Hematologic Observations of the Course of Erythroblastosis Fetalis

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The immunohemolytic nature of erythroblastosis fetalis is well recognized. It is our purpose to observe effects of the presence of this disease process on the hematopoietic system of the infant.

Methods and Material

During the first few days of life, determinations were made on venous blood collected in double oxalate. Later studies were done with either venous or capillary blood. Hemoglobin was measured by the oxyhemoglobin method with a Coleman Jr. spectrophotometer standardized by the Van Slyke procedure. Red cell counts were done in duplicate and the results averaged. Volume of packed red cells and white cell counts were done in the usual manner. Reticulocytes were enumerated on dry preparations. Platelets were counted directly by the method of Pohle. Serum bilirubin was measured by the method of Malloy and Evelyn.

Serologic studies on maternal blood included saline and albumin technics and indirect Coombs test. In cases involving anti-A, saline hemolysins were also observed. Serial observations of antibody titer were made during pregnancy. The same procedures as well as a direct Coombs test were carried out on the patient's blood.

Osmotic fragility of red cells was measured according to the technic of Shen, and the fragility curves were plotted as the increments of hemolysis at given saline concentrations. The following criteria were then compared: (1) the limits of hemolysis as defined by that point on the left side from which the curve originated and by the point on the right where it was completed, (2) the breadth of the curve covering that area in which there was an increment of hemolysis of at least 10 per cent, and (3) the number, location and height of the modes. A mode was considered to exist if the amount of hemolysis for a given concentration was greater than 5 per cent of the total and exceeded that noted on either side.

Mechanical fragility was measured by a modification of the technic of Shen et al. in which the flasks were rotated at 35 r.p.m. at a radius of 13 cm. When serial observations were made on a blood sample, a separate flask was used for each time interval.

The patients described in this report include 38 with erythroblastosis fetalis. Twenty-five of the mothers were observed during pregnancy. Thirty-three
cases were due to anti-D and 4 to anti-A. In one case both antibodies were demonstrated. Although a survey of cord blood samples revealed evidence of occurrence of this syndrome induced by anti-A in a form not clinically apparent, only those cases with clinical manifestations are included in this report.

**Results**

Eight infants received replacement transfusions during the first 6 hours after birth, 5 more within the first day, and 8 during a period from 24 to 72 hours. The indications included (a) a past history of kernicterus or of stillbirth in the family, or (b) degree of severity of the present illness with emphasis on the serum bilirubin values.

Certain immediate effects of replacement transfusion were apparent. These included a decrease in the level of bilirubin, reticulocytes and circulating nucleated red cells as well as a change in the hemoglobin value and platelet count.

The serum bilirubin which varied from 1.6 to 24.6 mg. per cent before therapy declined significantly and thereafter the level was never high enough to indicate the need for a second replacement transfusion.

![Graph](image)

**Fig. 1.**—Values for the nucleated red cells per thousand and reticulocytes per hundred red cells are plotted for the first 9 days in a patient who received replacement therapy. The reticulocytes are the open circles. The 14 hour values are those immediately prior to treatment and the “p” are those observed after transfusion.
Before transfusion, the number of circulating nucleated red cells averaged 13,000 cu.mm., varying from less than 100 to 138,000. Eliminating the two highest values, the average was 5,300. After being almost completely removed from the peripheral blood by the replacement therapy, they often reappeared within a day and then declined to normal during the succeeding two days. Such a case is demonstrated in figure 1.

The reticulocyte curve was similar to that of the nucleated red cells in its abrupt change after transfusion (fig. 1). After this sharp decline, the value reached a maximum within the following two days and then diminished gradually during the next week to less than 1 per cent. The curves simulate those of untreated babies except for a temporary break immediately following transfusion. The declining reticulocyte output parallels that seen in controls.

Hemoglobin concentration in the transfused group varied from 8.1 Gm. per cent to 16.7 with an average of 12.3 before treatment. Measurements 24 and 48 hours after transfusion averaged 12.7 with values ranging from 7.6 to 19.5 Gm. per cent.

Variations in platelet count were also observed during the neonatal period. Although in some the platelet count was low, there was no evidence of any

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**Fig. 2.—**The platelet counts are plotted in relation to replacement transfusion. “C” refers to values in cord blood. “A” refers to values immediately prior to transfusion, and “B” refers to the platelet count immediately following this. Thereafter the values are plotted according to the time after treatment.
bleeding tendency. The determination done at the conclusion of replacement was variable but by 24 hours the count was always lower and only gradually returned to normal after three days. A chart of these values may be seen in figure 2. Serial values in the untreated group showed no trend.

There was little difference in the course of those patients who received replacement transfusion and those whose disease was mild enough so that no therapy was given. Hemoglobin declined to an average minimal value of 7.4 Gm. per cent (5.4–9.6) in 19 treated babies and 7.8 Gm. per cent (6.6–9.0) in 9 untreated, both of which values are well below normal for the comparable age group. The degree of anemia at this time was unrelated to any single criterion of the disease. In those babies who received transfusion, no correlation was found between the post-treatment value or reticulocyte peak and the final low level of hemoglobin.

In those babies in whom replacement was accomplished, there was a correlation between the hemoglobin level observed the day after therapy and the time at which the minimal value was found, with the value decreasing at an approximate rate of a gram a week. Those patients who did not receive replacement transfusion differed from the treated group in that minimal values were reached more rapidly, as one would expect with hemolysis of the patient's own red cells.

This minimal value may be looked upon as the point at which the marrow is able to produce more red cells than are being destroyed. In those patients in whom the decline is going on at a normal rate of red cell destruction and in whom the reticulocyte count is at low levels, one may consider it as the point at which the marrow is finally able to respond by increasing production. This may be demonstrated more directly by the slight reticulocytosis which was seen approximately a week before the minimal value was reached. However, its onset was difficult to time, and occasionally it was missed.

In the babies whose blood was replaced, evidence of antibody disappeared in the post-exchange sample, but when the patients' own cells reappeared in adequate amounts, a positive Coombs test was found for periods up to 12 weeks after birth. There was no correlation between the duration of antibody and the degree of anemia or the time at which this lowest hemoglobin value was reached.

Neither was there any suggestion that “universal donor blood” was responsible for the seeming marrow depression in these babies. Twelve with A or B antigen who received O blood had a minimal value of 7.8 Gm. per cent and 7 receiving compatible blood had an average of 6.8 Gm. per cent.

To determine whether there was an abnormal response of the red cells to physical stress in the form of osmotic and mechanical fragility tests, serial observations were made at varying stages of the disease. Comparative figures describing osmotic curves of adults, of control infants less than a week old, and of erythroblastotic infants of the same age are presented in table 1. The controls differ from normal adults in that their curve is wider, lower, and bimodal. The left limit of hemolysis is more extreme while the major mode is more resistant to hemolysis. The osmotic fragility curves of samples of blood from erythroblastotic babies differed both from normal infants and from adults in the increased susceptibility of the major population to osmotic stress as evidenced by
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In this table average values are given for the osmotic curves of the blood of normal adults, normal newborn infants and infants with erythroblastosis fetalis. The width is measured in terms of 10 units between each 0.04 per cent NaCl decrement of the curve. Since half the patients had a single mode curve, values are listed separately for each type.

a shift of the major mode to the left. The curves were more often unimodal; the average curve was slightly narrower and higher than control samples but the range was wide. The limits were similar to normals in the tendency toward increased fragility compared to adult blood. Examples are plotted in figure 3 where they may be compared with normal newborn and adult curves.

Serial samples were drawn from 5 babies who did not receive replacement therapy. Throughout the course the curves were characteristically unimodal and in general somewhat higher and narrower than controls. They were all within normal limits by the age of 4 weeks.

In those patients who received replacement therapy, the immediately post-treatment sample showed evidence of the "storage lesion" with an increase in fragility. This was not present in later samples.

When osmotic fragility was observed at the time of minimal hemoglobin level, it became narrower without significant change in the location of the major mode. For a short time thereafter there was a tendency for bimodality of the curves, and then the single mode type predominated as in those of control samples at this age.

There was no correlation between the bilirubin level, hemoglobin value or reticulocyte count and the limits, width or modes of the osmotic fragility curve, with the exception that those curves with the limits most markedly to the left did have elevated reticulocyte counts. There was no suggestion that the survival of antibody in the infant's circulation influenced the tests.

Mechanical fragility of red cells in normal infants also differed from adults. In the first week of life, hemolysis ranged from 5 to 9.4 per cent after rotation of the sample for 80 minutes, and after 4 hours it varied between 8.8 and 17 per cent. The curve was similar for the first few months. In the adult, the values ranged from 2 to 6 per cent at 80 minutes with the 4 hour values ranging from 6.5 to 10.2 per cent.

The average mechanical fragility of blood from infants with erythroblastosis fetalis was greater than normal, but not strikingly so, and in 16 babies' specimens taken during the first 4 days, only 4 had elevated values after 80 minutes'
rotation. When the test was extended, more abnormal values could be demonstrated.

Mechanical fragility studies during the course of the disease were characteristically within normal limits after replacement therapy. In the untreated group, increased fragility was observed in a significant number until the minimal
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Fig. 4.—These curves demonstrate the mechanical fragility of the red cells at given time intervals. Percentage hemolysis is plotted on the abscissa and the number of hours of mechanical trauma on the ordinate. The number at the end of the curves represents the day of the test. In the first figure the normal range for the first three months is drawn. The mechanical fragility in a case of anti-A is presented in the second, where increased fragility is demonstrated at 4 days. In the case of anti-D (3.), abnormal fragility is still evident at 32 days with 4 hours of trauma. The fourth figure represents multiple tests on the blood of a patient after replacement therapy, all of which are within normal limits.

Hemoglobin level was reached. In figure 4, changes in the mechanical fragility during the course of the disease are charted.

There was no relation of the mechanical fragility to the severity of the disease as judged by bilirubin level, hemoglobin value or reticulocyte count. While the 4 early samples with elevated values were accompanied by higher bilirubin values, they all appeared in blood from babies over 40 hours of age with the normal results being found at an earlier age. In tests done later in the course of the disease, there was no relation between serum bilirubin and mechanical fragility.

Discussion

While replacement transfusion has modified the course of erythroblastosis fetalis by allowing the elimination of uncontrolled hemolysis with severe anemia and jaundice, it has allowed observation of more subtle manifestations of this disease. The post-transfusion reticulocytosis occurring in the absence of con-
continuing anemia demonstrates the completion of a process in action prior to therapy. In the milder untreated cases there was a similar curve with a peak during the first two days followed by a gradual decline. The nucleated red cells may also show this same brief return to elevated levels after replacement therapy, and it is probable that these arise from remaining extra-medullary hematopoiesis.

The platelets deviated significantly from normal after replacement. For several days, all values were strikingly low until a gradual rise began on the third day. This decline could be due to the short survival of platelets stored in glass, to an effect of the plasma transfused, or to the thrombocytopenia which accompanies hemorrhage. Thus the curve of platelet response may be depressed and therefore not a true picture of the usual platelet production in this state. Since no evidence of bleeding tendency was present in these babies, another factor must be superimposed on thrombocytopenia to account for the bleeding tendency reported in babies with this disease and also in adults who have received virtual replacement of their own blood by transfusion.

The late manifestation of anemia in erythroblastosis fetalis has been recognized in the past and Diamond has emphasized the lack of response of the disease at this stage to any known therapy. The picture was also described in a recent case report. It is seen in mild as well as severe cases and is not prevented by replacement therapy. This suggests that the defect, which is apparently a hypofunctioning marrow, is not merely the result of “strain” of excessive blood production but is a primary lesion in the disease.

The present study supplements other reports of red cell fragility in erythroblastosis fetalis. The striking change is in the shape of the curve, not in the limits, which are not abnormal in this disease when compared with controls of the same age group. There is a shift of the major red cell population to a more fragile zone. Instead of the bimodal curve seen in controls, a unimodal was often seen, and when a multimodal distribution was present there was a predominance of the red cell population at the “fragile” end of the curve rather than at the “resistant” end, which was the site of the major mode in the controls. In patients who did not receive replacement transfusion, this abnormality disappeared within 4 weeks, although antibody was present beyond this time.

The mechanical fragility probably reflects a defect in the red cell membrane allowing increased susceptibility to trauma. In those cases in which it was found, the abnormal samples were taken after the first day of life and prior to the time of minimal hemoglobin level.

The response to these two forms of physical stress in the red cells of infants with this disease demonstrates abnormalities in these cells. The lack of relation of these changes to other criteria of the disease suggests that they may not be directly related to the hemolytic mechanism.

**Summary**

Serial studies revealed characteristic changes in the peripheral blood of infants with erythroblastosis fetalis. In the presence of elevated nucleated red cells and reticulocytosis, replacement transfusion brought about a momentary decline to normal levels followed by a second rise during the succeeding three
days before the values returned to low levels. Platelet counts diminished within a day after replacement and only gradually rose toward normal after approximately three days. Such a picture was not seen in the mildly affected babies who did not receive replacement therapy, in whom the platelet values varied.

Within the first three months in both groups anemia developed in the presence of little evidence of compensating marrow activity. After a decline of the hemoglobin to an average level of 7.6 Gm. per cent, erythropoiesis became adequate for return to normal levels. This hypoplastic defect could not be correlated with any other parameter of the disease.

Abnormalities were noted in the osmotic and mechanical fragility of the red cells in this disease. The major population of cells was found to be more susceptible to osmotic stress while the limits of hemolysis were within the normal range. The osmotic curves of the blood of those patients who were not transfused returned to normal within 4 weeks. Mechanical fragility of the red cells was increased in a significant number, and this abnormality was observed until the time when minimal hemoglobin values were reached. There was no correlation of these variations in fragility with other laboratory or clinical manifestations of the disease.

**SUMMARIO IN INTERLINGUA**

Studios serial de neonatos con erythroblastosis fetal ha revelate characteristic cambiamentos in le sanguine peripheric. In le presentia de elevate contos de nucleate erythrocytos e de reticulocyteosis, transfusion de reimplicamiento causa un momentari declino a nivellos normal sequite per un secunde elevation durante le proxime tres dies ante que le valores retornava a basse nivellos. Contos de plachettas descendeva intra umi die post transfusion de reimplicamiento e ascedeva solo gradualmente verso le norma post circa tres dies. Iste situation non eseva incontrate in levemente afficite babies qui non recipeva therapia reimplicamential. In illes le contos del plachettas variava.

Durante le prime tres menses anemia se disveloppava in ambe gruppos con pauc evidentia de activitates compensatori del medulla. Post ummi declinio del hemoglobin a un nivello median de 7,6 g pro cento, erythropoiese deveniva adequate pro effectuar un retorno a nivellos normal. Iste defecto hypoplastic non poteva esser correlationate con ulle altere parametrom del morbo.

Anormalitades eseva notate in le fragilitate osmotic e mechanic del erythrocytos in iste morbo. Le majoritate del cellulas se revelava como plus susceptibile al stress osmotic durante que le limits del hemolyse se teneva intra variationes normal. Le curvas osmotic del sanguine de pacientes sin transfusion retornava al norma intra 4 septimeanas. Le fragilitate mechanic de erythrocytos eseva augmentate in un significative numero de casos, e iste anormalitate eseva observate usque le tempore quando valores minimal de hemoglobina eseva attingite. Nulle correlation eseva constatate inter iste variationes del fragilitate e altere manifestationes clinic o laboratorial del morbo.

**REFERENCES**

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