Clinicopathological Conference

A Case of Haemolytic Disease with Congenital Rubella
DEMONSTRATED AT THE ROYAL POSTGRADUATE MEDICAL SCHOOL

Clinical History
Dr. J. Davis: The patient (Case No. 317832, P.M. No. 11780) was a newborn male infant, birth weight 1,600 g., who died at the age of 6 hours, 32 weeks after conception.

The history of his illness began in 1947 when his mother broke her pelvis at the age of 11 and was given a transfusion of Rh-positive (D+ve) blood, her group being A Rh−ve (D−ve). In 1953 she married, her husband's blood group being CcDE with probable genotype R, R—i.e., probably homozygous Rh+ve.

During her first pregnancy in 1954 albumin antibodies (anti-C+D) to a titre of 128 against R,R, cells were found in the course of routine antenatal investigations. Following an unsuccessful attempt to induce delivery at term by artificial rupture of the membranes, she was delivered of a baby girl, birth weight 3,250 g., who developed moderately severe haemolytic disease, but survived after exchange transfusion.

Her next baby, born in 1956 at 38 weeks' gestation, was underweight for the dates (birth weight 2,400 g.), developed haemolytic disease, and died after an exchange transfusion, the necropsy findings suggesting a biochemical death.

Her third, fourth, and fifth pregnancies, in 1957, 1958, and 1962 all ended in the birth of stillborn premature infants with necropsy findings of severe haemolytic disease with hydrops foetalis (intrauterine anaemic heart failure).

In 1966 she became pregnant for the sixth time, L.M.P. 7 January, booked at Hammersmith Hospital nine weeks later. The antibody titre was 128. In view of her history it was decided to attempt an intrauterine marrow transplantation in the hope of establishing a strain of D-negative cells insusceptible to haemolysis by anti-D gammaglobulins. This was carried out at 11 weeks, 1 ml. of foetal AB D−ve marrow cells being injected into the peritoneal cavity at hysterotomy.

At 17 weeks she developed rubella, the diagnosis being confirmed by the demonstration of a high titre of rubella antibodies four weeks later (1:128). At 24 weeks amniocentesis was performed and the optical density peak at 4,500 Ångström units was found to be 0.14, which at this gestational age indicated a probable mortality of 28% should the infant go to term. At 26 weeks the optical density peak at 4,500 Ångström units had risen to 0.16 and it was decided to give an intrauterine peritoneal transfusion, 60 ml. of packed O/D−ve cells being injected. Following this procedure the membranes ruptured but labour was averted.

Further intrauterine peritoneal transfusions of 60 ml. and 35 ml. of O/D−ve packed cells were given at 27 and 30 weeks' gestation respectively. There was a vaginal bleed at 28 weeks, and following this incident the maternal antibody titre was found to have fallen to 64. At 31 weeks a further intrauterine transfusion was carried out. The needle was seen to enter the baby's gut on this occasion.

Delivery
At 32 weeks' gestation the mother went into labour and an elective caesarean section was performed under general anaes-

Clinical Diagnoses
(1) Severe haemolytic disease due to rhesus incompatibility.
(2) Respiratory distress.
(3) Possible runt disease.
(4) Possible intrauterine rubella infection.

Post-mortem Findings
Dr. J. Wigglesworth: The body was that of a premature male infant. The weight was 1,506 g., the crown–heel length 36 cm., and the crown–rump length 24 cm. Both weight and length were less than expected for the estimated gestation of 32 weeks.

The peritoneal cavity was completely obliterated by fibrous adhesions binding the liver to the anterior abdominal wall and...
matting the intestines together. Masses of meconium were present adhering to the coils of intestine, but no site of perforation was located.

Histology of the peritoneal surface of the intestine (Fig. 1) showed meconium as masses of eosinophilic granular material containing epithelial squames, with bile pigment within macrophages, occasional giant cells, and surrounding fibrous tissue formation, but no acute inflammatory reaction. There was a mass of about 20 ml. blood clot below the left lobe of the liver, probably representing the major part of the most recent transfusion.

The liver (113 g.) was grossly enlarged and bile-stained on section. In the posterolateral part of the left lobe (adjacent to the mass of clot within the peritoneal cavity) there was an area of infarction 4 cm. in diameter (Figs. 2 and 3). Histology of the non-infarcted areas of the liver showed intracanalicular bile stasis round the central veins and extensive extramedullary haemopoiesis (Fig. 4) which was excessive for the gestation. The infarcted portion showed complete necrosis in some areas, particularly in the subcapsular region (Fig. 5): elsewhere there were areas of partial necrosis with preservation of bile ducts, which appeared crowded together. Branches of the umbilical vein and hepatic arteries supplying the infarcted region were not occluded by thrombus, and it was concluded that the lesion was the result of local pressure from the intraperitoneal clot, which had caused functional obstruction to the umbilical venous blood supply.

The spleen (24 g.) was grossly enlarged and showed lack of Malpighian follicles (normally distinct at this gestation), extensive haemopoietic activity, and reticulum cell hyperplasia (Fig. 6).

The lungs (16 g.) were grossly hypoplastic, being only half the expected weight. Pressure volume curves performed on the left lung indicated, however, that the lung was stable (i.e., it contained adequate surfactant for normal function). Histological examination (Fig. 7) showed extreme immaturity of the lung parenchyma with widespread persistence of cuboidal alveolar lining cells (Type 2 cells), which, however, contained osmiophilic cytoplasmic inclusions. There was no alveolar duct dilatation, resorption atelectasis, or hyaline membrane formation.

The lymph nodes were enlarged up to 1 cm. diameter. Histology showed congestion with foci of haemopoiesis and many eosinophil myelocytes.

The brain (132 g.) was well below the weight expected for the gestation, but macroscopic and microscopic examination indicated that development was in keeping with the given gestation and there was no significant structural abnormality.

The pancreas was normal with no evidence of islet cell hyperplasia.

The bone marrow was very cellular with few megakaryocytes and many eosinophil myelocytes.

The heart, thymus, and adrenals showed structural development compatible with gestation but no abnormality.

The findings in the liver, spleen, and bone-marrow were considered to be compatible with either Rhesus isoimmunization or congenital rubella infection. The low brain weight in a small-for-dates baby was concluded to favour the latter possibility.

Pathologist’s Diagnosis

1. Pulmonary hypoplasia.
3. Hepatosplenomegaly with extensive extramedullary haemopoiesis; ? Rh isoimmunization; ? congenital rubella.
4. Infarction of left lobe of liver.

Discussion

Dr. Davis: I think we have to some extent to reconsider the diagnosis in this case, and the first question is whether this baby was in fact Rh—ve and unaffected by haemolytic disease. The possibility exists—it is about a 10% possibility—that the father of the baby was not homozygous but heterozygous, though in the face of the history of the family, with five affected babies in a row, this chance becomes considerably less than 1%. Even if one does accept that the baby was Rh—ve, certain discrepancies still exist; why, for instance, were the cells found in the baby not foetal cells, but adult cells? It is known that in haemolytic disease with increased red cell production, babies early on in gestation may produce larger quantities of adult haemoglobin than one would expect. In the situation that I was postulating, however—that is, the absence of haemolytic disease—or in the case of haemolytic disease that was present but had been adequately treated, one would not have expected increased red cell production and therefore one would have expected a normal quantity of foetal haemoglobin in non-donor cells. However, I am inclined to think that this baby was not suffering from haemolytic disease but was treated for a disease that it did not have, and that the disease that it actually had was caused by the rubella virus.

Rubella Syndrome

Here again there are difficulties because the rubella syndrome is almost entirely confined to rubella contracted in the first trimester of pregnancy. Going through a lot of recent literature on this subject, I have not been able to find an instance where a baby infected as late as 17 weeks (this was when the mother had rubella) developed the rubella syndrome and was born affected. But one could also ask the question—Was this baby less mature than we thought? This would mean that the mother booked at five weeks instead of nine weeks and received the intrauterine narrow transfusion at five or six weeks instead of eleven weeks. I think this is highly unlikely. If one considers the size that the baby would have been at five weeks gestation I cannot conceive that an operator, even with Professor Browne’s skill, could manage to penetrate the foetal peritoneal cavity through the uterus and make a satisfactory injection. All in all, I think it is probably more likely that the baby had rubella than that the baby had haemolytic disease, and all the clinical features could be explained on that basis—the enlarged liver and spleen, the low platelet count, the petechiae on the skin, and the enlargement of the glands are all characteristic of the rubella syndrome.

As suggested by Professor Harrison, I suppose we also have to consider that this baby may have been suffering from runt disease; that the lymphocytes took, even if the erythroblasts did not, and that the injected cells turned on the foetus and produced the features of rubella disease, some of which the baby showed. Dr. J. A. Dudgeon has shown that at birth the baby had a rubella neutralizing-antibody titre of 16 against the mother’s level then of <4. While this is not as high as at usual found with rubella infection, it is higher than the mother's (both sera had been thawed thrice—which can denature antibodies) and therefore has probably arisen within the baby. There is one last piece of evidence that is conceivably in favour of diagnosis of rubella, and that is the presence in the serum of a baby of 52 weeks or less gestation of 85 mg/100 ml. of gamma-M globulin, which babies normally do not manufacture in uterus until stimulated by injection—which in this case would have had to have been intrauterine infection. The baby, of course, receives a certain amount of maternal gamma-G globulin across the placenta, but this is not what I’m considering. However, some of this gamma-M globulin could have been acquired as a result of the intrauterine transfusions given to the baby, though the quantity would have been small as the cells were packed.
Fig. 1.—Ileum. H. and E. ×45. Organizing peritonitis involving masses of meconium on right of field.

Fig. 2.—Area of infarction in left lobe of liver, close up. Organizing fibrin deposits on upper, diaphragmatic surface, and intraperitoneal blood clot adherent to lower surface.

Fig. 3.—Section through liver showing areas of infarction near inferior surface of left lobe.

Fig. 4.—Liver. H. and E. ×115. Non-infarcted area showing widespread extramedullary haemopoiesis.
Fig. 5.—Liver. H. and E. ×115. Infarcted area. Necrosis of parenchyma with preservation of structures in portal tract only.

Fig. 6.—Spleen. H. and E. ×115. Showing lack of Malpighian follicles, extensive haemopoietic activity and reticulum cell hyperplasia.

Fig. 7.—Lung. H. and E. ×115. Immature structure with thick-walled air spaces showing widespread persistence of cuboidal epithelium.
Significance of Antibody Titres

Professor J. P. M. Tizard: I agree with what Dr. Davis has said about this child. It did of course have some form of haemolytic disease, but it is by no means certain that it was "haemolytic disease of the newborn" due to RH incompatibility. Perhaps we were a bit too quick to assume on a basis of an antibody titre of 128 that the baby was Rh+ve. I wonder what this mother's antibody titres were in between her last pregnancy and this one.

Dr. Davis: I can answer this. Nearly all the antibody titres done in the course of her previous pregnancies and in between were 128. Curiously and interestingly, the last titre done immediately before this baby was delivered was only 64.

Mr. H. Gordon: I am sorry to hear anybody talking about antibody titres. In these complicated cases where there have been one or more affected babies the antibody titres can be thrown out of the window, and no regard should be paid to them. Severe hydrops can occur with an antibody titre of 8, and rising antibody titres of over 2,000 may be associated with an Rh-ve infant. If you want to diagnose these cases it must be on the basis of analysis of the liquor.

Dr. Davis has put up a very interesting hypothesis, but I find it very difficult to believe. He has not explained two analyses of the liquor, which in our experience is an accurate method of diagnosis of haemolytic disease. We would expect an accuracy from this method without the history of about 95%, and an accuracy approaching 100% with the history we had at our disposal. He rather glossed over the Kleihauer acid elution technique, which showed only 8% of the foetal cells in the cord blood. He hasn't told us what happened to the best part of 300 ml. of very good O Rh-ve blood. Being something of a cynic it occurs to me that the mother's blood group was A Rh-ve; so that 8% of foetal cells could have been induced by manual removal of the placenta and the foeto-maternal haemorrhage, and I wonder if somehow the samples got themselves confused and the samples that have been very carefully analysed are, in fact, maternal samples and not the baby's cord blood at all. I'm sorry to be so cynical, but this is the only way in which it would make sense. With regard to the baby getting an infection; I would be most surprised if the baby did not have some infection—the membranes ruptured at 24 weeks after the first transfusion and this baby was not delivered until 33 weeks.

Possibility of Infection

Dr. Davis: First, as regards the findings at amniocentesis. I am assuming that this baby had erythroblastosis in this instance, caused by rubella infection; and since we do not know what pigment we're measuring, this might be produced by the baby, not in response to haemolytic disease but in response to rubella infection. Now, as regards bacterial infection, I believe that at caesarean section the baby's mother was found to have pyosalpingitis, and so it seems, as Mr. Gordon has said, most unlikely that the baby did not sustain any bacterial infection in the weeks immediately before delivery. However, all cultures taken from the baby—and there were a great many—were negative, and, though this is not an absolute bar to bacterial infection being present, I think it makes it somewhat unlikely.

The suggestion that the specimens were muddled would be as good an explanation as any of the difficulties of this case, except that we would have had to have muddled two specimens, not one, and that seems intrinsically rather unlikely.

Professor J. V. Dacie: I should have thought the baby had a haemolytic disease, as Professor Tizard has said. The extensive erythroblastosis in the organs and the peripheral blood is strongly in favour of this. The results obtained by amniocentesis suggest the occurrence of haemolysis, but this does not necessarily mean the presence of Rh haemolytic disease. If we accept that the Coombs test was negative, this is against the diagnosis of anti-D-produced haemolytic disease. This could be explained if the father's genotype was different from the one that had been supposed—namely, that he was not homozygous for the Rh antigen D. I would not have thought that it was necessary to suggest a mix-up of specimens or massive transfusion of maternal blood through the placenta. Could I ask Dr. Worledge to say something about the blood grouping of this baby, as she knows more about it than I do?

Dr. S. Worledge: The baby was grouped as A Rh-ve. We did two samples, so they would both have had to be muddled, and a blood sample was also sent to Dr. Kay with the same result. What percentage of the donor blood we would be able to detect is rather difficult to say—even if there had been 40% of donor cells there might not have been detected. They could have appeared as unagglutinated cells in the ABO grouping, but they might not have been noticed. The father's genotype means only that he was positive with all the anti-serum used—he was positive with anti-C, anti-D, anti-E, and anti-c. His type could be any one of nine possibilities, but 88% of people with this phenotype are R, R. There are, however, the other 12% made up of the eight other possibilities, and the commonest one is heterozygous D/d.

Professor Tizard: Mr. Gordon said he was sorry to hear people refer to antibody titres, but I don't think one should neglect them altogether. I agree that amniocentesis is more important than are antibody levels in following the course of an affected fetus. But the optical density at 450 millimicrons in this particular case gave if anything a good prognosis considering the mother's history, and if the obstetricians consider amniocentesis so important, why was this information not acted on?

Influence of the History

Mr. Gordon: You cannot rely on a single estimation. The second estimation showed some deterioration—slight, granted—but this taken in conjunction with the patient's past history made us very suspicious that this was going to be a rapidly progressing process. I think had this woman had one or no previously affected babies, we'd have said, "Right, we'll repeat the amniocentesis in another fortnight and see how things are going." With the case as presented to us, as soon as we knew that this baby was going to be affected all our evidence suggested that very rapidly there would be severe haemolysis and we should start our transfusion as early as possible.

Professor Tizard: In other words, we were relying on the history.

Mr. Gordon: We were not relying exclusively on an analysis of the liquor; we also considered the history but paid scant regard to the antibody titres after the first.

Professor Tizard: But you paid scant regard to the analysis of the liquor?

Mr. Gordon: No, the analysis of the liquor suggested this was an affected baby, and acting on that suggestion I think we were justified in treating the case as we did.

Dr. M. Berry: I have had the pleasure of hearing Dr. Liley, the New Zealander who introduced this technique, discuss the optical density of the amniotic fluid. As he points out, we don't really know what the pigments are. There is a mixture of pigments and it's fairly clear that it would be very desirable to try to characterize these a bit better. Obviously we would get a rather more accurate measure if we knew exactly what the
Rubella or Rhesus

Professor Booth: Can I just see where we're going? Dr. Davis thinks this is a rubella syndrome, and Professor Davis, Mr. Gordon, and Professor Tizard are taking the view that this is likely to be a haemolytic syndrome of some other sort.

Professor Dacie: No, I think we all take the view that rubella can produce a haemolytic syndrome, and I think that there is no evidence that the baby had haemolytic disease of the newborn due to anti-D.

Professor Booth: So this is rubella with a haemolytic syndrome not due to Rh incompatibility?

Professor Dacie: Yes, and Dr. Davis can tell you that this has been described before.

Dr. Davis: There's no question that rubella can produce a form of haemolytic disease of the newborn. But usually infection has to be in the first trimester of pregnancy and this was not the case here.

Dr. Wigglesworth: The histological findings would fit either rhesus incompatibility or rubella, but the organ-weight findings would be more in favour of rubella.

Dr. Hobbs: Can we just take the significance of the serum immunoglobulins a little bit further? The mother, at say 32 weeks, would have levels of gamma-G 800, gamma-A 250, and gamma-M 80 mg./100 ml., and the foetus would have gamma-G only at about 400 mg./100 ml. This baby had levels of gamma-G 700, gamma-A 15, and gamma-M 85 mg./100 ml. This could not have been due to a leak in the placenta, because gamma-A would have come across with a higher concentration than this. So, undoubtedly, this gamma-M had been made in the baby—but I don't know which cells made it. It is an enormous level at 32 weeks, as is also the gamma-A, which normally does not appear till two weeks after normal birth. We don't see this in erythroblastosis foetalis, so we don't see this with infection around the baby; most of the babies from grossly infected liquor get conjunctivitis and an occasional bit of pulmonary infection, but they don't get the infection we've just seen. Our results support an intrauterine infection within the baby.

Professor Booth: So this too is in favour of rubella.

Professor Dacie: Could I ask Dr. Worlledge a question about the Coombs test? Could the negative result be explained by the large admixture of group O Rh-negative blood present? We know the baby was forming some cells of its own—there were nucleated red cells in the circulation, and some of the cells contained foetal haemoglobin. Were they insufficient in numbers to give a positive Coombs test?

Dr. Worlledge: There were enough baby's cells there to give visual agglutination with anti-A. The Coombs test would definitely have been positive if the baby's cells had been sensitized.

Dr. I. Gillingland: This baby also had a meconium peritonitis—couldn't this reaction in fact cause these changes?

Dr. Davis: No, this was a chemical peritonitis.

Dr. Gillingland: Are you sure it's chemical? You've got a lot of infection elsewhere.

Dr. Davis: In an unborn infant, which is normally completely sterile, what is liberated into the peritoneum is meconium, which can cause peritonitis but is a chemical one. There was no infection; all cultures were sterile.

Dr. Hobbs: The gamma-M globulin didn't get into the baby with the transfusions, because they were packed cells. How fresh were those transfusions—were there live lymphocytes in them?

Dr. Worlledge: They were of freshly taken blood.

Marrow Grafts

Professor Booth: Can I ask anybody if any of the marrow cells survived?

Dr. Davis: I think we have to say the marrow cells did not survive. Dr. Kay looked very carefully and could find no evidence of the graft having taken.

Professor Booth: Have you got any patients in whom marrow cells have survived yet?

Mr. Gordon: We have never had definite proof of graft survival, though we have had surviving infants after this procedure. Professor Browne's explanation is that the graft may have a limited survival time, and may last for some time and then degenerate before the child is born.

Dr. Davis: This has been achieved in mice in Professor Polani's department with no difficulty at all, but mice become immunologically competent at a different stage.

Dr. J. W. Scopes: There's one point about the timing of the rubella. There are two sorts of rubella syndrome. One is characterized by cataract, deafness, and congenital heart disease—when rubella occurs in the first trimester. In the other sort the baby has the clinical signs of viriaemia with petechiae, jaundice, and hepatosplenomegaly at birth. Does anyone know whether in the latter sort the maternal infection is always in the first trimester?

Dr. Davis: After going through the extensive literature on the subject I cannot find any instance of it having actually happened. There are plenty of cases quoted where mothers did have rubella later in pregnancy but on follow-up the babies turned out not to be affected. So while I think theoretically it can occur, in practice I haven't found an instance where it has, except here.

Professor Tizard: Isn't it assumed that when there is persistent excretion of virus the infection must first have taken place before the embryo or foetus became immunologically competent? When the infection occurs later on in pregnancy the virus would be destroyed.

Dr. Hobbs: Nevertheless, such babies have enormous levels of gamma-M, which has high titre rubella antibody, when they're born, even premature ones, so they're not tolerant to the virus in that sense.

Professor Tizard: That doesn't necessarily alter the argument. What about gamma-A and gamma-G?

Dr. Davis: If the foetus was immunologically incompetent at 11 weeks, why didn't Professor Browne's marrow graft take?

Professor Booth: On that note I think we'll conclude.

We are grateful to Professor J. F. Shillingford and Dr. B. D. Williams for assistance in preparing this report, and to Mr. W. Brackenbury for the photomicrographs.