TREATMENT OF 160 CASES OF ERYTHROBLASTOSIS FOETALIS WITH REPLACEMENT TRANSFUSION*

BY

J. J. van LOGHEM, M.D.

J. H. van BOLHUIS, M.D.

J. M. SOETERS, M.B.

AND

G. M. H. VEENEKLAAS, M.D.

(From the Blood Grouping Department of the Central Laboratory of the Dutch Red Cross Blood Transfusion Service, Binnengasthuis, Amsterdam; Department of Paediatrics, University Hospital, Leiden; St. Ignatius Hospital, Breda; and Wilhelmina Children's University Hospital, Utrecht)

The discovery that Rh antagonism is a cause of erythroblastosis foetalis has revolutionized the treatment of this disease. Many authors consider early induction of labour to be a method of some importance in protecting the infant as much as possible against the attack of Rh antibodies, which pass the placenta mainly towards the end of pregnancy; even caesarean section is applied to prevent their penetration during labour. However, from several points of view this measure is open to discussion. Transfusions with packed Rh-negative cells or with Rh-negative whole blood have been widely applied with success. Maternal milk is often withheld because of the possibility of Rh antibodies being harmful in the first days of life through permeability of the intestinal tract. The application of vitamin K as a protection against haemorrhagic diathesis is still in use.

Owing to the studies of Wiener *et al.* (1947), Wallerstein (1947), Diamond (1947), and other workers a new method of treatment has been introduced. The principle of this method consists in replacing the Rh-positive infant's blood by Rh-negative blood of the homologous ABO group in order to eliminate the Rh-positive red cells, which will be rapidly haemolysed. At the same time Rh antibodies in the infant's circulation are washed out.

Although the different authors base their method of treatment on the same principle, the technique can be applied in various ways.

Wallerstein's method consists of infusion of blood into one of the greater veins, the blood being withdrawn with syringes out of the sinus sagittalis longitudinalis.

Wiener uses the saphenous vein for infusion of the blood and the radial artery for bleeding. It is very important to balance the rates of inflow and egress in order to prevent plethora by too rapid a transfusion, and to prevent shock from too rapid withdrawal of blood. In order to prevent clotting of blood the child is heparinized (Wiener and Wexler, 1946). Wiener, Wexler, and Shulman (1948) succeeded very well even with massive transfusions of 1,000 ml.

Diamond's method consists in introducing a plastic catheter into the umbilical vein. The exchange (or replacement) transfusion is performed by repeatedly replacing small quantities (20 ml.) of the infant's blood by citrated fresh donor's blood. He uses a closed system with the aid of three-way valves between catheter, syringe, and blood-transfusion ampoule.

In Holland exchange transfusion was started in January, 1947, as soon as the necessary information was obtained from Diamond and Wiener (van Loghem, 1948; van Loghem *et al.*, 1948). Exchange transfusion has been performed in 160 cases in two years by 43 physicians.* Nearly all the serological examinations were carried out in the Blood Grouping Department of the Central Laboratory of the Netherlands Red Cross.

Under the direction of the Working Committee for Rh Investigation courses of instruction were given; these greatly helped in acquainting physicians with simple methods of serological Rh-investigation and with the technique of exchange transfusion.

Diamond's method is very easy to perform, and was done even 40 hours after birth. Wiener's more complicated method does not offer any special advantage, except when the umbilical vein is obliterated, and even then it may be possible to use Diamond's method in one of the greater veins (vena femoralis). In only a few transfusions have we used Wiener's system.

Recently another method has been described which offers the possibility of performing the exchange transfusion through the umbilical vein a few days after birth, when the passage of the umbilical cord is obstructed. Pinkus (1948) described a new approach to the umbilical vein above the insertion of the umbilical cord.

Method of Treatment

Through lack of the plastic catheters recommended by Diamond one of us (Soeters, 1947) tried a rubber nelaton catheter which has now come into general use (No. 6—inner diameter 1 mm., outer diameter 2.6 mm. No. 8—inner diameter 1.5 mm., outer diameter 3.1 mm.—is also very useful). These catheters are very easy to insert into the umbilical vein. They are slightly modified—the end of the catheters being opened to facilitate the inflow of blood, or even cut off and well polished. One advantage they have over plastic catheters is that they are easy to sterilize by boiling and are very elastic. Furthermore, the end not being sharp, there is no danger of damaging the venous wall. A small glass tube is inserted between the rubber catheter and the adapter in order to control the inflow of blood and to prevent the inlet of bubbles of air. During the exchange of syringes the catheter is carefully closed by arterial forceps. One should be very careful in using the syringes. After every withdrawal and injection of blood they should be thoroughly washed in saline with heparin, and afterwards in pure saline. We use syringes of 20 ml. An assistant fills the syringes while the physician injects and withdraws the blood.

Heparinized blood is mostly employed for the exchange transfusion. " Heparine vitrum " (Brocades and Stheemann, Amsterdam), 20 mg. in 10 ml. of saline, is mixed with 500 ml. of donor blood of the homologous blood group. Heparinized blood should be administered within a few hours after taking, because heparin is apt to denaturate rather quickly. In general the infant is not heparinized. During the exchange transfusion the catheter is washed a few times with 1 or 2 ml. of a weak solution of heparin (2 mg. of heparin per ml.) in order to prevent clotting in the catheter. In our experience this is quite sufficient, although some prefer to heparinize the child by the injection of 10 mg. of heparin into the umbilical vein or intramuscularly before beginning the exchange transfusion.

If citrated blood is used, 90 ml. of 3.3% disodium citrate and 15% glucose is sufficient for 500 ml. of blood. Mollison

*Report of the Dutch Foundation for Blood Group Research.

*We are greatly indebted to all the physicians who have provided us with detailed clinical data of their exsanguinated babies and the necessary information about the histories of the pregnancies.

and Cutbush (1948) use the same quantity, 2.5 g. of disodium citrate and 3 g. of glucose, but dissolved in 50 ml. of distilled water, which has the advantage of preventing dilution of the blood so far as is possible. To increase the haemoglobin content of red cells of the donor blood Mollison eliminates about 50–100 ml. of the supernatant plasma citrate. By using citrated blood there is some risk of causing tetany by hypocalcaemia. About 5 ml. of a 10% solution of calcium gluconate is equivalent to the prescribed quantity of disodium citrate. To prevent tetany small quantities of calcium gluconate solution may be injected intravenously (about 1 ml. after every 100 ml. of citrated blood). It is also possible to administer calcium gluconate through the catheter, but previously the catheter should be washed carefully with saline in order to prevent clotting due to the presence of citrated blood.

As nearly all mothers of erythroblastotic children were submitted to an antenatal examination and were admitted to the hospital before delivery, it was possible to perform the exchange transfusion in nearly all cases immediately after birth, which has the advantage that the procedure goes very smoothly because of the free passage through the umbilical vein. It is also important from a therapeutic point of view to remove the Rh-positive blood and the Rh antibodies as soon as possible.

The infant is placed on an operation table in a well-heated room and held by a nurse. After disinfecting the abdominal skin with iodine the child is covered with sterile blankets and kept warm with electric cushions. Through the window (2 by 12 cm.) in the blanket the umbilical cord is brought outside. The operator, assistant, and nurse should take all the aseptic precautions necessary for an ordinary operation.

Before inserting the catheter the umbilical cord is cut off at a distance of about 1 or 2 cm. The cord is kept open with two forceps by an assistant, and the catheter is inserted into the great umbilical vein (6–8 cm.).

A stricture caused by fibrous tissue in the linea alba may obstruct the introduction of the catheter. It is always possible to pass this stricture—if necessary by dilatation by means of a sound. In any case it is advisable to cut off the cord very close to the skin to avoid unnecessary difficulty.

One of us (J.M.S.) studied the venous system of the newborn by means of *x*-ray photographs of cadavers after filling the venous system with thorotrast. In this way the catheter's route could be followed. It is possible to insert

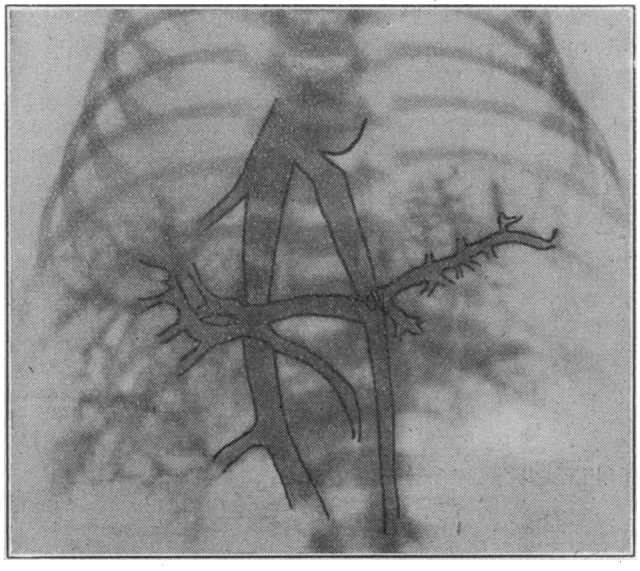


FIG. 1

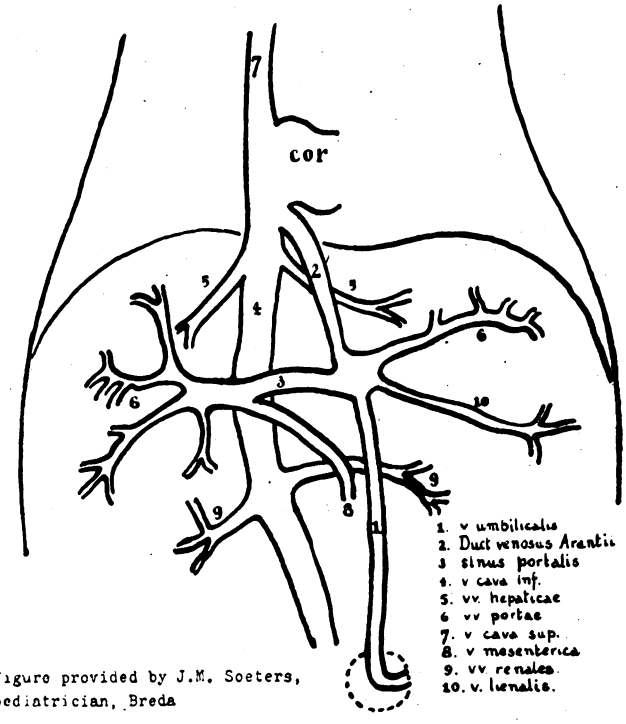


Figure provided by J.M. Soeters, pediatrician, Breda

FIG. 2

the catheter up to the vena jugularis. It passes the vena umbilicalis, sinus portalis, ductus venosus Arantii, vena cava inferior, vena cava superior, and the vena axillaris (see Figs. 1 and 2). Sometimes it is difficult to bring the catheter into the ductus venosus Arantii because of the stricture of the distal part of the duct, when the catheter is apt to turn off to the left or right side into the branches of the portal vein. When the catheter has been inserted to a length of about 12–14 cm. it has reached the vena cava inferior. A stoppage at about 11 cm. indicates that the catheter has entered the left or right portal branches.

The withdrawal of blood from the vena cava inferior proceeds very smoothly; in the sinus portalis it is rather difficult. Most of us have found that it is not necessary to insert the catheter up to 14 cm., but that the withdrawal and injection of blood is very easy to perform after inserting the catheter into the umbilical vein for 6–8 cm.

The catheter is filled with saline and closed with arterial forceps. When the catheter has reached its right place a syringe filled with saline is fitted on to the catheter. The arterial forceps is now removed and a small amount of saline is injected very carefully. When this can be done smoothly, an attempt is made to withdraw the baby's blood. Sometimes it takes a few moments to find the right place in the umbilical vein for the injection and withdrawal of blood.

The quantities of aspirated and injected blood are carefully measured in order to prevent overdosage. In cases of severe anaemia a supplementary injection of blood (maximum 50 ml.) is given. The quantity needed for the replacement depends on the infant's blood volume. The amount of circulating blood is about one-tenth of the birth weight. In general an exchange transfusion is done with about one and a half times the blood volume. In this way at least 70–80% of the infant's blood is replaced. In nearly all cases of exchange transfusion the percentages of replaced blood were controlled by one of us (J.J.v.L.).

Methods Used

(*a*) The best method for calculating the percentages of replaced blood is that of Ashby, modified by Mollison (1947). To 1 ml. of a red-cell suspension in saline an equal amount of a very powerful anti-Rh (anti-D) agglutinating serum is added. After an incubation period of one hour the tubes are centrifuged for one minute; the tube is then gently shaken and stood for a few seconds to allow sedimentation of the large clumps to start, and a drop of fluid from the column is examined for the content of non-agglutinating cells. By means of a control of the same red-cell suspension with an equal amount of saline instead of anti-Rh serum the percentages of Rh-positive and Rh-negative cells are calculated. Before this examination the infant's red cells have to be eluted thoroughly, as they are coated with incomplete antibodies, which sometimes prevent strong agglutination. The non-agglutinating cells in both tubes are counted in a counting chamber. In the control tube there are no agglutinating cells; in the other one there are a number, depending on the presence of Rh-negative cells, as nearly all the Rh-positive cells are clumped by the anti-Rh serum.

(*b*) It is also possible to obtain a rather rough impression of the replacement of blood by determining the titres of iso-agglutinins α and/or β. This is done in the following way.

The titres of α and β agglutinins in the donor's blood are carefully determined. In the blood of newborn infants these iso-agglutinins are usually absent or very weak. By comparing the increasing titres of the above-mentioned iso-agglutinins in the succeeding fractions during and after the exchange transfusion with the titre of iso-agglutinins in the blood of the donor it is possible to find how much of the baby's blood is replaced by that of the donor. If the exchange transfusion has been done correctly and about one and a half volumes of the baby's blood used for the exchange transfusion, the titre of the last fraction of the baby's blood will generally be a little lower than the titre of the donor's blood.

The Rh factor and Coombs's test are controlled during the replacement and are found to become negative.

The percentage of replaced blood (method of Mollison) corresponds well with the mathematical formula given by Wiener, Wexler, and Shulman (1948). When the exchange transfusion has been completed 20,000 units of penicillin are injected and the catheter is withdrawn. The umbilical vein is ligated and the cord sutured with catgut.

Indications for Exchange Transfusion

The following factors are considered to be indications for this therapy.

It is very important to have the previous history of affected children. The death of previous children *in utero* during the last months of pregnancy certainly calls for early induction of labour, but not before the eighth month of pregnancy, because prematurity adversely influences the clinical course. Clinical manifestations, such as anaemia, jaundice, and oedema, are of great help in arriving at a decision. In the same way oedema of the umbilical cord and hydropic placenta are also important. A pale placenta weighing more than one-sixth of the birth-weight must be regarded as a significant sign.

Sometimes it is very difficult to know whether an exchange transfusion is necessary. Serological investigations are often of great help (high titre of Rh antibodies of the incomplete form, homozygous genotype of the father). Occasionally there is a definite increase in the titre of Rh antibodies during pregnancy, but there may even be a fall in titre during the end of pregnancy. In general the control of the titre of Rh antibodies does not in itself give sufficient indication for exchange transfusion.

Sometimes the condition of the newborn may show a rather sudden and unfavourable change, so that even when the children seem to be in good condition it is necessary to apply exchange transfusion if the history of previous children is bad and the Rh factor and direct Coombs's test are positive.

Haematological examinations are of great value, especially when the infant shows no clinical symptoms shortly after birth. The findings may include hyperchromic anaemia, mild or strong leucocytosis with immature forms, erythroblastaemia, and hyperbilirubinaemia.

Mollison and Cutbush (1948) report that haemolytic disease was very mild in all infants whose cord-blood haemoglobin was above 15 g. per 100 ml. and cord-bilirubin concentration below 3 mg. per 100 ml., and that a stormy or fatal course would be likely in infants whose cord blood showed anaemia or a bilirubin value of over 4 mg. per 100 ml.

We observed a few cases in which the blood showed little abnormality shortly after birth and the bilirubin content was not increased in the cord blood, but kernicterus developed a few days after birth and the children died. We are inclined to believe that, apart from the haematological investigations, the other facts are of considerable importance.

The cord blood is used directly after birth for the necessary investigations preceding the exchange transfusion (direct Coombs's test, blood-group determination ABO and Rh, blood counts, blood smear, and bilirubin content).

The results of exchange transfusion are very favourable in cases of severe jaundice with anaemia, but it is difficult to decide whether it reduces the frequency of kernicterus.

The clinical symptoms gave the impression that haemolytic disease of the newborn is not merely restricted to the action of Rh antibodies on the red cells, but that liver, placenta, and other tissues also contain Rh antigen. The first investigations to establish the presence of Rh antigens in different tissues were done by Boorman and Dodd (1943). One of us has made a detailed study of this subject (van Bolhuis, 1948). The placenta of Rh-positive infants suffering from haemolytic disease of the newborn caused by Rh incompatibility nearly always had an inhibiting effect on Rh agglutinins. This influence was demonstrated in 27 out of 31 placentas.

Diseased parts of other organs, especially the basal ganglia of the brain in cases of kernicterus and the diseased liver in cases of icterus gravis, also have an inhibiting effect on Rh antibodies. On the other hand, suspensions of apparently normal organs do not absorb Rh agglutinins in any great quantity. It seems reasonable, therefore, to accept the view that kernicterus is due to the direct action of Rh antibodies on the brain, especially the basal ganglia, and that damage to the liver may be the result of Rh antibodies reacting with Rh antigen present in liver tissue.

Not until the diagnosis was firmly established on clinical and serological findings and the indication was clear was exchange transfusion performed.

Cerebral haemorrhage and congenital heart disease are, of course, contraindications to exchange transfusion.

Most cases were caused by D–d antagonism (replacement with cde/cde blood), one by C^W–C (cde/cde blood), one by c–C (replacement with CDe/CDe blood), one by B–O, and two by A–O antagonism.

Results of Exchange Transfusion

From Jan. 1, 1947, to Jan. 1, 1949, 160 newborn babies were treated with exchange transfusion*; 36 of them died—

*It is impossible to mention in detail here all the clinical and serological results of this material.

i.e., a mortality rate of 22.5%. The following were the causes of death.

Technical Failures.—Air embolism :—In one case the catheter was not closed during the exchange of syringes and caused aspiration of air. *Blood of the wrong group* :—Four children were treated with Rh-positive blood; two of them died shortly after the transfusion. It may be that Rh-positive blood was the cause of death, although the two other children had a normal clinical course. One case of A–O antagonism was treated with A rr blood: this child also died shortly after the operation. *Infection* :—One child died from sepsis. An abscess was found in the ductus Arantii. There were thrombosis of the portal vessels and multiple lung abscesses.

Possible Influence of Heparin.—In five children death was caused by : adrenal bleeding, 1 ; cerebral bleeding, 2 ; lung and cerebral bleeding, and bleeding in other visceral tissues, 1 ; and bleeding in all visceral tissues plus kernicterus, 1. It is not quite certain that heparin was the cause of death, but it may have had an influence on the haemorrhagic diathesis. Jorpes *et al.* (1939) proved that protamine sulphate synthesized in a special way is a harmless antidote against heparin. It is probable that it will be the medicament of the future to inactivate the action of this anticoagulant.

Treatment too Late.—One child was treated on the fifth day after birth. It already showed symptoms of kernicterus.

Treatment of Too-Premature Children.—Once exchange transfusion was applied to a baby 7½ months old. It is well known that prematurity has a bad influence on the diseased child.

Very Severe Cases of Haemolytic Disease of the Newborn.—Four exchange transfusions were applied to infants with hydrops foetalis, three of whom died. The child which survived was a milder case, with only oedema of the umbilical cord, hands, and feet, though it also had hepatosplenomegaly and a severe haemolytic anaemia (1,200,000 erythrocytes).

In the following cases there was nothing to suggest any relation to technical failures or other faults:

Eight children died with symptoms of kernicterus. One child died with cerebral symptoms (? kernicterus). Four children died from secondary infections about three to four weeks after birth—otitis plus gastritis, 1 ; bronchopneumonia, 2 ; scarlet fever or streptococcal sepsis, 1. Seven children died shortly after birth without the cause of death being known. One child died from rupture of spleen (the preceding child also died from spleen rupture, although untreated). In 36 children the treatment was unsuccessful.

In order to arrive at the results of the therapy it seems reasonable to exclude the following cases in determining the mortality rate:

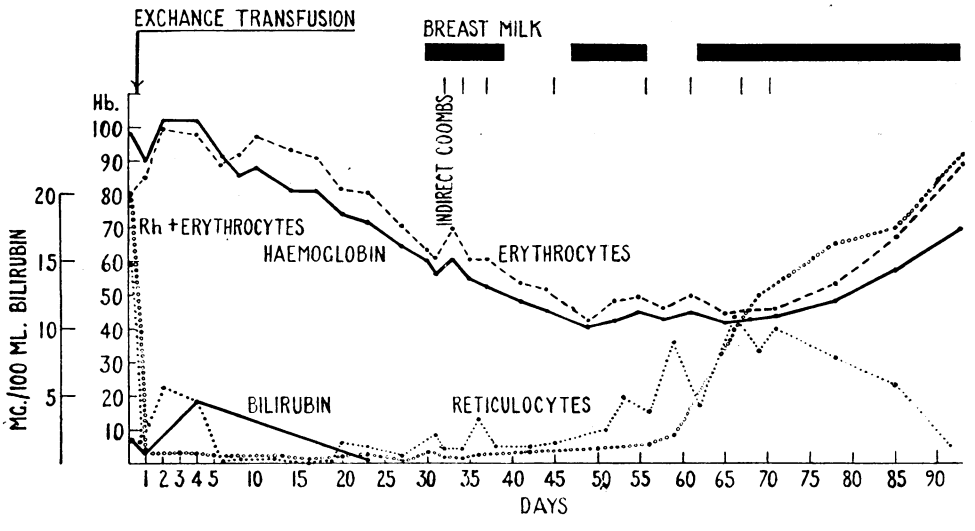
One case of air embolism ; one case treated five days after birth, when symptoms of kernicterus were manifest ; one case of sepsis ; four cases of secondary infection, death occurring three weeks after birth ; one case of spleen rupture.

By excluding these cases the mortality rate is reduced from 22.5 to 17.5%.

It is difficult to determine in what way the other above-mentioned factors such as heparin, Rh-positive blood, and symptoms of hydrops foetalis have had a direct influence on the lethal course.

We have received information concerning the subsequent clinical course of 135 patients treated with exchange transfusion. In five cases there were sequelae of brain damage. In a great number of cases a hypochromic anaemia occurred up to three to nine weeks after birth, in one case even combined with a fresh attack of jaundice. In a few cases it was necessary to give supplementary transfusions with Rh-negative blood.

One of us (G.M.H.V.) made a detailed haematological study of his exsanguinated babies. Haemoglobin content and erythrocytes decreased in nearly all infants. In a few of them who could be followed up for a period of three months a severe anaemia with a haemoglobin content of 6.4–8 g.% (40–50%) developed in three to nine weeks. In most cases the red cell count and haemoglobin content increased spontaneously. The following facts were noticed, as shown in the accompanying Chart.



The decline in haemoglobin content and number of erythrocytes correlates with the physiological death of the transfused Rh-negative erythrocytes. After three months these cells have nearly all vanished, and the infant's own Rh-positive erythrocytes have replaced them. It is remarkable that the bone marrow does not deliver Rh-positive cells sooner, for the total number of red cells is decreasing gradually. The nucleated red cells disappear in a couple of days. The bilirubin content increases in general after the exchange transfusion. Although the liver-cell damage and the obstruction of the bile ducts will in most cases add to the jaundice after exchange transfusion, the increase of bilirubin may be caused by the total destruction of the remaining 20% Rh-positive erythrocytes.

In order to determine whether the mortality rate of icterus gravis was reduced significantly by this therapy we compared in the same families the percentage mortality of icterus gravis cases not treated with exchange transfusion with that of the newborn infants who were treated.

In examining the obstetrical histories of 88 families, out of the same group of 160, with 465 pregnancies we found the results given in the following Table. It will be seen that the mortality rate of icterus gravis is 73.7%, and including mild cases 63.5%. After treatment with exchange transfusion it decreased to 22.5%.

Before exchange transfusion came into practice the death rate had already decreased by application of partus arte praematurus, plasma transfusions, and Rh-negative whole blood or packed red cells (Parsons, 1947).

It is impossible for us to compare the mortality rate of icterus gravis treated by simple blood transfusions with

Table showing Comparison between Newborn Infants Treated with Exchange Transfusion and Cases of Icterus Gravis not so Treated

No. of Families	No. of Pregnancies	Normal Children	Mild Cases of E.F. (Haemol. Anaemia and Mild Jaundice)	Icterus Gravis		Stillbirth (5th to 9th Month)	Abortion
				Alive	Died		
88	465	192 (41%)	25 (5·3%)	41	115	57 (12·2%)	38 (8·1%)
				156 (33·5%)			

Mortality rate of icterus gravis, 73·7%.
Mortality rate of icterus gravis, mild cases included, 63·5%.
Mortality rate of 160 cases of icterus gravis treated with exchange transfusion 22·5%.

that of cases treated by exchange transfusion, for we have not sufficient personal experience of the latter.

It is beyond doubt that the prognosis in icterus gravis of the newborn has improved considerably owing to the treatment with exchange transfusion, whether preceded or not by partus arte praematurus.

Summary

After a short introduction the method of exchange transfusion applied to 160 newborn infants suffering from haemolytic disease is described.

The indications for the application of exchange transfusion are discussed.

The mortality rate of 160 babies treated by this method was 22.5%.

In order to determine whether the mortality of icterus gravis was reduced by this therapy, the results of treatment with exchange transfusion were compared with those in icterus gravis of children not so treated. Although we have no data on the results of treatment with simple transfusion of Rh-negative blood, the difference (63.5 to 22.5%) seems to indicate that the prognosis of the newborn suffering from icterus gravis has improved considerably by the application of exchange transfusion.

We wish to express our gratitude to Dr. R. R. Race for all his kind help and much useful advice.

We are greatly indebted to Miss M. v. d. Hart and Miss A. Paulussen for their skilful help in serological investigations and statistical analysis.

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The Minister of Health intends to dispose of all hospital land and premises other than those which are being used or are likely to be needed for the hospital service. Hospital management committees have been asked to consider what property is suitable for disposal. The Minister will agree to the retention of endowed property only where there is a clear need for it—for example, when in the hospital board's opinion it should be reserved for future development. If the development and disposal of hospital property near a hospital is likely to injure the amenities of the hospital, the Ministry's attention should be drawn to the risk, so that it can consider including restrictive covenants in the conveyance of the property. In some cases boards may wish to make a case for the retention of such property.

A CASE OF ANAMNESTIC REACTION WITH Rh AGGLUTININS

BY

D. A. OSBORN, M.B., B.S.

Assistant Clinical Pathologist, Guy's Hospital

Since the recognition of the importance of Rh agglutinins in relation to pregnancy and haemolytic disease of the newborn the interpretation of the serological findings has become a rather complex problem. The discovery of Rh agglutinins during the antenatal period does not necessarily foreshadow damage to the foetus, as it may represent a "carry-over" from the previous pregnancy. Of much greater significance is the demonstration of a rising titre, which is generally accepted as evidence of specific antigenic stimulation by the Rhesus-incompatible cells from the foetus. The importance of this assumption hardly needs to be emphasized, since on it may depend the decision to induce premature labour.

The following case is recorded because of its rarity and to draw attention to a possible alternative interpretation of the serological findings in pregnancy.

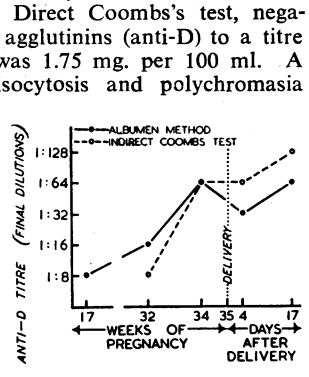
Case Report

The patient, a woman aged 34, had been married twice. By the first marriage she had a normal male child. Eight years later, in December, 1945, she was delivered of a normal male infant (by the second marriage). In December, 1947, she gave birth to a male child which was normal at birth but became jaundiced on the second day and died on the sixth day, deeply jaundiced and exhibiting kernicterus.

At this time the mother was found to be Rh-negative and her husband was thought to be homozygous positive. The infant was found to be R_2r (cDE/cde). The mother's serum contained albumin agglutinins to a serum dilution of 1:16.

In 1948 she became pregnant again and attended the antenatal clinic of this hospital. At the 17th week of pregnancy her serum was found to contain albumin agglutinins (anti-D) to a titre of 1:8. Examination of the patient's cells showed her to be group A_1,N,rr. At the 32nd week the titre was estimated to be 1:16, and two weeks later it had risen to 1:64. Taking into account the past history and the rising titre of antibodies, it was decided to induce labour in the 35th week. A normal female infant was subsequently delivered and has since made good and uneventful progress.

Examination of the cord blood yielded the following information: Group A_1,MN,rr. Direct Coombs's test, negative. Serum contained albumin agglutinins (anti-D) to a titre of 1:32. The serum bilirubin was 1.75 mg. per 100 ml. A blood film showed marked anisocytosis and polychromasia and a number of nucleated red cells (late forms)—approximately 9 per 100 white cells. A haemoglobin estimation performed on capillary blood was 18.9 g. per 100 ml. The picture was consistent with the degree of prematurity.



Examination of the mother's serum four and 17 days after delivery gave titres of 1 : 32 and 1 : 64 respectively—in albumin (see Chart). No saline agglutinins were detected, and the indirect Coombs's test gave fairly comparable results as shown. No anti-M could be detected in the mother's serum. Examination of the father's blood showed him to be group A_1, R_2r.

Discussion

The delivery of a D-negative infant came as a surprise in the face of the alleged genotype of the father, but, this