age 29, gravida 2, para 1, A, Rh negative, received 2 transfusions when ill with osteomyelitis at 15. The husband was A Rh positive. First child, boy, O Rh positive, was born 5 years prior to this examination. He had icterus gravis and anemia, received 3 transfusions and recovered fully. Anti-Rh
agglutinins of low titre (only in undiluted serum) were found in mother's blood a few days after the delivery, and again 3 years later (December, 1941). Patient returned in September, 1945, to be tested for Rh antibodies. She was then 3 months pregnant. The titre of agglutinins was still at the same level as in December, 1941. Six weeks later (October 22) the titre of agglutinins was unchanged but the titre of serum-albumin agglutinins had risen to 1:160. On December 10 the titre of serum-albumin agglutinins dropped to a level of 1:5 and persisted at this level until the end of the pregnancy and beyond it. There was a trace of blocking antibodies (only in undiluted serum). On February 6, 1946, 2 months after the precipitous drop of serum-albumin glutinins was noted, the patient gave birth to a hydropic stillborn fetus.

Was there any relation between the drop of antibodies and the changes in the fetus which led eventually to its death? The appearance of the fetus indicated that it had been dead in utero only about 7 days. It may be that the process which eventually destroyed the fetus may have started several weeks before delivery, and that the drop of the titre of antibodies may have been due to some such changes.

One more example of similar nature:

Case 55. During a routine prenatal blood grouping test the patient's serum was
found to clump cells of group O. At that time routine tests for the Rh factor had not been done. The irregularity was explained later by finding that the woman

**Table 6.—Drop of Antibodies in Maternal Serum Preceding Stillbirth**

<table>
<thead>
<tr>
<th>Case No. 35*</th>
<th>Saline Agglutinins</th>
<th>Serum-albumin Agglutinins</th>
<th>Blocking Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 29, 1945. Pregnant 8 months, due February 28</td>
<td>40</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>February 6</td>
<td>1</td>
<td>—</td>
<td>80</td>
</tr>
<tr>
<td>February 16. Birth of stillborn hydrops. Path.: Typical Erythroblastosis March 6</td>
<td>1</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>April 11</td>
<td>1</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>November 16</td>
<td>1</td>
<td>80</td>
<td>5</td>
</tr>
</tbody>
</table>

* Father—A Rh⁺ Rh⁺ Hr positive.  
Mother—A Rh negative, anti-Rh₀.  
Pregnancies: 1. ♂ A Rh positive. 3 years, normal.  

**Table 7.—Value of Periodic Tests for Antibodies**

| Case 30 A. Father ORh⁺Hr—  
Mother BRh—, anti-Rh₀  
Pregnancies 1. ♂ ORh⁻ 3 years, normal  
2. ♂ BRh⁺ F.E.I.G. died 3 days |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak antibodies during last weeks of pregnancy Normal child</td>
<td>High titer of antibodies throughout pregnancy. Severe icterus gravis.</td>
<td></td>
</tr>
</tbody>
</table>
| Case 31. Father ORh⁺Hr⁺  
Mother ORh⁻  
Pregnancies 1. ♂ 1938. ORh⁻, 5½ years, normal  
2. 1939. Abortion 2 months  
2 transfusions from husband  
3. 1941. Abortion 7 weeks  
4. 1943. Abortion 4 months |

<table>
<thead>
<tr>
<th>5 months pregnant</th>
<th>Saline aggl.</th>
<th>Blocking antibodies</th>
<th>3 months pregnant</th>
<th>Saline aggl.</th>
<th>Serum albumin aggl.</th>
<th>Blocking antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/11/44</td>
<td>o</td>
<td></td>
<td>4/25</td>
<td>80</td>
<td>320</td>
<td>o</td>
</tr>
<tr>
<td>9/15/44</td>
<td>o</td>
<td></td>
<td>6/5</td>
<td>80</td>
<td>0</td>
<td>o</td>
</tr>
<tr>
<td>10/12/44</td>
<td>o</td>
<td></td>
<td>7/15</td>
<td>40</td>
<td>10</td>
<td>o</td>
</tr>
<tr>
<td>11/17/44</td>
<td>+(1)*</td>
<td></td>
<td>8/22</td>
<td>160</td>
<td>310</td>
<td>o</td>
</tr>
<tr>
<td>12/4/44</td>
<td>Normal</td>
<td></td>
<td>9/24</td>
<td>160</td>
<td>1280</td>
<td>o</td>
</tr>
<tr>
<td>12/7/44</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/16/44</td>
<td>+(1)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Clumping only in undiluted serum.

was Rh negative and had anti-Rh agglutinins (titre 1:40). Two days later she reported to her obstetrician that she did not feel movements of the baby. Blood examined on February 6 showed agglutinins present only in undiluted serum and a
Rh antibodies

high titre of blocking antibodies (1:80). The same day a stillborn hydrops was delivered (table 6). This case poses the same question as the preceding. The relation between the drop of the titre and the stillbirth is even more suggestive. The impression is supported by the usual striking persistence of titres during pregnancy with only slight variations readily explained by technical factors and by fluctuations in the sensitivity of the Rh-positive test cells.

The value of periodic tests for Rh antibodies is also shown in the 2 cases recorded in table 7. In case 31, the mother had a bad obstetric history, with 3 abortions and 2 transfusions from the Rh-positive husband. Repeated tests were negative, till 17 days before delivery, when for the first time agglutinins were seen, but only in undiluted serum. Three and 12 days after delivery of a normal Rh-positive child the titre was the same. In addition, blocking antibodies were detected for the first time at the last examination (done later on a preserved frozen specimen). It is possible that accumulated experience may permit us to draw favorable conclusions from such a course.

For comparison, case 30A is recorded in the same table to show an entirely different course of antibodies in a pregnancy terminating in the birth of a baby with severe icterus gravis. The findings in the last 2 cases are in accord with the recent report by Page, Hunt and Lucia of a direct relationship between the duration of exposure to maternal antibodies and the occurrence and severity of erythroblastosis in the infant.

A series of patients in whom tests for Rh antibodies were done repeatedly during pregnancy was studied recently from the same point of view as presented in the paper of Page and associates and will be reported.

SUMMARY

Rh antibodies were studied during pregnancy at frequent intervals in the blood of 73 mothers of babies with fetal erythroblastosis. The serums containing antibodies were divided in 2 groups: (1) serums with blocking antibodies without saline agglutinins; (2) serums with saline agglutinins and serum-albumin agglutinins, but without blocking antibodies. The correlation of these 2 groups with clinical findings in the newborn showed striking differences: blocking antibodies present in 85 per cent of mothers of babies with hydrops or stillborn, and only in 9 per cent of mothers of babies with icterus gravis.

Predominance of saline agglutinins over serum-albumin favored survival, and vice versa.

In a small group of women with a history of transfusions who gave birth to erythroblastotic babies blocking antibodies were found in 92 per cent.

The number of cases used for this study is admittedly small, and only very guarded conclusions may be drawn. The results, however, are in general agreement with recent studies of Wiener on correlation of antibodies with clinical manifestations, and with results of Levine and others regarding the sensitizing influence of blood transfusions.

Periodic tests for antibodies during pregnancy may permit prognostic conclusions.

An anamnestic Rh antibody reaction is described.
REFERENCES

1. DAVIDSOHN, I.: Blood Groups in Kracke-Parker, Clinical Pathology, ed. 3, St. Louis, Mosby (in press).
6. DAVIDSOHN, I.: Unpublished data.

DISCUSSION

Dr. Levine: I enjoyed hearing these two very interesting papers. A number of points in Dr. Diamond’s paper* could have been anticipated on the basis of what we have known of isoimmunization by pregnancy, and what the immunologists know about minute quantities of blood and their action as immunizing agents. With reference to Dr. Davidsohn’s comments on terminology, I believe it is no secret that the suitable authorities in Washington are trying to lay down the standards for diagnostic anti-Rh serum. Some of us have already seen the preliminary draft. I am quite pleased with Dr. Davidsohn’s terms “hydrophilic” and “hydrophobic” antibodies for the so-called blocking tests and direct agglutination. We have seen a few cases where women had blocking antibodies at a moderate titre and I was, of course, disturbed as to the outcome in the infant. However, I was very much surprised to find that the infant was Rh positive and perfectly normal. The fact that the infants are Rh positive may indicate that perhaps the blocking antibodies did not go through the placenta, or perhaps the antibodies did go through the placenta, and the titre was not high enough, and there wasn’t sufficiently long intrauterine action to affect the cells.

Now these latter cases with blocking antibodies are in contrast to 3 cases we observed very early in our course of study in 1940 and 1941 when we found women with agglutinins and infants who were perfectly normal. At that time I suggested that we would never get erythroblastosis until we had prolonged intrauterine blood destruction. At the same time it is very important from the public health standpoint to detect those mothers who are already immunized in that particular pregnancy.

We can be certain that in subsequent pregnancies the immunization will be severe enough for the mothers to have an affected baby, and since that will be the first affected baby, I am not quite certain whether recommending longer intervals between pregnancies will be effective. It will be one of the only things we can suggest at this time to prevent severe reactions. So far as the nonspecific increases in agglutinins is concerned, years ago when Dr. Landsteiner and I were studying production of anti-M and anti-N sera, we occasionally found, curiously enough, a very weak anti-N response when we injected M. I can also confirm Dr. Davidsohn’s remarks that we found in pregnancies nonspecific increases in the anti-A and anti-B agglutinins when the affected infants were in group O. Now in regard to Dr. Diamond’s paper which I have been looking forward to hearing, especially his comments on the size of the 2 antibodies, perhaps he can tell us his impression of their sizes and possibly associate this with the permeability of the placenta of these 2 antibodies. In our early studies of the erythroblastosis of the first born, we made the guess that these women may have been immunized by previous transfusion, or by intramuscular injection of whole blood early in life. In a few cases we were able to demonstrate that the anamnestic reaction could persist for a long time between the intramuscular injection and the pregnancy. We also found evidence which confirms the findings that the number of transfusions prior to the first pregnancy did not influence the severity of the condition in the first infant, and I believe that it is correct to assume that a single transfusion is equivalent to multiple pregnancies.

Soon after we described the pathogenesis of erythroblastosis I was puzzled as to the mechanism of the immunization. I didn’t commit myself for a period of a few years, and in the meantime low Vitamin B was suggested before we demonstrated intraplacental hematomas with fetal blood on the maternal side.

*Dr. L. K. Diamond’s paper, “Physicochemical and Immunological Characteristics of Rh Antibodies,” presented at the Conference, was planned for appearance in this issue, but unfortunately was not received in time for inclusion. The Editors.
of the placenta. I suggested that isoimmunization begins at the latter part of pregnancy when there are certain structural changes in the villi and the position of the fetal blood vessels would suggest that they are separated from the maternal by only one layer of cells. We had calculated that it would require, on the basis of some experimental studies in rabbits, only about 0.07 cc. of blood in the course of the entire pregnancy to immunize the mother. On the basis of this, I am pleased to note that Dr. Diamond and Drs. Hill and Haberman found that minute amounts of blood, by intravenous injection, suffice to enhance the immunization in an already immunized woman. I believe that pregnancy offers very suitable conditions for immunization giving perhaps slow administration of an antigen over a long period of time. I would like to see someone attempt to immunize members of a family to determine what at present we are only guessing at, that is to explain the genetic capacity and the variations among individuals to respond to antigenic stimulus with the production of antibodies. If we could select individuals of a family and immunize the siblings of the 2 contrasting groups, those that immunize readily and those that do not, we could make a notable contribution to the problem of immunization by demonstrating the actuality of such an inherited characteristic. Anyone having such an opportunity could not only help produce the rare sera, but could also establish or disprove the inheritance of the response to the Rh antigenic stimulus. If this can be done, and result in the production of the rare anti-Hr sera in sufficient quantity, then the proper genetic studies among various races will determine the inheritance involved in the Rh Hr system. Until we have this we will flounder and be uncertain about our terminology and will probably have to use several terminologies.

Dr. Dameshek: I find it difficult to present a direct discussion of the papers of Drs. Diamond and Davidsohn in that they deal mostly with terminology as applied to the Rh factor. However, it would be wise to review some of the historical features and broader aspects of the problem. As I pointed out yesterday we have gone into this problem from a more basic standpoint because erythroblastosis fetalis, hemolytic disease of the new born is just one type of hemolytic anemia. There are other types of hemolytic anemia which should not be forgotten, even though this conference deals largely with the Rh factor. The specialty of immuno-hematology was first introduced by Chauffard, a very eminent French clinician, who published and studied hemolytic anemia during the period between 1905 and 1916. Chauffard was the first to describe the presence of hemolysins of a peculiar type in cases of hemolytic anemia following the report of Landsteiner, and he described cases of what he called hemolysinic anemia. Simultaneously with Chauffard, Widal, Abrami and Brullé described cases of hemolytic anemia in which they found autoagglutinins. As a result an active rivalry existed in the French school between the adherents of Chauffard and the adherents of Widal, Abrami and Brullé as to who would describe which type of case. Chauffard at that time introduced a concept of immuno-hematology and suggested that there might someday develop a specialty in this particular field. That idea lay dormant for many years until the recent developments, especially the Rh factor. This specialty is now coming to the fore especially as advanced by this conference. The developments which have taken place with reference to the Rh factor can be brought over directly to the subject of hemolytic anemias in general as I pointed out yesterday. Dr. Diamond's development of the bovine albumin technic has been utilized by us successfully for the determination of peculiar agglutinins and antibodies in various types of hemolytic anemia. In 1937, when we first encountered these peculiar cases of hemolytic anemia, Dr. Schwarz and I discovered these peculiar hemolysins and agglutinins in various types of hemolytic anemia. This was prior to the developments in the field of Rh antibodies and their determinations, and when Dr. Levine introduced his theory that erythroblastosis fetalis was due to an antibody antigen combination it was easy for us to understand that particular mechanism because we had been working along similar lines with the hemolytic anemias. I would like to point out that we shouldn't lose track of the fact that antibodies have reference not only to the Rh factor and hemolytic anemia but also to hemolytic anemia in general.

Dr. Race: I have only congratulations to offer to Dr. Davidsohn and Dr. Diamond for their excellent presentations. The scope of their studies is on a larger scale than the work in England. I am very grateful to Dr. Diamond for the extent of his technic for stimulating the antibodies already present. This proved very invaluable to us in getting enough of the 2 rare sera, the anti-e and the anti-Cw.

In the case of anti-Cw, the large amount of serum obtained was the result of restimulation of the immunized patient. I have never before appreciated the very high incidence of immunization in Rh negative men who have been transfused. In England, our incidence of the detection of antibodies is approximately 2 per cent.
Dr. Potter: I've been extremely interested in the difference in the number of patients in whom agglutinins have been determined in the English group and the American group. I think Boorman in that group reported agglutinins in 93 out of 97 Rh negative women, and I've been curious as to whether they are using some more sensitive technic which might have included blocking antibodies as an explanation of the difference between the percentage found by the English and American investigators.

Dr. Race: The only explanation I could offer is that they must have been reporting the presence of very small traces of agglutinins. This was done before the blocking or incomplete antibody was described. They found these only in their first tubes and they were really doing the so-called 'conglutinations.'

Dr. Levine: The tests on the mother's serum alone is not a very good criterion of the outcome of a pregnancy. It is very important to test the cord blood to determine the titre in the infant's blood at delivery and to follow the titre in order to obtain a true concept of the prognosis. In the slide I showed yesterday, 2 contrasting cases sent to me by Dr. Stillman of the New York Hospital are shown. In both cases the mother had positive albumin tests with a titre of 1/50,000. In one of them the infant had practically all of the mother's antibodies and it could have been expected that the baby would not survive. In the other one there is only a blocking antibody of 1/4 and a good prognosis was anticipated and the infant recovered with only 1 transfusion. I would like to find out the experience of the other workers here, with regard to following up the infant. It is difficult to obtain specimens of blood from infants, and developing tests could be done on a suspension of the infant's blood cells without obtaining serum. I would like to have the comments of Dr. Race or Drs. Hill and Haberman on this point.

Dr. Hill: I want to thank Dr. Diamond and Dr. Davidson for 2 very interesting papers; we're all so tremendously interested in these antibodies and their nature. I think that there is one thing I would like to make perfectly clear concerning the developing test. We used this term for convenience and we claim no credit or responsibility whatsoever for the development of this test. It's distinctly a test of Coombs, Mourant, and Race. The only responsibility, or perhaps the word I should use is culpability is in the use of this test to characterize third order antibodies (cryptagglutinoids) if such exist. We rather think that there are some antibodies still which are detected by this Coombs test, and which are not detected by the albumin test. I believe, however, further work must be done along this line. We have seen some such examples, and Dr. Race tells me of some examples over in England.

In response to Dr. Levine's question on the developing test and the progress of the affected infant, I can state that we have found this test invaluable. By daily or semiweekly tests on the child's erythrocytes we have been able to follow the progress of recovery. As the antibody and the adsorbed infant cells are disposed, the developing test becomes weaker, until finally it is negative when the child has completely recovered. In one case, the test remained positive for one month. The clinical condition improved as the test became progressively negative.

I would like to mention one thing in connection with the method of injection of the Rh positive cells that Dr. Diamond mentioned. We have been able to give the 5 cc. quite successfully to people with titres of even 1/50,000 to 1/100,000 with minimal or no reactions. This was reported in July, 1945, in the Journal of the American Medical Association. It's true that some slight reactions have occurred, a little headache or discomfort, but the more severe reactions have not occurred even in those cases where we did not give a diluted blood. We have usually diluted the 5 cc. with an additional 5 cc. of saline citrate taking care to use at least 10 minutes time in giving it. On one or two occasions we got a little curious and gave it more rapidly, particularly to some members of our own staff, who happened to be Rh negative and were receiving these injections, and it seemed to give some more difficulty.

In connection with this discussion of antibodies, I think it might not be inappropriate to bring up the question of method of typing for Rh. Dr. Chown, who will speak next, has a beautiful little test that we are very enthusiastic about for several reasons. It is the capillary test. We like it because it's simple, requires very little equipment, and is both rapid and specific. The beauty of it is that if you have a potent serum, so that you can use it saline diluted to avoid false positive reactions, you can utilize the third order antibodies (cryptagglutinoids). To that extent it is similar in its action to the albumin test. We are using it routinely and its specificity seems to be the same as the test tube method. Its sensitivity is very excellent. For the smaller laboratory this is a beautiful test.

Dr. Diamond. I should like to point out that Dr. Davidson's use of the term conglutinin varies somewhat from Dr. Wiener's use of the same term, since Dr. Wiener only applies the term conglutinin to that antibody which does not show the same titre in saline. Obviously if you use saline for typing of albumin on a saline or heat labile agglutinin, it will also give a positive test, and of the same titerable
value as with the saline. The only time Dr. Wiener has used the term conglutinin for a serum is when it contains either no saline agglutinin and positive agglutination with his plasma or serum, or when it contains a higher titre of agglutination with the plasma or serum that it does with the saline. So that with these charts which Dr. Davidsohn presented, if we adhered strictly to the Wiener nomenclature, we would have to use the term saline or simply agglutinins for the cases in which there was no difference between saline agglutination and albumin agglutination, and reserve the term conglutinin for the cases where there was a difference either complete or partial. As to Dr. Levine’s comments, he has always guarded and supported us and I am delighted that he approved of our work in the sense of having no harsh criticisms, since that was my one fear in presenting some of these observations in a field in which we are complete novices and he and Dr. Race and others are truly the experts. As to the fact of the albumin antibody I suggested that Dr. Onkley and his group had found or at least had preliminary observations (and we hope that they will carry these further), and the fact that the albumin agglutinin tends to be a heavier molecule. Now whether or not this means that it cannot be a univalent antibody is again beyond my experience or knowledge, and immunologists like Dr. Heidelberger and Dr. Cabot of New York may possibly eventually decide this important theory. Dr. Cabot has been a great help in many of the discussions. We have presented him with some of the material, and I hope in due time he will be able to give us the answer as to whether the pure albumin antibodies are multivalent or univalent and as to their true chemical structure, and particularly their weight. I mentioned that we have at least 4 women who have received multiple transfusions of Rh positive blood and though they were Rh negative, and had pregnancies following it, showed no antibody reaction at any time and had no difficulty with their infants. In only 1 case were we able to try any tests on a member of that family, this case being a woman whose father had been the donor of blood for her transfusion. He was the only other living member of her family, and he came in and subjected himself to injection with Rh positive blood against which he developed no antibodies, again suggesting as Dr. Levine mentioned that this may be a familial characteristic that some humans are just poor guinea pigs in the sense of antibody producers and, with a relatively poor antigen such as the Rh factor, do not respond by antibody production. So that despite the real danger we all must realize that some humans will not develop antibodies even though subjected to the same insults as others by the injection of Rh positive blood. Dr. Dameshek mentioned that this opens up a much wider field of study particularly in hemolytic anemia. We have had that brought to our attention, and I think Dr. Witebsky will substantiate this by the fact that many of our allergies are now reviewing their work on antibody production and antibody protection in allergic patients who are sensitive to ordinary antigens better known and better studied like egg albumin and rag weed pollens, and so on. These investigators have assured us that they find exactly comparable states in such individuals in that the heat stable antibody develops at the multiple sensitization. In fact the whole theory of desensitization of allergic individuals by repeated injections of very minute doses of the pollen or the antigen to which they are sensitive is probably dependent not on desensitization but on the development of a more complete antibody which does not permit the symptoms of allergy like hay fever or asthma, but protects the individual from such symptoms after a long series of injections. Loveless in 1941 in New York, worked on the subject, and Dr. Ableson, I think, was the first to bring it to my attention. Dr. Loveless published a beautiful series of papers on heat stable antibodies produced against one of the common antigens after multiple injections which wiped out the skin sensitivity in sensitive individuals who had previously been tested with the antigen and reacted violently with weals. The whole field of allergy, immunology, and certainly hemolytic disease will bear further study and restudy in the light of some of the newer technics.

Dr. Scherer questioned the use of donors who had received transfusions, and might not have been recognized as carrying antibodies, because they were not tested by the more sensitive Coombs test or albumin test. We have only one very good example of this, in that a very patriotic woman who had given blood many times to the Red Cross came to see us with the story that she was interested in our work because she had lost several babies with erythroblastosis. This was the beginning of our albumin titration technic and we demonstrated that she had a titre of 1:500 of albumin agglutinins. She had given blood at least 4 times to the Red Cross, her plasma having been used in various pools, and at this time we mixed 100 cc. of her serum with 10 times that amount of normal plasma and then injected a small sample of 50 cc. of the mixture into an Rh positive individual and demonstrated that there was some destruction of the recipient’s cells even from this small amount because she had such a high titre. Cer-
certainly the use of an individual such as she, as a donor for an Rh positive recipient would have been extremely dangerous, and we therefore should add to our blood bank the question of the possible donors having signs of sensitization either from previous transfusions or from previous pregnancies. I am sure Dr. Hill and others who are responsible for blood banks probably do this because occasionally a donor of that type would truly be dangerous, if by mistake he or she were used for an Rh positive recipient. Of course, in most instances such an Rh negative donor would be used for an Rh negative recipient and therefore no harm would be done. But if the plasma were pooled as it was in the Red Cross campaign, possibly that might explain some of the untoward effects from giving plasma that were thought to contain pyrogens or nonspecific disturbing antibody. We routinely test the cord blood of every new born baby when we suspect sensitization, and test it by 3 methods immediately. That is the Rh typing is done immediately to determine whether the child is Rh positive or Rh negative, and we found both Dr. Chown’s very simple and effective test or our slide test is adequate for determining within 10 minutes whether the new born baby is Rh positive or Rh negative. If the baby is positive, and we do not have the mother’s blood available for checking, we also test the baby’s serum to determine whether there are antibodies carried over from the mother. Finally we also apply the reagglutination test of Dr. Abelson to demonstrate whether the baby has antibody adsorbed red cells which will reagglutinate other Rh positive cells when the two are mixed. Incidentally that is a good demonstration of the danger of using Rh positive blood for the transfusion of the babies with erythroblastosis especially during the first 2 days of life. If the baby’s cells are enveloped or coated with the mother’s antibodies, and then more Rh positive blood is put into the baby’s circulation, not only will that positive blood be destroyed more rapidly, but the baby’s own coated cells will be completely agglutinated in most instances. I think this in vitro as well as in a few cases we have studied in vivo mechanisms is the reason for avoiding Rh positive blood in children with erythroblastosis certainly during these first 10 days or 2 weeks of life when we know such coated cells may exist in circulation. We have been fortunate in being able to follow up the blood tests of infants with erythroblastosis for 2 weeks or more. We obtained sufficient blood not by venipuncture which is often difficult, but by using a Wright tube and collecting from a heel puncture up to 1 to 2 cc. of blood which is immediately mixed in the tube with heparin or oxalate. By this method we can prove that the mother’s antibodies may persist in the child’s serum for as long as 10 days or 2 weeks or more, which is difficult to understand when we realize that the baby’s Rh positive blood is there all the time and should theoretically be taken out or neutralizing the antibody in the serum. Some other mechanism is probably operating and it is hoped that some laboratories working on this will be able to disclose why such antibodies are not removed by the Rh positive cells in the baby’s blood stream.

We persist in trying to substantiate Dr. Hill’s talk that there might be a third variety of antibodies. We have subjected the serums which give a good Coombs test to the same principle and chemical destructive agents as we have used for the antibodies detected by the albumin tests and have as yet found no antibody which would give a negative albumin test and then give a Coombs test that is positive. Possibly for this experience we can assume that there is such an antibody or possibly further tests on the part of Dr. Hill and his group or Dr. Race will confirm our own findings. Therefore I wish to commend Dr. Chown for his development of this beautifully simple and very efficient and also very saving test. This economical test is a very nice way of being able to handle the rarer serums rather than our very wasteful method of using a whole drop of serum and possibly obtaining only 15 to 20 tests from 1 cc.

**Dr. Davidsohn:** I want to express my appreciation to Dr. Hill and the staff and administration of the Baylor Hospital for the privilege of being able to be here at this remarkable meeting. I think it is truly remarkable that we consider, I think, for the first time in history that within about 6 years after a discovery of a new phenomena a congress was called together and arranged so successfully. A large number of people interested in one way or another have had an opportunity to hear first hand about the new developments. Regarding the question that was brought up by Dr. Diamond in his paper as to the origin of the statement that only from 2 to 4 per cent of Rh negative individuals receiving Rh positive blood may become sensitized, I too have been interested in this statement, and it seems to me the time to track it down. This is one of those events which make up a medical mythology. The best I could find out was that it was first expressed by analogy to the frequency of fetal erythroblastosis in Rh negative women. Then it was simply put in as a fact which need not have any further confirmation. I think you ought to be very careful in accepting such medical myths because they cannot be substantiated. Dr. Diamond’s observations are extremely valuable. Dr. Dameshek’s discussion suggested to me that we at
Rh ANTIBODIES

This conference may also take up here in Mexico City the question of terminology, that of erythroblastosis versus hemolytic anemia. He refers to hemolytic disease, hemolytic anemias, as being a related subject. Suggestions have been made in the literature that erythroblastosis and the terms we apply to erythroblastosis be discarded in favor of hemolytic disease of the newborn and fetus. I am not so sure that it is the right thing to do, although this term seems to be gaining in popularity. I think that it probably is just as bad and just as indefinite as fetal erythroblastosis because at least all of us know what we mean when we say erythroblastosis and the term hemolytic disease of the newborn may be misleading unless we add to it "resulting from Rh incompatibility." So for the present time it may be desirable to continue to use the term erythroblastosis and to see which term will eventually gain more popularity.

Dr. Potter questioned Dr. Race, regarding the use of this term by the British workers. I reported in the American Journal of Clinical Pathology in 1945 a study in Rh antibodies in which we investigated 3 groups of cases. One group where we had only 1 specimen of blood obtained sometimes long after the baby with erythroblastosis was born. And there the incidence of agglutinins was very low. Then the third group where the patient was in our own hospital where we could do repeated tests and especially tests about 8 to 10 days after the birth of the child, we went up to as high as 81 per cent. Now in this table, however, you may recall that I had only 61 per cent and that is explained in the same way as Dr. Race suggested for British results that we included their low titres of agglutinins which actually we interpret now as being heat stable agglutinin and therefore they were excluded from the present list of 61 per cent. I agree with Dr. Levine regarding the advisibility of investigating the antibodies in the cord blood as a prognostic finding. We did it in some cases and we didn't include it here because we did not have sufficient figures, but so far I must say that our impression has not been that there is a striking parallelism between the titre these passively transmitted antibodies and the severity of the disease in the child or even of the occurrence of the disease in the child. So I think that we must gather more information of the type of Dr. Levine's report. Recently when Dr. Coombs and his associates first published their paper on the test, and then when Dr. Hill published his, we began using it and we had recourse to old precipitin serums produced years ago by injection of the whole serum. And we found the titre of anti-globulin pretty high. We got into trouble probably because we at that time were not concerned with the use of only old serum, but we used a mixture of all serums. I am interested in the report of Dr. Race, but it seems to me that if one is to produce a good anti-globulin serum with albumin, then the adsorption of that serum with albumin might also remove the globulin as well as the albumin if that is the homologous antigen used for the immunization.
Rh ANTIBODIES; CORRELATION WITH CLINICAL FINDINGS
I. DAVIDSOHN