THE TREATMENT OF ERYTHROBLASTOSIS FOETALIS

BY

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Since 1941, when Levine, Katzin, and Burnham demonstrated that haemolytic disease of the newborn may be due to immunization of the mother by the Rhesus antigen, the investigations of Wiener and his colleagues (1946), and of Coombs, Mourant, and Race (1945), have seen prediction brilliantly fulfilled. The most recent aid in haematological diagnosis has been the anti-human globulin rabbit serum of Coombs et al. (1945), with which non-specific sensitization of the baby's cells by a maternal antibody may be shown.

The mechanism of the production of erythroblastosis is now understood, at least in the majority of cases, but attention has been focused on the etiology to a greater extent than on the treatment of the condition. There remains a large field for investigation into the best means, firstly, of treating the haemolytic aspect, and, secondly, of preventing the late sequelae of the disease. Among the latter, hepatosplenomegaly (Drummond and Watkins, 1946), kernikterus, and mental defect are known to occur, and it is probable that other conditions, notably spastic diplegia, will be shown on further investigation to be part of the aftermath of so-called erythroblastosis foetalis.

Treatment at the present time is properly concerned with the transfusion of Rh-negative blood, providing the baby with a vehicle for oxygen-carrying which should be immune from the influence of the agglutinin causing haemolysis of its Rh-positive cells. Preservation of life is the primary consideration, and the maternal agglutinin present in the baby is left to take care of itself. It is this antibody, however, present either in its complete or 'incomplete' form, which is responsible for the haemolysis and the later phenomena, and Wiener (1946) has suggested that the incomplete form may be more potentially dangerous than the complete antibody.

Only rarely has the anti-Rh agglutinin been demonstrated in the child's serum (Coombs et al., 1946) although Baar (1945) has shown that the incomplete antibody is present in roughly 50 per cent. of babies in a small series of cases. Wiener has postulated different molecular sizes for complete and incomplete agglutinin, and there is agreement that, having been called forth in the mother, they pass across the placental site to exert their effect on the baby's cells. Thereafter little is known of their fate, but they presumably may last in the baby's circulation and tissues for two to three weeks, as do other immune bodies.

Although maternal antibody is not often directly demonstrable in the foetal serum, its presence may be inferred from the fact that at birth the foetal cells are sensitized, as shown by the anti-globulin serum test. How long its presence continues is not known, and so the blood of affected babies was investigated to ascertain how long antibody survives in sufficient quantity to sensitize the red cells. It is possible that some sensitized red cells will outlast the presence of 'free' antibody in the serum.

Investigation

Ten consecutive babies admitted to this hospital with erythroblastosis were investigated. In every case there was a parental Rh incompatibility and a maternal agglutinin was present. The red cells of each baby were found to be sensitized on admission, and direct sensitization tests were performed at weekly intervals until the cells gave a negative reaction. The results are shown in fig. 1. It will be seen that in nine out of the ten cases the antibody had ceased to sensitize the cells after twenty-eight days, and that in the last case the test had become negative after a further week.

There was some superficial relationship between the maternal antibody titre and the duration of foetal sensitization, and this is illustrated in fig. 2. In all cases the antibody titre was estimated on the fourteenth day after parturition, when the titre is normally

FIG. 1.—Duration of sensitization.

FIG. 2.—Maternal titre in relation to duration of sensitization.

at its highest. It is not intended to draw any conclusions from such small figures, but the findings are in contrast to the lack of correlation between maternal antibody titre and the clinical severity of erythroblastosis.

From this investigation it appears that maternal antibody cannot be demonstrated in the foetal serum, either directly or indirectly, later than a month after birth. After the antibody has disappeared the haemolysis should cease and recovery in the blood picture should take place, but this improvement notoriously does not occur in many cases. The following case illustrates this failure.

Case 1. Baby P., the second child of healthy parents, was admitted jaundiced at three days old on June 2, 1946, with clinical erythroblastosis. The first child was normal. On admission the red cell count was 1,620,000 per c.mm., haemoglobin 30 per cent. (Sahli) or 4.2 g. per 100 c.cm. The white cells numbered 24,000 per c.mm. and late normoblasts 19,000 per c.mm., reticulocytes 11 per cent. The red cells were sensitized, but no agglutinin was demonstrable in the foetal serum. The child was group A CDe cde, the father group A CDe cde, and the mother group A cde cde with an anti-CD titre of 8 on the fourteenth day after birth. The subsequent blood history of the baby is shown in table 1.

How much longer this child will need blood replacements remains to be seen. He has already had nearly twice his birth volume of whole blood, and the count is still only about half what it should be.

In cases similar to this one, an actual marrow dysplasia appears to be present, and in several such children a series of marrow counts was made in parallel with the blood counts. A typical example is the following, only the more important of the many investigations carried out being included.

Case 2. Baby M., a second child, was admitted with erythroblastosis on February 23, 1946. The blood group was A CDe cde, and the maternal antibody titre on the fourteenth day after birth was 256. Between the date of admission and April 1, 1946, the child received eight transfusions, totalling 750 c.cm. of Rh-negative blood, or more than twice his own blood volume. In spite of this the blood count on April 2, was still falling, although no Rh-positive cells were present, and, due to donor's blood, the

TABLE 1

Date	Red cells per c.mm. (millions)	Treatment and condition
2.6.46	1.6	Transfused with 120 c.cm. of Rh-negative blood.
5.6.46	4.3	
9.6.46	3.4	Transfused with 80 c. cm. of blood.
10.6.46	5.03	
11.6.46	4.5	The jaundice had disappeared.
22.6.46	4.1	The cells were still sensitized.
30.6.46	3.0	The cells were no longer sensitized.
7.7.46	2.2	Transfused with 80 c.cm. of blood.
13.7.46	2.7	
15.7.46	2.1	Transfused with 90 c.cm. of blood.
20.7.46	3.3	
27.7.46	2.5	Transfused with 150 c.cm. of blood.
30.7.46	4.9	
7.8.46	2.9	

peripheral blood group was O (Cathie, 1946). At this stage the marrow was counted and was found to contain only 2 per cent. of late normoblasts and 0.5 per cent. of intermediate normoblasts. In view of this hypoplasia, further transfusions were withheld to see if the degree of anaemia would cause the marrow to undergo some hyperplasia (table 2).

TABLE 2

Date	Red cells per c.mm. (millions)	Marrow nucleated reds (per cent.)
2.4.46	4.2	2.5
25.4.46	3.6	
27.4.46	2.6	
30.4.46	3.1	13.0
2.5.46	3.2	
16.5.46	3.3	24.0
20.6.46	4.5	26.0

Transfusion

These two cases raise a series of pertinent questions concerning the transfusion of blood. They are not isolated, but cases representative of the majority of the babies admitted to this hospital with erythroblastosis. The more important questions are: at what level of anaemia shall blood be given, how much shall be given, at what speed and by what method, and, lastly, what type of blood?

Whitby and Britton (1946) say that twelve days after birth the red cell count is 5 to 5½ millions, although personal experience would place the count somewhat lower. Chuinard, Osgood, and Ellis (1941) give the average count as 4.6 million, with a normal range from 5.8 to 3.4 million. If this low figure of 3.4 million is taken to be the lowest limit of normality, then any count below it in the neonatal period indicates anaemia, and correction of this anaemia

when the baby is first seen in the stage of active haemolysis, and later when a varying number of donor cells are present, calls for somewhat different treatment.

In the early stages of erythroblastosis there is usually some degree of anaemia; many of the red cells are reticulocytes, while others are basophilic macrocytes. Normoblasts may be present in small numbers or up to 100,000 or more per c.mm. and the general blood picture suggests deficiency of oxygen-carrying powers. Experience has shown that the anaemia is progressive, and there is little point in withholding immediate transfusion of Rh-negative blood. Enough should be given to bring the red cell count up to about 4.5 million. Theoretically these Rh-negative cells should be unaffected by Rh-agglutinins and should survive for a normal time apart from obsolescence. In many cases, however, continued haemolysis, after more than enough Rh-negative cells have been given entirely to replace the circulation, suggests that other factors apart from agglutinins and normal wear and tear are involved in the production of anaemia.

After the initial transfusion, the blood count usually falls slowly to a level when more blood is indicated, but this level is difficult to decide upon. In case 2, quoted above, the count was allowed to fall to 2.6 million, after which it rose of its own accord to within normal limits. Probably about 2.5 million is the lowest to which the count should be allowed to fall, on account of the possible dangers of tissue damage due to anoxaemia, and at this level a further transfusion should be given.

As a general principle the count should not be raised to 5 million, for two reasons. Firstly, the count is not maintained at this level, and the resulting haemolysis may throw an added strain on an already potentially damaged liver. Secondly, the bone marrow must be assisted in every way to produce its own cells, and while a small blood transfusion may act as a stimulus to production a normal peripheral count will tend to allow the marrow to lie fallow.

The majority of babies seem to fix for themselves a rather low red cell count, usually between 3 and 3.5 million, and on this they thrive and put on weight. Above this level they lyse the peripheral red cells and return to their individual level. Therefore enough blood should be given after the initial transfusion to bring the count to about 3.5 million as often as it has fallen to 2.5 million. Many babies have been treated on these lines and have done uniformly well, the count eventually stabilizing in the region of 3 million for some weeks before starting to rise slowly towards the figure of 4.5 million.

The following case of affected twins shows that low blood figures may be maintained without exciting attention. The male child was a case of moderate erythroblastosis, while the female was apparently clinically unaffected.

Case 3. Baby John S., was born on June 2, 1946, and admitted as a case of clinical erythroblastosis on the sixth day after birth. His group was B CDe cde.

The mother was B cde cde, and the father O CDe CDe. The female twin's group was O CDe cde. The red cells of both twins were sensitized. After a stormy blood history, in which several transfusions were necessary, improvement in the boy's condition started (table 3).

TABLE 3

Date	Red cells per c.mm. (million)		Treatment and condition
	Boy	Girl	
12.7.46	3.3		Cells of both babies now gave a negative sensitization test, and both were agglutinated by maternal serum to a titre of 1 in 256. Boy transfused with 60 c.cm. Rh-negative blood.
17.7.46	2.8	3.6	
29.7.46	3.3	3.0	
8.8.46	3.9	3.4	

Comment

There is no doubt that here both twins were affected. Both twins' cells were sensitized, and the maternal serum agglutinated the cells of each to the same titre. It is not clear why the boy manifested erythroblastosis clinically, while the girl had a transient so-called physiological jaundice which had disappeared before her brother was admitted to hospital. And while the blood count of the boy was considered low and he was not thriving, the girl continued to put on weight; it was not until her blood group was checked that her count was found to be lower than that of the brother, who was receiving all the attention.

The amount of blood to be given to obtain a desired rise may be calculated from the red cell count or the haemoglobin percentage, and, as the anaemia is normocytic, either may be used. The blood volume in infants is assumed to be between 40 and 50 c.cm. per lb. of body weight, and to err on the safe side the lower figure of 40 may be used for calculation. Gimson (1943), using haemoglobin levels, gives the formula

$$\frac{\text{desired percentage rise in haemoglobin} \times \text{blood volume}}{100}$$

for estimating the amount of blood to be transfused. As, however, hyperbilirubinaemia is so frequent in erythroblastosis and gives rise to such faulty haemoglobin readings, blood amounts based on the red cell count are preferred. Using the red count the amount of citrated donor blood to be transfused is given by the formula

$$\frac{\text{desired count} - \text{actual count}}{\text{desired count}} \times \text{blood volume.}$$

The formula is not mathematically correct, but works in practice. For instance, suppose it is desired to raise a count of 3 million to 4 million. The desired

count of 4 million minus the actual count of 3 million is 1 million. This, divided by the desired count of 4 million, gives $\frac{1}{4}$. If the baby weighs 7 lb. the approximate blood volume will be 7×40 , or 280 c.cm., so that the necessary amount of blood is $\frac{1}{4}$ of 280, or 70 c.cm.

It has recently been shown that some donor cells may survive after transfusion as long as 120 days, after which they are no longer demonstrable. When taken from the donor these cells will be in all stages of maturation, and, while the longest surviving cells may reasonably be assumed to be the most recently produced by the donor, the older transfused cells will have a shorter life. If the graph of days and number of cells surviving is a straight line, there will be a small reduction daily in the recipient's red cell count. In case 2, none of the child's own cells remained, all the circulating red cells being donor cells, which brought the blood count to 5 million. In such a case a daily fall of, roughly, 1 per cent. of haemoglobin would be expected if normal obsolescence occurred with no replacement from the recipient's marrow. That other factors are involved in the production of the anaemia is shown by the fact that more than double the blood volume of whole Rh-negative blood had been given to this baby in six weeks and the blood count was then only about half of what it should have been. Differential cell survival estimations were not necessary, as, had all the Rh-negative cells transfused survived, the peripheral blood count would have been in the region of 12 million.

Blood may be transfused by cannulization of a vein, or directly into a scalp vein with a small intravenous needle. For the initial large transfusion the blood is best given slowly, by means of a cannula tied into a vein, at the rate of 15–20 c.cm. per hour. The total volume of blood to be transfused is seldom more than 200 c.cm., so that it may conveniently be given during the course of a day. This slow rate allows the baby to deal with the excess of fluid by normal excretion. During the transfusion the baby's spleen may be felt to enlarge, and presumably becomes engorged with blood, and for this reason a blood count to check the result of the transfusion is best delayed for twelve hours, when the spleen has shrunk and a representative count may be obtained.

There is a limited number of veins in the infant which can be cannulized, and transfusions subsequent to the initial one may be given into a scalp vein. The amount of blood to be given for maintenance is usually small, and the method results in a considerable saving of the time of the operator and that of the nursing staff. It is preferred to the marrow infusion method of Gimson (1944) because of the risks of sepsis attaching to the latter.

The apparatus necessary for a scalp vein transfusion is a 20 c.cm. syringe and a two-way adaptor connecting to a blood reservoir on the one hand and a Luer-Kaufman syringe on the other. The Luer-Kaufman syringe is of about 2 c.cm. capacity and has a side arm half way along the barrel; the plunger has a small metal chain connecting it to the barrel, so

that pressure through the side arm cannot expel the plunger. After washing the apparatus through with saline, blood is propelled from the 20 c.cm. syringe through the connecting rubber tubing into the small syringe. The small syringe is then emptied, and sterile saline taken into it so that the plunger stops short of the blood-containing side arm.

The largest and straightest scalp veins are usually found above the ear, and one of these is entered with a small needle on the Luer-Kaufman syringe. When blood is seen entering the syringe the plunger is drawn back past the side arm, and gentle pressure on the 20 c.cm. syringe allows blood to flow into the vein. By this technique blood may be given at the rate of 60 to 80 c.cm. in fifteen to twenty minutes.

The potential drawback to the method is that the rapid increase in circulating volume may embarrass the recipient's heart action, although very many transfusions have been given without untoward effects. In view of this possibility, however, latterly concentrated cells have been given, concentration being achieved by removing the supernatant plasma after standing the bottle of blood for between twelve and twenty-four hours.

Economic and sociological factors lend weight to the advantages of scalp vein transfusion. Firstly, it is often three months or longer before erythroblastotic infants can be released from strict supervision, but to keep every affected baby in hospital for this length of time would be impracticable. Control of the blood and clinical conditions can be maintained while they are out-patients, and a small transfusion may be given as necessary, the baby being allowed home after a rest of an hour or so. Secondly, erythroblastosis usually occurs in second and later children, which means that there are other children at home needing maternal care, and the admission to hospital of a nursing mother while her baby is given a formal transfusion throws a strain on the home life and the indulgence of the neighbours.

The standard treatment of Rh incompatibility is the transfusion of Rh-negative blood. As the baby's serum contains anti-Rh agglutinin derived from the mother, which will react with Rh-positive cells, the treatment is essentially sound, and consists in replacing lysed blood by inert cells until such time as the agglutinin is no longer active. The suggestion has frequently been made that Rh-positive blood should be given in order to be agglutinated by the antibody, in the hope that thereby the antibody would be more rapidly removed from the circulation.

Investigation. In order to examine the possibilities of this suggestion the following experiments were carried out. Unsensitized CDe CDe cells were lysed by alternate freezing and thawing, and the stroma, after being centrifuged down, was treated with anti-CD serum. The titre of the serum after incubation with the stroma fell from 1 in 32 to 1 in 4, showing that the stromal antigen was still capable of absorbing agglutinin. CDe CDe cells were next treated with homologous antibody at 37° C. for two hours. They were then washed three times in saline to remove all trace of agglutinating serum, and were resuspended in saline. After freezing and thawing

to lyse the cells, the stroma was removed by centrifuging. The supernatant fluid was found to agglutinate CDe cells, showing that some antibody had been freed from the lysed red cells.

These experiments indicate that Rh-positive blood should not be given in erythroblastosis, as, although some of the agglutinin will be retained on the cells and stroma, some will be released from the cells when they are lysed and will be free to act upon further Rh-positive cells, so that the antibody comes to act almost as a catalyst.

A further suggestion with regard to treatment is that where a pregnant woman is known to have antibodies the infant should be exsanguinated at birth, the circulation being replaced by inert Rh-negative blood. On theoretical grounds such treatment is not likely to be successful, apart from its difficulties and dangers. The foetus is subject to the action of the maternal antibody for the whole nine months of pregnancy, during which time much of the fixation of the antibody in the tissues should have taken place. As was seen in the sensitization investigation reported above, the antibody lasts in the infant only a short time after birth, the longest period being for a month. Thus the antibody can act for a total period of ten months, and the prospects of improved results by cutting this time down to nine months must be slight, while the heroic measure of exsanguination cannot be without risk. The distressing sequelae of erythroblastosis, such as kernikterus and mental defect, have probably been determined during intra-uterine life, but exsanguination theoretically should help the condition of haemolytic anaemia by removing circulating antibody. But that the anaemia is not due entirely to antigen-antibody reaction is shown by the cases in which there is clear evidence that not only Rh-positive cells are lysed, but that Rh-negative cells also disappear from the circulation, sometimes almost as fast as they can be transfused.

Weaning of erythroblastotic infants on account of the high titre of antibody often present in the maternal milk has been recommended. A few experiments have been made in which high-titre Rh-antibody was drunken, and in no case has antibody been demonstrable in the blood thereafter, either by direct or sensitization tests. The anaemic child is notably susceptible to infection, and the weaned child even more so, and the theoretical disadvantages of breast feeding are so overwhelmingly outweighed by the practical advantages that the suggestion of weaning cannot be supported as long as breast feeding is possible.

Comment

Some cases of erythroblastosis make an uneventful recovery after one transfusion, while others are so mild that no treatment other than expectant is necessary, as, for example, the untreated twin in case 3. These are rare, however, and the majority of cases call for more intensive treatment. The foregoing remarks on this treatment are the conclusions drawn from an analysis of the last 38 cases

of Rh incompatibility seen in which a maternal antibody was present. There is a small group in which no incompatibility or antibody can be shown but which in every other respect have erythroblastosis, and in these cases efforts to maintain a higher blood level by transfusion have been more successful than in the demonstrable incompatibility cases.

Excluding those infants who were admitted moribund, and who usually died before treatment could be instituted, there was no case of sudden death in the series. This may have been due to the close supervision that was maintained over all the children until their blood counts stayed spontaneously within normal limits, but, unlike the acute haemolytic crises of the Lederer type of anaemia, the fall in the blood count in erythroblastosis is gradual and progressive and an impending dangerous degree of anaemia can usually be foreseen and rectified.

Blood transfusion is at present the only form of treatment available and appears to correct only the anaemic aspect of the disease. Kernikterus, spasticity, and many other afflictions which will doubtless be shown to have their origin in Rh incompatibility, do not seem to be averted by transfusion. In the light of the recent work of Drummond and Watkins (1946), a ten-year follow-up would appear to be necessary before any reliable figures of the incidence of sequelae can be obtained.

Jaundice may be haematogenous or hepatogenous in origin. It has been suggested that liver damage and not haemolysis is the cause of central nervous disease, and the blood and liver components of the disease syndrome have come to be regarded almost as separate entities. It is impossible to subdivide the syndrome into clear-cut compartments in this fashion. Kernikterus can occur with no history of jaundice, while the worst jaundiced babies may never show any sign of nervous involvement. That the anaemia is based on a deeper pathology than a mere intravascular haemolysis is shown by the marrow depression present in so many infants, and it is reasonable to suppose that the erythroblastosis syndrome is, in fact, the result of a series of widespread antigen-antibody reactions in the various tissues, any or all of which may be affected. The immediate effects and late sequelae are merely manifestations of localized tissue damage. That not all the damage is irreversible is shown by the occasional unblocking of blocked bile ducts and by the recovery of the bone marrow hypoplasia under treatment.

It should be borne in mind that blood transfusion is only a replacement therapy. As long as incompatible matings occur, erythroblastosis will be seen, and eventually the ideal treatment of the condition must be its prevention by fixation or elimination of the maternal antibody as it is formed. But until all pregnant women can be accurately blood-typed such a prophylactic ideal, even if there were any method of dealing with the antibody, is unattainable, and for the affected children transfusion of Rh-negative blood must continue as the treatment of choice.

Summary

The indications for and methods of giving blood to erythroblastotic babies are discussed and described. Illustrative cases are cited.

Experimental work is reported to show, in cases of erythroblastosis: (a) the duration of sensitization of foetal red cells by maternal antibody; (b) that the transfusion of Rh-positive blood is contra-indicated; (c) that the dangers of feeding breast milk containing atypical antibodies have been over-estimated.

I am indebted to the physicians of the Hospital for Sick Children, Great Ormond Street, London, for access to their cases, and to Dr. A. D. Barlow for demonstration of transfusion techniques.

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