Hemolytic Disease of the Newborn Due to Anti-A

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The diagnosis of hemolytic disease of the newborn is made by demonstrating incompatibility between the mother's serum and the infant's red cells, associated with signs of red cell destruction in the infant. When the disease is due to anti-Rh it is not uncommon to find evidence of serologic incompatibility, including a positive direct antiglobulin test in the infant, without evidence of a hemolytic process, but signs of a hemolytic process are never found in the absence of a positive direct antiglobulin test. In hemolytic disease of the newborn due to anti-A, the diagnosis is made far more difficult by two facts: first, that the direct antiglobulin test may be negative; and second, that the demonstration that the mother's serum contains anti-A and the infant belongs to group A has by itself little significance.

The present paper summarizes observations on 11 infants in whom the diagnosis of hemolytic disease of the newborn due to anti-A was made. It is demonstrated that there are two signs which are of value in establishing the diagnosis: the presence of a hemolysin in the mother's serum and of a raised osmotic fragility of the infant's red cells.

Clinical Material

Seven infants (Cases A 1 to A 7) were first tested because they became jaundiced within twenty-four hours of birth. Three infants (A 8, A 9, and A 11) were tested because their mothers had previously given birth to infants suspected of having hemolytic disease of the newborn; 1 infant (A 10) was tested because a potent ρ hemolysin was found in its mother's serum during pregnancy. In all 11 infants there was evidence of a hemolytic process. In every case the infant was group A and the mother group 0; no incompatibility other than that due to anti-A could be demonstrated.

Several other infants who had become unusually jaundiced during the first forty-eight hours of life were also investigated. Although in most of these cases the infant's red cells were found to be incompatible with the mother's serum, no evidence of increased red cell destruction was obtained. The difficulty of making a diagnosis in such cases is discussed.

Several groups of normal infants were tested: first, to define the range of the osmotic fragility of the red cells in normal infants during the first week of life; second, to see whether any of the signs observed in hemolytic disease of the newborn due to anti-A were detectable in apparently normal group A infants with group 0 mothers; and third, to provide control observations on the strength of the reactions of group A cells in newborn infants, compared with those in adults.

The osmotic fragility of the red cells of 9 infants with hemolytic disease due to anti-Rh was also determined, for comparison.

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Submitted November 22, 1952; accepted for publication December 15, 1952.

We should like to thank Drs. M. A. Arthurton, Josephine Lord, D. de la C. MacCarthy, T. P. Mann, J. A. G. McSorley, D. O'Brien, and C. Upjohn for drawing our attention to some of these cases and Drs. J. R. P. Tizard and S. Yudkin for inviting us to make observations on infants under their care. It is a pleasure to acknowledge the constant help of the Departments of Child Health and of Obstetrics at the Postgraduate Medical School of London.
Methods

Blood Samples

Venous samples were used for all determinations; blood was usually obtained from a scalp vein or the external jugular vein.

Hematologic Tests

Hemoglobin was determined as oxyhemoglobin, either in a photo-electric colorimeter using Ilford filter 625, with a grey screen of known optical density as a standard, or in the M.R.C. Photometer. Hematocrit was determined on heparinized blood spun in Wintrobe hematocrit tubes for 30 minutes at 3000 R.P.M. in a centrifuge of radius 15 cm. Reticulocyte counts were determined by the method described by Dacie. Blood films were prepared from blood mixed with Wintrobe's oxalate and stained with May-Grünwald-Giemsa combination. In many cases, films were also prepared from fresh blood, unmixed with anticoagulant, to confirm the presence of spherocytosis. Plasma bilirubin concentration was estimated by the method of King and Coxon, modified for 0.1 or 0.2 ml. of plasma.

Osmotic fragility was measured by the method of Parpart and co-workers with the following modifications: heparinized rather than citrated blood was used; the range of saline solutions was extended to include NaCl concentrations up to 1.0 per cent; the addition of complementary solutions was omitted, the mixtures of blood and hypotonic saline being centrifuged after standing for 45 minutes in a water-bath at 23 C. and the color densities of the supernatant solutions then measured in a photo-electric colorimeter; the quantities of blood and saline solution were measured with automatic pipets (based on Wright) designed to deliver approximately 0.04 and 5.0 ml. respectively.

For conciseness, results are usually expressed as the “median corpuscular fragility” (M.C.F.); this was deduced by plotting on arithmetic probability paper the percentage lysis found in the various concentrations of hypotonic saline, fitting the best straight line to the points in the range 0 to 80 per cent by inspection, and then reading off the saline concentration corresponding to 50 per cent lysis. In the cases of hemolytic disease of the newborn due to anti-A, only points falling in the range 20 to 80 per cent lysis were used to deduce the M.C.F. (see results).

When testing the osmotic fragility of an infant with hemolytic disease of the newborn, a control sample was always tested at the same time. At first, the blood of a normal newborn infant was used, but later it was found more convenient to use the blood of an adult who had frequently been tested before. With this method the osmotic fragility of the red cells of any particular adult showed relatively little variation. Thus, one adult tested twenty-five times at intervals over a period of thirteen months had a mean M.C.F. of 0.434 per cent NaCl with an S.D. of 0.009 and a second adult tested twenty-five times at intervals over a period of thirteen months had a mean M.C.F. of 0.433 per cent NaCl with an S.D. of 0.008.

Survival of transfused red cells. The method used was a modification of Ashby’s original method. Two antisera were used: anti-A produced in rabbits by the method described by Morgan, and anti-M powdered globulin obtained commercially.

Serologic Tests

Direct antiglobulin test. The test was carried out as described by Mollison. In four cases (A1, A2, A3, and A7) the antiglobulin serum was used at a dilution which gave the strongest reactions with Rh-sensitized cells. In the remaining 7 cases, the infant’s cells were tested against serial dilutions of the antiglobulin serum.

Detection of free anti-A. The serum of the infants with hemolytic disease of the newborn due to anti-A, and of ten control group A infants with group O mothers, was tested against adult A cells, first, in saline and second, by the indirect antiglobulin test.

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Reactivity of infant's cells with anti-A. The reactivity of the red cells of the infants with hemolytic disease of the newborn due to anti-A was compared with that of cells from normal adults and normal infants. The tests used were: agglutination by an \( \alpha \) serum, prepared by absorbing a group O serum with A\(_2\)B cells; hemolysis by an immune anti-A serum; and an indirect antiglobulin test, using an immune anti-A serum partially neutralized with saliva from a group A secretor.\(^{11}\)

A substance in saliva. Saliva was obtained and tested by the method described by Race and Sanger.\(^{12}\)

Isoagglutinin titrations. The method used was that described previously.\(^{11}\) In some cases the mother's serum was tested against the cells of her own infant as well as those from A\(_1\) and A\(_2\) adults.

Detection of a hemolysin. The hemolysin titer of a serum was determined as follows: To serial, halving dilutions of the test serum were added first a volume of fresh neutral serum and second a volume of a 5 per cent suspension of A\(_1\) cells. The neutral serum, added as a source of complement, was freshly drawn from the same donor throughout the work; this donor belonged to group O; her serum had a low \( \alpha \) agglutinin titer (16) and did not itself hemolyse A\(_1\) cells. Hemolysin titers are expressed as the reciprocal of the highest dilution of the test serum in which more than a trace of lysis occurred. In calculating the dilution, the volumes of neutral serum and of test cells have both been taken into account so that the dilution of test serum in the first tube of the titration is considered as 1 in 3, that in the second tube as 1 in 6, etc. This differs from the usual convention, in expressing isoagglutinin titers, of ignoring the volume of cell suspension and considering the dilution in the first tube as 1 in 1.

Indirect antiglobulin test using partially neutralized serum. The method used was that described previously; in a number of cases the mother's serum was tested against cells from her own infant as well as those from A\(_1\) and A\(_2\) adults.

RESULTS

Most of the findings in the 11 infants with hemolytic disease of the newborn due to anti-A are set out in table 1.

The Mother's History

Only 1 mother in the series had not previously been pregnant (A 2). Two mothers (A 5 and A 6) had had normal infants and 2 others had had infants who were stillborn but apparently not affected with hemolytic disease of the newborn (A 4 and A 10).

The remaining 6 mothers had previously given birth to infants with an unusual degree of jaundice and in 4 of these 6 a diagnosis of hemolytic disease of the newborn due to anti-A had been made (A 7, A 8, A 9, and A 11).

None of the mothers gave a history of having had injections of bacterial vaccines or toxoid or anti-serum.

Hemoglobin Concentration

Of the 4 infants whose cord blood was tested, 3 (A 1, A 8 and A 11) had values below normal and 1 (A 9) a low normal value. (The range of hemoglobin concentration of the cord blood of normal infants was taken as 13.6 Gm./100 ml. to 19.6 Gm./100 ml.\(^{13}\) The relationship of these figures to those of other authors has been discussed elsewhere.\(^{19}\)

Of the remaining 7 infants first tested some hours after birth, 3 (A 2, A 4, and A 6) had values below the normal range and the remaining 4 (A 3, A 5, A 7, and A 10) had values in the lower half of the normal range. (The hemoglobin
concentration in venous blood of normal infants during the first two to three
days of life was taken as 14.5 to 22.5 Gm./100 ml.  

Mean Corpuscular Hemoglobin Concentration (M.C.H.C.)

The mean M.C.H.C. in normal newborn infants has been found to be approximately 32 per cent. In hemolytic disease of the newborn due to anti-Rh, M.C.H.C. often appears to be lower than in normal infants, presumably because the cell column includes a significant proportion of nucleated, non-hemoglobinized red cells. Thus in table 3 there are 3 infants whose M.C.H.C. was below 30 per cent.

As shown in table 1, M.C.H.C. in the cases of hemolytic disease of the newborn due to anti-A tended to be higher than normal. It will be noted that the 2 cases with the highest osmotic fragility (A 4 and A 6) had an M.C.H.C. of approximately 36 per cent.

Reticulocyte Counts

In this laboratory, reticulocyte counts in normal infants are found to range from 2 to 7 per cent during the first two days of life and to be less than 5 per cent from the third day of life onwards. By these standards all the infants in the present series had an increased number of reticulocytes, values ranging from 8 to 21 per cent on the first day of life and gradually diminishing thereafter. In the control series of 10 apparently normal group A infants with group 0 mothers, values in the first thirty-six hours of life ranged from 2 to 6 per cent.

Blood Films

Erythroblastemia. Of 9 infants examined within forty-eight hours of birth, 7 had 30 nucleated red cells or more per 100 W.B.C.—a proportion which is well above the upper limit of normal.

Spherocytosis was present in the films of all 11 infants with hemolytic disease due to anti-A and was particularly striking in Cases A 4, A 6, A 10, and A 11. In Cases A 6 and A 11, spherocytes were still present during the third week of life.

Blood films of 6 of the 9 infants with hemolytic disease due to anti-Rh were also examined. Only 1 case showed a striking degree of spherocytosis (R 1); a second infant (R 9) showed doubtful spherocytosis; the remaining four (R 3, R 4, R 5, and R 7) showed no spherocytosis.

Jaundice and Plasma Bilirubin Concentration

Cases A 1 to A 7 were first tested because of early jaundice. One infant (A 1) became only slightly jaundiced, the maximum bilirubin concentration being 7 mg./100 ml. In the remaining 6, the maximum bilirubin concentrations recorded varied from 11 mg. to 26 mg./100 ml. In some of these infants, anxiety was felt because of the depth of the jaundice but none of them developed any signs of damage to the central nervous system.

Amongst the cases tested because of the mother’s previous history or serologic findings (A 8 to A 11), only 1 (A 9) became jaundiced.
<table>
<thead>
<tr>
<th>Case no.</th>
<th>Previous pregnancies</th>
<th>Present infant</th>
<th>Direct anti-globulin test</th>
<th>Reaction with</th>
<th>Free anti-A: infant's plasma</th>
<th>Age of infant when tested</th>
<th>Plasma bilirubin mg./100 ml</th>
<th>Hb g./100 ml</th>
<th>P.C.V. %</th>
<th>M.C.H.C. %</th>
<th>Retic. %</th>
<th>Osmostic fragility % NaCl</th>
<th>NaCl cr. h.c. per 100 w.h.c.</th>
<th>50% Lysis</th>
<th>5% Lysis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 1 Cla.</td>
<td>1. J.</td>
<td>F 7/8</td>
<td>+ +</td>
<td>+</td>
<td>Cord blood</td>
<td>3</td>
<td>16.1</td>
<td>80.3</td>
<td>34</td>
<td>34</td>
<td>230</td>
<td>230</td>
<td>230</td>
<td>230</td>
<td>230</td>
<td>Simple transfusion of 70 ml. cell suspension at 16 hrs.</td>
</tr>
<tr>
<td>A 2 Tra.</td>
<td>Nil.</td>
<td>...</td>
<td>0</td>
<td>...</td>
<td>23 hours</td>
<td>3</td>
<td>12.9</td>
<td>38.3</td>
<td>34</td>
<td>21</td>
<td>81</td>
<td>0.461</td>
<td>0.461</td>
<td>0.461</td>
<td>0.461</td>
<td>Simple transfusion of 135 ml. blood at 24 hrs.</td>
</tr>
<tr>
<td>A 3 And.</td>
<td>1. J.</td>
<td>F 7/4</td>
<td>0</td>
<td>0</td>
<td>3 days</td>
<td>15</td>
<td>17.7</td>
<td>55.5</td>
<td>32</td>
<td>9</td>
<td>2</td>
<td>0.428</td>
<td>0.428</td>
<td>0.428</td>
<td>0.428</td>
<td>No treatment</td>
</tr>
<tr>
<td>A 4 Lyo.</td>
<td>1. S. B.</td>
<td>F 8/0</td>
<td>+</td>
<td>+</td>
<td>2 days</td>
<td>22</td>
<td>13.3</td>
<td>37.0</td>
<td>36</td>
<td>19</td>
<td>35</td>
<td>0.482</td>
<td>0.482</td>
<td>0.482</td>
<td>0.482</td>
<td>No transfusion, but ACTH, 170 mg injected in 4 days starting at age of 44 hrs.</td>
</tr>
<tr>
<td></td>
<td>2. N.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 5 Dow.</td>
<td>1. N.</td>
<td>M 8/4</td>
<td>+</td>
<td>+</td>
<td>3 days</td>
<td>26</td>
<td>16.4</td>
<td>44.0</td>
<td>37</td>
<td>37</td>
<td>1</td>
<td>0.450</td>
<td>0.450</td>
<td>0.450</td>
<td>0.450</td>
<td>No treatment</td>
</tr>
<tr>
<td></td>
<td>2. N.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HEMOLYTIC DISEASE OF NEWBORN**

**TABLE 1.—Details of Eleven Cases of Hemolytic Disease of the Newborn due to Anti-A**

(Except for estimation of maximum bilirubin concentration observations made after exchange transfusion are omitted)
| A 6 Byr. | 1. N. | M | 8/0 | 0 | + | + | 0 | 2 days | 8 | 10 | 14.1 | 39 | 38 | 18 | 47 | 0.822 | 0.765 | Simple transfusion of 70 ml. blood on 9th day |
| | | | | | | | | | | | | | | | | | | | |
| A 7 Bev. 1 | 1. J. | M | 5/0 | + | + | + | + | 14 hours | 10 | 14.7 | 44 | 33 | 8 | 30 | 0.450 | 0.538 | Exchange transfusion at 36 hrs. |
| | 2. J: died | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| A 8 Bev. 2 | 1. J. | M | 5/2 | + | 0 | + | + | Cord blood | 3 hours | 12.7 | 38 | 34 | 16 | 58 | 0.463 | 0.566 | Exchange transfusion at 3 hrs. |
| | 2. J: died | | | | | | | | | | | | | | | | | | |
| | 3. J (Case A 7) | | | | | | | | | | | | | | | | | | |
| A 9 Kin. | 1. Misc. 3/12 | M | 6/8 | + | 0 | 0 | + | Cord blood | 10 hours | 14.1 | 42 | 34 | 12 | 2 | 0.397 | 0.477 | Exchange transfusion at 10 hrs. |
| | 2. S. B. | | | | | | | | | | | | | | | | | | |
| | 3. J: died | | | | | | | | | | | | | | | | | | |
| A 10 Joy. | 1. S. B. | F | 9/6 | + | + | + | 0 | 14 hours | 2 | 17.0 | 48 | 35 | 15 | 6 | 0.435 | 0.585 | No treatment |
| | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| A 11 Col. | 1. J. | F | 7/12 | 0 | 0 | + | + | Cord blood | 3 days | 12.0 | 30 | 34 | 19 | 30 | 0.659 | 0.756 | No treatment |
| | 2. J. | | | | | | | | | | | | | | | | | | |

* N. = normal; J. = jaundiced first day; S.B. = stillbirth.
† Tested by method of Dacie and Vaughan.
Osmotic Fragility

Normal infants. The results of osmotic fragility measurements in normal infants, expressed as the concentrations of NaCl in which 50 per cent lysis and 5 per cent lysis occurred are summarized in table 2. For comparison with the infants with hemolytic disease, the normal range of 50 per cent lysis and 5 per cent lysis, calculated as "Mean ± 2 S.D.,” is shown against each age group. A group of normal adults has been included for comparison. The group of seventeen infants tested at two to twenty-four hours includes 8 infants whose cord blood was tested; the group of infants tested at two to five days includes 4 infants whose blood was tested at two to twenty-four hours, but does not include any of the infants whose cord blood was tested.

Eight infants were tested at birth (cord blood) and again three to five hours later; in every case the M.C.F. was lower on the second occasion, the mean change being 0.011 per cent NaCl.

The observations on the normal infants are also presented in figure 1, in which average percentage lysis is plotted against NaCl concentration. It will be noted that in the three age groups, the percentage lysis in the higher concentrations of saline is that expected from extrapolation of the line from which the M.C.F. was deduced.

The normal infants in these age-groups were chosen at random and the blood groups of the infants and their mothers were not determined. It was thought advisable to test some apparently normal group A infants whose mothers were known to be group O. In 10 such infants tested between two and thirty-six hours after birth, M.C.F. varied from 0.387 to 0.417 per cent NaCl, and 5 per cent lysis occurred in NaCl concentrations ranging from 0.477 to 0.511 per cent; all values thus being well within the range found in unselected infants.

Infants with hemolytic disease of the newborn due to anti-A. (See table 1.) In Cases A 3 to A 11 osmotic fragility was measured by the method of Parpart and co-workers, and the results can be compared with those found by the same method in normal infants (see table 2). Only in Case A 9 were the findings definitely within normal limits. Fragility was just above the upper limit of normal

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Table 2.—Osmotic Fragility of Red Blood Cells in Normal Infants

<table>
<thead>
<tr>
<th>Age of infants</th>
<th>No. of cases</th>
<th>50% Lysis</th>
<th>5% Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± 2 S.D.</td>
<td>Mean ± 2 S.D.</td>
</tr>
<tr>
<td>Cord blood</td>
<td>16</td>
<td>0.422 ± 0.02</td>
<td>0.380-0.464</td>
</tr>
<tr>
<td>2-24 hours</td>
<td>17</td>
<td>0.408 ± 0.015</td>
<td>0.378-0.438</td>
</tr>
<tr>
<td>2-5 days</td>
<td>14</td>
<td>0.395 ± 0.016</td>
<td>0.363-0.427</td>
</tr>
<tr>
<td>Normal adults</td>
<td>18</td>
<td>0.424 ± 0.01</td>
<td>0.404-0.444</td>
</tr>
</tbody>
</table>

*tests on M.C.F.*

Cord blood vs. 2-24 hours, p = 0.07;
2-24 hours vs. 2-5 days, p = 0.04;
Cord blood vs. 2-5 days, p < 0.01.
in 1 case (A 3), was only slightly increased in 3 (A 5, A 7, and A 8), but was moderately or greatly increased in 4 (A 4, A 6, A 10, and A 11).

In 6 untransfused infants in whom osmotic fragility changes were followed for one week or more after birth, the results were within normal limits at the age of 1 week in two cases (A 3 and A 5) and became normal at some time between 16 and 51 days in the other 4 (A 4, A 6, A 10, and A 11).

The first 2 infants (A 1 and A 2) were tested by the method of Dacie and Vaughan. It may be assumed that for normal infants more than a few hours old the upper limit of the M.C.F. by this method is less than 0.40 per cent since in a small series of normal infants tested in this laboratory the upper limit of

**Fig. 1.—Osmotic fragility of the red cells of normal infants during the first five days of life.** The 3 groups are those referred to in table 2. Each point on the chart is the average percentage lysis of the red cells in any particular NaCl concentration. Percentage lysis is plotted on a scale of arithmetic probability.

M.C.F. for cord blood was found to be 0.405 per cent NaCl, and in another series of infants tested by this method one to seven days after birth, the upper limit of normal for M.C.F. was found to be 0.36 per cent NaCl. In Case A 1, the M.C.F. at the age of 15 hours was 0.42 per cent NaCl (slightly increased), and in Case A 2 at the age of 23 hours the M.C.F. was 0.46 per cent NaCl (considerably increased).

To summarize: osmotic fragility was outside normal limits in 10 out of 11 infants with hemolytic disease of the newborn due to anti-A.

Figure 2 shows that expression of the results in terms of M.C.F. gives an inadequate idea of the findings and underestimates the departure from normality. In particular, the amount of lysis in concentrations of NaCl above 0.55 per cent
was much greater than that expected from extrapolation of the upper part of the curve.

**Infants with hemolytic disease of the newborn due to anti-Rh.** Some of the findings in 9 infants are shown in table 3. In all, the direct antiglobulin test was moderately or strongly positive and all had hemoglobin concentrations below normal limits. Six of the 9 infants had a hemoglobin concentration of less than 10.5 Gm./100 ml. (cord blood in 5 cases, venous blood nine hours after birth in one case) and must be regarded as a severely affected group.

Fig. 2.—Osmotic fragility in 9 infants with hemolytic disease of the newborn due to anti-A. Percentage lysis is plotted on a scale of arithmetic probability. The case numbers are marked against their respective curves; except in Cases A6 and A9, the experimental points are omitted. In each case, the estimations on only one blood sample are shown, viz: at age of 3 to 14 hours in Cases A 7, A 8, A 9, and A 10; at age of 2 to 3 days in Cases A 3, A 4, A 5, A 6, and A 11.

Osmotic fragility was normal in 3 cases (R 5, R 7, and R 8); was near the upper limit of normal in 2 cases (R 4 and R 6); just raised in 2 cases (R 2 at three hours and R 3 at thirty-six hours) and considerably increased in only 2 cases (R 1 and R 9). The results are plotted in figure 3. It will be observed that on the whole the percentage lysis in the less hypotonic saline solutions is that expected from extrapolation of the upper part of the curves.

In none of the 4 cases with a raised fragility was there incompatibility due to anti-A between the infant’s red cells and the mother’s serum.

**Survival of Transfused Cells**

In 2 cases, transfusions of group A and group O cells were given in order to obtain evidence about the specificity and severity of the hemolytic process. The
findings in Case A 1 have been reported previously.\textsuperscript{10, 19} During the six days following transfusion, more than two thirds of the A cells were eliminated while the O cells survived normally.

**TABLE 3.—Osmotic Fragility Measurements in 9 Severe Cases of Hemolytic Disease of the Newborn due to Anti-Rh**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>No. of previously affected siblings</th>
<th>Sex</th>
<th>B.Wt.</th>
<th>Present infant</th>
<th>Age at time of examination</th>
<th>Bilirubin mg./100 ml.</th>
<th>Hb. Gm./100 ml.</th>
<th>P.C.V. %</th>
<th>M.C.H. %</th>
<th>Retic. %</th>
<th>NaCl %</th>
<th>Osmostic fragility % NaCl 50° Lysis (M.C. F.) 3° Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 1 Pag.</td>
<td>0 M 8/0</td>
<td>Cord blood</td>
<td>7</td>
<td>9.0</td>
<td>32</td>
<td>28</td>
<td>35</td>
<td></td>
<td>400</td>
<td>0.510</td>
<td>0.638</td>
<td></td>
</tr>
<tr>
<td>R 2 Cha.</td>
<td>2 F 7/14</td>
<td>Cord blood</td>
<td>3 hr.</td>
<td>9.4</td>
<td>31</td>
<td>30</td>
<td>25</td>
<td></td>
<td>200</td>
<td>0.453</td>
<td>0.523</td>
<td>0.455  0.519</td>
</tr>
<tr>
<td>R 3 Coo.</td>
<td>0 M 7/10</td>
<td>9 hr.</td>
<td>14</td>
<td>9.6</td>
<td>34</td>
<td>28</td>
<td>34</td>
<td>180</td>
<td>0.431</td>
<td>0.534</td>
<td>0.441  0.560</td>
<td></td>
</tr>
<tr>
<td>R 4 Ric.</td>
<td>1 M 6/14</td>
<td>Cord blood</td>
<td>3 hr.</td>
<td>9.6</td>
<td>28</td>
<td>34</td>
<td>...</td>
<td></td>
<td>...</td>
<td>0.440</td>
<td>0.496</td>
<td></td>
</tr>
<tr>
<td>R 5 Lan.</td>
<td>0 M 6/10</td>
<td>Cord blood</td>
<td>5</td>
<td>10.2</td>
<td>32</td>
<td>32</td>
<td>20</td>
<td>60</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>R 6 Fos.</td>
<td>1 M 7/1</td>
<td>Cord blood</td>
<td>3 hr.</td>
<td>10.2</td>
<td>34</td>
<td>30</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>R 7 Dua.</td>
<td>1 F 6/10</td>
<td>Cord blood</td>
<td>3 hr.</td>
<td>11.7</td>
<td>40</td>
<td>29</td>
<td>19</td>
<td>67</td>
<td>0.425</td>
<td>0.518</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>R 8 Hoa.</td>
<td>0 F 8/0</td>
<td>Cord blood</td>
<td>9 hr.</td>
<td>13.0</td>
<td>37</td>
<td>35</td>
<td>7</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.431  0.501</td>
<td></td>
</tr>
<tr>
<td>R 9 Lak.</td>
<td>0 M 7/7</td>
<td>Cord blood</td>
<td>3 hr.</td>
<td>13.2</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.480  0.565</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R 3 and R 5 were treated with A.C.T.H. or Cortisone and transfused on the second day of life or later; the remaining infants were treated by exchange transfusion.

In Case A 6 a transfusion was given on the ninth day of life because the infant's P.C.V. had fallen to 32 per cent; both A\textsubscript{1} and O cells were given in order to discover whether the hemolytic process was still active. Carefully measured
amounts of blood were transfused by scalp vein and samples were withdrawn during the subsequent five weeks. The estimates were corrected by the factor

\[
\text{Body weight on day of estimation} \times \frac{\text{Body weight on day of transfusion}}{\text{Body weight on day of estimation}}
\]
to allow for changes in blood volume. The survival of the transfused cells is shown in figure 1; on the day after transfusion, the relative numbers of A and O cells in the infant's circulation were almost exactly those expected from the numbers transfused (ratio A/O: expected 1.07; found 1.08). However, thereafter the number of A cells rapidly diminished so that within the following eight days approximately 60 per cent of them were eliminated. By contrast the O cells were eliminated at the expected rate of approximately 1 per cent a day.

**Direct Antiglobulin Test**

In 7 out of the 11 cases the test was positive, though the reactions were weak; in no case did the test remain positive after the first seven days of life.

The possible value of testing the cells against a range of dilutions of antilobulin serum was demonstrated in Case A 9. Although there was some agglutination of the infant's cells with dilutions of antilobulin serum ranging from 1/4 to 1/1024, the maximum agglutination was observed at a dilution of 1/256. By contrast the same antilobulin serum gave maximum agglutination.
at a dilution of 1/32 when tested with Rh positive cells weakly sensitized with Rh antibody.

In 2 of the 4 cases in which a negative result is recorded, the cells were tested against a range of dilutions (1/4 to 1/1024) of two different antiglobulin sera, without eliciting any trace of reaction (A 6 and A 11).

**Spontaneous Clumping of Whole Blood**

In Cases A 5 and A 6 it was observed that blood freshly drawn from the infant formed large clumps which were easily seen when the blood was allowed to flow down the side of a tube or was examined on an opal glass tile. In Case A 5 the clumping had become weak by the fifth day of life; in Case A 6 it was still demonstrable on the fourteenth day of life but was not demonstrable on the seventeenth day. The phenomenon may well have been present in the other cases in this series but it was not specially looked for and was not noted.

**Free Anti-A**

In 8 out of 11 cases, the infant's serum, collected within the first three days of life, sensitized adult A1 cells to an antiglobulin serum although it would not agglutinate or hemolyse A1 cells. No free anti-A could be demonstrated in Cases A 3, A 6, and A 10.

In the control group of 10 normal A infants with group O mothers, there were 3 cases in which the mother's serum was capable of sensitizing A1 cells; in 1 of

![Graph](attachment:image.png)
HEMOLYTIC DISEASE OF NEWBORN

these 3 cases the infant’s serum sensitized A1 cells although the infant’s own cells gave a negative direct antiglobulin test.

Reactivity of A Cells

Control group. Twenty-six apparently normal group A infants were tested firstly with an α1 serum and secondly with a partially neutralized immune anti-A serum, in parallel with A1 and A2 cells from adult donors. The results are shown in table 4.

Note that among the 17 infants whose cells were agglutinated by α1, there were only 4 whose cells reacted as strongly as adult A1 cells in the indirect antiglobulin test and among the 9 infants whose cells were not agglutinated by α1 there were 6 whose cells reacted even more weakly than adult A2 cells.

Further evidence of the weaker reactivity of the infant’s cells was obtained from hemolysin tests. Among the 10 apparently normal group A infants with group 0 mothers, there were 2 cases in which the mother's serum lysed cells from A1 and A2 adults but failed to lyse those of the infant, although the infant’s cells were weakly agglutinated by an α1 serum. In the same 2 cases the mother’s serum gave a strong indirect antiglobulin test with adult A1 cells but failed to sensitize the infant’s cells.

Cases of hemolytic disease of the newborn due to anti-A. As shown in table 1, cells from 10 of the 11 cases were tested with an α1 serum within the first few days of life and 4 of the 10 failed to react; of the 4 who were negative at birth (A 3, A 8, A 9, and A 11), 3 were re-tested later. In Case A 11 the reaction had already become positive at the age of 2 months; of the 2 infants (A 8 and A 9) tested at the age of 10 months, one was now positive but the other still negative.

Thus, in all, 8 infants could be classified as A1 and 1 as A2; the remaining 2 infants could not be classified since 1 (Case A 2) was never tested with an α1 serum and the other was tested only at birth (Case A 3).

In 6 cases, the reactions of the infant’s red cells with its mother’s serum were compared with those from adults of group A1 and A2 (see table 5). Note that in all 6 cases the hemolysin titer when the infant’s cells were used was as low as, or lower than, that with adult A2 cells; the results of indirect antiglobulin tests also demonstrate the low reactivity of the infant’s cells.

Secretion of A Substance in Saliva

All 11 infants were secretors of A substance.
The Mother's Serum

All the mothers belonged to group 0; only 2 were Rh negative and neither had anti-Rh in her serum.

Some of the characteristics of the anti-A in Cases A 3, A 4, A 6, A 9, A 10, and A 11 have already been shown in Table 5. Table 6 shows the isoagglutinin titer in saline and the hemolysin titer of samples from all 11 mothers, with a record of the time before and after delivery when the samples were taken. Although in most instances the isoagglutinin titer was not exceptionally high, in all cases the serum was capable of lysing A₁ cells and of sensitizing them to an antiglobulin serum.

Treatment

There were 7 infants in whom the diagnosis of hemolytic disease of the newborn was made within the first twenty-four hours of life (A 1, A 2, A 7, A 8, A 9, A 10, and A 11). Cases A 1, A 10, and A 11 did not appear to be severely affected; Cases A 10 and A 11 received no treatment; Case A 1 received a simple transfusion of group 0 and group A blood, as already described. Case A 2, (treated at another hospital), received a simple transfusion of group O blood. In the remaining three cases (A 7, A 8, and A 9) the mother had previously lost an infant from hemolytic disease of the newborn due to anti-A, and it was thought desirable to carry out exchange transfusion using a concentrated suspension of group O red cells, to which had been added AB substance.

Amongst the 4 cases in which the diagnosis was made after the first twenty-four hours of life, no treatment was given to Case A 3 who appeared to be very mildly affected; although two infants (A 4 and A 5) were deeply jaundiced, they were considered to be too old when first seen to benefit from exchange transfusion; one of these (A 4) was given a course of A.C.T.H. without any obvious effect on its hemolytic process, although a sharp rise in the reticulocyte count was noted twenty-four hours after starting treatment. In Case A 6, as already described, a transfusion of group A and of group O blood was given on the ninth day of life.

Subsequent Progress of Infants

Five of the infants who were not treated by exchange transfusion were tested on several occasions during the first month of life. In the 2 infants (A 4 and A 6)
HEMOLYTIC DISEASE OF NEWBORN

who were tested most frequently, the lowest hemoglobin concentrations (9.9 and 9.7 Gm./100 ml) were observed at sixteen and twenty-two days after birth respectively; both infants showed a spontaneous rise thereafter. In the remain-

Table 6.—Agglutinin and Hemolysin Titers of the Mother's Serum in Cases of Hemolytic Disease due to Anti-A

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Days after delivery</th>
<th>Tests with A cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Agglutinin titer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 1</td>
<td>0</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>+10</td>
<td>512</td>
</tr>
<tr>
<td>A 2</td>
<td>+1</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>+7</td>
<td>2048</td>
</tr>
<tr>
<td>A 3</td>
<td>+4</td>
<td>256</td>
</tr>
<tr>
<td>A 4</td>
<td>+2</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>+9</td>
<td>1024</td>
</tr>
<tr>
<td>A 5</td>
<td>+3</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>512</td>
</tr>
<tr>
<td>A 6</td>
<td>+2</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>+12</td>
<td>1024</td>
</tr>
<tr>
<td></td>
<td>+20</td>
<td>1024</td>
</tr>
<tr>
<td>A 7</td>
<td>0</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>+7</td>
<td>1024</td>
</tr>
<tr>
<td>A 8</td>
<td>−50</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1024</td>
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<td></td>
<td>+10</td>
<td>256</td>
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<td></td>
<td>+120</td>
<td>128</td>
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<tr>
<td>A 9</td>
<td>−30</td>
<td>2048</td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>512</td>
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<tr>
<td></td>
<td>+5</td>
<td>2048</td>
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<tr>
<td>A 10</td>
<td>−60</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>1024</td>
</tr>
<tr>
<td></td>
<td>+12</td>
<td>1024</td>
</tr>
<tr>
<td>A 11</td>
<td>+10</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>+2</td>
<td>8192</td>
</tr>
<tr>
<td></td>
<td>+15</td>
<td>2048</td>
</tr>
</tbody>
</table>

ing 3 infants of this group (A 5, A 10, and A 11) the lowest value recorded was 10.3 Gm./100 ml., but these infants were tested less frequently.

No infant in the series developed signs of kernicterus during the first week of life. Moreover the 2 infants in whom the highest bilirubin concentrations were recorded were seen again at the age of 6 months and appeared to be entirely normal (A 4 and A 5).
Suspected Cases

As mentioned earlier, many infants were seen in whom the diagnosis of hemolytic disease of the newborn due to anti-A was suspected but not established. These were infants who became jaundiced during the first twenty-four hours of life and were found to belong to group A and to have group O mothers. However they had no signs of increased red cell regeneration and no increase in red cell osmotic fragility. It appears that the role of anti-A in these cases can be demonstrated only by examining large groups and showing that anti-A incompatibility is significantly more frequent than in a control group of non-jaundiced infants.20 21

In one infant the diagnosis of hemolytic disease of the newborn due to anti-A was suspected because the mother had previously lost an infant, apparently from this cause. However, investigations failed to reveal unequivocal signs of a hemolytic process:

Mrs. D's first infant became jaundiced at the age of 12 hours. The jaundice rapidly deepened and the infant was found to be very anemic and to have nucleated red blood cells in the peripheral blood. It died at the age of 31 hours. The mother was found to be group O, Rh negative but her serum contained no Rh antibody; the infant's blood group was not determined. The mother's serum was examined by the present authors seven months later; no Rh antibody could be detected. Twelve months after the birth of her first infant, Mrs. D became pregnant again; during the last few weeks of this pregnancy her serum was examined once more; no Rh antibody was found but the serum hemolyzed A, cells to a titer of 12. The infant was born at term; cord blood findings were as follows: group A, Rh positive; direct antiglobulin test, negative; cord hemoglobin concentration, 20.5 Gm./100 ml.; reticulocyte count, 6 per cent; film, 50 nucleated red blood cells per 100 white blood cells, suggestion of spherocytosis; osmotic fragility—M.C.F. 0.438 per cent NaCl.; plasma bilirubin, 2.3 mg./100. ml.

The number of nucleated red cells in the peripheral blood was suggestive of a hemolytic process but the other findings were all within normal limits. The peak bilirubin concentration was only 4.0 mg./100 ml. and the infant never became jaundiced. The nucleated red cells disappeared from the peripheral blood after forty-eight hours.

This case is of interest in showing that a group A infant with no definite signs of disease can be born to a woman whose serum contains an α hemolysin and who has previously lost an infant from hemolytic disease, presumably due to anti-A.

Discussion

Much of the previous work on hemolytic disease of the newborn due to anti-A (or anti-B) has been reviewed elsewhere.10 The present paper, based on a study of 11 cases in which there were definite signs of red cell destruction, emphasizes the features of the fully developed syndrome.

Signs of Increased Red Cell Destruction

The findings in all cases were consistent with those of a mild or moderate hemolytic process. Although 4 of the 11 infants had hemoglobin values within the normal range, these 4 infants, like the remainder, had an increased number of reticulocytes.

It appears that red cell destruction continues for a shorter time after birth in
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cases due to anti-A than it does in cases due to anti-Rh, since severe anemia occurring two to four weeks after birth was not noted in untreated cases in the present series. However, 2 infants (A 4 and A 6) had hemoglobin values below 10 Gm./100 ml. in the first three weeks of life and in one of these infants (A 6) the persistence of red cell destruction during the third week of life was demonstrated.

Although early jaundice appears to be a common feature in hemolytic disease due to anti-A, there were 2 infants (A 10 and A 11) in the present series who never became jaundiced despite having evidence of a moderately severe hemolytic process (reticulocyte counts of approximately 15 per cent). For this reason the term hemolytic disease of the newborn due to anti-A seems preferable to "icterus praecox".

Osmotic Fragility Changes

Grumbach and Gasser drew attention to the presence of microspherocytes in the blood films of 2 cases of hemolytic disease of the newborn due to ABO incompatibility; in another case they found osmotic fragility to be increased although they gave no details of their method and no normal range of values for newborn infants. Robinson and associates also noted spherocytosis and found osmotic fragility to be raised in 4 out of 4 cases, lysis beginning in 0.63 to 0.69 per cent NaCl compared with 0.45 to 0.47 per cent NaCl in normal adults; at the end of fourteen or twenty-one days, lysis began in 0.48 to 0.51 per cent NaCl; no normal range for healthy infants was given. Vogel considered spherocytosis to be a very useful sign in diagnosing the syndrome of hemolytic disease of the newborn due to ABO incompatibility.

In the present series, osmotic fragility has been studied by the method of Parpart and associates which has the great merit of making it possible to obtain closely standardized results. Thus the median corpuscular fragility (M.C.F.) of 15 normal adults reported in the present paper, namely 0.424 per cent NaCl, is almost exactly the same as that found by Parpart et al. in 12 normal young males, namely 0.425 per cent NaCl. The finding that the M.C.F. of the red cells in the cord blood of normal infants is practically the same as that of adult red cells agrees with that of Waugh and co-workers as does the finding that M.C.F. diminishes during the first few days of life. The present observations show that this diminution in fragility begins within five hours of birth.

In the present series of cases of hemolytic disease of the newborn due to anti-A, M.C.F. was above normal limits in 10 out of 11 cases, and in all 11 the fragility curves had an abnormal shape (see figure 2); the blood films of all 11 cases showed spherocytosis. By contrast, in a series of cases of hemolytic disease of the newborn due to anti-Rh, of greater than average severity, osmotic fragility was raised in only 4 out of 10 cases and spherocytes were noted in only 2 cases out of 6 that were examined. Only one extensive series of observations on fragility changes in hemolytic disease of the newborn due to anti-Rh has been published previously: Shapiro found that osmotic fragility was sometimes considerably increased in severe cases (3 out of 9) but was not usually increased in mild cases—findings which are essentially in agreement with the present ones.

The association between a raised osmotic fragility and a high M.C.H.C. noted
in the present series can also be observed in some of the cases of hereditary spherocytosis in adults described by Vaughan.

Direct Antiglobulin Test

Boorman and associates found the direct antiglobulin test to be positive on the fourth day after birth in a severe case of hemolytic disease of the newborn due to anti-A, though the test was negative in another case examined on the tenth day after birth. Robinson et al. reported that the direct antiglobulin test was negative on the first day of life in 2 cases. In the present series the direct antiglobulin test was definitely, though not very strongly, positive in 7 out of 11 cases and negative in the remaining 4. The weakness of the reactions seems to be due rather to some quality of the antigen than to the lack of antibody, since in 2 instances in which the direct antiglobulin test was negative the plasma of the same infant would quite strongly sensitize adult A, cells to an antiglobulin serum.

Free Antibody in Infant's Plasma

In the present series, free anti-A, detected by the indirect antiglobulin test using adult A, cells, was present in the infant's plasma in 8 out of 11 cases. Presumably the amount of free anti-A in the infant's serum depends partly upon the amount of antibody passing across the placenta and partly upon the ability of the infant's cells to absorb antibody; it is therefore not surprising that no obvious relationship between the presence of free anti-A and severity of the disease could be demonstrated. Free antibody was demonstrated in 2 cases of hemolytic disease of the newborn, 1 due to anti-B and 1 to anti-A, by Wiener and co-workers; the antibody was demonstrated by agglutinin tests made in a medium of plasma or acacia.

Spontaneous Clumping of Red Cells

In the case due to anti-A described by Wiener and associates, the infant's cells formed a smooth suspension in saline but clumped spontaneously when suspended in plasma or acacia. In the present series spontaneous clumping of whole blood was noted in 2 cases and may have been present in others. In 1 case this clumping persisted for two weeks after birth. The appearance closely resembled that seen in the blood of group A recipients transfused with group O blood containing potent A isoantibody, and that seen in some cases of acquired hemolytic anemia in adults.

Reactivity of Infant's Cells

The relative weakness of the A receptor in newborn infants was first demonstrated by Kemp; its possible role in protecting the newborn infant from anti-A has been discussed by Tovey and by Wiener et al. Witebsky and Engasser showed that this relatively low reactivity of group A cells in newborn infants was well demonstrated by testing the cells with a partially neutralized immune anti-A serum. They found that 18 out of 34 group A infants tested by this method reacted as weakly as, or more weakly than, adult A, cells. In the present work a
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slight modification of their method was used but almost identical results were obtained, 14 out of 26 infants reacting as weakly as, or more weakly than adult A2 cells. The present findings also show that the relative weakness of the A receptor in newborn infants can be demonstrated by testing the cells with a hemolytic anti-A serum, using adult A1 and A2 cells as controls.

Immune Anti-A in the Mother's Serum

In all 11 cases in which hemolytic disease of the newborn due to anti-A was diagnosed, the mother's serum was capable of hemolyzing her infant's cells in vitro. It is suggested that in a doubtful case the failure of the mother's serum to hemolyse her infant's cells in vitro should be taken as evidence that the infant is not affected by hemolytic disease of the newborn due to anti-A.

SUMMARY

Eleven cases of hemolytic disease of the newborn are described in which the only blood group antibody in the mother's serum, incompatible with the infant's cells, was anti-A. The direct antiglobulin (Coombs) test on the infant's red cells was weakly positive in 7 cases and negative in 4 cases. In every case the mother's serum displayed immune characteristics, in particular the ability to lyse A cells.

Osmotic fragility was increased in 10 out of 11 cases. This finding is contrasted with those in a series of cases of hemolytic disease of the newborn due to anti-Rh.

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Hemolytic Disease of the Newborn Due to Anti-A

HAL CRAWFORD, MARIE CUTBUSH and P. L. MOLLISON

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