A-B Hemolytic Disease of the Newborn

Analysis of 1480 Cord Blood Specimens, with Special Reference to the Direct Antiglobulin Test and to the Group O Mother

By RICHARD E. ROSENFIELD

With statistical analysis by FLORENCE EISINGER*

Despite the great advances that have been made in the diagnosis and treatment of Rh hemolytic disease of the newborn, the role of the A and B factors as a cause of a similar disease has not received sufficient attention.1

Four facts have hindered a general appreciation of A-B hemolytic disease. (1) Whereas Rh disease is readily recognizable with the aid of the direct antiglobulin test, A-B disease may not be. The direct antiglobulin test in A-B disease is extremely weak when positive, and may be negative. (2) An "unusual" antibody is not present in the serum of the mother, since anti-A and anti-B are normally present. (3) The usual form of the disease has little or no anemia and a variable amount of jaundice, which at times is clinically indistinguishable from physiologic icterus. (4) Less than 10 per cent of all severe cases of hemolytic disease of the newborn are due to the A-B factors, so that there is little clinical material to attract attention.

Any evaluation of the problem of A-B hemolytic disease is restricted by the limitations of our knowledge of Rh disease and of physiologic icterus.1 The latter condition is not well understood and may be mistaken clinically for mild Rh or A-B disease. It has been assumed that the liver of the newborn has a variable and usually unsatisfactory mechanism for excreting bilirubin, and that this dysfunction may result in some clinical jaundice at 48-72 hours of life. Premature infants commonly develop icterus, and with complications such as sepsis, are subject to kernicterus. Liver insufficiency as the sole cause of physiologic icterus in the newborn is not altogether proved, and there may be other possible explanations. Any mechanism, however, must have an effect on the course of frank hemolytic disease. For example, the prognosis in Rh disease is usually correlated with the titer of Rh antibodies in the mother (especially those detected by the indirect antiglobulin test2), but the exceptions that are common clinical experience may be the result of the factors responsible for physiologic icterus, including the efficiency to excrete bilirubin. The problem of A-B
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hemolytic disease must also be affected by these same unknown factors. In addition, A-B hemolytic disease is complicated by the presence of A and B substances in body tissues other than red blood cells. This situation creates a number of special considerations such as the amount of available A and B substance and the reversibility of the antibody-antigen complex.

Moderate to severe A-B hemolytic disease is fairly well characterized and the diagnosis can be made with relative certainty shortly after birth. At this time moderate spherocytosis with marked reticulocytosis is found, and the direct antiglobulin test by our technic is usually weakly positive when read microscopically. This is in contrast with the findings in Rh hemolytic disease where spherocytosis is rare, reticulocytosis is minimal to moderate, and the antiglobulin test is strongly positive. Treatment of severe cases of A-B hemolytic disease by replacement transfusion with group O blood is effective, and the survival of transfused blood of homologous group has been shown to be short.

The marked reduction in morbidity and mortality in Rh hemolytic disease has been due largely to the prenatal detection of immunized Rh negative women. Thus, if an expectant mother has no demonstrable Rh antibodies, the infant will be free of this disease even though Rh positive; but if an expectant mother is immunized to Rh, the infant, if Rh positive, will usually be affected to some degree. Such a readily diagnosable situation does not exist with the A-B problem because of the normal occurrence of anti-A and anti-B in individuals negative for the respective factors.

Although variable anti-A and anti-B titers are found in the mothers of infants with A-B hemolytic disease, usually these titers are higher than the average normal and the antibodies are at least partly “incomplete”, difficult to neutralize with specific substance, and somewhat lytic in the presence of adequate human complement. However, the disease can rarely be predicted from either the variety or the titer of antibodies found in antenatal serum. Furthermore, in marked contrast with the Rh problem, a large proportion of cases occur in primipara with no increase in severity in the subsequent pregnancies.

MATERIAL AND METHODS

In an attempt to establish an early diagnosis and to evaluate the weakly positive direct antiglobulin test, an oxalated specimen of umbilical vein blood was obtained, when possible, from every infant born at The Mount Sinai Hospital from January 1, 1953 to September 14, 1953. There were 1,486 specimens available for examination. The following tests were performed on each satisfactory specimen: (1) direct antiglobulin test, (2) blood group and Rh factor, (3) hemoglobin, (4) reticulocyte count and examination of erythrocyte morphology, (5) plasma bilirubin, and (6) screening osmotic fragility with 0.52 per cent NaCl. Maternal blood was always available for testing and thus the infants have been classified as group compatible and group incompatible with the mother. Six cases of Rh-Hr disease were encountered and have not been included in this series. The sera of all mothers have been examined for intragroup antibodies by saline, trypsin, and antiglobulin technic, and (except for the six cases of Rh-Hr disease) were found to be negative. The direct antiglobulin test was negative with all the group compatible infants, whereas an appreciable number of group incompatible infants was found to have a weakly positive test. The sera of mothers of group incompatible infants with positive direct antiglobulin tests have been titrated for anti-A and anti-B by saline, trypsin, and antiglobulin methods, and against normal cord red cells for anti-A and anti-B hemolysins.
Umbilical Vein Blood

At the time of birth, 5 ml. of blood was drawn by syringe from the umbilical vein and gently mixed with dried balanced potassium ammonium oxalate to prevent coagulation. Specimens were stored at 4°C until tested (usually within 24 hours).

Determination of Hemoglobin

With pre-calibrated pipets, well mixed oxalated blood was laked in 0.1 per cent Na₂CO₃ and the hemoglobin determined as oxyhemoglobin with a Model 6A Coleman Spectrophotometer at 550 μm. The instrument was standardized with hemin (pyridine ferrohemochromagen), oxygen capacity, and iron.

Group and Rh

Red cells washed four times with large volumes of 0.9 per cent NaCl, were resuspended to 2 per cent concentration. Anti-A, anti-B, and anti-Rh, typing serum, meeting or exceeding N.I.H. specifications, was distributed in suitable small test tubes in 0.05 ml. quantities with 0.05 ml. of red cell suspension added. These tubes were then shaken and centrifuged without incubation. The anti-Rh serum was of the incomplete variety and contained 25 per cent bovine albumin. All Rh negative reactions were incubated 1 hour at 37°C and then subjected to the antiglobulin test.

Direct Antiglobulin Test

To 0.05 ml. of antiglobulin serum in a suitable small test tube, 0.05 ml. of washed red cell suspension was added, mixed and immediately centrifuged at 2,000 r.p.m. for 10 seconds. The red cells were then resuspended under 6X magnification and re-examined under 100X magnification. Agglutination of as little as 20 per cent of the red cells into aggregates of 3 or more red cells was considered as positive.

The antiglobulin serum was obtained from rabbits immunized by intravenous injections of alum precipitated human gamma globulin. The sera of selected rabbits were diluted with 0.9 per cent NaCl to an optimal concentration derived by suitable cross-titrations with incomplete Rh antisera, and these selected rabbit sera were pooled. The rabbit serum was not absorbed with washed human red cells since simple dilution was sufficient to prevent agglutination of unsensitized human red cells of all blood groups. Two such reagent sera were used throughout but did not always give results of identical avidity; one of the reagents had been submitted to, and approved by the National Institutes of Health.

Bilirubin Determination

The micro-method of Hsia, Hsia, and Gellis, using 0.2 ml. of plasma, was used. Readings were made with a Model 6A Coleman Spectrophotometer, at 640 μm. The instrument was standardized with a number of different commercial bilirubins.

Reticulocyte Counts

New Methylene Blue was used to stain reticulocytes by the method of Brecher. At least 1,000 erythrocytes were counted in each determination.

Screening Hypotonic Osmotic Fragility

1.0 ml. of washed 2 per cent red cell suspension was centrifuged and the supernatant saline carefully removed with a fine Pasteur pipet. 1.0 ml. of 0.52 per cent NaCl was then added, the red cells carefully resuspended and then recentrifuged. Any visible hemolysis of the supernatant 0.52 per cent NaCl was considered positive. All positive results were rechecked with a full series of hypotonic NaCl solutions ranging from 0.72 per cent to distilled water, and a repeat screening with 0.52 per cent NaCl was performed with a drop of fresh capillary blood obtained from the infant's toe.
**Titration of Anti-A and Anti-B**

Maternal sera were titrated in twofold dilutions beginning with a volumetric 1:5 dilution. Each dilution was distributed in 0.05 ml. (two drop) quantities, an equal volume of red cell suspension added, and the tubes incubated for 1 hour at 37°C before reading (not centrifuged) and before conversion to the antiglobulin test (centrifuged).

Trypsin treatment of red cells was by the method previously described.

**Hemolysin Titer Against Red Cells**

This was performed by a modification of the method of Crawford, Cutbush, and Molli-son. Maternal serum was inactivated at 56°C for 20 minutes, so that the low-titer group O serum which was added furnished a uniform amount of complement to each tube. The test red cells used were obtained from fresh cord specimens with negative antiglobulin test; A cells were selected from those reacting with anti-A (absorbed B) serum. All titrations were incubated for 1 hour at 37°C and centrifuged before reading. The following controls were always included: (1) Negative controls—(a) maternal serum + saline + cells, (b) saline + low-titer group O serum + cells; (2) Positive controls—(a) standard lytic anti-A and anti-B sera diluted in saline so as to yield 10 per cent hemolysis under conditions of the test + low-titer group O serum + cells. The purpose of the positive controls was to ensure the complement activity of the low-titer group O serum.

**RESULTS**

Of 1,480 specimens, only 1,127 hemoglobin determinations, 1,112 reticulocyte counts, and 929 plasma bilirubin determinations could be done due to clotting of the specimen, insufficient quantity of blood, undue hemolysis, etc. The data from these determinations were divided into three categories, (1) group compatible infants with negative antiglobulin test, (2) group incompatible infants with negative antiglobulin test, and (3) group incompatible infants with positive antiglobulin test. The arithmetic means were obtained for each category and compared for significance of difference. Table 1 summarizes these findings.

Statistically it certainly appears as if a weakly positive direct antiglobulin test detects an abnormal class of group incompatible infants. In comparison with the other incompatible infants, those with positive direct antiglobulin test have a lower mean hemoglobin (14.66 Gm. per cent vs. 15.66 Gm. per cent; P ≈ .003), a higher mean reticulocyte count (5.71 per cent vs. 4.33 per cent; P < .0006), and a higher mean bilirubin (3.08 mgm. per cent vs. 1.86 mgm. per cent; P < .0000006). However, the direct antiglobulin test does not appear to detect all involved cases since incompatible infants with negative direct antiglobulin test have a higher mean reticulocyte count (4.33 per cent) than compatible infants (4.05 per cent), and the probability that the difference between these two mean values is due solely to chance is only about 0.015. Mean hemoglobin and bilirubin values do not show this distinction.

None of the group incompatible infants with positive direct antiglobulin test required exchange transfusion, and only three infants received one or two simple transfusions of packed group O red cells for anemia. There was considerable variation in the cord blood values (table 2).

As can be seen in table 2, 31 out of 38 infants had increased fragility in hypotonic NaCl. This simple procedure can be performed on capillary blood obtained from the infant’s toe, and may be helpful in making a diagnosis of A-B hemolytic disease. Two cases, however, with normal fragility had what appeared to be
TABLE 1.—Comparison of the Mean Hemoglobin, Reticulocytes, and Bilirubin of Umbilical Vein Blood of (1) Group Compatible Infants, (2) Group Incompatible Infants with Negative Direct Antiglobulin Test, and (3) Group Incompatible Infants with Positive Direct Antiglobulin Test

<table>
<thead>
<tr>
<th>Infants</th>
<th>Hemoglobin (Gm.%)</th>
<th>Reticulocytes (%)</th>
<th>Bilirubin (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Group compatible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x_1)</td>
<td>15.83</td>
<td>4.05</td>
<td>1.81</td>
</tr>
<tr>
<td>(n_1)</td>
<td>885</td>
<td>846</td>
<td>719</td>
</tr>
<tr>
<td>(\sigma_1)</td>
<td>1.63</td>
<td>1.54</td>
<td>0.73</td>
</tr>
<tr>
<td>(\sigma_{x_1})</td>
<td>0.055</td>
<td>0.053</td>
<td>0.027</td>
</tr>
<tr>
<td>(2) Group incompatible with negative direct antiglobulin test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x_2)</td>
<td>15.66</td>
<td>4.33</td>
<td>1.86</td>
</tr>
<tr>
<td>(n_2)</td>
<td>232</td>
<td>233</td>
<td>190</td>
</tr>
<tr>
<td>(\sigma_2)</td>
<td>3.37</td>
<td>3.14</td>
<td>1.43</td>
</tr>
<tr>
<td>(\sigma_{x_2})</td>
<td>0.113</td>
<td>0.105</td>
<td>0.053</td>
</tr>
<tr>
<td>(3) Group incompatible with positive direct antiglobulin test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x_3)</td>
<td>14.66</td>
<td>5.71</td>
<td>3.08</td>
</tr>
<tr>
<td>(n_3)</td>
<td>32</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>(\sigma_3)</td>
<td>3.90</td>
<td>5.02</td>
<td>3.12</td>
</tr>
<tr>
<td>(\sigma_{x_3})</td>
<td>0.357</td>
<td>0.452</td>
<td>0.365</td>
</tr>
</tbody>
</table>

\[
\sigma(x_1 - x_2) = 0.122 \\
P(x_1 - x_2) \geq 0.165 \\
\sigma(x_2 - x_3) = 0.332 \\
P(x_2 - x_3) \geq 0.003 \\
\sigma(x_1 - x_3) = 0.008 \\
P(x_1 - x_3) \geq 0.000006
\]

spherocytosis on the blood smear. Examination of the red cell morphology in these cases, therefore, was more valuable than the determination of hypotonic fragility.

A striking finding (table 2) is the fact that 38 of 39 mothers of these “involved” infants are of blood group O. When compared with the distribution of the blood groups of the mothers of other incompatible infants (table 3), this is found to be of marked significance (P \(\geq\) .0001).

Considering compatible and incompatible infants from mothers of blood groups A, B, and AB only, the arithmetic means of cord hemoglobin, reticulocytes, and bilirubin have been obtained and compared for significance of difference. As shown in table 4, no significant difference was found.

The arithmetic means of cord hemoglobin, reticulocytes, and bilirubin have been calculated for the compatible and incompatible infants from mothers of blood group O only. A third category of infants, those incompatible and with a positive direct antiglobulin test, are available for comparison. Table 5 summarizes these calculations and shows higher significance of mean differences than was obtainable in table 1 where the infants of all mothers were analyzed. This is a further indication that most of the A-B hemolytic disease problem centers about mothers of blood group O.
### Table 2.—Group Incompatible Infants with Positive Direct Antiglobulin Test

| Case number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 |
|-------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **Mother's group** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Infant's group** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Cord hemoglobin (Gm.%)** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Cord reticulocytes (%)** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Cord bilirubin (mgm.%)** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Spherocytosis on blood smear** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Partial hemolysis in hypotonic NaCl** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Maternal antibodies** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Anti-A₁** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Saline** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Trypsin** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Antiglobulin** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Anti-B** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Saline** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Trypsin** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Antiglobulin** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Hemolysin titer vs. normal cord cells** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
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Table 3.—The Distribution of the Blood Groups of the Mothers of Incompatible Infants

<table>
<thead>
<tr>
<th>Blood group of mother</th>
<th>Direct antiglobulin test of infant</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>O</td>
<td>176</td>
<td>38</td>
</tr>
<tr>
<td>A</td>
<td>74</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>298</td>
<td>39</td>
</tr>
</tbody>
</table>

χ² = 21.316; n = 2; P ≅ .0001.

Table 4.—Comparison of the Mean Hemoglobin, Reticulocytes, and Bilirubin of Umbilical Vein Blood of (1) Group Compatible Infants and (2) Group Incompatible Infants with Negative Direct Antiglobulin Test, when the Mothers are of Blood Groups A, B, and AB only

| Infants                                      | Hemoglobin (Gm.%) | Reticulocytes (%) | Bilirubin (mg.%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Group compatible</td>
<td>15.804</td>
<td>4.103</td>
<td>1.781</td>
</tr>
<tr>
<td></td>
<td>565</td>
<td>557</td>
<td>483</td>
</tr>
<tr>
<td>(2) Group incompatible with negative antiglobulin test</td>
<td>15.778</td>
<td>4.214</td>
<td>1.844</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>98</td>
<td>80</td>
</tr>
</tbody>
</table>

σ(\bar{x}_1 - \bar{x}_2) = 0.179, 0.167, 0.088
P(\bar{x}_1 - \bar{x}_2) = 0.89, 0.51, 0.48

Table 5.—Comparison of the Mean Hemoglobin, Reticulocytes, and Bilirubin of Umbilical Vein Blood of (1) Group Compatible Infants, (2) Group Incompatible Infants with Negative Direct Antiglobulin Test, and (3) Group Incompatible Infants with Positive Direct Antiglobulin Test, when the Mothers are of Blood Group 0 only

| Infants                                      | Hemoglobin (Gm.%) | Reticulocytes (%) | Bilirubin (mg.%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Group compatible</td>
<td>15.893</td>
<td>3.946</td>
<td>1.869</td>
</tr>
<tr>
<td></td>
<td>298</td>
<td>289</td>
<td>236</td>
</tr>
<tr>
<td>(2) Group incompatible with negative antiglobulin test</td>
<td>15.581</td>
<td>4.419</td>
<td>1.877</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>135</td>
<td>110</td>
</tr>
<tr>
<td>(3) Group incompatible with positive antiglobulin test</td>
<td>14.66</td>
<td>5.71</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>33</td>
<td>20</td>
</tr>
</tbody>
</table>

σ(\bar{x}_0 - \bar{x}_1) = 0.174, 0.167, 0.084
P(\bar{x}_0 - \bar{x}_1) = 0.073, 0.0046, 0.93
σ(\bar{x}_0 - \bar{x}_2) = 0.360, 0.351, 0.223
P(\bar{x}_0 - \bar{x}_2) = 0.0108, 0.00032, 0.0000006
Analysis of these data for fetal loss is not possible since the only mothers included are those with live infants. Since a positive direct antiglobulin test was obtained in approximately 11 per cent of group incompatible infants, one might postulate a fetal loss due to A-B incompatibility. The extent of such fetal loss, as well as the inferred instability of the gene frequencies A, B, and O will have to await a thorough study of the blood groups of matings involved in abortions and stillbirths.

The frequency of Rh positivity does not differ significantly in mothers and infants, nor is it affected by the blood groups or the direct antiglobulin test (except for the 6 cases of Rh-Hr disease).

The distribution of the blood groups, A, B, O, and AB, of the infants does not differ significantly from the expected distribution and is not affected by the direct antiglobulin test.

**DISCUSSION**

The result of the direct antiglobulin test in A-B hemolytic disease has been in dispute. Many serologists have been of the opinion that this test is almost invariably negative when this condition is present, but others have claimed that the test was usually positive, although very weak. In the present study, a weak but recognizably positive antiglobulin test was encountered in over 11 per cent of incompatible infants but in none of the compatible infants except the six cases of Rh-Hr disease. Furthermore, from statistical analysis of the cord blood hemoglobin, reticulocytes and bilirubin, the infants in whom a positive direct antiglobulin test was obtained had significantly abnormal values. This appears to validate the previous reports of weakly positive direct antiglobulin tests in A-B hemolytic disease.

The mean values for cord hemoglobin, reticulocytes, and bilirubin obtained in this study do not differ markedly from previously published reports. Molliison\(^2\) reported the mean cord hemoglobin of 133 infants as 16.55 Gm. per cent ± 1.5 Gm. per cent. Statistically this is significantly higher than the mean herein reported and may be due to inaccuracies in sampling at time of delivery or to a 2–3 per cent error in standardization. Reticulocytes for the newborn have been reported to average 4.35 per cent,\(^2\) a figure practically identical with the present study if a mean for all the infants is obtained. Average normal cord serum bilirubin has been previously reported as 1.7 mg. per cent,\(^2\) a figure not seriously lower than the value obtained in the present study. Johnstone\(^2\) recently reported on the bilirubin values of 1,459 random cord specimens. He found a mean value of 1.25 mg. per cent for compatible infants, a figure considerably lower than reported in this study. This discrepancy probably results from a difference in standardization. Johnstone, however, found that incompatible infants have a mean bilirubin value 9 per cent higher than compatible infants, and this increase is identical with what has been found in the present study.

Of striking importance is the fact that in all but one of the cases with positive direct antiglobulin test, the mother was of blood group O. In reviewing 25 of our most recent cases of moderate or severe A-B hemolytic disease, 24 of the mothers were found to be group O.\(^2\) Wiener et al.,\(^2\) reported 14 similar cases, all with group O mothers, and Cutbush et al.,\(^2\) reported 11 similar cases and the mothers
were all group O. It can be calculated from the gene frequencies, A, B, and 0, of all the infants, that only 69 per cent of mothers with group incompatible gestation can be expected to be group O. It is probably because of this high expectation that the role of the group O mothers in A-B hemolytic disease was not appreciated in earlier reports.

If, with rare exception, all of the A-B hemolytic disease problem centers about mothers of blood group O, there should be some peculiarities about the isoagglutinins in individuals of group O not shared by individuals of group A or group B. No striking peculiarities have been noted heretofore, but the subject is now under reinvestigation. Even if group O individuals are found to have significantly higher anti-A and anti-B titers than group A and group B individuals, some explanation will be necessary.

Since group O women have group compatible infants (group O) as well as group incompatible infants, it is important to know something about the passage of anti-A and anti-B antibodies across the placenta when the fetus is not incompatible. In 1929, Polayes et al.,28 studied the anti-A and anti-B content of 500 cord sera and assorted the results according to the blood groups of the mothers. From the published table, the following information can be extracted: Of 187 compatible infants of group O mothers, 113 had both anti-A and anti-B in their serum, whereas of 232 compatible infants of mothers of group A and group B only 113 had the single expected antibody. Subjecting this information to 2 × 2 analysis, \( \chi^2 \) is found to be 5.7 and \( P < .02 \). Again, in 1949, Wiener et al.,24 compared the anti-A and anti-B titration values of cord sera with the titration values in the sera of the mothers. The data, too, were conveniently arranged in table form by mother's blood group, and it is rather obvious that, even disregarding compatibility, infants of group O mothers show considerably more anti-A and anti-B activity in their sera than do infants of mothers of groups A and B. Current studies of this problem indicate that the natural isoagglutinins are more regularly transferred across the placenta when the mother is group O.24 Data for a future report show that almost 90 per cent of group O infants of group O mothers have both anti-A and anti-B in their cord sera, whereas less than one-third of the cord sera of the compatible infants of group A and group B mothers have the single expected isoagglutinin.27

Recently, Wiener has cited additional evidence for the existence of another A-B-O blood factor which is shared by A and B but absent in O, and he has termed this factor, C.28 If this point of view is correct, then infants of group O mothers would be subject to two antibodies rather than one: anti-C plus anti-A or anti-B.

Another explanation, which does not require the postulation of an additional A-B-O factor, revolves solely about the cross-reactivity of the naturally occurring antibodies for A and B. It is known that anti-A and anti-B cross-react with many substances,29 and that in group O individuals these antibodies are usually not fully separable from each other.30 Thus the incompatible fetus of the group O mother may be subject to the effects of more than one antibody: anti-A plus cross-reacting anti-B or anti-B plus cross-reacting anti-A.

Regardless of the ultimate explanation, the available evidence points strongly to a peculiarity (or peculiarities) inherent in the anti-A and anti-B activity in
group O mothers, that is responsible for most of the A-B hemolytic disease problem.

SUMMARY AND CONCLUSIONS

A study was made of oxalated umbilical vein blood of nearly every infant born at The Mount Sinai Hospital in a nine month period. A specimen of maternal blood was available for intragroup antibody screening and six cases of Rh-Hr hemolytic disease were eliminated from the data. The umbilical vein blood was tested, where possible, for: (1) group and Rh, (2) direct antiglobulin test, (3) hemoglobin, (4) reticulocyte count and examination of red cell morphology, (5) plasma bilirubin, and (6) osmotic fragility in 0.52 per cent NaCl. From the mothers’ blood groups, the infants were classified into group compatible and group incompatible, and the arithmetic means of the hemoglobin, reticulocyte count, and plasma bilirubin obtained for each class. A third class of infants, those with positive direct antiglobulin test, were analysed separately for comparison.

1. A weakly positive direct antiglobulin test was obtained on the umbilical vein blood of over 11 per cent of group incompatible infants but in none of the group compatible infants.

2. It appears that the weakly positive direct antiglobulin test detects an abnormal class of group incompatible infants, since their mean hemoglobin is low, their mean reticulocyte count is high, and their mean bilirubin is high, when these means are compared with those of the other group incompatible infants.

3. Thirty-eight of thirty-nine mothers of incompatible infants with positive direct antiglobulin test were group O. In comparison with the distribution of the blood groups of the mothers of other incompatible infants, this disproportion is of significance.

4. The mean reticulocyte count of incompatible infants with negative direct antiglobulin test is slightly (but with statistical significance) higher than the mean reticulocyte count of compatible infants. This difference was found to be associated almost entirely with group O mothers.

5. Thirty-one out of thirty-eight infants with positive direct antiglobulin test had increased osmotic fragility in hypotonic NaCl. Two of the negative cases appeared to have slight spherocytosis on blood smear.

SUMMARIO E CONCLUSIONES IN INTERLINGUA

Esseva studiate oxalate specimens de sanguine venal ab le cordon umbilical de quasi omne infantes nascente al Hospital Monte Sinai durante un periodo de nove menses. Specimens del sanguine materne esseva disponibile pro le assortiti in intragruppal del anticorpores, e sex casos de morbo hemolytic Rh-Hr esseva detegite e eliminate ab le datos includite in le presente studio. Le sanguine umbilical, in tanto que possibile, esseva subjicite a sex differente tests e reactiones: (1) Determination del gruppo sanguinee e del factor Rh. (2) Reaction antiglobulinic directe. (3) Determination de hemoglobina. (4) Contation del reticuloctytes e examine del morphologia erythrocytic. (5) Determination de bilirubina plasmatic. (6) Test del fragilitate osmotic in un solution de 0,52 pro cento de NaCl. In relation al gruppous sanguinee del matres le infantes esseva classificate como compatibile e incompatibile. In ambe iste classes le valores median arith-
metic esseva obtenite pro hemoglobina, reticulocytos, e bilirubina plasmatic. 

Un tertie classe de infantes—illes qui habeva un positive reaction antiglobulinic directe—esseva analysate separatamente pro objectivos de comparation.

(1) Resultatos levemente positive esseva obtenite in le reaction antiglobulinic directe in plus que dece-un pro cento del infantes incompatibile sed in nulle caso inter le infantes compatible.

(2) Il pare que un resultato levemente positive in le reaction antiglobulinic directe segrega un typo anormal inter le infantes incompatibile proque in comparation con le altere infantes incompatibile illes es characterisate per un basse valor median de hemoglobina, un alte contation median de reticulocytos, e un alte valor median de bilirubina.

(3) Inter 39 infantes incompatible qui habeva positive valores in lor reaction antiglobulinic directe, 38 habeva matres del gruppo O. In comparation con le distribution del gruppos sanguinee inter le matres del altere infantes incompatibile iste disproportion es significative.

(4) Inter le infantes incompatible qui habeva negative valores in lor reaction antiglobulinic directe le contation median del reticulocytos esseva levemente (sed a un grado statisticamente significative) plus alte que le contation median de reticulocytos inter infantes compatible. Il esseva constatate que iste differentia se associava quasi integrementemente con matres del gruppo O.

(5) Trenta-un inter le trenta-octo infantes con positive resultatos del reaction antiglobulinic directe habeva un elevate fragilitate osmotic in solutiones hypothomic de NaCl. In duo inter le casos negative, frottis sanguinee pareva revelar un leve spherocytosis.

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RICHARD E. ROSENFIELD and FLORENCE EISINGER

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