

**OBSTETRICAL ASPECTS OF RHESUS ISOIMMUNISATION.**

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by

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## OBSTETRICAL ASPECTS OF RHESUS ISOIMMUNISATION

### I. INTRODUCTION

Rhesus isoimmunisation very rarely causes any upset in the pregnant woman but may have a profound and serious effect on her child. For this reason, the condition is of considerable importance to the obstetrician, especially as its presence is likely to be detected during the time that he is responsible for the patient's care and well-being. Although consultation with the serologist and paediatrician are advantageous, it is the duty of the obstetrician to decide upon the optimum management for the patient.

The consequences to the foetus and infant in the presence of Rhesus isoimmunisation had been known for many years when the Rhesus factor was identified by Landsteiner and Wiener in 1940. These were hydrops foetalis or general dropsy or anasarca of the foetus, icterus gravis neonatorum with kernicterus, and congenital haemolytic anaemia of the newborn, and it was found that in each there was evidence of varying degrees of excessive production of immature red blood cells. This gave rise to the name 'erythroblastosis foetalis', but later, when it was realised that red blood cell destruction was the underlying process, the name 'haemolytic disease of the newborn' was suggested.

At the present time, the correct management of the

patient with Rhesus isoimmunisation is concerned with the accurate prediction of the degree of haemolytic disease present in the foetus in utero. Basically, if the mother and foetus are well able to deal with the 'toxic' breakdown products of red blood cell haemolysis, the more mature the foetus is at the time of delivery the better the prognosis. On the other hand, if the haemolytic process has reduced the number of circulating red blood cells to such an extent that cardiac failure has supervened, the foetus may die in utero, and should be delivered before this has occurred. The obstetrician has to predict the severity of the condition from the evidence available to him and, in this thesis, the value of this evidence has been studied.

The factors involved in the occurrence of Rhesus isoimmunisation and haemolytic disease of the newborn are important when the prevention of the condition is considered and some of these factors have also been studied. The treatment of the infant with haemolytic disease of the newborn is a paediatric problem and a detailed study of this has not been made, but obviously close co-operation between the obstetrician and paediatrician is essential and the overall results obtained are necessarily joint ones.

Firstly, a historical review is presented.

## II. HISTORICAL REVIEW

In an historical review of haemolytic disease of the newborn and Rhesus isoimmunisation, it is possible to consider three periods of time. The first ended about 1939 and 1940 with the identification of the Rhesus factor and publication of the evidence associating it with haemolytic disease of the newborn (Levine and Stetson, (1939); Landsteiner and Wiener (1940); Levine and Katzin (1940). The second period was concerned with reports on the nature and properties of the factors making up the Rhesus complex, and with methods of detecting the antibodies to these factors. During this period, some clinical reports were published and the treatment of both the mother and the infant were considered. In the third period, which commenced at the time of publication of the results of the national trial in Great Britain by Mellison and Walker (1952), larger series of results were reported and more attention was directed to the causation and prevention of the occurrence of haemolytic disease.

### 1. Before 1939

Hydrops foetalis has been known of for centuries and Ballantyne (1892a) wrote an excellent review of the literature on the subject. He believed that Hippocrates may have described a case of hydrops when he referred to a 'fleshy foetus' (foetus carnosus), but in a more recent

review, Pickles (1949) suggested that Plater (1641) had given the first clear account of a hydropic foetus, when he described the subcutaneous fluid found in one who had been aborted at about the fourth or fifth month of pregnancy. He also reported that the urinary tract of the abortus was normal on post-mortem examination. Severin (1643) reported a hydropic foetus with fluid in the abdomen and thorax, and also referred to the development of 'icterus'.

Ballantyne (1892a) quoted a number of case reports in the Continental literature and also a case described by Simpson (1838). Simpson's patient (Case 6) was aged 42 and had had 3 children, the second and third being stillborn. Her fourth pregnancy ended in a stillbirth also and examination of the foetus revealed fluid in the pleural, pericardial and peritoneal cavities and the presence of an enlarged spleen. Difficulty at delivery was encountered in the case reported by Walker (1858), who had found hydramnios during the pregnancy and 'dirty water' in the foetal abdomen after delivery. A few years later, Burton (1861 and 1863) described 2 stillbirths in the one family, each foetus being found to have 'straw-coloured' fluid in the serous sacs, and the second of the pregnancies described being complicated by hydramnios. At a meeting of the Obstetrical Society of London in 1875, Smith and Tait, described cases of general dropsy of the foetus

and suggested a derangement of the hepatic system, cardiac disease and syphilis as possible causative factors.

Jakesch (1878) described a case of hydrops foetalis and an hydropic placenta in detail. He thought that some blood factor was responsible for the condition - 'hydraemia', leukaemia or pernicious anaemia. He then described his findings on microscopic examination of the liver and spleen, and concluded that a leukaemic diathesis was proved. In the actual case reported, premature labour, hydramnios and oedema were all present, and the liquor was golden in colour. The foetus was born alive but died almost immediately and was seen to be hydropic. The placenta was very large, and the cotyledons were described as pale and oedematous. The description of the delivery of the placenta was also very explicit - 'Wolle aus einem uberfullten angerissenen Wollsacke vergleichen konnte'. Simpson (1880) showed a hydropic foetus to the Edinburgh Obstetrical Society and noted that in a previous pregnancy the patient had been delivered of an apparently normal infant, but that the placenta had been oedematous. The first report of a hydropic foetus in the American literature was by Lengaker (1889) when a patient with hydramnios was delivered of a hydropic foetus and a diseased placenta, and the case presented to the Obstetrical Society of Philadelphia.

As well as fully reviewing the literature, Ballantyne (1892b) described cases of general dropsy of the foetus and defined the condition. He considered that in 'general dropsy' there was general anasarca, fluid effusions in the peritoneal, pleural and pericardial sacs, and oedema of the placenta. This resulted in the death of the foetus before, during or immediately after his birth. Ballantyne also made a number of clinical observations which are still pertinent. He found that the patients were almost always parous and had usually had 1 or 2 healthy infants previously, and he had noted that a healthy infant could be born between two with dropsy. Premature onset of labour, antepartum haemorrhage and hydramnios were commonly associated with the condition. The mother was usually healthy but sometimes anaemia was reported. Ballantyne described the placenta as being large, pale, oedematous and friable, and showed an increased incidence of third stage and postpartum haemorrhages. The foetus, as well as being dropsical, was found to have an enlarged liver and spleen, and the ascitic fluid was yellow, due to the presence of bilirubin. Ballantyne also referred to a clinical syndrome which is now known as the 'maternal hydrops syndrome', although he did not directly associate pre-eclampsia with hydrops. He suggested that the fluid in the serous sacs should be aspirated and attributed the condition to some abnormality of the blood-

forming organs, with leukaemic infiltration of the liver.

Crozier (1913), quoted by Capon (1922), had found reports of maternal and foetal oedema occurring together. Rautmann (1912) and Liegner (1919) both thought that maternal toxæmia influenced the foetal marrow to produce erythroblasts, and erythroblasts had been found in the blood of a patient who had had eclampsia. The picture in syphilis was similar but no spirochaetes were found. Maternal hydramnios and oedema were not uncommonly associated with hydrops, but Capon (1922) pointed out that toxæmia was relatively common, while foetal oedema was rare. No evidence of any paternal cause for the condition could be found, but there were two foetal theories. The first, a mechanical one, referred to obstruction in the biliary tract and was based on the pathological findings at autopsy. In the early part of this century many reports from the Continent offering explanations along these lines were published. The non-mechanical theory was concerned with excessive haematopoiesis and, as this was also found in patients with icterus gravis, it was considered justifiable to link the two conditions at this time. The placental theory suggested that there was a failure of foetal excretion due to circulatory back pressure and secondary involvement of the placenta. The oedema fluid present in the placenta had certainly caused impairment of the blood supply to the villi. Schridde (1910: 1911) demonstrated the presence of

large numbers of nucleated red cells and immature myeloid cells in the foetal blood, and also showed cardiac hypertrophy to be present, secondary to the anaemia.

Rautmann (1912) had noted the excessive proliferation of red blood cells and first used the term 'erythroblastosis foetalis' when describing his findings in a foetus who was hydropic. He thought that maternal nephritis was the causative factor with the active production of erythroblasts as a result, not appreciating that this proliferation was a secondary feature following cell destruction.

Hoeck (1925) described two hydropic foetuses and placentae, the weight of one of the placentae being 1075 g. The autopsy on the second foetus revealed the usual findings in hydrops, and Hoeck noted the yellow fluid in the abdomen. He quoted Nykoff (1911) as suggesting that the toxic signs during the antenatal period were the result of a diseased foetus and not the cause. Hoeck's own view was that damage to the placenta as a result of toxæmia, syphilis or mechanical congestion due to foetal movements or uterine contractions, caused a hindrance to the circulation with resultant hydrops.

Thus, although it was known that anaemia and cardiac failure were the causes of death in the foetus with hydrops, the reason for the excessive destruction

of red blood cells and the causes of the changes seen in the placenta were not yet even suspected.

Early in the Twentieth Century, Rolleston and Hayne (1901) described a case of congenital hepatic cirrhosis with obliterative cholangitis. In the same year Bloomfield (1901) reported a family of 5 in which all the male children - numbers 2 to 5 - had died of extreme jaundice. The first child - a female - was jaundiced for 48 hours after birth. Bloomfield said that 3 of the others had convulsions after the jaundice and died within a month of their birth. The only explanation suggested was that the maternal placenta contained a 'poison', which was absorbed by the mother causing sickness and that the placental failure resulted in jaundice in the infant. Smith (1902) reported a family in which all the infants had jaundice and only the first survived. The liquor amnii, membranes and placenta were all stained with bile in these cases.

Arkwright (1902) gave details of a family of 15 of whom all but 1 had jaundice, and 11 had died. The jaundice was never present at birth and in some instances did not develop for 48 hours, but the time was not accurately noted. Again many of the infants were recorded as having had convulsions. It is of interest to note that the 14th and 15th children survived despite the deaths of all the children after the 4th. Both males and females were affected in this family. Busfield

(1906) reported a family in which 3 infants survived out of 10. Although the 7th and 8th infants recovered, the condition showed no diminution in intensity and seemed to become more rapidly fatal. Nason (1910) described a family in which some of the infants were jaundiced while others were apparently normal and survived. Infection seemed to be the most likely explanation, the organisms affecting the fetus in utero and not by means of the mother's milk after delivery. This must have been an extremely puzzling case in 1910, while a heterozygous Rhesus positive husband would be the simple explanation now.

Buchan and Comrie (1909) gave an excellent review of icterus gravis neonatorum, both clinical and pathological, and thought they could see two courses which the disease might follow. The first was a long-continued anaemia associated with jaundice in the neonatal period, but with occasional recovery and no residual effects. The second course was that of an obstructive jaundice which resulted in the death of the infant in the first few days of life and was associated with anaemia with evidence of rapid blood destruction and regeneration. The haemorrhagic tendency due to thrombocytopenia was also recorded. The most likely causes were thought to be obstructive jaundice associated with congenital fragility of the red cells, or a toxic substance causing liver damage, with particular reference to the blood-

forming properties of that organ.

Rolleston (1910) reported 'a case of recurring jaundice in 4 successive pregnancies with fatal jaundice in 3 successive infants'. The fourth infant survived after the mother had been treated with urotropin and sodium salicylate. Rolleston stated that, as the infants were not born jaundiced, the condition must be a familial one and not hereditary. It was very unusual to find a recurring jaundice in the mother and usually the first child escaped the condition. The view expressed by Pfannensteil (1908) that the familial jaundice was an intensified form of physiological jaundice found favour with Rolleston, who did not consider maternal toxæmia or infection to be aetiologically important. McGibbon (1912-13) also described a family with jaundice occurring in the infants of successive pregnancies (except in the first pregnancy) and he noted a relationship between the jaundice and anaemia. He thought that this was caused by the haemolytic action of bile or by some toxic action, possibly from a syphilitic infection. Ylppö (1918), on the other hand, found no reason to differentiate familial jaundice from ordinary icterus neonatorum.

Rolleston (1920) fully reviewed the information then available about icterus gravis and suggested that it was an hereditary disease in which the mother might be jaundiced during her pregnancy. The infant became

jaundiced a few hours or days after birth and, in 70 per cent of cases, became drowsy and then died with convulsions. Rolleston did not agree with the syphilitic or infective theories and emphasised that the condition was unlikely to occur in the first or even the second infant in a family. Klemperer (1924) described 3 infants with icterus gravis and, in each family, found that there had been previously jaundiced children, although the first child was unaffected. The changes found in the liver were a diffuse, and not focal, necrosis and destruction of liver cells, especially in the central areas of the lobules, and also fatty changes. In 2 of the 3 he noted a haemorrhagic condition while the third case was of 'anhepatocellular origin'. An indirect van den Bergh test was reported positive in the latter condition and Klemperer thought this indicated that the condition was less severe than 'hepatic icterus' where the direct test was positive. He suggested that glucose therapy might help to protect the liver.

Hart (1925) described a typical family with icterus gravis and discussed the treatment which had been advised for the condition up to that time - Apart from glucose, the main aim in treatment had been to attempt to eradicate any infection present. Hart thought the features observed in icterus gravis were due to an unknown toxin circulating in the blood, possibly gaining

access from the gastro-intestinal tract. Meningeal irritation was not uncommon, and was usually followed by convulsions and death. In an attempt to remove this toxin, he performed an exsanguination transfusion on the infant soon after birth. Spiller (1915) had previously suggested the use of blood transfusion in this condition, while Pitfield (1912) had given injections of whole blood. Klemperer had given transfusions without exsanguination and his patients had died. Hart wished to remove sufficient 'toxin' to prevent the progression of the disease, and so he removed 300 ml. of blood from the anterior fontanelle and inserted 335 ml. of blood into the internal saphenous vein. After the removal of some of the blood, synchronous withdrawal and transfusion were performed, and an additional 35 ml. was then injected. The blood donor was a healthy male who was unrelated to the infant. The jaundice improved initially and although it became worse at a later stage, the infant made a good recovery. Struthers (1926) described an infant with haemolytic jaundice of the newborn with an increased fragility of the red blood cells to hypotonic solutions and a prolonged bleeding time. In view of this, 10 ml. of the father's blood was injected intra-muscularly into each buttock to stop the bleeding. Subcutaneous saline was also given, but as the child's condition deteriorated, further

intramuscular injections were given into each buttock, and eventually an infusion of 50 ml. of the mother's blood. The child died, and Struthers suggested that an immediate transfusion, preferably an exsanguination one, should be performed, and a splenectomy should also be performed in view of the red cell fragility.

Hampson (1929) gave a very clear account of the mechanism of the production of jaundice in the foetus and newborn and stated that, although the premature infant had less than the average amount of bilirubin in his blood, the amount might rise quickly at birth and cause difficulties in the management of the baby. The reason for this excess production of bilirubin was certainly not the method of feeding, but it was clear that the efficiency of the liver in removing bilirubin from the blood by conjugation was very important. Before birth, the placenta removed the bilirubin, but after delivery, when the infant's liver assumed this responsibility and was unable to function adequately because of immaturity, indirect reacting bilirubin accumulated in the blood. In icterus gravis of the newborn, jaundice was found early and might have been present at birth. The jaundice and drowsiness increased and death was often associated with convulsions after 2 or 3 weeks of life. The products of red cell destruction were thought to be

extremely toxic causing the death of the foetus in utero. Because of the haemolytic nature of the disease the anaemia was seen to be progressive, and because the liver was not excreting these toxic products of haemolysis, symptoms and signs developed. Hampson suggested that maternal serum should be injected into these infants as this contained an ante-haemolytic agent, which he presumed to be lacking in foetal blood and present in the mother. The serum was given by intramuscular injection and whole blood was advised against because the cells were not absorbed.

Smyth (1931) described one of his cases where the patient had had 9 children, and lost 6 infants with jaundice and a seventh who was stillborn. During the pregnancy Smyth prescribed a diet rich in protein and lipins, and, as it had been stated that vaginal delivery might predispose to excessive red cell destruction in the puerperium, elective Caesarean section was performed. The child died on the fourth day despite these measures.

A number of the infants who developed jaundice and survived for some time were found to develop symptoms of brain damage, including choreiform movements, deafness and mental retardation. (Guthrie (1914) Greenwald and Messer (1927), Fitzgerald, Greenfield and Kounine (1939).) Orth (1875) had previously noted areas of discolouration in the brains of infants

dying from icterus gravis in the first few days of life. Schmorl (1904) described two types of lesion. In one, the whole of the brain was discoloured with 'bile' and microscopic areas of degeneration were seen. In the other, there was yellow discolouration of the basal ganglia and medulla, especially in the lenticular and subthalamic nuclei. This type he referred to as 'kernicterus'. Guthrie (1914) pointed out that disturbances of the nervous system had not been described up to that time because the infants failed to survive. His patient survived, with neurological disorders. Guthrie quoted Kinnier Wilson as suggesting that the lesions in kernicterus were similar to those found in progressive lenticular degeneration which is associated with cirrhosis of the liver. Abt (1917) described the symptoms and course of icterus gravis neonatorum and noted that meningeal irritation with crying and whining occurred. These were sometimes followed by convulsions and opisthotonus. He reported the details of 2 families in which the 5th child in each died after convulsions and whereas in one family the subsequent children also died, in the other the 6th child survived.

Zimmerman and Yannet (1933) described 2 cases of kernicterus including the autopsy reports, and they also examined 2 patients with extreme dysfunction of the central

nervous system following severe neonatal jaundice. They favoured the "sepsis" theory as the cause of the condition rather than intoxication due to dysfunction of the foetal liver, but associated the condition with icterus gravis neonatorum. Fitzgerald et al. (1939) described two patients with post-mortem evidence of sequelae of kernicterus, including involvement of the extra-pyramidal system. These patients had had rigidity and choreo-athetoid movement, mental retardation and defective vision.

To summarise, by 1939, it was known that some process, possibly an infection, led to the destruction of foetal red cells with an increase in the amount of circulatory 'indirectly-acting' bilirubin and resultant jaundice. The later children in the family tended to be involved and although the infants were not born with jaundice, it appeared very shortly after birth. Sometimes, despite the jaundice and associated anaemia, recovery was possible. At other times, death occurred and in some cases was associated with damage to the basal nuclei of the brain - a condition known as kernicterus. If a child with kernicterus survived, neurological damage became apparent. Blood transfusion, especially exsanguination transfusion, had been used with success in the treatment of icterus gravis neonatorum, and when the blood donor was unrelated to the child's father, there was a greater chance of a successful outcome. The view

that familial jaundice was a more severe manifestation of 'physiological jaundice' found in certain families, had some support and had certainly not been completely disproved.

Congenital haemolytic anaemia is the third clinical syndrome included in haemolytic disease of the newborn. As already stated, Buchan and Comrie (1909) described an anaemia associated with jaundice, but it was Ecklin (1919) who first described a case of severe anaemia in an otherwise healthy infant born to healthy parents. There were 5 children in the family and the fourth had died as a result of fits while the fifth died from icterus gravis. The sixth child became jaundiced within 12 hours of delivery and 8 days later was admitted to hospital with a haemoglobin of 32 per cent and 1 erythroblast in every 400 red cells seen. By the sixteenth day, the jaundice had cleared but the anaemia persisted. As the jaundice was thought to be the result of haemolysis of the infant's red cells, so the accompanying anaemia was also due to haemolysis. The condition of the child improved gradually without treatment. Ecklin thought that the case described was similar to congenital splenic anaemia, and suggested that the haemolysis in the infant was caused by toxic products from the placenta or unknown micro-organisms passing through the placenta.

Donnelly (1924) described cases of severe congenital anaemia associated with icterus gravis and thought that the anaemia which appeared after a few weeks was due to a lack of stored iron in the infant, especially if he were premature. Sanford (1925) discussed possible causes of anaemia in the newborn, including haemolytic jaundice and familial icterus. In the case he described, the haemoglobin at birth was 48 per cent. Jaundice developed but then cleared and one week later the haemoglobin was 28 per cent. No treatment other than x-rays to the extremities had been given. Despite the low haemoglobin and red cell count, the haemopoietic system was active, with numerous nucleated red cells seen, but no marked evidence of blood destruction. Bonar (1927), when describing a similar case, suggested that haemolytic drugs might have been responsible, and stated that anaemia was not very common in association with icterus gravis or congenital dropsy. Abt (1932) recorded a case in which the anaemia appeared within 2 weeks of birth and for which no cause could be found. 'Menenuclear erythrophagecytosis' was also reported and Abt suggested that a transfusion might have helped.

Parsons (1932) thought that if 'anaemia' was present at birth, it was not haemolytic, but probably nutritional, and recommended yeast and iron therapy.

Parsons et al (1933) related anaemia of the newborn to icterus gravis and to hydrops foetalis, and remarked that anaemia alone is rare and usually detected about 6 weeks after birth.

Congenital haemolytic anaemia, therefore, was sometimes associated with icterus gravis neonatorum and less commonly with hydrops foetalis, but if it occurred alone, it had been suggested that it was due to a slow-acting, but long-acting haemolytic agent in the foetal blood. This 'agent' was certain to gain access to the foetus through the placenta but its nature was not known. Infection and drugs had been suggested as well as placental degeneration products. The amount of haemolysis which occurred was variable, but it eventually ceased, and the active bone marrow attempted to restore the number of circulatory red cells to normal by an out-pouring of erythroblasts.

Thus, three conditions had been identified and defined, but there were certain points of similarity among them which suggested that they were in some way associated in the condition known as erythroblastosis foetalis. Fordyce and McAfee (1924) studied the features of icterus gravis neonatorum and, in one case, found on post-mortem examination that the changes in the liver were identical with those described by Capon (1922) in general oedema of the foetus. Gregory (1928)

reported a case in which the infant was 'white' at birth and jaundiced after 10 hours. Seven days later the jaundice was very marked and the spleen was enlarged. The haemoglobin level was recorded as 30 per cent before the marrow reacted successfully. Icterus gravis and congenital anaemia were thus co-existing in the one patient. De Lange and Arntzenius (1929) referred to the dark yellow liquor and vernix found at delivery where the infant subsequently developed icterus gravis and associated the condition with congenital hydrops.

Heuper and Mullen (1930) examined a foetus with hydrops and found a marked anaemia and erythroblastosis, while Ferguson (1931) noted abnormal extra-medullary haematopoietic activity with enlargement of the spleen and liver in 6 cases, of which 3 had jaundice and 2 had generalised oedema. Buhrman and Sanford (1931) reviewed the literature on erythroblastosis and because of the similarity of the findings in 2 cases, concluded that familial jaundice of the newborn and congenital anaemia were manifestations of erythroblastosis. As well as anaemia and jaundice, both cases showed a marked extra-medullary erythropoiesis with hyperplasia of the marrow, with numerous erythroblasts in the circulation. The authors ruled out prenatal influences in the aetiology of the condition. Clifford and Hertig (1932) wrote an excellent summary of the pathological findings

and clinical types of erythroblastosis, and drew attention to the continuation of or reversion to the embryonic level of blood formation in the condition. They estimated that the incidence of hydrops foetalis was 1 in 2,000 or 3,000 pregnancies, while that of icterus gravis was 1 in 340 pregnancies. Because of the familial incidence they considered the condition to be due to a defect in the primitive germ plasma or fertilised egg, the defect involving the haemopoietic system. They were unable to correlate it with any maternal conditions, including toxæmia and anaemia, and found no predilection for either sex or any particular race. The first born was usually spared, however. The pathological processes concerned the excessive destruction of red cells as well as the presence of immature blood cells in the circulation. The authors decided that hydrops was the more chronic form of the condition with widespread erythropoiesis, and that anaemia was more common in this form, despite the attempts to replace the blood destroyed. The placenta as well as the infant was oedematous and the liver and spleen were enlarged. In icterus gravis, there was widespread erythropoiesis but more mature cells were present and cell destruction was more acute. Here there was little oedema and the placenta was only slightly enlarged, although the liver and spleen were larger than normal. Kernicterus was a serious compli-

cation in this form. The authors recommended the transfusion of blood from either parent, without 'cross-matching', in the treatment of both conditions.

In the same year, Diamond and his associates (1932) established beyond doubt the association of the three conditions with each other, but retained the name 'erythroblastosis foetalis'. They quoted many case-histories from the literature on this subject, and defined the three forms of the disease. They reported 20 cases, 2 with hydrops, 12 with icterus gravis and 6 with anaemia, and showed that immature red blood cells and an enlarged liver and spleen were found to a greater or lesser degree in all the cases quoted. They also described one family in which all forms of the disease had occurred and considered the cause to be a disturbance of the metabolism of the haemopoietic system with failure of maturation of the erythrocytes or over-production of the immature forms and their release into the circulation at an earlier stage than normal. The disturbance also caused the excessive destruction of red blood cells and their breakdown products over-burdened the immature liver, or alternatively the increased haemolysis produced pressure on the liver cells with resultant atrophy.

Hawksley and Lightwood (1934), when considering the cause of erythroblastosis foetalis, showed that transfused blood given in the treatment of the condition

was also haemolysed so the primary factor in the disease was the haemolytic process. They also noted 2 different types of haemoglobin in the infant during the first month of life - one 'foetal' and the other 'adult'. The possibilities were that the condition was a primary haemolytic process with secondary erythroblastosis or that the red cells were defective or diseased, and the haemolysis was secondary. Previously there had been support for both these views, but the findings with transfused blood strongly suggested a primary haemolytic process, although all the features were not explained, especially in hydrops, and a hereditary factor, inherited as a Mendelian recessive, was not completely ruled out. Toxins might be causative or produced as a result of the haemolysis, and they might be endogenous or exogenous. Treatment consisted of repeated blood transfusions and the authors quoted Opitz (1922) who had used intramuscular blood for 'stimulation' and intravenous blood for 'substitution'. They thought that early discharge of the infant was important because of gastro-intestinal and respiratory infections which these infants were liable to contract in hospital. As sequelae to erythroblastosis they quoted hepatic fibrosis, kernicterus with signs of damage to the central nervous system, and mental deficiency.

Macklin (1937) discussed the possibilities of the

mode of inheritance of erythroblastosis and concluded from the reports published in the literature that it could not be as a Mendelian recessive gene but was more likely to be a dominant mutation. Darrow (1938) reviewed the whole subject of erythroblastosis paying particular attention to the aetiology of the condition. The inter-relationships between the various forms of the disease were illustrated and the points which had to be explained by any aetiological theory were enumerated. These included the absence of heredity, the birth of healthy children before and after affected ones, the absence of parental illness or prenatal conditions, the clinical features and post-mortem findings in the three forms of the disease. Darrow's theory was one of anaphylaxis, with sensitization of the mother by a protein possessed by the foetus and not by the mother. Foetal haemoglobin was suggested and the antigen-antibody reaction offered the best explanation for all the findings. This was an excellent review of the subject and the explanation offered was, in fact, very nearly the correct one. Only the identification of the Rhesus blood group system was lacking and was made two years later.

In the light of our present knowledge as to the causation of haemolytic disease of the newborn by Rhesus isoimmunisation, it is pertinent to consider some theories propounded before 1940, regarding blood

groups and their role in the causation of disease. Dienst (1905) showed that in pre-eclampsia the placenta was 'porous' and postulated that agglutinins and haemolysins could cross from the mother to the foetus and vice versa. He thought that if small amounts of foetal blood entered the maternal circulation and if the two bloods were incompatible (with reference to the ABO groups), albuminuria resulted. If more incompatible blood crossed the placenta, pre-eclampsia and eclampsia resulted. In support of this view, placental cells had been reported in the lungs of pregnant women, most commonly in those with eclampsia. Chorionic villi had also been found in the maternal circulation, and some of the features of eclampsia resembled those occurring when a patient had been given incompatible blood. As haemagglutinins and haemolysins had been found in post-eclamptic women, it was thought that these could have crossed to the foetus and destroyed his blood cells with resultant haemoglobinuria in the mother. Dienst did not suggest that the destroyed foetal red cells gave rise to any other disease, however. Ottenberg (1923) postulated direct tissue damage as a consequence of a circulating antibody produced by the transference of incompatible (ABO) blood from the foetus to the mother. This antibody was difficult to demonstrate but he suggested examination of the maternal red cells for clumping or phagocytosis in early toxæmia. Ottenberg

also suggested that this blood incompatibility might be the explanation of other diseases including jaundice of the newborn and haemorrhagic disease of the newborn. McQuarrie (1923) also showed that where the blood groups of the foetus and mother differed (again referring to the ABO system), there might be 'clots' found in the liver of the mother who had pre-eclampsia. Foetal blood, having crossed the placenta was agglutinated in the maternal circulation. McQuarrie found that it was unsafe to transfuse a baby with blood from his mother on occasion, and he calculated that toxæmia was 16.5 times more frequent when the maternal and foetal bloods were incompatible than when they were compatible.

Landsteiner and his co-workers (1927-28) reported that although transfused blood might be compatible with regard to the ABO groups, reactions sometimes occurred, especially if more than one transfusion had been given from the same donor. An abnormal isæagglutinin was found in a Group O patient after a transfusion and this reacted more strongly with other random bloods than with the donor's blood. No agglutination was obtained when the serum of the recipient was tested with the red blood cells of the donor before the transfusion, so it was postulated that an agglutinin of slight activity was originally present in the serum of the recipient, and the amount had been increased by the transfusion. The reactions which occurred were similar to those reported when the blood groups in toxæmia were

being studied. It is quite likely that the reactions were due to Rhesus positive blood being given to a Rhesus negative patient. Parr and Krischner (1932) and Johnson and Conway (1933) described intra-group transfusion reactions during pregnancy and the puerperium, and Neter (1936) reported the production of abnormal isoantibodies following transfusion with ABO compatible blood. Other specific antigens had been found but antibodies for these could only be identified after rabbit immunisation and not in the 'normal' person.

To summarize our knowledge of haemolytic disease of the newborn at the time that the Rhesus factor was identified, it can be said that the forms in which the disease presented, both clinical and pathological, were well understood and the inter-relationship of these of the conditions fully appreciated. The cause of erythroblastosis foetalis was thought to be related to an antigen-antibody reaction in the mother with subsequent destruction of the foetal red cells by this maternal antibody. The causative antigen was related to the foetal blood cells in some way and it was presumed that the antigen crossed the placenta to the maternal circulation, and that the antibody crossed the placenta from the mother to the foetus. A blood group incompatibility had been considered as a cause, but the actual blood group antigen had not been identified. The disease was treated by multiple transfusions or

transfusion, but the results were not good and the infants readily died of intercurrent infection.

2. 1939 - 1953.

Until 1939, the greater part of the literature on erythroblastosis foetalis had been contributed by obstetricians, paediatricians and pathologists. With the identification of the Rhesus factor, the subject became very much within the province of serologists both in America and in other countries, including Great Britain. As the experimental work leading to the identification of the Rhesus blood group complex was carried out initially in the United States of America, much of the early work was reported from there, particularly by Levine and Wiener. In Britain the names of Race, Fisher, Coombs, Sanger and Mourant were prominent in blood group studies during the same period.

In 1939 Levine and Stetson reported that blood from a female patient, who had never received blood by transfusion or injection, was found to contain an iso-agglutinin which agglutinated 80 per cent of the bloods of her own group (Group O). The patient had had one previous normal pregnancy and then developed signs of pre-eclampsia in her second pregnancy. The foetus died in utero and one week later the patient began to bleed vaginally, and labour commenced. After the delivery of a stillborn foetus, one pint of blood was administered, the donor being the patient's husband.

Although his group was also O, she had a transfusion reaction, while Group O blood from two other donors was given successfully and without any upset. On subsequent testing, only 21 out of 104 Group O blood specimens were found to be compatible, and the intensity of the agglutination reaction had diminished greatly 2 months after delivery. These occurrences were similar to those reported by Landsteiner et al (1927-28) and Neter (1936) but their patients had been previously transfused. In the case reported by Levine and Stetson, the foetus in utero - either the first or the second, or both - was considered to be the likely source of the sensitizing antigen. As this was lacking in the mother and blood from the father caused the transfusion reaction, it was thought likely that the antigen was inherited by the foetus from the father. The case reported illustrated the occurrence of a stillbirth possibly due to erythroblastosis and associated with pre-eclampsia, and probable hypofibrinogenaemia with intrapartum and post-partum haemorrhage of such severity that hysterectomy was required.

Landsteiner and Wiener (1940) then made their celebrated contribution to the understanding of the problem by reporting that immune sera, produced by the injection of blood from Rhesus monkeys into rabbits, was capable of reacting with human bloods that contained an agglutinin, which they had previously

named M (Landsteiner and Wiener (1937)). This test was used to determine whether human blood possessed the agglutinogen, 'Rh', as the immune sera agglutinated cells which were Group O M and also others which did not possess the M factor. Wiener and Peters (1940) found that some patients, who had had intra-group transfusion reactions, (i.e., despite the fact that donor's and recipient's bloods were ABO compatible, a transfusion reaction occurred) possessed an immune serum with similar properties to the anti-Rh serum produced by Landsteiner and Wiener. Thus, if the patient's blood was positive for the Rh antigen, no reaction would occur after a transfusion with blood containing 'Rh', but if the recipient was Rhesus negative or lacked the Rhesus antigen, a weak anti-Rh haemolysin might be present and after 2 or 3 transfusions with blood containing 'Rh', a reaction would occur. Naturally-occurring anti-Rh agglutinins were expected as with naturally-occurring anti-A and anti-B, but it then became apparent that not all Rhesus negative patients reacted to Rhesus positive blood. It was thus necessary to suggest an acquired immunity to the 'Rhesus group' with a time interval elapsing between the initial transfusion and the transfusion causing the reaction. It was also found that a transfusion to a puerperal woman might result in a reaction without any previous sensitizing transfusion, and it was postulated that the foetus in utero might have caused sensitization of his mother. The foetus obtained the antigen, which was lacking

in his mother, from his father, and if the patient had been transfused with her husband's blood, an antigen-antibody reaction was likely to occur. Levine and Katzin (1940) showed this to be the case and described 7 instances in which the 'atypical' antibody in the mother agglutinated blood from the father, although they were ABO compatible. The authors thought that the mother might be immunised by the foetus or by the foetal parts of the placenta. Although several varieties of isoagglutinin were observed, one serum containing warm-agglutinins corresponded with the 'anti-Rh' immune serum of Landsteiner and Wiener, and this was further reported by Levine, Katzin and Burnham (1940).

The association of anti-Rh antibody with erythroblastosis foetalis was suggested by Levine, Katzin and Burnham (1941). They had previously reported 12 patients who had had atypical agglutinins in their blood and obstetrical histories which included toxæmia, macerated foetuses, repeated abortions or stillbirths, and they then reported 5 additional patients with atypical agglutinins, 3 of whom were delivered of infants with erythroblastosis foetalis. The authors suggested that the anti-Rh agglutinins in the maternal circulation passed the placental barrier and that when they came into contact with the blood and tissue cells of the foetus, they gave rise to changes

which resulted in erythroblastosis. If there was a sufficiently high concentration of agglutinins, the foetus might die in utero. They also suggested that blood donors to a post-partum woman should be selected from among her blood relatives but not from among her husband's relations. They also disapproved of the administration of blood to an anaesthetised patient because of the danger of a reaction being masked.

In the same year, Levine, Burnham, Katzin and Vogel (1941) established beyond doubt the role of Rhesus iso-immunisation in the pathogenesis of erythroblastosis foetalis. They defined 'isoimmunisation' as immunisation which occurred within the same species - the individual being immunised and the source of the antigenic stimulus both belonging to the same species. Blood transfusion was one method of stimulation but the inheritance of the dominant Rhesus factor by the foetus from his father, when the mother lacked the antigenic factor or was Rhesus negative, was another method. The mother was sensitized by the Rhesus positive foetal blood (which crossed the placenta by some method as yet unknown) and was stimulated to produce anti-Rhesus agglutinins. These crossed the placenta, destroyed the foetal red blood cells and gave rise to erythroblastosis. The factor involved in the majority of cases of erythroblastosis was the same as the Rhesus agglutinogen described by Landsteiner and Weiner. The agglutinin was found to be more active

at 37°C., i.e., a warm agglutinin, and so agglutination was sometimes missed when blood samples were cross-matched in vitro. By studying the obstetric histories of patients with atypical agglutinins in their serum, it became evident that a very significantly higher number of erythroblastotic infants were born than would be expected. Ninety per cent of the mothers in the series were 'Rh' negative, the expected number being 15 per cent, while all the husbands and babies were Rhesus positive. In addition, they found that agglutinins could be detected more readily if the woman was recently delivered. The authors discussed the question of ABO - heterospecificity in pregnancy and the possible destruction of foetal cells which were ABO incompatible with the mother. They also considered the possibility of neutralisation of anti-A and Anti-B antibodies by the foetal tissues in utero when the foetus was a secretor of A and B substances. It did not seem that the Rhesus factor was secreted into the tissues because anti-Rh caused haemolysis of the foetal blood cells and no Rhesus substance had been found in saliva, seminal fluid or the other body fluids which had been investigated. Although agglutination occurred in vitro, in the patient the antigen-antibody reaction resulted in haemolysis of the red blood cells. The writers considered that the different clinical forms of erythroblastosis foetalis were the result of variations

in the degree and duration of the isoimmunisation during the course of the pregnancy. The time of onset of isoimmunisation in pregnancy was not known and the possibility of abortions occurring soon after conception was considered. It seemed possible that prolonged exposure of the foetus to Rhesus antibodies might cause more damage than agglutinins late in pregnancy, and the delayed onset of neonatal jaundice might be due to maternal agglutinins stored in the tissues of the foetus returning to the foetal circulation with the occurrence of further haemolysis. It was thought possible that the antibodies might be transmitted to the baby by means of colostrum, but some of the infants developing jaundice after 24 hours had not been breast fed. In this excellent paper, it was suggested that Rhesus negative blood be transfused to the baby because his blood group was Rhesus positive and this blood was being destroyed. The genetics involved in the inheritance of the Rhesus factor were studied, and homozygous and heterozygous Rhesus positive fathers were seen to be important in the development of the condition. As the first baby was rarely affected, it was postulated that more than one Rhesus positive pregnancy was usually required before a sufficient degree of isoimmunisation had been attained to produce erythroblastosis. As a higher incidence of the condition was expected than actually occurred, the amount of blood

required to stimulate the production of antibodies was considered to be inadequate on occasion, and the size of the family and the ability of the patient to respond to the sensitizing antigen also became very important; apart from the identification of other antigens in the Rhesus complex, there have been few advances in our knowledge of the mechanism of Rhesus isoimmunisation, which were not postulated or proved in this paper.

Wiener (1941) showed that the 'Rhesus antigen' was inherited as a simple Mendelian dominant and also that if compatible blood was transfused to a recipient, the cells survived for 3 or 4 months, whereas incompatible blood after transfusion was slowly eliminated at first and antibodies appeared in the recipient's serum. In the latter event, a subsequent transfusion resulted in a reaction, unless it had been given in the 'negative phase' following the first incompatible transfusion, during which antibodies were developing but were not active. Wiener thought that the antibodies disappeared in time, or alternatively that the technique for their detection was not very sensitive. He also suggested that there was more than one type of anti-Rhesus antibody.

Vogel, Rosenthal and Levine (1943) considered the problem of haemolytic reactions resulting from isoimmunisation following repeated transfusions of apparently homologous blood, and showed that these

reactions occurred in both males and females. It had been reported that repeated stimuli were sometimes necessary before a reaction occurred, and that this had occurred after 2 or 3 donations from the same donor. Levine (1943a) reviewed the pathogenesis of erythroblastosis foetalis and attempted to answer some of the unsolved problems. At least 3 common varieties of anti-Rh sera were available by this time (Levine, Burnham, Katzin and Vogel (1941) and Wiener (1941)). Other Rhesus antigenic factors were identified and a nomenclature for these antigens and antibodies was evolved, with statistical estimates of their incidence in the community. From these discoveries it was possible to understand why some apparently 'Rhesus positive' women had erythroblastotic infants, the incompatibility being concerned with another antigen in the Rhesus complex. One major problem still unsolved was the explanation as to why erythroblastosis was found in only 1 pregnancy in 200, while one-seventh of random matings resulted in a Rhesus positive man marrying a Rhesus negative woman. The risk of an erythroblastotic infant being produced apparently increased with parity, and the incidence of erythroblastosis in a community depended on the incidence of Rh negative individuals in the community. (Rhesus negative persons were rare among the Chinese (Levine and Wong (1943)) and American Indians

(Landsteiner, Wiener and Matson (1942)) where they had possibly been 'bred out' as natural selection was seen to favour the Rhesus positive individual by removing the heterozygous positive one (Wiener (1946b)). The mechanism of transplacental iso-immunisation was also a problem. It seemed that the Rhesus antigen was limited to the red blood cells only and the passage of these cells to the mother probably occurred to a varying degree in all pregnancies. After delivery, the foetus was removed from the source of the maternal antibodies and further destruction of foetal red cells should have ceased, but it was presumed that antibodies present in the foetal tissues continued the haemolytic process.

Kariher and Spindler (1943) described 6 cases of erythroblastosis supporting the Rhesus isoimmunisation theory and each case illustrated an important point in diagnosis or management. These included a so-called 'anamnestic reaction' (which they defined as an exaggerated response to repeated antigenic stimuli), the dangers of giving blood from either parent to an infant with haemolytic disease, the fact that the antibody might disappear or be undetectable by the sixth week post-partum, the possibility that some cases of habitual abortion were the result of isoimmunisation and the fact that haemolysis was the important finding in the condition

and not erythroblastosis, which might be minimal. They suggested that the term 'Haemolytic Disease of the Newborn' be used to describe the 3 forms of the disease, and not erythroblastosis foetalis.

Levine (1943b) continued to study the association between Rhesus isoimmunisation and ABO compatibility. He pointed out that the proportion of ABO compatible matings (the mother being the recipient and the father and infant being the donors) was significantly greater in families in which erythroblastosis had occurred. It was then suggested that if the foetal cells entering the maternal circulation were ABO incompatible with the mother, they would be rapidly destroyed before the maternal reticulo-endothelial system was able to produce antibodies to any Rhesus antigen which might have been introduced. He also considered (Levine (1944)) that the capacity to produce Rhesus antibodies differed in individuals and that this depended on certain genetic properties. In pregnancy, there might be a slow administration of antigen over a long time with a resultant increase in the amount of antibodies present in the circulation during the pregnancy. However, he showed that an 'anamnestic reaction' was possible, defining this as 'the production, in response to an antigenic stimulus, of an antibody that had been produced in the tissues on some previous occasion. The increase in antibodies in the mother during

a pregnancy might have been due to general stimulation of the antibody-producing mechanism as a result of the pregnancy and not necessarily due to the presence of the specific antigen. When the method of sensitization of the mother was considered, Levine suggested that microscopic 'breaks' in the chorionic villi might be present and not gross lesions, and he also thought that toxic symptoms in the mother were only likely to occur when an hydropic infant was present in utero. In order to prevent isoimmunisation he postulated that it would be necessary to block or induce fatigue in the reticulo-endothelial system.

In the meantime, work continued on the identification of the different antigens in the Rhesus complex. In America, this work was reported by Levine, Burnham, Katzin and Vogel (1941), Landsteiner and Wiener (1941) and Wiener and Sonn (1943). Then Wiener (1943) published a theory concerning the identification and inheritance of the Rhesus factor, distinguishing 5 'partial antigens' in the Rhesus positive gene and using the 3 anti- 'Rhesus' antisera available at that time. In Britain, Race, Taylor, Cappell and McFarlane (1943) had reached similar conclusions as Wiener and changed their nomenclature to fit the American one. Then Race (1944) reported Fisher's theory in which there were thought to be 3 pairs of allelomorphic antigens or genes, which were called Cc, Dd and Ee. As the Rhesus genotype consisted of one gene from

each allelomorphic pair, there were 8 theoretical Rhesus types produced by combining these antigens. The 6 predicted anti-sera were named from the antigen letters and the gene combinations were also given names. All the gene combinations and antibodies (with the exception of anti-d ) have been found as predicted by Fisher. (A number of other alleles of C, D and E have been reported since the theory was published.) The fundamental difference between the Wiener and Fisher-Race nomenclature concerned the presence of multiple allelomorphs at a single locus on the chromosome as suggested by Wiener, or of three closely linked genes on the chromosome as proposed by Fisher and Race. It is now becoming evident that neither nomenclature can be used satisfactorily to explain the various actions and interactions in the Rhesus complex and another nomenclature may be required (Race and Sanger (1962)).

Of more practical importance to the obstetrician were the methods of detecting the presence and amount of antibodies in the blood of pregnant women, and the significance of these antibodies. It has already been pointed out that antibodies could be identified more readily if they were looked for within two months of delivery, and it had also been noted that the titre of antibody was often highest a fortnight after delivery. Anti-Rh agglutinins were demonstrated by incubating cells of a known blood group suspended in saline with the serum

to be tested. The incubation at 37°C lasted for 2 hours and if agglutination had occurred, the antibody to the known antigen was present (Levine (1943a)). In some patients who were known to be Rhesus negative and to have had erythroblastotic infants, no antibody could be detected by the 'saline method'. It was then found, simultaneously by Wiener (1944) and Race (1944) that certain forms of Rhesus antibody were capable of inhibiting the agglutination of Rhesus positive cells by the ordinary saline or agglutinating antibody. Wiener called this the 'blocking' antibody and Race referred to it as 'incomplete' antibody. The antibody was shown to have other properties and was specific for the D antigen. If two or more demonstrable antigens were present in cells which were incubated with 'blocking' serum, only the D locus was blocked and the other antigens could still be agglutinated by their specific antisera. Wiener also thought that the blocking antibody was of more significance in erythroblastosis and failure to detect it previously explained why there was an absence of correlation between the titre of agglutinins and the severity of the disease (Dockeray and Sachs (1944), and Gallagher, Danis and Jones (1943)).

Wiener (1945a) believed that future work on the prevention of haemolytic disease of the newborn would be concerned with the treatment of sensitised Rhesus negative women so that they could bear healthy Rhesus

positive infants. To this end, he (Wiener (1945b)) considered the question of the competition of antigens in the production of antibodies and confirmed the importance of Levine's observation regarding ABO compatibility, as the A and B antigens were more effective than the Rhesus antigen in stimulating the production of antibodies. This led him to consider the possibility of desensitization by a Rhesus hapten, which he thought was impracticable, and of counter-immunisation during pregnancy by potent but innocuous vaccines to suppress the formation of Rhesus antibodies. Typhoid and pertussis vaccines were used but no convincing proof of their efficacy in the suppression of Rhesus antibodies was obtained.

By the following year, Wiener (1946a) was able to go further in explaining the pathogenesis of haemolytic disease of the newborn. He showed that the agglutinating antibodies were bivalent, while the blocking antibodies were univalent, and were thus able to block the combining sites on the red cells without agglutinating them. The blocking antibodies were found to have a smaller molecular size and were able to cross the placenta to the foetus. This made them of great importance in obstetrics, as the larger molecule of the 'saline antibody' had prevented it from reaching the foetal circulation. Wiener advocated the 'conglutination' method for the detection of blocking

antibodies. This consisted of suspending the known 'test' cells in serum instead of saline. He also expounded his 'X protein theory' in which he stated that a protein formed in the infant after birth was capable of destroying the infant's red cells which had already been sensitised by the absorption of antibody. If this X protein was formed while the foetus was in utero, a stillbirth resulted. To explain the rarity of haemolytic disease in a first pregnancy, Wiener suggested that the leakage of foetal cells into the maternal circulation only occurred after delivery, particularly during the delivery of the placenta. Wiener and Sonn (1946) next described typical cases of haemolytic disease in which they discussed the management which had been attempted. An exchange transfusion of a mother using large quantities of blood was performed in an attempt to reduce the antibody titre, but this soon returned to its pre-transfusion level. They concluded that fresh blood should be used for foetal exchange transfusions and that Caesarean section was ineffective in the prevention of haemolytic disease.

In a further paper on his nomenclature, Wiener (1946b) discussed the Hr factors which corresponded to the c, d and e antigens in Fisher's classification. He considered that a high titre of univalent antibodies would produce a stillbirth, while a weaker titre of these antibodies resulted in a less severe form

of the disease. (Wiener 1946c) He also suggested that the bivalent antibodies might enter the foetal circulation during labour and delivery, and produce multiple thrombi with resultant brain and liver damage, and jaundice with little or no anaemia. In a personal communication from Professor L. H. Snyder, quoted in this paper, it was suggested to Wiener that one variety of spina bifida was produced by Rhesus sensitization, but this has not been subsequently confirmed.

During this time blood transfusion centres in Britain were examining the data available to them from blood donors and from families in whom cases of haemolytic disease of the newborn had occurred. Boorman, Dodd and Mollison (1944) reported a series of 100 families all including one or more infants affected by haemolytic disease. By testing the mothers and children and the husbands whenever this was possible, they were able to conclude that the disease process was not necessarily more severe in successive pregnancies, that the antibody titre increased after the delivery of a Rhesus positive infant, and that a diagnosis of haemolytic disease was made when anti-Rh agglutinins were present in the mother's blood and the infant was Rhesus positive. Antibodies were sometimes found when the mother was Rhesus positive, and the authors presumed that these were related to the

Rhesus system if they were incompatible with foetal red cells at 37°C. They were unable to state that the presence of antibodies in the mother indicated a Rhesus positive foetus, which was affected by haemolytic disease, in utero, nor were the changes in the titres obtained of prognostic value. The writers felt that the effect of the antibodies on the foetus depended on the titre of antibody (but also some other factor might be involved here), the permeability of the placenta and the amount of extra-corporeal group - specific antigen present - particularly A and B, but also Rh. if it existed. In some instances, the patient seemed unable to produce antibodies to Rhesus positive blood and they thought that this might be related to the amount of blood transfused or entering the circulation from the foetus.

In Britain a new test for the detection of weak and 'incomplete' Rh agglutinins had been devised and was known as 'the Coombs' test. (Coombs, Mourant and Race (1945 a and b, 1946) and Coombs and Mourant (1947).) The test had been developed to 'amplify the effect of weak (or subliminal) Rhesus agglutinins and incomplete antibodies by the action, on the sensitised cells, of a rabbit anti-human globulin serum. Red cells sensitised with weak antibodies could be strongly agglutinated by using the test. It seemed to be more effective than Wiener's conglutination test, and Coombs

and his co-workers described a direct and indirect test. The anti-human globulin was prepared by injecting human serum into rabbits. In the direct test, the red cells to be tested for the presence of antibody were inoculated with anti-human globulin (A H G) serum, and if agglutination occurred, antibody was present. In the indirect test, red blood cells were incubated with the serum to be tested for the presence of antibodies, and then with A H G serum. If the serum contained antibodies, agglutination occurred. The test was not specific for Rhesus antibodies but detected the presence of any one of a number of blood group antibodies, although a strong reaction was usually obtained with anti-Rh antibody (anti -D).

Plaut, Barrow and Abbott (1945) reported the introduction of ante-natal blood testing for the ABO and Rh groups, and for the presence of antibodies. They were successful in detecting antibodies in blood from the majority of patients with a history suggestive of haemolytic disease in their children. Boorman and Dodd (1947) found that certain agglutinins gave a higher titration value when serum was used instead of normal saline. This applied to anti-Rhesus agglutinins, but was not found with the 'naturally-occurring' anti-A and anti-B agglutinins. It did apply, however, to the 'immune' anti-A and anti-B agglutinins and a definite qualitative difference was thus observed between 'immune' and 'naturally-occurring' agglutinins,

which led to further investigation of the properties of the agglutinins.

Since 1939 the main emphasis in the published work quoted here has been on the identification of components of the Rhesus complex and on the relationship between the Rhesus complex and haemolytic disease of the newborn. A fourth type of haemolytic disease was reported by Henderson (1942a and b), however, when he described 4 foetuses all of which were intra-uterine deaths but were not found to be hydropic. The foetuses showed diffuse hepatic cirrhosis and splenomegaly and the placentae were greatly enlarged and pale pink in colour. The degenerative changes reported in the liver were the same as those found in infants with hydrops foetalis and icterus gravis neonatorum, and cirrhosis was not uncommonly reported in infants who had survived after haemolytic disease of the newborn but had not received adequate treatment. Henderson thought that the condition could be distinguished from congenital syphilis by the history, serological tests and microscopic examination of the placenta.

Potter (1943) when considering hazards associated with the first two days of life, drew attention to the fact that too many infants dying with anaemia and effusions were diagnosed as having 'erythroblastosis foetalis'. Extra-medullary erythropoiesis was an essential finding for such a diagnosis at autopsy, and antibodies should also have been detected in the mother.

Potter stated that the reason that haemolytic disease of the newborn did not occur more frequently was related in part to the limitation of family size and to other factors concerning the transmission of foetal cells to the mother and the subsequent response of the maternal and foetal tissues. She also reported that males were frequently born in a family before the birth of an infant with erythroblastosis, and a history of allergy was not uncommonly found in the mothers of such infants. Both Potter (1943) and Gimson (1943) advised that treatment of the condition should be by transfusions with Rhesus negative blood as the Rhesus positive blood cells of the foetus were being destroyed by the antibody. They advocated simple, and not exchange, transfusions, and Gimson did not think that transfusions would influence the occurrence of kernicterus.

Diamond (1946-47) reviewed the whole question of haemolytic disease of the newborn in the light of the identification of the Rhesus factor, and pointed out that the risk of a woman developing anti-Rhesus antibodies increased from 5 per cent to 50 per cent if she had been given an incompatible transfusion. When he considered the types of antibody which had been identified, he suggested that in early isosensitisation, saline antibodies or agglutinins developed, while later there were also hyperimmune or incomplete antibodies, and later still only the hyperimmune antibodies remained.

He stated, however, that the form of Rhesus antibody and its concentration or titre bore no direct relation to the severity or type of erythroblastosis which developed, and consequently the prognosis based on antibody tests or titres in any given case had to be offered with reservations. Although Diamond did not think that transfusions could prevent kernicterus, the mortality rate from haemolytic disease had been reduced from 40 per cent to 10 per cent by exsanguination transfusion with Rhesus negative blood and the induction of labour 2 or 3 weeks before term.

Murray and Taylor (1949) were not impressed with the prognostic value of antibodies titres nor with haptan therapy, but believed that the induction of labour before term and exchange transfusion were beneficial. They also noted that an infant born to an immunised mother, after a previous infant had been affected by haemolytic disease or if the mother had been immunised by an incompatible transfusion, had a very poor prognosis unless adequate treatment was given. In the first pregnancy in which antibodies were found, the infant could be mildly affected, particularly if the antibodies appeared late in pregnancy. In some cases, however, the first affected infant might be severely affected or be stillborn with hydrops foetalis. Allott and Holman (1949) reviewed 200 cases of isoimmunisation, none of the infants being treated by replacement transfusion. They considered induction of labour to be

undesirable before 36 weeks gestation had been reached because of the dangers of prematurity, and reported a survival of 50 per cent among first affected infants and 25 per cent among subsequently affected infants. They also confirmed the occurrence of an anamnestic reaction with a Rhesus negative foetus in utero and a rising antibody titre.

Boorman, Daley and Dodd (1947) set out to ascertain the relationship between the clinical condition of the infants and the serological findings in a large number of mothers. Only 35 per cent of infants with haemolytic disease survived in the series. They could find no relationship between isoimmunisation and abortions, toxæmia of pregnancy and congenital defects in the newborn. They were unable to confirm the findings of Halperin, Jacobi and Dubin (1945) that the prognosis was worse if the infant was a male, and they pointed out that a 'normal' healthy Rhesus positive foetus could be found in the presence of Rhesus antibodies, although they presumably meant that the infant was clinically unaffected but serologically affected by haemolytic disease. They also made a number of recommendations which were of importance. They advised against the sterilization of the immunised woman in case a solution to the problem was found, or in case the woman remarried a Rhesus negative husband. In the ante-natal clinic routine re-checking of the blood of parous Rhesus negative women was advised at 34 weeks, so that induction of



labour might be considered, and routine genotyping of the family was advocated when haemolytic disease was known to have occurred. One of their suggestions was that labour should be induced when the weight and maturity of the foetus were compatible with extra-uterine life, and the indication for this was a late rise in antibody titre.

Allen, Diamond and Vaughan wrote a number of articles on erythroblastosis during 1950. In the first, Vaughan et al (1950) discussed the changing mortality in haemolytic disease with relation to different methods of treatment - transfusion with Rhesus negative blood, early induction of labour and exchange transfusion. Induction of labour before the 39th week of pregnancy had resulted in an increase in the incidence of kernicterus and the authors found only minimal improvement with exchange transfusion. Allen, Diamond and Watrous (1949) reported that female blood was more successful when used for exchange transfusion. Allen and his colleagues (1950a) considered the prognosis for successful pregnancies in women with Rhesus antibodies when related to the history, the antibody titres and the length of gestation. They found an incompatible blood transfusion to be a more potent stimulus to iso-immunisation than pregnancy, and they concluded from their results that the greater the susceptibility of a Rhesus negative woman to sensitization by pregnancy, the worse

the prognosis for her infants. With regard to antibody titres, they did not think these were accurate in any given case, but in general the outcome was related to the level of titre recorded. The authors believed that if the titre was high in a subsequent pregnancy, the likelihood of a stillbirth in that pregnancy was increased, and that induction of labour was only justified if the outcome was fairly certain to be a stillbirth without this intervention. The risk of kernicterus in the immature infant was very considerable. Diamond et al (1950) related the prognosis of haemolytic disease to the clinical and serological findings in the infant at birth and noted that although the liquor amnii, the vernix and the umbilical cord might be yellow, the infant was not jaundiced at birth. Persistent jaundice was associated with kernicterus, and anaemia with the death of the infant. Maleness was still considered to be unfavourable, especially with regard to kernicterus, but Allen and his co-workers (1950b) were able to report that the use of exchange transfusion had virtually removed the dangers of kernicterus, while their conservative policy with regard to induction had not resulted in an increased stillbirth rate. Diamond et al (1950) pointed out that serological errors could occur when the antibody present in the blood of a newborn infant might interfere with agglutination by the typing serum, giving rise to a false Rhesus negative result, and by similar

interference the Coomb's test might also be negative when the infant was severely affected.

In 1947, Merton and Pickles suggested a refinement in the method of detecting incomplete antibody. They exposed the red cells used in the test to the enzyme, trypsin, which hastened and enhanced agglutination by the 'unknown' serum containing the antibody. Stratton (1953) and Goldsmith (1955) suggested that papain be used in the preparation of the red cells.

A good deal of work on antibody titres and prognosis has been carried out in Australia and Kelsall and Vos (1952) related the antibody titre in the mother and the infant to the treatment required when the infant was affected by haemolytic disease. They considered that the albumen titre was of more value than the saline one, and that the Coombs antiglobulin titre was the most reliable provided the same laboratory worker always carried out the titrations. More accurate results were obtained if enzyme-treated cells were used. If, at birth, the antibody titre in the infant was the same as in the mother, the infant was Rhesus negative and unaffected by haemolytic disease. When the infant was affected, however, the severity of the condition was related to the absolute height of the titres, and the difference between the higher titre found in the mother and the lower titre found in the infant. These workers suggested two practical points in connection with exchange transfusion technique. They recommended that

more blood be withdrawn than was transfused during the exchange, and that sodium citrate be avoided as the anti-coagulant for the blood to be transfused. Rhesus negative blood was used for transfusion. Induction of labour at 36 weeks was recommended and it was suggested that the antigen - antibody action on the foetal tissues contributed more to the death of some of these infants than the anaemia did.

The finding of a rising antibody titre in a Rhesus negative woman when the foetus in utero was also Rhesus negative has been already referred to as an anamnestic response. Pickles (1949) suggested that this might be associated with the increase in protective antibodies noted by Von Haam and Rosenfeld (1942) during pregnancy in mice, and probably related to the increased production of oestrogen and its excretion as oestrone. Certainly the anamnestic response has proved, then as now, to be a very misleading finding when a prediction has been required regarding the condition of a child in utero when the father is known to be heterozygous Rhesus positive.

One of the theoretical methods of preventing haemolytic disease of the newborn was by the injection into the maternal circulation of a preparation of the Rhesus antigen in order to neutralise the anti-Rhesus antibodies in the mother's blood and tissues. For this to be safe and successful, it would be necessary, as pointed out by Calvin, Evans, Behrendt and Calvin

(1946) to overwhelm the mechanisms for the production of antibodies or to use a non-antigenic hapten. These workers attempted to isolate the Rhesus antigen from the stroma of erythrocytes and to study its properties. Belkin and Wiener (1944) had already demonstrated A, B and Rh antigens in the stroma of red cells, and the tests of Calvin et al (1946) showed the Rhesus antigen to be inactivated by heat. They were able to extract a lipoprotein - 'elinin' - from erythrocyte stroma and found this to contain a Rhesus substance which inhibited anti-Rh serum. Carter (1947) suggested that this ether-soluble inhibitor of anti-Rh antibody was a hapten which was incapable of stimulating the production of antibody unless a protein 'carrier' were added. Carter also described the method of preparation of this 'hapten' substance. Later (Carter 1949) she described the nature and method of assay of her 'Rhesus hapten' and recommended that it be used early in pregnancy or before the start of a pregnancy to inhibit the Rhesus antibodies. Infants might also be given this to remove maternal antibodies from their circulation. The lipids obtained by Carter's method were found to be without specificity for the Rhesus (D) antigen by Howe and Rustigian (1950) and they found no clear therapeutic rationale for its use in Rhesus sensitized women. Various workers have attempted to repeat Carter's work but were unsuccessful either in vitro - Stratton and Renton (1950) and Coombs (1950) - or in vivo - Greenwalt (1949). Wolf and his associates

(1950) agreed that Carter's substance could be a hapten but found that it brought no conclusive benefit when used therapeutically. Possibly larger doses would have been more beneficial. Murray et al (1950) were unable to find any evidence that a hapten was involved in the lipid extracts obtained by Carter and to which powers of inhibition had been attributed. Osborn (1951) was unable to isolate the hapten.

Finally, during this time, attempts were being made to find alternative or confirmatory methods for predicting the severity of the haemolytic process while the foetus was in the uterus. This allowed arrangements to be made for confinement in a maternity hospital, equipped for the care of the affected infant, and also for the induction of labour before term. In 1930, Gill and Auld suggested that an x-ray of the pregnant uterus and foetus might be of diagnostic help. In the case which they described, they noticed that the ribs of the foetus were 'flared' out, and that the arms were thick and pushed out in front of him. A large baby and placenta were delivered. Snow and Powell (1934) thought that they could see the placenta on X-ray and decide whether it was very large or not. Samuel and Cohen (1950) referred to the 'halo' sign reported by Javert (1942), the military attitude of the spine as a result of oedema, and the 'Buddha' position in which the knees are flexed and the hips are extended and rotated

laterally. Bishop (1961) reviewed the radiological findings and thought the foetal position, the elevated ribs and displaced arms, and the straight spine were the most helpful findings, along with a large placenta. Unfortunately, some or all of these observations may be noticed when the foetus is perfectly normal.

Summary 1939 - 1953

Following the identification of the 'Rhesus factor', other antigens, with their antibodies were isolated in the Rhesus complex and the relationship of these with transfusion reactions and haemolytic disease of the newborn or erythroblastosis foetalis was thoroughly investigated. The pathogenesis of haemolytic disease was now established. It seemed certain that Rhesus positive blood cells in the foetus crossed the placenta and sensitized the Rhesus negative mother so that anti-Rh antibodies were produced. These then recrossed the placenta and destroyed varying numbers of foetal blood cells, producing one of the forms of haemolytic disease. The diagnosis of the condition depended on the detection of antibodies in the blood of the pregnant woman, and more particularly on the estimation of the titre of 'albumen' or 'incomplete' antibody, or the anti-human globulin titre. Some unexplained results were obtained when the titres were used to predict the severity of haemolytic disease in utero. In the management of patients with isoimmunisation, three measures were tried, of which exchange

transfusion with Rhesus negative blood was the most successful. Hapten therapy had been generally unsuccessful, and the place of induction of labour before term was disputed. With active treatment, the survival rate was improving.

### 3. After 1953

During the decade from 1953 to the present time, clinicians have laid less emphasis on the serological aspects of Rhesus isoimmunisation and have given their attention to attempting to explain the causation of the immunisation, methods of predicting the severity of haemolytic disease in the newborn and the management of the mothers and infants concerned. More reports have been published by paediatricians, obstetricians and pathologists, as well as those by serologists.

With the publication of the preliminary findings of the Medical Research Council's National Trial (Mollison and Walker (1952)) the best treatment for the infant was seen to be exchange or exsanguination transfusion without doubt, if any treatment was required at all. The value of premature induction of labour was less certain, however, and the controversy on this aspect of management still continues.

It seems certain that the mother is sensitized by red blood cells crossing from the foetal circulation to the maternal circulation. Javert and Reiss (1952) suggested that foetal and maternal blood might mix under

certain conditions and lead to the formation of intervillous coagulation haematomas. The largest number of haematomata were found in hydropic placentas associated with haemolytic disease of the newborn. Nucleated red blood cells have been found in these haematomas and as the foetus, at term, has nucleated cells, at least a part of the haematoma was foetal in origin. The authors thought that the foetal cells gained access to the maternal circulation at the 'trophoblastic-endothelial' junction, as this junction could be stretched by a rise in capillary pressure, as a result of spasm, degeneration or ageing of the placenta, or by trauma. In premature separation of the placenta, it was considered that if decidual vessels were damaged, decidual haemorrhage could occur. The foetal cells, having crossed the placenta, might then enter the maternal circulation, and Javert and Reiss thought that it might be possible to demonstrate their presence by qualitative methods. When haematomas were present, a high incidence of Rhesus isoimmunisation was found, and erythroblastosis was found four times more often in association with infarcts than in their absence. In an attempt to prevent this capillary damage, a diet rich in citrus fruit and Vitamin C was recommended, and the avoidance of traumatic rupture of the placental attachment strongly advised. Lee, Goebel and Fulton (1955) came to similar conclusions about the leakage

of foetal red blood cells.

Rucknagel and Chernoff (1955) suggested that the amount of foetal blood in the maternal circulation could be calculated by estimating the amount of foetal haemoglobin present in the mother's blood. In some of the patients they examined, the amount increased in the second trimester and then decreased during the remainder of the pregnancy and post-partum. Despite the finding that in 10 patients out of 91, significant changes in the amount of foetal haemoglobin in the circulation were observed, none of the Rhesus negative women developed antibodies. The writers did not think that a rapid leakage was the likely explanation because of the large volume of blood which would be required. On the other hand a slow leakage over a long period also seemed unlikely because of the thick cytotrophoblast or Langhans' layer in the villi. The authors believed that the increase in foetal haemoglobin in the third trimester might be due to an increased production of this haemoglobin in the adult in response to the stress associated with pregnancy, and the increased level of chorionic gonadotrophin.

Bromberg, Salzberger and Abrahamson (1957) found that the normal retroplacental haematoma, associated with the separation of the placenta in a normal delivery contained minimal amounts of foetal blood. This foetal blood might be forced into the maternal circulation during the delivery of the infant

and the third stage of labour, and it had been found that the concentration of Rhesus antibodies increased markedly during the puerperium. Levine (1944) calculated that 0.0672 ml. of Rhesus positive packed cells were required to immunise a Rhesus negative mother. This might be found in 10 to 70 ml. of retroplacental clot, and the reason for the low incidence of isoimmunisation might be due to the infrequency with which such a comparatively large volume of blood entered the maternal circulation.

Zepursky, Hill, White and Israels (1959) used a modification of the method of Kleihauer, Hildegard and Betke (1957) and were able to detect small numbers of foetal cells in the blood of recently delivered women. This method depended on the elution of adult haemoglobin from the red blood cells by a citrate-phosphate buffer. Foetal haemoglobin resisted this elution, and so the foetal cells were readily seen, whether or not counter-staining was used. During the first 6 days of the puerperium, up to 3 ml. of foetal blood were calculated to be in the maternal circulation. Brody and Engstrom (1960) studied the different types of haemoglobin present in newborn infants with erythroblastosis and concluded that the larger and less mature the cells, the higher the relative content of foetal haemoglobin. They were unable to substantiate the idea that there was preferential regeneration of adult haemoglobin in infants with erythro-

blastosis, but there might have been preferential destruction of foetal haemoglobin.

Wimhofer, Schneider and Leitenberger (1962) estimated the amount of foetal blood in the circulation of puerperal women and found that an appreciable amount had been transferred in 10 per cent of those who had normal deliveries, but a similar amount had crossed to the mother after 40 per cent of operative deliveries. Finn and his co-workers (1961) found varying numbers of foetal cells in the circulation of 11 per cent of mothers who had recently been delivered. In only 1.5 per cent did they consider the amount of haemorrhage to be more than 5 ml. They found that 2 out of 3 women who had received large foetal 'haemorrhages' developed Rhesus antibodies, and only 1 out of 75 in whom no foetal cells were detected became immunised. They concluded that the placental 'barrier' was the most important factor in preventing Rhesus isoimmunisation.

A number of writers have blamed operative and manipulative procedures in pregnancy and labour for causing the leakage of red cells from the foetus to the mother. Gainey, Nicolay, Keeler and Doyle (1954) found that there was a high incidence of second and third stage complications in pregnancies prior to a diagnosis of Rhesus isoimmunisation being made. They found that there were more abortions in immunised women

than in a group of controls and they recommended conservative delivery in all Rhesus negative women, avoiding curettage, oxytocin drips and manual removals of the placenta. It is difficult to see how this can be achieved in general obstetric practice. Aaro (1959) also urged the avoidance of obstetric procedures, including Caesarean section, in Rhesus negative women, if at all possible. Kelsall, Vos and Kirk (1959) thought that separation of the placenta might be associated with the transference of foetal blood cells to the maternal circulation.

Knox, Murray and Walker (1961) found an association between Rhesus sensitization and pre-eclampsia, the risk of sensitization increasing with the severity of the condition. They also found that surgical procedures which were likely to disturb the chorio-decidual junction, abnormalities of the placenta and small babies were associated with subsequent Rhesus sensitization. Reilly (1960) recommended that the 'hypotensive syndrome' be avoided as much as possible because of the risk of abruptio placentae, and also that the membranes be ruptured early in labour. He was against the use of oxytocin drips and thought that the clamp on the placental aspect of the divided cord could be omitted, in an attempt to prevent the transference of foetal cells to the maternal circulation.

On the other hand, Lawrence, Dieferbach and Ehrenberg (1956) could not show that abortions and other

'abnormal obstetric experiences' increased the subsequent risk of isoimmunisation in Rhesus negative women. They considered that there was a natural tendency for foetal blood to cross the placenta and they cited the work of Mengert and his associates (1955) who showed that cells in the mother were able to cross to the foetal side of the placenta and assumed that the reverse was true. Novell and Taylor (1961) found that abruptio placentae, Caesarean section and manual removal of the placenta produced no significant increase in the number of women who developed antibodies after delivery.

If it is assumed that foetal red blood cells which are capable of producing Rhesus isoimmunisation are found in the maternal circulation during pregnancy or after delivery, it is obvious from the observed incidence of Rhesus isoimmunisation that not every woman at risk becomes immunised. Knox and Walker (1957) presented data to show that all or almost all Rhesus negative women were susceptible to isoimmunisation. They found that once a woman had been sensitized, she was necessarily immunised to the next Rhesus positive child. Also, the failure of immunisation to occur in an early pregnancy was no guarantee that it would not occur in later ones.

Kelsall, Vos and Kirk (1959) reported that 'episodes of immunisation' occurred during pregnancy, an episode being the time at which antibodies appeared during the pregnancy. This might occur more than once

in a pregnancy, and might give a pattern which would be useful for predicting the severity of haemolytic disease in the current and any future pregnancy. Antibodies found early in a second pregnancy usually indicated a 'carry over' from the first pregnancy, even although the first infant was Coomb's negative at birth. The amplitude of the antibody response is related to the mass of foetal cells entering the maternal circulation, and also the ability of the mother to form Rhesus antibodies.

In 1943 Levine (1943b) noted that Rhesus antibodies were much more likely to occur in women who lacked the natural iso-agglutinins for the red blood cells of their offspring (homospecific pregnancies) than in others who had iso-antibodies (heterospecific pregnancies). Since that time this preponderance of ABO compatible matings in patients with Rhesus isoimmunisation has been remarked upon very frequently. Three explanations had been offered for this, the first by Wiener (1945b) who thought that there was competitive selectivity between the A and B antigens and the Rhesus antigens, and if A or B incompatibility was present between the foetal cells and the maternal serum, the incompatible Rhesus antigen did not act as an effective stimulus to antibody formation. Fisher (1954) suggested that ABO incompatible foetuses might be lost from early abortion with a decreased chance of sensitizing the mother to the Rhesus antigen,

while Race and Sanger (1954) considered that the chances of antigenic stimulation by the Rhesus antigen were greatly reduced in ABO incompatibility because the naturally-occurring ABO agglutinins destroyed the cells entering the circulation before the Rhesus antibodies could be produced.

Stern, Davidson and Masaitis (1956) were puzzled by the infrequency with which Rhesus negative women developed antibodies when all were exposed to identical antigenic stimuli. Some of the possible explanations suggested included genetic factors which determined the immune response, anatomical features that influence the permeability of the placenta, differences in hormonal and nutritional factors and acquired tolerance to the Rhesus factor during intra-uterine life. In order to determine the effect of ABO incompatibility in the sensitization of the mother to foetal cells, these workers injected Rhesus positive blood into Rhesus negative males. In some cases the ABO groups were compatible - injected cells and recipient's serum - while in others they were not. They found that the injected blood stimulated the production of antibodies more readily when the ABO groups were compatible than when incompatible, and also they reported that the antibodies produced were all of the 'incomplete' type. They also deduced from their experiments that once a woman had been sensitized, the antibody titre rarely increased irrespective of the number of subsequent pregnancies.

This meant that a maximal response was achieved with relatively few additional stimulations by the antigen concerned, but is not borne out by the findings in successive pregnancies when the antibody titre is seen to increase. In a number of instances anti-C antibodies were also detected (as well as anti-D), but the C antigen appeared to be a less powerful stimulus. They pointed out that the blood injected was homozygous Rhesus positive while the foetal blood must be heterozygous positive, and was, therefore, possibly a weaker stimulus to the development of antibodies. As no saline agglutinins were found it seemed that the theory that these appeared early in isoimmunisation was disproved. Fisher's theory of early abortion was obviously untenable in man, and while Wiener's view might be the correct one, the writers favoured the view of Race and Sanger. They were sure that ABO compatibility was not the only factor favouring Rhesus isoimmunisation, however.

The finding that ABO incompatibility between the foetal cells and maternal serum greatly reduced the chances of sensitization to the Rhesus antigens was confirmed by Novell and Taylor (1961), Bornew (1961), Stern (1958), Knox, Murray and Walker (1960a and 1961), and Caughey (1960a&b). Bresler (1960) when studying the effect of ABO and Rhesus group interaction on the foetal haemoglobin level, noted a deficiency of ABO incompatible matings in the Rhesus sensitised group, and an excess of ABO incompatible matings in Rhesus negative

women who had not been sensitized. In the non-sensitized series, there were more Group O mothers and an excess of Group A and AB fathers over the expected percentages. They reported that if the parents were compatible or incompatible for both the ABO and Rhesus groups, there was a greater chance of the infant having a normal haemoglobin level than if there was incompatibility for one or the other group when the haemoglobin level was less likely to be normal.

Nevanlinna and Vainia (1956) also investigated the effect of ABO compatibility between the mother and foetus, and found that if the foetus possessed the A or B antigens and the mother did not, her chances of becoming immunised were markedly reduced. Among the husbands of Rhesus immunised women there is no selection towards the heterozygote in the ABO system, as if foetal A or B antigens always inhibited immunisation, none of the husbands of Rhesus immunised patients would be homozygous for A or B. They also found that an ABO incompatible foetus provided a weaker stimulus than a compatible foetus, whether immunisation had occurred or not. The inhibitory effect of ABO incompatibility persisted until detectible evidence of immunisation was seen to have occurred. After this the A and B antigenic properties of the foetus ceased to prevent the Rhesus antigens acting as a stimulus when there was incompatibility, but they might have weakened the stimulus. The writers had, thus,

explained the rarity of pregnancy immunisation by considering the factors inhibiting sensitization or 'sensibilisation'. They thought that after sensitization, immunisation must occur in the next pregnancy with a Rhesus positive foetus, and they believed that the secretor status of the foetus might also be important. They concluded that an ABO incompatible marriage with a husband who was heterozygous for the A and B antigens required twice as many Rhesus positive children as a 'compatible' marriage to produce Rhesus iso-immunisation. The number of affected children born after immunisation was smaller in incompatible marriages also. This was a very important work and established statistically what had been suspected by other works on this subject.

No satisfactory explanation had been offered as to why a red cell destroyed by anti A or anti B was incapable of acting as an Rhesus antigenic stimulus by virtue of the stroma of the cell. Andersen, Bentzen and Larsen (1961) suggested that foreign blood cells (foetal cells) were immunologically inactive while they were in the circulation. If antibodies were present to the antigens in these cells, the cells would be destroyed. If there were no antibodies, however, the cells were not sensitized and eventually disintegrated. The Rhesus antigen might be destroyed in the circulation when the cell was destroyed, because destruction of red cells by anti-A or anti-B might be by a different mechanism.

If the cells were not destroyed, they would eventually be removed on disintegration by the reticulo-endothelial system and stimulate the production of antibodies at that time. The writers thought that the magnitude of the preceding Rhesus positive stimulus has a decisive influence on the probability and strength of immunisation in a subsequent Rhesus positive pregnancy.

The relationship between neonatal jaundice ('icterus praecox') and ABO incompatibility was considered by Dyggue and Munk-Andersen (1960), and they found a 2 per cent incidence of anti-A and anti-B antibodies in 3,500 unselected cord bloods in one year. The amount of bilirubin in the infant depended on the highest titre of anti-A and anti-B antibody in the mother, and the haemoglobin of the infants was lower in such patients than in controls. Exchange transfusion was required on 2 occasions and stillbirths, hydrops and severe anaemia were rare.

Haemolytic disease of the newborn is occasionally reported in the infant of a first pregnancy, when there is no previous history of blood transfusion or injection. It was suggested that the mother of the affected infant might have been immunized in utero by Rhesus antigens from her mother. As it was believed that an embryo is unable to produce antibodies to foreign antigens, this theory was unlikely to be the correct one. It was known, however, that antibodies

could not be produced to antigens to which the organism or person has been exposed sufficiently early in his or her development, as tolerance was acquired. Booth, Dunsford, Grant and Murray (1953) considered that if maternal blood antigens as well as antibodies could reach the foetus, antibodies should never be developed by the foetus to either its own or its mother's antigens. However, on examining the blood of the grandmothers of infants affected by haemolytic disease, the Rhesus group distribution was as expected, and there was no evidence that the chance of a Rhesus negative woman developing antibodies was influenced by the Rhesus group of her mother.

Owen and his co-workers (1954) studied two groups of Rhesus negative women in the first of which were women with no evidence of Rhesus sensitization after 3 Rhesus positive infants, while in the other group sensitization had occurred within 3 pregnancies. The tolerant group (no sensitization) had a 25 to 9 ratio of Rhesus positive to negative mothers, while the intolerant (early sensitization) had a 4 to 13 ratio of Rhesus positive to negative mothers. When erythroblastotic infants were considered there was no evidence of any preponderance of Rhesus positive grandmothers in the families studied. Tolerance acquired by the mother from her Rhesus positive mother may be over-ridden by an early Rhesus positive pregnancy, or by prolonged or excessive exposure to the antigen.

This might occur if a large number of foetal cells entered the maternal circulation. Where there was no tolerance, antibodies developed readily. Wiener (1949) injected Rhesus positive blood into Rhesus negative volunteers and 4 out of 10 were easily sensitized. It can be calculated that 4 out of 10 Rhesus negative persons should have Rhesus negative mothers.

Ward, Walsh and Koopzoff (1957) tested the 'Rhesus positive grandmother' hypothesis but were unable to correlate the likelihood of an infant developing haemolytic disease, with the Rhesus group of the maternal grandmother. The type of antibody and the number of pregnancies may be relevant in this study, however.

It has been suggested that foetal tolerance is not maintained in extra uterine life unless there is a continuance of exposure to the antigen as in a true chimera. Woodruff and Lennox (1959) described a pair of twins showing blood chimerism, as erythropoietic cells had been exchanged and produced characteristic serological findings. They suggested that immature foetal tissue might be used to induce tolerance to homografts without the danger of "host versus donor" disease. Billingham, Brent and Medawar (1953) stated that the exposure of animals to antigenic stimuli before the maturation of the faculty of immunological response caused a specific weakening or suppression of reactivity during later life. This was acquired immunological tolerance and was acquired only during foetal life in man. After this there was a null

period when no immunization could take place (Mitchison 1961.). The tolerance was specific and the stimulus an antigenic one, so the induction of tolerance involved changes in the cells which would normally have responded to the antigen by the formation of antibodies. Antigens were found to be able to induce immunological unresponsiveness under certain conditions and an excess of antigens might mask the production of antibodies. For paralysis to occur, there must have been a central failure of response, and a large dose of antigen was required. In order to maintain this paralysis the antigen must remain in the body but this is not apparently necessary for the state of tolerance. At a Symposium in the Royal College of Obstetricians and Gynaecologists, Tovey and Lennon (1960) and McClure Browne (1960) described the methods they had used in their attempts to produce a blood group chimera in a Rhesus negative woman with antibodies and a history of early stillbirths. Rhesus negative haemopoietic tissue was injected into the umbilical cord or peritoneal cavity of the foetus at 16 weeks gestation in the hope that sufficient Rhesus negative blood cells would be produced to prevent the death of the foetus because of the excessive destruction of Rhesus positive cells. The follow-up of any infants successfully delivered would be of great immunological interest.

The diagnosis of the occurrence of isoimmunisation is not difficult provided routine tests of the maternal blood are performed during pregnancy. Vaughan (1959) stressed that the maternal blood should be tested in every pregnancy, as 'clerical and technical errors are too common to permit last year's report to stand for all time'. Boronow (1961) recommended that an indirect Coombs' test and genotype be performed on all antenatal patients as a test for the presence or absence of D was not sufficient. This arose from the author's description of three patients with isoimmunisation to c with affected infants, and in one case no blood transfusion had been given previously. Boronow suggested that the husband's blood should also be genotyped as a routine, but this seemed an expensive and time-consuming procedure. Krieger (1955) did not believe that single estimations of the antibody titre during pregnancy were of much value except as a warning or diagnostic sign. In an Annotation in the Lancet (1958) the writer strongly recommended that antenatal testing for antibodies between the 32nd and 36th weeks in Rhesus negative patients became a routine procedure.

The significance of the antibody titre in the severity of haemolytic disease in the unborn child has been the subject of much debate. It is agreed that saline titres are of little value but albumen or

incomplete antibody titres and anti-human globulin titres are believed by many to be of prognostic value. Vaughan (1959) suggested that the appearance of antibodies during a pregnancy usually indicated that the infant was affected by haemolytic disease of the newborn, but probably not severely, although there were exceptions. Bourke(1955), however, reported cases in which antibodies appeared during the last 4 weeks of pregnancy for the first time and the infant was severely affected or even dead at the time of delivery. Rigsby, Verys, Copeland and Ullery (1961) stated that if no antibodies were found during the first few months of pregnancy and developed later, it invariably meant that sensitization had taken place during the pregnancy. This has not been our experience.

Krieger (1955) drew attention to the cryptagglutinoïd antibodies in addition the saline and albumen types. He suggested that there were 3 forms of univalent antibody, the blocking antibody for saline antibody, i.e., albumen antibody, the non-blocking antibody which was the cryptagglutinoïd antibody, and a blocking antibody for the cryptagglutinoïd antibody.

In 1958, Kelsall, Vos and Kirk in Australia wrote that they were so certain of their antiglobulin method of obtaining antibody titres that they induced labour and performed exchange transfusions on the infant being largely guided by the antenatal titre. They proposed

a 'titre index' in which the titre values were given a code number and this was multiplied by the weeks of gestation at which the value was obtained. The lower the titre index, the lower the mortality. They did say, however, that Rhesus negative infants could not be predicted and that severe maternal oedema was often associated with a falling titre and an affected infant. In two annotations in the Lancet (1958 and 1959) in which the prevention of stillbirths due to haemolytic disease was discussed, it was agreed that antibody titres could not be correlated with the severity of the disease, and the numerous exceptions in the behaviour of the titre made it unreliable for prognosis in a particular case.

Wiener (1959) would not accept an anamnestic reaction in which the antibody titre rose despite the presence in utero of an unaffected Rhesus negative foetus, and considered that this was solely due to faulty laboratory technique. Wiener, Wexler and Brancato (1956) had previously stated that they had never seen a significant rise in antibody titre when the foetus reached the blood factor to which the mother was sensitized. Jacobs (1959c) agreed with other writers that an anamnestic reaction did occur and was misleading in prediction in this condition.

Predicting the severity of haemolytic disease is of the utmost importance, particularly if induction of labour before term is considered in the management

management of the isoimmunised patient. Although some workers believed that the antibody titre had a place in deciding the management few were prepared to rely on it completely. Kelsall and Vos (1955) were prepared to induce labour if there was a 'harmful rise' in titre, particularly after the 36th week of pregnancy. Tovey and Valaes (1959) and Tovey (1961) found a definite relationship between the height of the anti-globulin titre and the severity of the haemolytic disease in a first affected pregnancy. In subsequent pregnancies the height of the titre was of less help but could still be used in prognosis and they advocated the induction of labour three weeks before term. Townsend and his co-workers (1961) believed that the antibody titres were not completely reliable but, along with the colour of the liquor amnii and the genotype of the husband, they could be used in the prediction of the severity of the condition and also in deciding the optimum time for the induction of labour. Wiener et al (1956) relied on the value of the antibody titre at term or the average of titres obtained during pregnancy but they do not seem to have favoured induction and the determination of severity antenatally is rather academic in such circumstances. Stern (1958) thought that serological tests were of value in prognosis and Krieger (1955) found a correlation between a rise in titre and the severity of haemolytic disease in the infant. Aarstrand and Hope (1960) found that a low

titre which rose and then fell again indicated a bad prognosis.

On the other hand, Davies and her co-workers (1953) had not found that antibodies were helpful and relied on the patient reporting less vigorous foetal movements, and the amount and colour of the liquor amnii when predicting the severity of the condition. Gainey and his associates (1954) were not impressed with antibody titres as a method of prediction. Evans (1954) thought that a rising titre in the last trimester increased the likelihood of an affected baby, but thought the past obstetric history and zygosity of the patient's husband were more helpful than the antibody titres generally when the question of pre-term delivery was being considered. Walker (1959) thought that examination of the liquor might give more reliable information than the antibody titre had done, and Jacobs (1959a) could only correlate titres with severity in first affected pregnancies.

Murray (1956) found that the genotype  $R_2$  ( $\bar{c}DE$ ) produced more severe haemolytic disease than the genotype  $R_1$  ( $CD\bar{e}$ ) and considered that this might influence the management of the patient.

Kelsall, Vos and Kirk (1959) believed that episodes of immunization take place during pregnancy and that more than one of these might occur during a pregnancy. They suggested that the foetus might be able to compensate for a single episode by the production of red cells to

replace those destroyed by the antibodies, but if there was more than one episode, the foetus would show signs of haemolytic disease.

The previous history was found by Davies and her associates (1953) to be very important. They pointed out that the assumption that the severity of haemolytic disease increased in succeeding pregnancies was not always correct. If the first affected infant had been only mildly affected, the prognosis for subsequent infants was better than if there had been no previously affected infants. A severely affected infant, on the other hand, had a very much greater chance of being followed by another severely affected infant than by a mildly affected one, although this was not impossible. The difficulty of assessing severity presents a problem in this type of study. Evans (1954), however, stated that usually succeeding infants were more severely affected. Chewn (1948) had previously shown that a severely affected infant might be followed by a mildly affected one. Walker and Murray (1956) considered haemolytic disease of the newborn to be a family problem and hoped to be able to predict the outcome if the disease followed a pattern. They found an incidence of 10 to 15 per cent for stillbirths and also showed that 7 to 8 per cent of apparently first affected infants were stillborn. The writers found that a previous incompatible blood transfusion

did not increase the risk of stillbirth, thus disagreeing with the writer of the annotation in the Lancet (1954) on this subject, but that it increased the severity of the disease. They gave prognostic estimates from their series of the chances of severely affected and stillborn infants being delivered after mildly and severely affected infants in a previous pregnancy. The view that the longer the time interval between pregnancies, the better the outcome after an affected infant was not supported by Stratton (1955) who believed that the longer the delay, the worse the prognosis. Goperud (1961) and Petter (1958) both related the prognosis to the previous history and agreed that the more severely affected the baby was, the worse the prognosis in general. After a stillbirth due to haemolytic disease the chance of a live infant was between 10 and 25 per cent despite active intervention and exchange transfusion. Townsend and his colleagues (1961) reached similar conclusions having 'grouped' their patients in a similar manner.

The routine genotyping of the blood of husbands of Rhesus negative women has been advocated by Aaro (1959), and Berenew (1961) recommended this as a routine in every pregnancy. Roy (1959) suggested that the husband's genotype should be identified and proved by examining the blood groups of other children in the family. There is a small but definite error in the determination of the genotype, because anti-d antisera is not available for testing, and this may be of some

importance as it is frequently information about the zygosity of the Rhesus complex which is most required. Also the genotype of the husband does not identify the blood group of the fetus in utero in a current pregnancy.

Diamond, Vaughan and Allen (1950) found that male infants were more susceptible to severe haemolytic disease of the newborn than females, and this was particularly so if the infant was immature. Armitage and Mollison (1953) agreed with this finding in their analysis of the M. R. C. trial of management in Rhesus isoimmunisation. Knox, Murray and Walker (1961), however, were unable to associate the sex of the infant with the severity of the condition in their large series.

The value of radiography in the diagnosis of severely affected infants was referred to previously. Some other workers, notably Goperud (1961), have attempted to diagnose an affected infant by x-rays with varying degrees of success.

In the literature, reference has been made from time to time to the apparent association between pre-eclampsia and blood group incompatibility. More recently Campbell (1955) stressed the importance of associated pre-eclampsia when predicting the severity of haemolytic disease. Walker, Murray and Russell (1957b), who were reluctant to induce labour in Rhesus isoimmunisation, were prepared to do so when pre-eclampsia

was present and the patient gave a history of a previous stillbirth as a result of haemolytic disease. They found that the risk of hydrops in these circumstances was considerable. Knox, Murray and Walker (1961) found that the risk of Rhesus sensitization increased with the severity of associated pre-eclampsia. Scott (1958) found that pre-eclampsia was not more common in patients with Rhesus isoimmunisation than in a control group, but that 50 per cent of patients with hydrops foetalis developed pre-eclampsia. Generally the pre-eclampsia was severe and developed comparatively early in pregnancy. He postulated that this might be due to over-distension of the uterus with an accompanying alteration in placental function. He noted that in hydrops foetalis the cytotrophoblast cells persisted in late pregnancy and this observation was confirmed by Crawford (1959). The placenta was over-active and an increase in the production of chorionic gonadotrophin had been found. Scott suggested that the pre-eclampsia associated with these conditions might be the result or the cause of over-activity of the gonadotrophin-producing cells. Speck (1960) described a case in which the patient had had eclamptic fits at 16 weeks gestation. She was known to have Rhesus antibodies as a result of a previous incompatible transfusion. Hysterotomy was performed and the placental tissue was vascular and oedematous, but it did not resemble

a hydatidiform mole. Jeffcoate and Scott (1959) had found that when the ratio of the placenta to the foetus by weight was over 0.7 (approximately 1:1½ or less) the incidence of toxæmia was 80 per cent.

Lewine (1944) had referred to toxic symptoms developing in the mother when the foetus became hydropic in utero. O'Driscoll and Lavalley (1955) and Geedlin (1957) have described a 'maternal hydrops syndrome' in which a pregnant patient with Rhesus antibodies in her circulation develops oedema, hypertension and albuminuria, along with hydramnios, skin irritation and absence of foetal movements. These features usually occurred immediately prior to the death of the foetus in utero and persisted to a varying degree until delivery. The mechanism was not known but it was presumed that the placenta was being overwhelmed by bilirubin and oedema, as a result of the anaemia and foetal cardiac failure, and when it failed, it poured fluid into the amniotic sac, bilirubin into the maternal circulation and possibly gonadotrophin also with resultant pre-eclampsia. Vaughan (1959) also referred to the 'maternal hydrops syndrome' and drew attention to the risk of hypofibrinogenaemia developing when an intra-uterine death occurred.

It is logical to consider examining the amniotic fluid when an attempt at predicting the severity of haemolytic disease is made. The fluid and membranes are 'part of' the foetus and are in close contact with him, therefore products of metabolism, which are not

removed from the circulation by the placenta, may be excreted into it.

In 1945, Witebsky and Mohn reported that A and B group-specific substances had been found in the liquor (and in other body fluids) when the woman was a 'secretor', a property which was inherited by a dominant gene. The Rhesus factor, however, was thought to be limited to the red cells and was insoluble in water. They had found that the group of the child and not the mother determined the group-specific substance in the liquor, and the Rhesus substance could also be determined. They suggested that severely affected infants might be non-secretors of Rh substance but this has not been confirmed.

Westin, Lind and Teger - Nilsson (1960) examined the liquor in early pregnancy in legally induced abortions, and calculated the electrolyte and protein content. Sodium and potassium were found to be present in the same concentration in liquor as in maternal plasma but higher in fetal plasma. The concentrations of calcium and protein were lower in the liquor than in maternal or fetal blood.

Oram (1960) considered that liquor was a transudate from the maternal vessels and from the fetal circulation, and also a secretion by the amniotic epithelium. Oram found that fetal urine contributed to the liquor and that it was possible to calculate the electrolyte concentrations and turnover. Hydramnies and an unstable

lie were indications for aspirating liquor and it was possible to induce labour by injecting various substances into the liquor. Danforth and Hull (1958) suggested that the exchanges in the content of the liquor might be effected by stomata, and canaliculi in amniotic cells, which they were able to define by the electron microscope.

Bevis (1956) published a report on blood pigments in haemolytic disease of the newborn. He found that bilirubin and oxyhaemoglobin were present in the liquor and he tried to relate the concentration of these substances to the severity of the haemolytic disease. The bilirubin found was mainly indirect-acting, but up to 20 per cent direct-acting bilirubin had been found. Bevis (1952) had previously described the technique of obtaining liquor by 'tapping' the uterus, and after centrifuging, the liquor was examined in a spectrophotometer (Unicam S P 600) using 10 m.m. cuvettes. The optical density was then plotted on a logarithmic scale against the wavelength of light transmitted through the liquor. The shape of the curve produced on the graph was a qualitative identification of the substances present, and the vertical height of the curve a quantitative indication of the concentration. The concentration of bilirubin is measured as optical density at 460 m $\mu$ .

Walker (1957) used this method for the prediction of 'affected' and 'unaffected' babies, carrying out

the paracentesis between 33 and 35 weeks gestation if possible. He showed that the timing of the test was important because the 'curve' or 'bulge' above the 'normal line' on the graph became less as the pregnancy approached term. In the first 74 cases, a correct forecast was made in 52 (70 per cent). If no distinction between unaffected and very mildly affected infants were made and if the timing of the test was correct, the accuracy of the test increased considerably, (91 per cent). Prediction of the severity of haemolytic disease was not mentioned in this report, nor the management of patients after prediction. Walker did state that the test was safe and fairly simple and if blood is examined for the presence of antibodies in the 32nd week of pregnancy, the paracentesis can be arranged without difficulty.

Interest in this procedure was aroused by this report and since that time reports have been published in Australia and New Zealand and in the United States of America. Liley (1960) described his technique of amniocentesis and the complications he had encountered during 200 tests. He had difficulty in obtaining liquor when the placenta was anterior and if the foetal back was anterior. He reported premature spontaneous labour in 7 patients and 2 foetal deaths from infection. Other investigators (Carey (1960)), Robertson (1961) and McBride (1961) were impressed by the ease with which the procedure could be carried out and

thought that examination of the liquor would help in the prediction of severity in patients with Rhesus isoimmunisation.

Macbeth and Robertson (1961) obtained liquor from a point between the anterior shoulder and the occiput of the foetus and performed two tests during a pregnancy complicated by Rhesus isoimmunisation. This site differs from the one used by Bevis and Walker and is rather near the foetal head. Bevis and Walker aspirated liquor from the side of uterus opposite to that occupied by the foetal spine, and at about the level of the umbilicus, so that limbs only could be encountered by the needle. Macbeth and Robertson suggested that the test was reliable for detecting the presence of a Rhesus negative infant in utero and for indicating the progress of the disease. They found that the height of the optical density indicated the severity of the disease and the management with regard to induction.

Liley (1961) described a series of 101 liquor tests and commented that if he obtained blood on paracentesis, this was examined to determine its origin - foetal or maternal - and the blood group. His findings on examination of the liquor were similar to Walker's (1957) but he found it beneficial to carry out 2 or more tests and based his prediction on the height of the optical density at 450  $\mu$  and whether this was rising or falling. His results were plotted in three zones, the lowest zone indicating the least affected and the upper zone

the most severely affected. He was able to correlate the cord haemoglobin and the height of the peak at 450 m $\mu$  when this was known within one week of delivery. Although Liley was unable to distinguish between the unaffected infant and the mildly affected infant, he found that the severity of the condition as predicted was a satisfactory guide to the optimum time for the induction of labour. The test was particularly useful in preventing the loss of the foetus in a 'first-affected' pregnancy.

Mackay (1961) performed 1 test on each of 233 patients and studied the liquor for bilirubin staining. He noted 6 grades of staining and found that the prediction of severity given by these was better than that obtained from the optical density at a peak, or the chemical estimation of the bilirubin concentration in the liquor. Again, it was found difficult to distinguish between unaffected and very mildly affected infants but the management of such patients was the same. The grade the patient was placed in determined the time of her induction. Townsend et al (1961) were also influenced by the colour of the liquor when deciding the optimum time of induction. Mackay pointed out that there were other causes for the presence of bilirubin in the liquor, such as rarer blood group incompatibilities and chronic intra-uterine hypoxia.

Walker and Jennison (1962) reported a further series

of 156 patients tested and a 'correct' prediction rate of 91 per cent. Fewer cases were tested after 35 weeks, and occasionally the error was due to failure to distinguish between an unaffected infant and a mildly-affected one. They also chemically estimated the liquor bilirubin but the results were not as reliable as the spectro-photometric estimations in predicting the severity of the infants.

A preliminary report on amniotic fluid studies in Rhesus sensitized women was published by Beecham, Molthan, Boutwell and Rohrbeck (1962) but they were not impressed by the results of these tests. They attempted to evaluate the severity of haemolytic disease in the foetus in 11 patients, including 3 controls, by studying the anti-D antibody concentration, the amount of direct and total bilirubin estimated chemically, and also the amount of iron, haemoglobin and leucine aminopeptidase. They did not carry out spectrophotometric analysis, however.

Moncrieff (1960) condemned paracentesis as he felt that a marked rise in antibody titre could occur following the insertion of the needle. Kelsall, Vos and Kirk (1959), Mackay (1961) and Walker and Jennison (1962) were all able to present evidence that a titre rise in the last six weeks of pregnancy occurred just as frequently when no paracentesis was performed.

Mackay (1961) studied the protein content of the

liquor and found that this was increased when the infant was severely affected by haemolytic disease. The increase in total protein was due to an increase in the albumen fraction. Wild (1961) found an association between the protein and bilirubin content of the liquor amnii, the protein being particularly high in two patients who had hydropic infants. A fall in both the protein and bilirubin occurred after the 35th week and occurred even when the foetus was unaffected, provided the foetus was alive. If death in utero had occurred, the values increased. Indirect bilirubin was found by Wild to enter the liquor bound to the serum albumen, and as the foetal bilirubin was invariably found to be higher at birth than the liquor bilirubin at 34 weeks, the liquor bilirubin and protein were probably derived from the foetal serum. Unlike Liley (1961) Wild was able to correlate the liquor bilirubin before 35 weeks with the cord bilirubin at delivery.

Before considering the recent literature on the management of patients with Rhesus isoimmunisation, it must be remembered that other antibodies besides anti-D may cause haemolytic disease of the newborn. Grundorfer (1953) reported 2 patients who had received repeated blood transfusions before pregnancy and who both gave birth to infants affected by

haemolytic disease. The antigen concerned was  $hr^c$  or c and although he considered that this was a weak stimulus, Grundorfer felt that it was dangerous to give 'Rhesus negative' blood to Rhesus positive patients. Wiener, Wexler and Brancato (1956) considered that  $hr^c$  or c was the most common antigen causing isoimmunisation after  $Rh_0$  or D and the ABO groups. They pointed out that anti-c antibodies might not be detected by the Coombs test and that enzyme preparations might be required to enhance the action of the antibody. Duffy, Kidd and anti-A and anti-B antibodies were also not detected by using enzyme preparations. Boronow (1961) reported 3 patients who had anti-C antibodies, 2 of whom had had previous transfusions. The third patient had no transfusions but a previous infant had required exchange transfusion.

Additional antigens were described by Mourant (1957) and at that time he thought that a fourth and fifth locus for genes had been identified. However, combinations of the basic genes were found to occur, and the two new antigens, called f and V were in fact c and e, and c and e<sup>s</sup>. Allen and Tippett (1958) described the G antigen and explained why Rhesus negative women, who had not been transfused, were able to produce anti C + D antibodies, although their husbands lacked the C antigen. The patients were producing anti D + G, the G antigen being present along with the C or D antigen.

Another anomaly was reported by Murray and Clark (1952). Heat extracts of cells lacking the D antigen were able to produce anti-D antibodies in guinea pigs, These workers suggested that the antibody might have been directed at the substance which forms the basis of all the Rhesus antigens. In 1959, Dunsford and Stapleton reported that anti-D antibody had been taken up by cells apparently lacking the D antigen. No anti-D was found in serum separated from a Rhesus negative clot at 4°C, but anti-D was detected at 37°C.

During this past decade, attempts have been made to treat patients with antibodies with a number of substances, and planning the management of immunised women is of great importance. As the haematological findings in the infant at birth suggested a haemolytic anaemia, and as it is known that the acquired form benefits from the administration of cortisone and adrenocorticotrophic hormone, it seemed reasonable to try the effect of these hormones in the previously immunised pregnant woman. Anderson (1952) reported a reduction in the severity of the haemolytic disease, while De Costa, Gerbie and Potter (1954) found nothing to recommend it as a form of therapy. Hunter (1954) was more hopeful but still reported the occurrence of intra-uterine deaths and neonatal deaths.

Carter and her co-workers (1956) were still claiming success for their Rhesus hapten therapy. This

substance was extracted from Rhesus positive blood, and administered intramuscularly, preferably before the start of a pregnancy, and continued daily throughout pregnancy. During pregnancy the dose was increased but induction of labour was not necessary. Many infants did not require exchange transfusion. Lewis and Carter (1958) reported that they had not lost a foetus or infant from haemolytic disease for 3 years by using a hapten capsule orally. In view of these excellent results it is unfortunate that other workers are unable to produce the hapten or to obtain comparable results.

The study of immunochemistry has resulted in a new approach being made to the treatment of isoimmunisation. Rhesus substances have not been successfully extracted so attempts have been made to find substances which will inhibit the action of antibodies on Rhesus antigens - Hackel, Smolker and Fenske (1958) showed that anti-Rhesus sera (anti-D, C and E) was specifically inhibited by a number of ribonucleic acid derivatives, suggesting that Rhesus antigens were partly nucleotide in composition. Hackel and Spolyar (1960) further showed inhibition of anti-c and anti-e antibodies by derivatives of ribonucleic acid, and showed that some of the substances used had a strong inhibiting effect on some of the antibodies, in their saline or albumen

form, while the effect on others was much less strong. They concluded that as an isolated blood group substance could prevent its specific antibody from causing haemagglutination, specific inhibition of the antibody indicated a similarity between the blood group substance and the antigen for which the antibody was specific. In view of this, it was deduced that part of the specificity of the Rhesus antigens was due to abonucleic acid derivatives.

On the other hand, Boyd, McMaster and Waszczenko - Zacharczenko (1959) were unable to confirm this inhibitory effect by nucleic acid derivatives. They found that L-glucose, L-mannose and D-gulose weakly but specifically inhibited agglutination of Rhesus positive cells by anti-D serum. They concluded that the D- receptor of Rhesus positive red cells contained, as a terminal unit of the active portion, one of the 'unnatural' hexoses, or their derivatives. Anti-D was also inhibited by streptomycin and rutinose. Sialic acid had been suggested as an inhibitor by Dodd, Bigley and Geyer (1960) but this was not confirmed (Springer et al (1961)). Of possibly more practical importance was the report by Boyd and Reeves (1961) that colominic acid, produced by certain strains of *E. coli* inhibited anti-D in relatively low concentration. As this substance was reported to be non-toxic and non-antigenic (Barry (1958)), Boyd and Reeves thought that it might be of value in the treatment of Rhesus

sensitized pregnant women, but they have experienced some difficulty in the preparation of the acid and so have not yet been able to give it a trial (Boyd (1962)).

As the foetal blood cells apparently enter the maternal circulation as a result of damage to the placental capillary vessels (Javert 1955), it was thought by Jacobs (1956) that bioflavonoids might prevent this damage and the increased capillary fragility in the baby. In 6 patients the results were encouraging and the treatment was still being given in 1959 (Jacobs (1959a)).

A novel approach was that suggested by Finn and his co-workers (1961). Having identified variable numbers of foetal cells in the maternal circulation after delivery, they suggested that when the mother was Rhesus negative and her infant positive, and foetal cells were seen in the maternal circulation soon after delivery, these cells could be destroyed by the administration of anti-Rh antibody. The cells would be removed from the circulation before they stimulated the production of antibodies, and the foreign antibodies would disappear in time. Obviously this treatment could not be employed when the patient was pregnant.

In general terms, Walker (1959) emphasised the need for centralisation of immunized patients and for adequate arrangements being made during pregnancy for blood testing. If an infant were born outside hospital and the mother's blood had not been checked, cord blood should be taken for grouping and Coomb's test.

The infant should be transferred to a hospital which has experience in exchange transfusion techniques if haemolytic disease had been suspected or diagnosed.

Induction of labour is the only other method of treatment of a woman with Rhesus isoimmunisation.

The rationale of induction of labour before the expected date of delivery is that as the foetus is affected by haemolytic disease and is in danger of dying in utero from hydrops foetalis, it is safer to deliver the baby when his maturity gives him a better chance of survival than the uterine environment does. The difficulties are that one can never be absolutely sure that the infant is affected by haemolytic disease, that the severity is such that death from hydrops foetalis is likely and that the induction will be completely safe and satisfactory.

Mollison and Walker (1952) reporting on the results of the Medical Research Council trial showed that induction reduced the number of stillbirths but increased the number of neonatal deaths. The authors calculated that the survival rate could only be improved by one per cent by induction of labour. Prematurity was found to be a definite hazard and to aggravate the hyperbilirubinaemia of icterus gravis neonatorum. Armitage and Mollison (1953) emphasised that the inability to predict severity was the major drawback to a policy of induction. Davies and her co-workers (1953) recommended induction of labour or Caesarean section between 34 and 36 weeks gestation if

a previous infant had been very severely affected. Early induction increased the risk to the infant of kernicterus and was condemned in an annotation in the Lancet (1954).

Evans (1954) was in favour of 'preterm' induction of labour as opposed to premature induction, because in the latter the dangers of prematurity outweighed the advantages of early delivery. He therefore advised induction after the 36th week if a previous infant had been stillborn or severely affected. If the husband were heterozygous, Evans considered that medical induction only was justifiable. Watson, Crosse and Hatchuel (1954) recommended premature delivery if a previous infant had been severely affected, but they felt that each case must be treated individually on its merits.

In Australia, a policy of induction in patients with Rhesus isoimmunisation was being carried out in a number of centres. Campbell (1955) conducted a trial, inducing half the patients at term and the other half at 38 weeks, and considered that the induction group fared better. Kelsall and Vos (1955) compared their results following induction with those of Mollison and Walker (1952). In the spontaneous groups the mortality was the same - 24 per cent, while in the induced group, the Australian mortality was 10 per cent and the trial mortality was 36 per cent. The timing of induction depended on the height of the antibody titre.

Walker, Murray & Russell (1957 a & b) found induction before 35 weeks to be of no real value, while later induction saved a very small number of infants because of the hazard of prematurity. The authors recommended induction only to prevent a stillbirth, and then only if the husband was homozygous Rhesus positive. They had been unsuccessful in delivering more than one live-born child after a stillbirth. Fisher (1957) induced labour for the same reason as Walker et al - to reduce the foetal mortality from haemolytic disease - and his results showed that induction reduced the mortality despite the fact that the severity of the disease was greater in the induced group. He also emphasised that each pregnancy should be considered individually.

Allen (1957) thought that induction at 37 weeks was justified when the titre was low, in case it rose dramatically, but the induction had to be consistent with the safety of the mother and baby. Earlier induction was performed if the albumen antibody titre was high, and exchange transfusion was utilised to counteract the affects of prematurity. Kelsall, Vos and Kirk (1958) induced labour before term depending on the antibody titres obtained.

Two annotations appeared in the Lancet in 1958 and 1959, and the writer of the first felt that induction of labour was justified if a stillbirth had been forecast. In 1959, the writer drew attention to the dangers of induction, and stated that where the

indication for induction was clearest, the prospect for benefit was small, because of the danger of immaturity as well as any haemolytic disease. Dique and Wrench (1959) induced labour at 36 weeks in patients with an albumen antibody titre of 1/128 and over and found that the percentage of surviving infants increased proportionately with the percentage of inductions performed during any year in their series.

Wiener (1959) recommended preterm delivery provided that the mother and baby came to no harm, but he was reluctant to advise Caesarean section. Aaro (1959) also recommended preterm delivery, induction being carried out between 37 and 40 weeks.

Tovey and Valaes (1959) considered that premature delivery was the only rational method of preventing stillbirth, with kernicterus being prevented by the use of exchange transfusion. Using an anti-human globulin titre, they found that in a first affected pregnancy the prognosis was good when the titre was under 1 in 40. If it was over this and if<sup>a</sup> induction had been performed at 37 weeks, two-thirds of the stillbirths which occurred in the high titre group would have been avoided. If there had been antibodies present in a previous pregnancy, the prediction was more difficult to make, but induction was justifiable if stillbirth was expected. Jacobs (1959b) believed that induction at 37 or 38 weeks was the correct management of patients with Rhesus isoimmunisation, provided the obstetric conditions were favourable. He did not think that Caesarean section

was of value, but he did not make it clear whether this had been performed for an obstetrical reason or to benefit the foetus in some way. Ziel and Smith (1960) reported 3 cases in which planned early delivery with multiple transfusions was successful in severe haemolytic disease. They also stressed that each instance of maternal Rhesus sensitization must be individually considered for early termination of pregnancy.

Townsend and his co-workers (1961) still believed in the induction of labour in Rhesus isoimmunisation after a careful analysis of their results over three years. They were influenced by the antibody titre, the colour of the liquor amnii and the genotype of the husband, as induction was only performed if a Rhesus positive infant was anticipated. The authors commented on the difficulty they had in some cases in deciding the exact maturity because of the doubt which some patients had as to the date of the commencement of their last menstrual period. This was obviously of importance in deciding the optimum time for induction.

Norman (1960) recommended the induction of labour, even early induction, and McClure Browne (1960) suggested that routine induction at 38 weeks was a worthwhile practice as the foetus gained little in size and a number of stillbirths and severely affected infants would be avoided. Rigby et al (1961) favoured early induction if anaemia of the foetus was suspected but not if an excess of bilirubin was likely

to occur. The latter type was better treated by exchange transfusion.

Gairdner (1961) was in favour of early induction to lessen the time of exposure of the foetus to the antibodies, while Goplerud (1961) advocated induction at 38 to 39 weeks if there had been no previously affected infant, and 37 to 38 weeks or even earlier if a previous death had occurred.

Jouvencaux, Brizard, Michaud and Revel (1959) believed that the prognosis for the child was better when the birth weight was over 2500 g. and they found that radiological examination was helpful in the estimation of maturity. The authors gave qualified approval to a 'harmless' induction, but were not in favour of Caesarean section. On the other hand, Morley, Anderson and Forsythe (1961) did not think that there was any indication for preterm delivery in the first affected infant, but they did agree that treatment should be decided on the merits of individual cases. If previously affected infants had been delivered and the husband was homozygous, earlier induction might be justifiable.

After delivery, a number of tests are carried out on the baby, including tests on the cord blood. These include the determination of the infant's ABO and Rhesus groups and a direct Coomb's test. Vaughan (1959) drew attention to the possibility of 'false negative' results being obtained with regard to the Rhesus group and Coomb's test, and to the value of

bilirubin estimations after birth in an attempt to prevent the occurrence of kernicterus. Krieger (1955) had also drawn attention to the possibility of the foetal cells being coated with antibodies and being unable to react with the testing serum, thus giving a 'false' Rhesus negative result, when the group is Rhesus positive. Dique and Wrench (1959) found that the cord serum protein values were lower in infants affected by haemolytic disease than in normal infants, and in those with the lowest values, the mortality was highest. The writers believed that this was due to interference with the liver function of the foetus by the action of antibodies on the liver cells.

In the comparison of results from different centres, the method of assessment of severity of the haemolytic disease becomes important. No one method has been generally accepted, and there are individual criteria for the carrying out of exchange transfusions. Brody (1960) based his assessment of foetal severity on the level of bilirubin in mg. per cent at birth and after exchange transfusion, and he also pointed out that the plasma cholinesterase activity was depressed. Pierce, Rigor and Luken (1958) assessed severity at birth and later by estimating the cord haemoglobin and the Coomb's test, and relating this to the clinical condition of the infant, including his colour and the time at which jaundice appeared. Walker and Neligan (1955) and Walker and Murray (1956) assessed the severity of the haemolytic process by estimating the haemoglobin

and the serum bilirubin. Walker and Jennison (1962) were required to predict the severity of the haemolytic disease at 34 weeks whereas the infant might not be delivered for 4 to 5 more weeks, and they tended to assess the severity by the number of exchange transfusions required.

The indications for exchange transfusion were stated by Walker (1958) to be the treatment of anaemia and the prevention of kernicterus. If the serum indirect bilirubin concentration was seen to be rising at the rate of 1 mg. per 100 ml. per hour and was in danger of reaching 20 mg. per 100 ml., kernicterus would be likely to occur unless the concentration was reduced by exchange transfusion. Repeated transfusions were sometimes necessary to keep the bilirubin concentration below the danger level. Claireaux et al (1953) and Vogel (1953) had shown that indirect bilirubin could cross the blood-brain barrier and be taken up by brain cells which had been damaged by anoxia and anaemia. This gave rise to the yellow staining seen on post mortem examination. Wiener (1959) also quoted 20 mg. of bilirubin per 100 ml. of serum as the accepted upper limit and performed exchange transfusion to keep the bilirubin level below this. Simple 'top-up' transfusions were given when the haemoglobin level reached 45 to 50 per cent (approx. 7 to 7.5 g. per 100 ml.). Jouvenceaux and his colleagues (1959) advised early exchange transfusion and stated that the

procedure was not without risk as 14 infants out of 418 were lost during or immediately after transfusion in their series.

There is now no controversy about the value of exchange transfusion as opposed to simple transfusion. Exchange transfusion was advocated by Allen et al (1950b), and Mollison and Walker (1952) and Armitage and Mollison (1953) showed the definite advantages of this procedure in the National Trial. It was found to be particularly advantageous when the infant was mature and had a low haemoglobin level, and also when the infant was immature and had a high haemoglobin, and usually a rising bilirubin. Campbell (1955) stressed that a severe anaemia and a high bilirubin could both be treated well by exchange transfusion. Kelsall and Vos (1955) preferred to give a larger volume of blood than the 80 ml. per pound of body weight recommended by Walker and Neligan (1955), and tried to avoid repeating the procedure. Wiener (1959) has always carried out the transfusion by the saphenous vein and radial artery. Most paediatricians, however, have favoured the umbilical vein for the procedure. Wiener also recommended that blood be cross matched with the mother's blood and administered to the infant without cross-matching.

Wiener, Wexler and Brancato (1956) considered that two exchange transfusions were sufficient, the first to arrest the progress of the disease by removing the red cells coated with antibody and to keep the bilirubin at

a low level. More bilirubin would diffuse from the tissues into the circulation and a second transfusion was necessary to bring about a permanent fall. Other workers have had to perform more transfusions to keep the serum bilirubin below 20 mg. per 100 ml. Vaughan (1959) also referred to the value of exchange transfusion in keeping the bilirubin level low and thus preventing kernicterus.

Since the time of Jakesch (1878) the placenta in haemolytic disease of the newborn, and particularly in hydrops foetalis, has aroused much speculation and interest. Crawford (1959) studied the growth of the normal placenta and noted that it increased in size by enlargement of individual cotyledons, the total number remaining constant. This growth process took place at the edge or fringe of the cotyledon and was still taking place at term. In erythroblastosis foetalis, the degree of enlargement of the placenta was found by Crawford to be related to the severity of the condition. In hydrops there were fewer cotyledons, due to a reduction in the number of small and medium-sized cotyledons. The overall placental size was increased by hypertrophy and the fringes of the cotyledons consisted of less mature 'growing ends'. These were seen to be actively dividing and gave rise to the yellow discoloration seen on the maternal surface. In icterus gravis, the author found the placenta to be less hypertrophied.

Chernyak and Rabtsevich (1959) studied a number

of placentas associated with varying degrees of haemolytic disease. They noted a decrease in the average diameter of the chorionic villi, the largest being found in the placenta of hydropic infants, while the immunised women with normal infants unaffected by haemolytic disease, had villi of normal size. The ratio of placenta to foetus by weight ranged from 1:1 to 1: 3.5 in hydrops foetalis, from 1: 3.5 to 1: 4.3 when the infant died as a result of haemolytic jaundice, and from 1: 4.4 to 1. 5.4 in patients with haemolytic disease with a favourable outcome. In the clinically unaffected group the ratio was the same as in the control group, ranging from 1:5.8 to 1:7. Goplerud (1961) found that in his series when the placenta to foetus ratio was less than 1 in 4, the infant died. Exceptionally the infant was so immature that the placenta to foetus ratio would normally have been less than 1:4, whether haemolytic disease was present or not.

Walker and Neligan (1955) laid down certain criteria which they thought could be used as a basis for comparison of the severity of haemolytic disease in different series. These included the cord haemoglobin, the birth weight and the sex of the baby. In their series no infant had been lost who had not been given an exchange transfusion, and none of those who did have an exchange transfusion developed kernicterus. The authors considered that 98 per cent of infants born alive with haemolytic disease should

survive if adequately treated.

Shelton (1955) drew attention to the tendency among paediatricians to omit the number of stillbirths and the number of Rhesus negative infants born to immunised mothers. To the obstetrician all these women presented a problem of management. He had a stillbirth rate of 20 per cent in his series and accepted this as inevitable, while 5 per cent of infants were born alive but died within 24 hours.

Walker and Murray (1956) found a stillbirth rate of 10 to 15 per cent quoted, and noted that 7 to 8 per cent of apparently first affected pregnancies ended in stillbirth. After a mildly affected infant, the risk of stillbirth subsequently was 2 per cent, but after a stillbirth, the risk was 68 per cent. Walker, Murray and Russell (1957b) stressed that it was difficult to calculate the incidence of stillbirths due to haemolytic disease unless the overall incidence of haemolytic disease was known. Hospital series might be unfairly loaded with stillbirths sent in for delivery, while mildly affected infants remained undiagnosed outside the hospital.

Tovey and Valaes (1959) had an overall foetal survival rate of 87.7 per cent with a stillbirth rate of 8.4 per cent. The neonatal death rate was 3.1 per cent.

Poster (1958) studied a large number of case records and concluded that after a previously affected infant, there was a 50 per cent chance of survival, but

only 20 percent after a neonatal death from haemolytic disease, and 10 per cent after a stillbirth. Porter considered that this rather gloomy prognosis could be improved by more vigorous and active treatment. In 1958 Walker went further than previously and stated that, with adequate care, the neonatal mortality rate should be as low as 6 per 1,000.

Jacobs (1959a) calculated that, in 'first affected' infants, the survival rate should be 95 per cent with treatment, in families with previously affected but surviving infants, the rate was 66 per cent, but after a 'Rhesus' stillbirth or neonatal death, the prospects were hopeless. Townsend et al (1961) divided their cases into 3 groups, - first affected, previously affected but alive, and previous neonatal death or stillbirth. The perinatal mortality in the three groups was as follows - 7 per cent, 17 per cent and 54 per cent of Rhesus positive infants.

#### SUMMARY.

During the last decade many of the problems associated with Rhesus isoimmunisation have been investigated. The mechanism of isoimmunisation is fairly well understood, but the reasons why some women respond to the Rhesus positive antigen by forming antibodies while others do not has not been satisfactorily explained. As regards the management of immunised patients, the technique of exchange transfusion has been perfected and the electrolytic changes associated with it have been thoroughly studied, and

so if a baby is born alive and is able to tolerate an exchange transfusion, it has a very good chance of survival. Unfortunately there are still a large number of stillbirths from haemolytic disease - 10-15 per cent in most series, or 500 each year - England and Wales (Lancet 1958) - and despite a number of promising lines of research, no satisfactory method is available for treating these severely affected infants in utero. The only possible treatment is the delivery of the infant before term so that he is removed early from exposure to antibodies and takes his chance in the nursery. No one wishes to add prematurity to the risks which the baby requires to run, and so some workers are unwilling to induce labour unless it is certain that a stillbirth will occur if labour is not induced. Unfortunately the means of ensuring that a stillbirth will occur are not available. Antibody titres are generally considered unreliable except possibly in a first affected pregnancy, and anomalous results have been obtained. The genotype of the husband is only a probability and can be wrong, while the previous history is not a sufficiently reliable index to invite the performance of heroic procedures. The clinical history during the pregnancy is not of much help, nor are X-rays considered to be of help in prediction. Some workers have examined the liquor amnii obtained by uterine paracentesis at about 34 weeks. The examinations have been both naked eye and spectrophotometric and although there have been some

false results reported, the procedure has proved to be of some value.

Having predicted an affected or unaffected infant, or a mildly affected or severely affected one, the timing of the induction of labour, if it is to be performed, has to be decided upon. It is generally agreed that, only in very exceptional circumstances and without much hope for the infant, is induction likely to be of value before 35 to 36 weeks gestation. Routine induction at 37 and 38 weeks, and by term, has been recommended, but others have stressed that each patient must be considered on her merits and that no statistical deductions should be applied to particular cases.

It is comforting to know that efforts continue to be made to find new methods for the treatment of this condition, and that many are prepared to devote their time to diagnose and manage these isoimmunised patients.

METHODS AND RESULTS.

The patients who have been studied in the present investigation fall into 2 groups. The first group is a very small one, consisting of women who have been attending the Ante Natal Clinic of the Simpson Memorial Maternity Pavilion of the Royal Infirmary of Edinburgh during the last 8 months of 1962 and whose blood has been examined for the presence of foetal red blood cells. The second group is much larger and includes all the women who have attended the Simpson Maternity Hospital during the period 1956 to 1962, and in whom Rhesus antibodies have been demonstrated or who have given birth to an infant found to have haemolytic disease of the newborn due to Rhesus isoimmunisation.

The Simpson Memorial Maternity Pavilion (S.M.M.P.) has 180 obstetric beds and is the largest obstetric hospital in the South-Eastern Region of Scotland. It consists of 3 separate units but the patients with Rhesus isoimmunisation are distributed among the units, although since 1958, when the prospective part of this study commenced, there has been a tendency for patients known to have Rhesus isoimmunisation to be referred to and delivered in the Professorial Unit.

Routinely blood is obtained from every patient, in every pregnancy, on first attendance at the ante-natal clinic (or on admission to hospital as an obstetric emergency). This blood is grouped for

ABO and Rhesus factors and is 'screened' in the Regional Blood Transfusion Centre for the presence of immune antibodies. If an abnormal or unexpected agglutination reaction occurs during 'screening', an indirect Coombs' test is carried out and the specificity of the abnormal antibody is identified. Initially the concentration or titre of this antibody was determined against cells suspended in saline ('saline antibodies') and against cells in a protein medium ('albumen', 'incomplete' or 'blocking' antibodies). During the last 3 years titrations using Coombs' serum and red blood cells have been carried out, giving a quantitative estimate of the antibody concentration. The techniques used are standard ones, enzyme (papain) treated cells being used on occasion in the detection of antibodies but not in quantitative estimations. It is not proposed to consider these techniques any further as they were carried out exclusively by technicians in the Transfusion Centre and the reports issued form the basis for the diagnosis of Rhesus isocimmunisation.

Further blood specimens are obtained from all Rhesus negative patients at the 32nd and usually the 38th weeks of pregnancy, and are screened for antibodies in the same way. Repeat tests are also carried out on blood obtained at about 32 weeks gestation if a patient is Rhesus positive and has had a blood transfusion, an unexplained stillbirth or neonatal death, or an infant with unexplained jaundice. If no antibodies are detected in these patients, no further action is

required with regard to the possibility of Rhesus isocommunisation, but if the infant after delivery, shows any sign of developing icterus gravis neonatorum, blood investigations on the mother and infant are carried out.

If antibodies are detected, the report is sent to the University Departmental Sister in the S.M.M.P., and arrangements are made for further investigation of the patient. If the antibodies are detected early in pregnancy, a further specimen of blood may be taken at about the 20th week for antibody titre estimation. Also a convenient date at about 34 weeks gestation is arranged for performing a uterine paracentesis to obtain amniotic fluid for spectrophotometric analysis. If possible, arrangements are made to test blood from the patient's husband. Blood is taken off at the hospital or by the family general practitioner and is sent to the Blood Transfusion Centre for ABO grouping and Rhesus genotyping.

After analysis of the liquor, and after the previous history of the patient with regard to Rhesus isocommunisation have been scrutinised, the Rhesus genotype of the patient's husband and the level of and changes in the antibody titres have been studied, the clinical condition of the mother and foetus has been ascertained and the radiological appearance of the foetus and placenta has been examined, a prediction is made by me as to whether the infant is affected or unaffected by haemolytic disease, the severity of the disease and the

optimum time for delivery of the infant.

This prediction is then passed to the Unit under whose care the patient is being confined and the management decided upon after consultation between the members of the obstetric and paediatric staffs. The method of induction used and the management of the patient's labour are decided by the obstetrical unit concerned and the treatment of the infant by the appropriate paediatricians. At birth cord blood is obtained and sent to the Blood Transfusion Centre for ABO and Rhesus grouping, direct Coombs' test and estimation of serum bilirubin, by the method of Molloy and Evelyn (modified)<sup>(1937)</sup>. The haemoglobin content of the blood is also determined. Blood is made ready for exchange transfusion if this is indicated.

The S.M.M.P. receives patients from Edinburgh and the surrounding districts through its own Booking Ante Natal Clinic, but patients with obstetric complications including Rhesus isoimmunisation are also referred from the Eastern half of the Scottish Borders area including Hawick, Galashiels, Jedburgh, Duns and Berwick-on-Tweed. A considerable number of patients with Rhesus isoimmunisation are to be found in Fife and patients are referred to the S.M.M.P. from East Fife, because the Maternity Hospital in that region is not equipped to carry out exchange transfusion in an emergency, should this be necessary.

In the series of patients with Rhesus isoimmunisation (Table I) approximately one-third of the patients live in Fife. As occasionally patients with Rhesus isoimmunisation are, in fact, delivered in Fife, or in the Borders, and as sometimes these patients are referred to other hospitals in Edinburgh,



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IMAGE QUALITY POOR IN ORIGINAL

TABLE I

Year	Number of Mothers (a)	Number of Abortions (b)	Number of Abortions with haemolytic disease	Number of Twin Pregnancies (c)	Number of Viable Infants (a-b + c)
1956	61	4	1	2	59
1957	64	3	1	1	62
1958	69	5	3	3	67
1959	82	1	1	1	82
1960	82	4	3	1	79
1961	110	7	4	3	106
1962	82	6	3	1	77
<b>TOTAL</b>	<b>550</b>	<b>30(5%)</b>	<b>16</b>	<b>12(2%)</b>	<b>532 (97%)</b>

TABLE I Patients with Rhesus Isoimmunisation delivered in  
Simpson Memorial Maternity Pavilion, Edinburgh.

or outside the area, the incidence of haemolytic disease in this Region is difficult to estimate. A possible explanation for the unexpectedly high incidence in Fife is that there is a large Roman Catholic element in the population, and large families are common rather than the exception. It is not possible to say, from available figures, whether there are more Rhesus positive men and Rhesus negative women in Fife than in the rest of the country, or whether there is any susceptibility to the development of antibodies in these women. A full population study might throw some light on this.

During the 7 years under review, (Table I) the numbers have been gradually increasing to an exceptional 'peak' in 1961. There is no obvious explanation for this peak except that the number of parous patients delivering in the hospital was very high and possibly more patients with unsuspected antibodies were seen and the condition diagnosed after routine blood testing in the clinic.

During the 7 years under review, the number of deliveries in the S.M.M.P. was 26,891 (Table II) and the incidence of Rhesus isocimmunisation among multiparous patients delivered in this hospital was 4.1 per cent.

This figure has only local significance, however, as the immunised patients form a highly selected group.

In Table III the patients in the whole series are classified according to their age and parity and as might be expected with a condition occurring almost exclusively in multiparous patients, the highest incidence was the 'Para 1' and 'Para 2' groups; and in the age groups 25-29 and 30-34. In

TABLE II

	<u>Primigravid patients</u>	<u>Multiparous patients</u>	<u>Total</u>
1956	2084	1479	3563
1957	2061	1669	3730
1958	2029	1766	3795
1959	2028	1808	3836
1960	2039	1873	3912
1961	2002	2019	4021
1962	2000*	2034*	4034
Total	<u>14,243</u>	<u>12,648</u>	<u>26,891</u>

Number with Rhesus  
antibodies

532 viable infants ----- 2% of total admissions

8 primigravid mothers

524 multiparous mothers ----- 4.1% of parous admissions.

TABLE II

Numbers of primigravid and multiparous patients delivered in the Simpson Memorial Maternity Pavilion from 1956 to 1962, and incidence of Rhesus isoimmunisation.

\* Provisional figures.

TABLE III

Age	Primigravida	'Para 1'	'Para 2'	'Para 3'	'Para 4'	'Over Para 4'	Total
Under 20	1	0	0	0	0	0	1
20 - 24	3	34	24	12	6	2	81
25 - 29	1	51	60	30	18	19	179
30 - 34	3	33	44	38	17	37	172
35 - 39	0	10	18	17	11	32	88
Over 40	0	2	6	6	4	11	29
<b>TOTAL</b>	<b>8</b>	<b>130</b>	<b>152</b>	<b>103</b>	<b>56</b>	<b>101</b>	<b>550</b>

TABLE III

Patients with Rhesus Isoimmunisation by age and parity.

(Note - in this table, an 'abortion' is considered

a 'pregnancy' for calculation of parity.)

Table IV are detailed the age and parity of women in whom antibodies were detected for the first time, and 39 per cent of immunizations were found in the second pregnancy. In some of these patients an unaffected infant was delivered, so sensitization definitely occurred in the first pregnancy. This occurrence will be referred to again. The age group with the maximum number of 'first-immunized' patients is 25 to 29, but immunization was detected for the first time in 17 women over 40 and on 5 occasions, the patient had had 4 pregnancies previously. In Table V, the parity of the patient, when antibodies were first detected, is considered with the severity of the haemolytic disease found in the infant. In 7 per cent the infant was lost because of haemolytic disease, and, of possibly more importance, in 8 per cent the infant lost was in the patient's second pregnancy. If the first pregnancy had ended in abortion or a stillbirth or neonatal death because of a congenital abnormality or pre-eclampsia, the chance of the patient having a healthy living infant in a subsequent pregnancy is greatly reduced.

In presenting the results obtained in this series, various methods of grouping of the patients have been employed and these require definition.

i. 'Booked' and 'Not Booked'.

As it is considered unlikely that an infant will survive if delivered before 34 weeks gestation with haemolytic disease, and as this was the optimum time for carrying out the paracentesis test, a 'Booked' patient was one who was seen at an ante natal clinic in the S.M.M.P. before the 34th week of pregnancy. Some of these patients had been attending the

**TABLE IV**

Age	Primigravida	'Para 1'	'Para 2'	'Para 3'	'Para 4'	'Over Para 4'	Total
Under 20	1	0	0	0	0	0	1
20 - 24	3	33	18	6	1	0	61
25 - 29	1	49	34	8	5	6	103
30 - 34	3	31	22	17	6	10	89
35 - 39	0	7	10	8	6	11	42
Over 40	0	2	4	3	3	5	17
<b>TOTAL</b>	<b>8</b>	<b>122</b>	<b>88</b>	<b>42</b>	<b>21</b>	<b>32</b>	<b>313</b>

**TABLE IV** Patients with no previous history of Rhesus Isoimmunisation  
by age and parity.

(Note:- In this table, an 'abortion' is considered a 'pregnancy'  
for calculation of parity).

**TABLE V**

Severity of Haemolytic Disease	Primigravida	'Para 1'	'Para 2'	'Para 3'	'Para 4'	'Over Para 4'	Total
Unaffected	1	14	13	6	3	13	50
Mildly affected	2	31	16	16	7	8	80
Moderately affected	2	49	43	12	7	8	121
Severely affected	2	13	9	3	3	0	30
Stillbirths and Neonatal Deaths due to Haemolytic Disease	1	10	4	5	1	1	22
Abortions and Infants Not Tested	0	5	3	0	0	2	10
<b>TOTAL</b>	<b>8</b>	<b>122</b>	<b>88</b>	<b>42</b>	<b>21</b>	<b>32</b>	<b>313</b>

**TABLE V**

**Patients with no previous history of Rhesus Isoimmunisation by severity of haemolytic disease and parity.**

(Note:- In this table an 'abortion' is considered a 'pregnancy' for calculation of parity).

Hospital Clinics from early pregnancy, while others had intended to be confined under the care of their own doctor or a smaller hospital, and ante natal supervision had been undertaken elsewhere. When antibodies were detected, the patient was referred to the S.M.M.P. In a few instances the patient had had no ante natal care and usually was referred to us after the 34th week of pregnancy, or came into hospital in labour. In such cases, if maternal blood had not been tested during the pregnancy, cord blood was sent for testing routinely.

ii. Grouping by Previous History of Rhesus Isoimmunisation.

Group I:- Patients found to have Rhesus antibodies apparently for the first time, but including those with antibodies known to have been stimulated by an injection or transfusion of incompatible blood.

Group II:- Patients known to have had Rhesus isoimmunisation in one or more previous pregnancies, but all the affected infants had survived.

Group III:- Patients with Rhesus isoimmunisation in a previous pregnancy, but on at least one occasion the infant had been lost because of haemolytic disease - either a stillbirth or neonatal death. These patients were grouped together because the numbers with previous stillbirths and neonatal deaths is small.

The grouping suggested by Goplerud (1961) has been used, his Groups III and IV being combined as Group III in this classification. This differs from the grouping used by Jacobs

(1959a) and Townsend et al (1961) with regard to Groups I and II but not III. The number in Group I where antibodies have been stimulated by an incompatible transfusion is small.

iii. Severity of Haemolytic Disease.

Unaffected:-

The Rhesus group of the infant is the same as that of the mother in all respects, or if different, the direct Coomb's test on the infant's blood is negative, and the infant is clinically unaffected.

Mildly affected:-

The Rhesus group of the infant differs from that of the mother and the direct Coomb's test is positive or there is clinical evidence of haemolytic disease, but no treatment is required in the form of exchange or 'top-up' transfusions.

Severely affected:-

The infant is serologically affected with a very low cord haemoglobin - anaemia group - or the indirect serum bilirubin rises rapidly - hyperbilirubinaemia group - usually more than once, to 20 mg. per cent. or over, despite exchange transfusions. The infant survives the neo-natal period.

Moderately affected:-

The infant is affected by haemolytic disease and requires treatment, but is not as severely affected as the 'severe' group.

Rhesus deaths:-

The child is stillborn and haemolytic

disease confirmed by post mortem examination as the cause of death or a very important contributory factor: or the child dies in the neonatal period from haemolytic disease or from a cause directly associated with exchange transfusion.

Other deaths:-

The infant is stillborn or dies in the neonatal period as a result of a condition unrelated to haemolytic disease or its treatment, even although haemolytic disease is present.

Rhesus abortions:-

Post mortem examination of the abortus shows definite evidence of gross haemolytic disease.

Others:-

Includes 14 abortions, and 5 living infants born to immunised mothers but from whom no blood was obtained for testing and who showed no signs of haemolytic disease.

This classification is not ideal but is a practical one. The distinction between 'unaffected' and 'mildly affected' is unimportant from the point of view of management of the pregnancy, while the difference between mildly affected and moderately affected is often dependant on the gestation of the pregnancy at the time of delivery and the views of the paediatrician regarding the indications for exchange transfusion. In many tables in this thesis, the two are grouped together. There is also some dispute about the severely affected group.

If the cord haemoglobin is very low, the infant is severely affected, but unless kernicterus develops, the 'high bilirubin' group might not be considered to be severely affected. As the aim of treatment, obstetrical and paediatric, is to deliver living infants who survive the neonatal period, both types of severe case would seem to be important.

It is difficult to devise a scheme predicting the degree of severity which takes into account the patient's maturity at the time of delivery, the foetal birth weight, the foetal haemoglobin at birth and subsequently and changes in the level of bilirubin. The treatment seems to be a fairly good guide and although it is of no value in comparing the results with those of different series, it allows a fairly constant assessment of severity to be made.

The problems considered in this thesis are the method of sensitisation of the Rhesus negative mother, (omitting Rhesus positive patients who are usually sensitized by blood injection or transfusion) any mechanism which might prevent a patient being immunised, prediction of the severity of haemolytic disease before the delivery of the infant, the advantages and disadvantages of induction of labour, and the timing of induction, and the state of the infant at delivery. Some observations on the placenta are also made and cases illustrating difficulties in prognosis or treatment are quoted.

1. Sensitization of the Rhesus negative woman by foetal cells during pregnancy.

It has been established that foetal blood cells can be detected in the maternal circulation (Zipursky et al 1959), Finn et al (1961) and Wimhofer et al (1962). Although

Rucknagel and Chernoff (1955) suggested that foetal haemoglobin detected in maternal blood might be produced by 'foetal haemoglobin-producing' tissue in the mother's own marrow, in response to chorionic gonadotrophin, it seems likely that the 'foetal cells' seen have in fact come from the foetus.

Most workers have suggested that the red cells of the foetus gain access to the maternal blood during labour or at delivery, and this accounts for the rarity with which isoimmunisation is observed during the first pregnancy (as opposed to the post natal period). These writers associate the occurrence of isoimmunisation particularly with a high incidence of operative procedures or manipulations during the delivery of the infant or of the placenta during a previous pregnancy (Gainey et al (1954) Aaro (1959), Kelsall, Vos and Kirk (1959) Reilly (1960) and Knox, Murray and Walker (1961). However, Lawrence et al (1956) and Novell and Taylor (1961) were unable to find such an association.

Zipursky et al (1959) described a modification of the method of Kleihauer et al (1957) for the detection of the foetal haemoglobin in foetal blood cells and this method has been used, with some modifications, in an attempt to demonstrate the presence of foetal red cells in the maternal circulation during pregnancy.

a. Material and method.

Initially a few patients who had developed antibodies during their pregnancy were selected and blood was examined from them. More recently, a group of patients - any primigravida, and also multipara who were known to be Rhesus negative and to

have no antibodies - were chosen at 'random' from Booking Ante Natal Clinic and blood was obtained at their first visit (before 20 weeks gestation), at 20 weeks, at 28 weeks, 32 weeks and 36 weeks. Blood was also obtained during labour, after delivery, in the puerperium and in the post natal clinic, and umbilical cord blood was obtained for ABO and Rhesus blood grouping.

Peripheral blood was obtained from an arm vein and clotting prevented by potassium oxalate crystals in the tube. In the laboratory the blood was initially diluted by 3 times its own volume of normal saline, and a blood film made on a slide. After the film had dried, it was fixed in absolute ethyl alcohol for 2 minutes and, again after drying, it was washed for 90 seconds in citrate phosphate buffer (0.16 M  $K_2 HPO_4$  and 0.18 M citric acid, PH 3.4 - 3.6 at 37°C.). The film was then stained with May-Gruenwald stain and washed until all the visible stain had been removed. After drying, the slide was examined under the low power lens of the microscope. Foetal cells were seen as pink-staining refractile cells among erythrocyte 'ghosts', and the slide was scanned for 3 minutes, a count being made of the number of foetal cells seen in that time. At least two slides were made from each patient.

The technique was later modified so that the blood sample was diluted with only twice its own volume of saline, the slide was fixed for 3 minutes in alcohol

and the buffer was in contact with the cells for 3 minutes. We found that it was unnecessary to stain the slide, (Figure 1), and that counting of the cells with refractile nuclei was easier under high power. Counting was carried out in each case for 3 minutes, and the average of the counts calculated for all the slides from that patient was thereafter known as the patient's 'score'.

A third small group of patients have had blood taken before and after delivery, the first specimen being taken in labour and the second 48 hours after delivery. It is hoped to determine whether the method of delivery influences the 'leakage' of foetal cells into the maternal circulation, and these studies are continuing.

b. Preliminary report on Results obtained.

The number of cases in which the investigation is complete is very small and the only reason for the inclusion of these results in this thesis is to indicate a study which might in time yield some significant information. Two interesting cases are also described.

In the small group of patients known to have Rhesus isoimmunisation, foetal cells in any appreciable number were only found in one patient (Mrs. G.A.). Presumably any foetal cells entering the circulation before antibody production is stimulated will be destroyed when the antibodies enter the circulation. Any red cells entering after immunisation will be removed from the circulation rapidly by the antibodies already

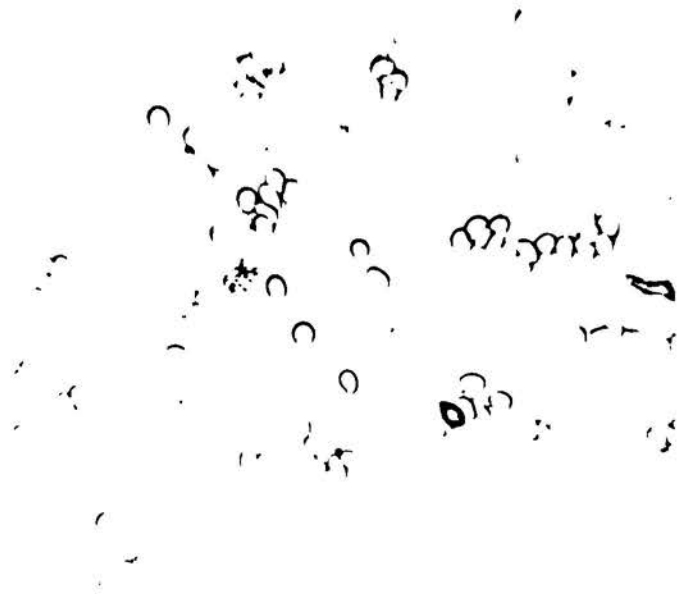


Figure 1. (a) Foetal cells in maternal circulation  
X 330.

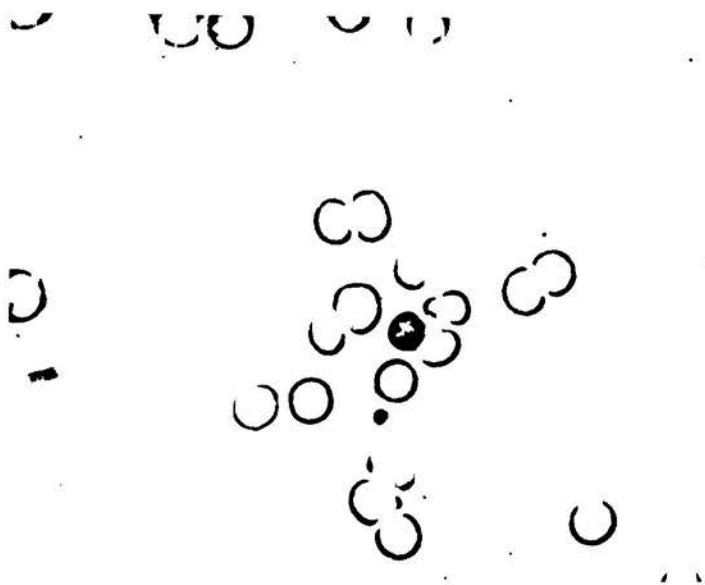


Figure 1. (b) Foetal cells in maternal circulation  
X 750.

present. The exception was a very interesting case.

Mrs. G.A. was a primigravida aged 34 and was in Australia when she became pregnant. Antibodies were detected when she first attended the ante natal clinic at 23 weeks gestation, the albumen titre being recorded as 1 in 512. She was found to be Group O, Rhesus negative, while her husband and infant were Group B, Rhesus positive. Foetal red cells were recorded in the maternal circulation at 30 and 32 weeks gestation, the 'scores' on both occasions being about 150 (148 and 151). It is necessary in this case to postulate a continuous, quite large, leakage of foetal cells to the mother as presumably the cells were being, continuously destroyed, or at least 3 'haemorrhages' into the mother. Labour commenced spontaneously at 36 weeks gestation, and the infant, a male, weighed 4 pounds 9 ounces (2065 g.) and the placenta 1 pound 13 ounces (820 g.), a placenta to foetus ratio of 1:2.5. The cord haemoglobin was 10.2 g. per cent, and the cord serum bilirubin 6.85 mg. per cent, indicating that some haemolysis had occurred. No other placental abnormality was noted.

This patient had had no injections or transfusions of blood, but had received gamma globulin to prevent Rubella infection. It is, therefore, likely that primary sensitization and immunisation occurred during the first pregnancy, and caused the infant to be severely affected by haemolytic disease.

In the second group, studied during pregnancy, 15 patients have now been delivered. Ten of these were primigravida and were subsequently found to be Rhesus positive. Although 4 patients were reputed to have a score of 9 or 10 at their Booking Clinic visit (8-14 weeks gestation) the scores were generally low in early pregnancy. The highest scores obtained in each case ranged from 7 to 166 (at gestations from 28 to 36 weeks), but in every case the score after delivery was zero.

In the 5 parous patients, the number of cells seen in early pregnancy was 0-2 but in the last trimester the number ranged from 6 to 136. These patients were all Rhesus negative and their infants were found to be Rhesus positive. In the blood of only 1 patient was an appreciable number of foetal cells seen after delivery (160), but she has not yet developed Rhesus antibodies. One patient developed antibodies during her pregnancy.

Mrs. E.W. was Group O, Rhesus negative, and her first infant, who weighed 11 pounds 10 ounces (5270 g.) had been delivered by ventouse extraction 2 years previously. This child was group O, Rhesus positive, and the direct Coomb's test was negative. In her second pregnancy, no antibodies or foetal cells were detected at 6 weeks gestation but a further test for the presence of foetal cells at 28 weeks gestation gave a score of 186. The patient was admitted to hospital with an accidental haemorrhage at just over 30 weeks

gestation, and immediately after this, antibodies were found in her blood - the albumen titre was 1 in 512 and the antiglobulin titre 1 in 80. At 32 weeks gestation, no foetal cells could be seen in the maternal blood and the foetal heart was no longer heard. X-ray examination at that time confirmed the presence of an hydropic foetus in utero. (Figure 2 shows a marked 'halo'). Labour commenced spontaneously at 34 weeks gestation and the patient was delivered of a stillborn male infant weighing 5 pounds 9 ounces (2520 g.). The placenta weighed 2 pounds 3 ounces (990 g.), a placenta to foetus ratio of 1:2.75. The antibody titre remained high after delivery, and no foetal blood cells were found in the maternal circulation during the puerperium or 6 weeks after delivery. The foetus was obviously hydropic on examination and there was evidence of placental separation.

The patient's husband was Group O, Rhesus positive, with a probable genotype of CDe/cDE, and the patient had antibodies to C and D. A transplacental haemorrhage seems likely in this case, possibly associated with an undetected mild degree of accidental haemorrhage. The 'clinical' accidental haemorrhage may have been the result of isoimmunisation with hydropic changes occurring in the placenta and predisposing<sup>to</sup> its separation. After immunisation, foetal cells were removed from the maternal blood.

It can be said from these few cases that foetal cells certainly enter the maternal circulation during

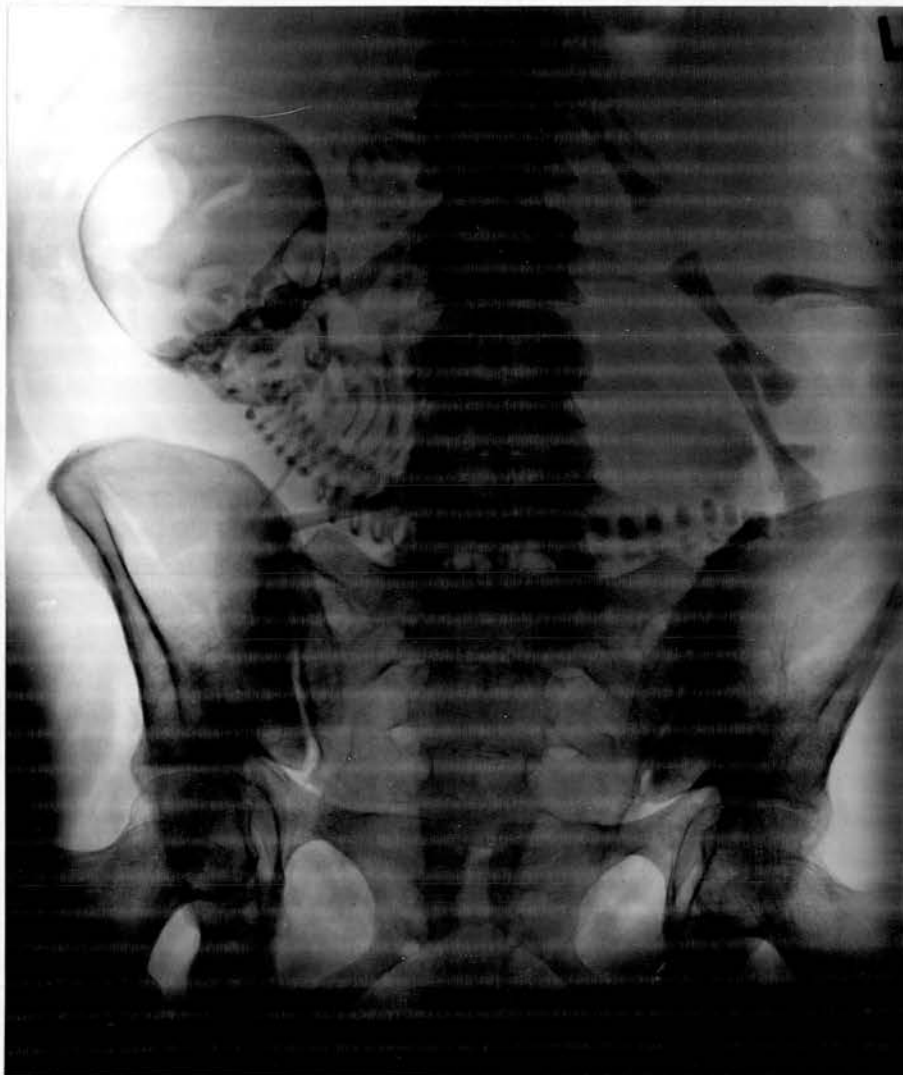


Figure 2. X-ray - hydropic intra-uterine death with 'halo'.

pregnancy, and if the patient is Rhesus negative antibody formation may result. The presence of foetal cells in the maternal circulation in a Rhesus negative woman does not necessarily indicate that isoimmunisation will occur, and the volume and rapidity of the leakage, as well as the gestation at which it occurs may be of some relevance.

In a small third group of patients, whose blood was studied during labour and in the puerperium, the scores obtained after 11 spontaneous deliveries were zero, except on one occasion when 6 cells were seen. The blood was obtained from 5 primigravid patients and 6 multipara. Three patients were delivered by forceps and 1 gave a score of 8 afterwards. One twin delivery has been studied, the first infant being delivered by forceps and the second by the ventouse, but no foetal cells were found. A manual removal of the placenta was performed on one primigravid patient, but no foetal cells were seen after this. In 1 of 3 patients delivered by Caesarean section, the 'score' was high (76) before the operation commenced, and rose to 103 after the placenta had been delivered. The patient was Rhesus positive and no antibodies were detected. The results obtained in the other two patients are suspect because of the staining technique being used at that time.

Again, from the small number of patients studied it can be seen that operative delivery may be responsible for foetal cells entering the maternal circulation but

this is quite likely to occur during a spontaneous delivery and may be related to the labour - normal or 'abnormal' - more than to the actual method of delivery. The third stage of labour may also be very important.

2. Results obtained in Patients with Rhesus Isoimmunization.

The overall results obtained in the series are presented in Table VI. The stillbirth rate among viable infants in the series is 7.3 per cent and among affected viable infants (infants unaffected by haemolytic disease and those not tested being excluded) it is 9 per cent. The neonatal death rates expressed as a percentage of live births - total and affected by haemolytic disease - are 2.7 and 3.3 respectively. These results compare very favourably with those obtained in other centres but no comparison is valid unless the number of severely affected infants is also compared. This again leads to difficulty over definition of severity, and so the overall figures for the series are presented.

It will be seen in Table VI that 13 infants were lost in the series from causes unrelated to haemolytic disease. Details of these 13 are given in Table VII.

It is necessary to blame the management of the patient's Rhesus isoimmunization in 2 of these cases for causing or contributing to the death of the infant. In 1 pregnancy, surgical induction of labour failed and the patient was left for one week, before other measures were taken to bring about delivery. The infant was moderately affected by haemolytic disease, but died of

TABLE VI

Severity of Haemolytic Disease	Number of Infants	Percentage of Viable Births (532)	Percentage of affected Viable Births (435)
Unaffected infants	92 (6)	17.5	-
Mildly affected infants	105 (3)	19.5	24.1
Moderately affected infants	198 (3)	36.8	45.5
Severely affected infants	80 (1)	15.0	18.4
Stillbirths from haemolytic disease	39	7.3	9.0
		Percentage of live births (490)	Percentage of affected live births (396)
Neonatal deaths as a result of haemolytic disease	13	2.7	3.3
Other deaths (3 Stillbirths and 10 neonatal deaths)	(13)		
Rhesus abortions	16		
Other abortions	14		
Living infants not tested	5		
Total viable infants	532		

TABLE VI Foetal results in the series.

(Note - figures in brackets = deaths from causes other than haemolytic disease - See TABLE VII.)

TABLE VII

<u>Stillbirths</u>	3 - Unaffected -	1 Prolapse of umbilical cord.	Spontaneous onset.
		1 'Anoxia' at 36 weeks.	Spontaneous onset.
		1 'Anoxia' at 39 weeks.	Spontaneous onset.
<u>Neonatal deaths</u>	10 - 3 Unaffected -		
		1 Prematurity; under 2 pounds.	Spontaneous onset.
		1 Respiratory distress syndrome (twin.).	Spontaneous onset.
		1 'Anoxia'.	Induction of labour.
	3 Mildly affected -		
		1 Hydrocephalus.	Spontaneous onset.
		1 Respiratory distress syndrome.	Induction of labour (for antepartum haemorrhage).
		1 Congenital diaphragmatic hernia.	Induction of labour.
	3 Moderately affected -		
		1 Lung abscesses.	Induction of labour. (delivered 7 days after induction)
		1 Congenital heart disease.	Spontaneous onset.
		1 Respiratory distress syndrome.	Induction of labour.
	1 Severely affected -		
		Hyperbilirubinaemia.	
		Congenital abnormalities.	Spontaneous onset.

TABLE VIIDeaths from causes other than haemolytic disease.

pulmonary infection and lung abscesses. A second patient was thought to have a moderately affected infant and labour was induced at 38 weeks gestation. An oxytocin drip was required after artificial rupture of the membranes and the baby died twelve hours after delivery of 'anoxia'. The infant's blood group was Rhesus negative and induction was unnecessary on this occasion.

In neither case in which the infant died as a result of the respiratory distress syndrome (Table VII) was induction carried out because of Rhesus isoimmunisation.

3. Factors concerned with the Previous History of the Patient.

It is very important to obtain as complete information as possible about blood-tests, clinical features and the foetal outcome in previous pregnancies, when the patient is found to have Rhesus antibodies in her blood. This was not always possible in the present series, but if no information was available about previous pregnancies it was necessary to assume that the current pregnancy was the one in which antibodies were first demonstrated.

The results obtained when patients are allocated to one of 3 Groups based on the previous history are shown in Table VIII. The fact that 50 patients had unaffected infants in Group I is explained by the assumption that the patients were sensitized in a previous pregnancy (Knox et al (1961) and either that

TABLE VIII

Severity of Haemolytic Disease	Group I	Group II	Group III	Total	Other deaths (Table VII)
Unaffected	50 (1)	35 (4)	7 (1)	92	6
Mildly affected	80 (3)	25	0	105	3
Moderately affected	121 (1)	69 (2)	8	198	3
Severely affected	30	38 (1)	12	80	1
Deaths due to haemolytic disease	22	20	10	52	-
Abortions and infants not tested	10	10	15	35	-
Total	313 (5)	197 (7)	52 (1)	562	13

TABLE VIII Foetal results by Groups.

(Group I - First pregnancy with Rhesus isoimmunisation.

Group II - One or more previous infants affected by haemolytic disease.

Group III - One or more previous infants lost because of haemolytic disease.)

the patient's husband is heterozygous for the D antigen, or that the husband (and foetus) does not possess the antigen to which the patient has been immunised in a previous marriage or by blood injection or transfusion. Occasionally it was known that the patient's husband was not the father of the child concerned.

In Group I, 30 infants (9.5 per cent) were severely affected by haemolytic disease and 22 (7 per cent) were lost because of it, a mortality rate of 8.7 per cent among affected infants, (Table IX). This loss occurred despite a more active policy of induction during the last 5 years, and so it is never justifiable to say that, because a pregnancy is the first one in which antibodies have been detected, the infant will survive. These results are much better than those quoted by Goplerud (1961) but similar to the results of Townsend et al (1961). A disturbingly high mortality rate (13.2 per cent) was found among infants who were affected by haemolytic disease and whose mothers had previously shown evidence of Rhesus isoimmunisation. On the other hand the mortality rate in Group III (33.3 per cent) is considerably better than that reported in other series. Porter (1958) reported an 80 per cent foetal loss after a neonatal death due to haemolytic disease, and a 90 per cent loss after a stillbirth from haemolytic disease, unaffected infants being included in these figures. Jacobs (1959) considered the prognosis hopeless in this

TABLE IX

Groups	Mortality excluding abortions			Mortality of infants affected by haemolytic disease.		
	Number of infants	Foetal deaths	Percentage Mortality	Number of infants	Foetal deaths	Percentage Mortality
I	308	27	8.8	253	22	8.7
II	187	27	14.4	152	20	13.2
III	37	11	29.7	30	10	33.3
Total	532	65	12.2	435	52	12.0

TABLE IX      Mortality rates by Groups.

group.

4. Blood Group Incompatibility between Foetus and Mother in ABO system.

Levine (1943) showed that Rhesus antibodies were more likely to develop in women who lacked the natural isoagglutinin for the red blood cells of their offspring (homospecific pregnancies) than in others who have isocantibodies (heterospecific pregnancies). Reasons for this were suggested by Wiener (1945), Fisher (1954) and Race and Sanger (1954) as already stated previously. The most favoured view - Race and Sanger - suggested that the foetal red cells were destroyed by the naturally occurring anti-A or anti-B/antibodies in the maternal blood and the Rhesus positive component ceased to be antigenic after the destruction of the cells. This view was shared by Andersen, Bentzon and Larsen (1961). Nevanlinna and Vainia (1956) and Knox, Murray and Walker (1960) considered that the important pregnancy as far as ABO compatibility was concerned was the 'sensitizing' pregnancy - when the mother was sensitized by foetal red cells - and that ABO incompatibility had much less of a protective effect after sensitization had occurred.

In the present series the compatibility of foetal blood with the appropriate maternal blood (foetal cells and maternal serum) is correlated with the foetal result in Table  $\bar{X}$ . The results have been grouped according to the previous history of Rhesus isoimmunisation and it is obvious that there is a gross discrepancy

**TABLE X**

Group I	Total	Unaffected	Mildly and Moderately affected	Severely affected	Rhesus deaths	Not tested
Mother and baby groups compatible	247	43	169	29	6	30
Mother and baby groups incompatible	36	4	31	1	0	
<b>Total</b>	<b>283</b>	<b>47</b>	<b>200</b>	<b>30</b>	<b>6</b>	
<b>Group II</b>						
Mother and baby groups compatible	152	32	77	34	9	25
Mother and baby groups incompatible	20	1	15	3	1	
<b>Total</b>	<b>172</b>	<b>33</b>	<b>92</b>	<b>37</b>	<b>10</b>	
<b>Group III</b>						
Mother and baby groups compatible	24	6	6	10	2	24
Mother and baby groups incompatible	4	0	2	2	0	
<b>Total</b>	<b>28</b>	<b>6</b>	<b>8</b>	<b>12</b>	<b>2</b>	
<b>Group Totals</b>						
Mother and baby groups compatible	423	81	252	73	17	79
Mother and baby groups incompatible	60	5	48	6	1	
<b>Grand Total</b>	<b>483</b>	<b>86</b>	<b>300</b>	<b>79</b>	<b>18</b>	

**TABLE X** Foetal results, by Groups, when mother and baby ABO blood groups compatible and incompatible.

in the 'mother and baby groups incompatible' results, in each of the 3 'previous history' groups, although the difference is most marked in Groups I and II. In Group I the foetus is compatible with his mother 6 times more often than incompatible, and yet there are 10 times as many compatible as incompatible combinations in the unaffected group. In both Groups I and II, the foetus is compatible with his mother in the severely affected group many more times than might be expected, suggesting that as well as producing sensitization to the Rhesus factor more frequently, the foetal cells which are ABO compatible with the mother's cells, may give rise to more serious haemolytic disease. These observations would require a very much larger series with less selection of cases to be statistically valid, but a trend can be seen from the figures presented.

In Table XI<sup>the</sup> severity of haemolytic disease in each case is correlated with the maternal ABO blood group. The percentages in the different degrees of severity for each group are compared with the known distribution of blood groups in the whole population of this area of Scotland (Mourant and Kopec (1958)). It is seen that in all degrees of severity there are more Group A mothers and fewer Group O mothers, even in the unaffected group, presumably reflecting the protection offered by the mother's naturally-occurring antibodies in a previous pregnancy. The differences were only significant for the mild and moderately affected group and for all the affected infants taken together.

TABLE XI

Maternal blood group	Percentage distribution for Edinburgh	Percentage distribution for East and North Scotland	Unaffected		Mild and Moderate		Severe and Rhesus deaths		Total affected	
			No.	%.	No.	%.	No.	%.	No.	%.
A	36	35	43	47	121	40	50	40	171	40
B	11	11	8	9	40	13	10	8	50	12
AB	3	3	2	2	15	5	6	5	21	5
O	50	51	39	42	123	41	58	47	181	43
Total			92		299		124		423	

TABLE XI Distribution of maternal ABO blood groups with severity of foetal haemolytic disease.

5. Rhesus genotype of Patient's Husband.

Whether the husband of the patient is homozygous or heterozygous for the D antigen (or the antigen against which antibodies have developed) is of some importance in predicting the chance of a healthy baby being delivered after a stillbirth from haemolytic disease. If the husband is said to be homozygous for D, the chances are not good, while if he is heterozygous they are at least 50-50. Sometimes the probable genotype is of value along with analysis of the liquor amni and the patient's previous history. If, for instance, a patient had had a stillbirth due to haemolytic disease, her husband was reported as probably heterozygous and the liquor curve indicated the presence of very little bilirubin, it is almost certain that the infant would be unaffected by haemolytic disease.

Along with the antibody titres, the genotype of the husband adds to the difficulty of prediction because of the different possibilities when the reported titre is low in a second or subsequent immunised pregnancy.

The genotype is not accurate because there is no anti-d antiserum to permit identification of the 'd' antigen thus confirming heterozygosity. An intelligent guess is all that can be made and with certain phenotypes the chance of an incorrect genotype being reported is quite high. In Table XII the foetal results by Groups and genotypes is recorded. It will be noted that 12 patients had unaffected infants although their husbands had been reported to be homozygous for the D antigen.

TABLE XII

Severity of haemolytic disease	Homozygous positive husband. Groups				Heterozygous positive husband. Groups				Genotype of husband not known. Groups			
	I	II	III	Total	I	II	III	Total	I	II	III	Total
Unaffected	4	6	2	12	20	20	4	44	26	9	1	36
Mild and moderate	76	44	4	124	37	25	2	64	88	25	2	115
Severe	15	23	11	49	7	5	1	13	8	10	0	18
Haemolytic disease deaths - Stillbirths	11	9	4	24	3	3	2	8	3	2	2	7
Neo-natal deaths	2	3	2	7	1	2	0	3	2	1	0	3
Abortions and not tested	2	3	10	15	2	3	2	7	6	4	3	13
<b>Total</b>	<b>110</b>	<b>88</b>	<b>33</b>	<b>231</b>	<b>70</b>	<b>58</b>	<b>11</b>	<b>139</b>	<b>133</b>	<b>51</b>	<b>8</b>	<b>192</b>

TABLE XII Genotype of husband for D antigen with Foetal Results by Groups.

Proportionately more stillbirths and severely affected infants were found in the homozygous group, but the results could be chance findings.

In 1957 Murray reported that the genotype  $R_2R_2$  (cDE/cDE) was associated with more severe haemolytic disease than other genotypes, the inference being that foetal red cells with the genotype  $R_2r$  ( $\bar{c}DE/\bar{c}de$ ) stimulated the production of antibodies in the mother more readily than  $R_1r$ , and a higher titre resulted with more serious effects on the foetus in utero. In the present series the expected frequency (Race and Sanger (1962) of the common genotypes has been compared with that observed in unaffected infants and those with haemolytic disease. As expected there is a significant increase in the percentage of homozygotes and a reduction in the percentage of heterozygotes when the infant has been affected by haemolytic disease. As regards severity associated with any one genotype, there is no evidence in this small series that the genotype  $R_2$  is a more powerful stimulus, or that the genotype  $R_2r$  in the foetus is more susceptible to destruction by the anti-D antibody. (Table XIII).

#### 6. Clinical Course of the Pregnancy.

A study of the clinical course of the pregnancy in cases of Rhesus isoimmunisation is not usually rewarding, as the diagnostic features only become apparent when it is too late to obtain a live infant. It has been said that there is an association between pre-eclampsia and

TABLE XIII

Rhesus Genotype of husband	Expected frequency %	Unaffected		Mildly and Moderately affected		Severely affected and Rhesus deaths		Total affected	
		No.	%.	No.	%.	No.	%.	No.	%.
R <sub>1</sub> R <sub>1</sub> (CDe/CDe)	17 (20.5)	-	-	57	31.5	34	34.3	91	32.5
R <sub>2</sub> R <sub>2</sub> (cDE/cDE)	2 (2.4)	-	-	5	3.3	3	3.0	9	3.2
R <sub>1</sub> R <sub>2</sub> (CDe/cDE)	12 (14.5)	-	-	56	30.9	40	40.4	96	34.3
R <sub>1</sub> r (CDe/cde)	32 (38.6)	37	77.1	47	26.0	13	13.1	60	21.4
R <sub>2</sub> r (cDE/cde)	11 (13.3)	8	16.7	14	7.7	6	6.1	20	7.1
Others	9 (10.8)	3		1		3		4	
Total	83 (100)	48		181		99		280	

TABLE XIII

Severity of haemolytic disease with corrected Rhesus genotype of patient's husband (anti D antibodies).

(As 17% of expected genotypes are d/d, 'expected frequencies have been adjusted in table - within brackets.)

hydrops foetalis (Scott 1958) and the same writer showed a correlation between the severity of haemolytic disease and the amount of chorionic gonadotrophin excreted in the urine. Unfortunately, the 'normal range' for urinary chorionic gonadotrophin in late pregnancy is wide and only gross alterations in the amount excreted are likely to be significant.

Recognition of the 'maternal hydrops syndrome' (O'Driscoll (1956), Goodlin (1957) is not usually of practical help in management, as the foetus is already dead in utero, or the stage of gestation precludes even heroic intervention. In this condition, the patient complains of feeling unwell and is found to have a variable degree of hydramnios, but it is usually quite marked. The foetal movements are reported as being violent and then ceasing and sometimes the patient complains that her skin is 'itchy' or that she has a generalised irritation. There may or may not be signs of pre-eclampsia and these usually disappear soon after the death of the foetus, as does the skin irritation. The foetus is stillborn and there is a risk of hypofibrinogenaemia developing.

The results in patients with pre-eclampsia and the 'maternal hydrops syndrome' are recorded in Table XIV.

#### 7. Radiological examination of the Foetus in Utero.

As long ago as 1934, Snow and Powell noticed that the 'black line' due to the greater penetrability of the fat-containing, subcutaneous tissues was obliterated if the soft tissues were oedematous. From this the 'halo'

TABLE XIV

Foetal Results	Number of cases of pre-eclampsia by Groups			Total	
	I	II	III		
Unaffected	1	1	0	2	<u>Maternal 'hydrops'</u> <u>syndrome</u> <u>Group III</u> Rhesus abortions 2 Rhesus stillbirths 3 Total <u>5</u>
Mildly affected	4	2	0	6	
Moderately affected	10	4	0	14	
Severely affected	1	2	0	3	
Stillbirth due to haemolytic disease	2	0	3	5	
<b>Total</b>	18	9	3	30	

TABLE XIV Number of patients who developed pre-eclampsia and maternal hydrops syndrome, with foetal outcome.

effect was developed in hydrops foetalis. The Buddha position, in which the femora are externally rotated at the hips to appear in the directly antero-posterior view of the foetus as if the foetus was sitting on his femora, was not entirely reliable, and the halo sign was not always associated with the death of the foetus from hydrops foetalis. Samuel and Cohen (1950) described an abnormally straight spine which was thought to be due to the protuberant abdomen, and the large liver and spleen which were invariably found. For the same reasons, the ribs may be elevated and horizontal. Because of subcutaneous oedema, the legs and arms are displaced from the body and in appropriate views the scapulae are seen to be some distance from the rib cage. A large 'placental space' may sometimes be seen and, if any of these findings are present and the patient has circulating Rhesus antibodies, it may be indicative of a severely affected infant. Figure 3 shows an X-ray of a severely affected foetus, with a protuberant abdomen, elevation of the ribs and a Buddha position of the legs. The scapulae are some distance from the thoracic cage and the fat line is clearly seen. A large placenta is also present.

Figure 4 shows a severely affected foetus with a straight spine and hydramnios. The same patient had a severely affected infant in an earlier pregnancy and X-ray showed elevation of the ribs and a large placental 'space' (Figure 5).

Figure 6 is an X-ray of a severely affected infant

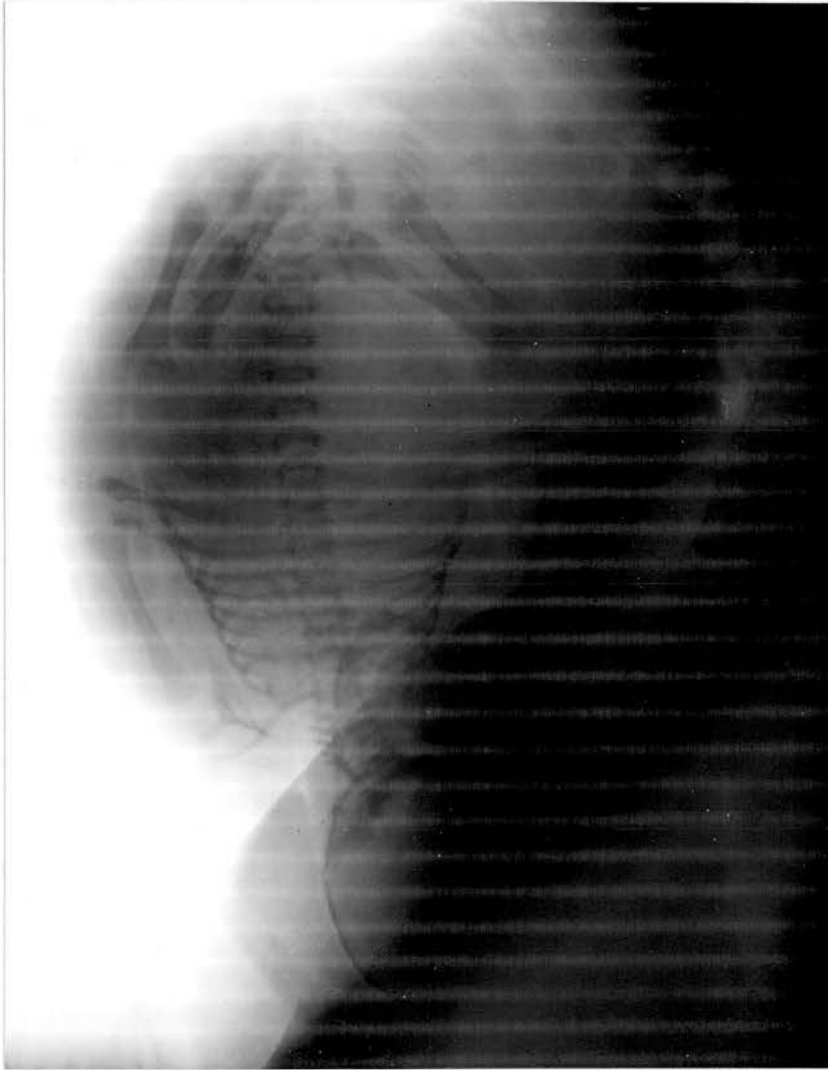


Figure 3. X-ray - severely affected foetus, with protuberant abdomen, Buddha position and scapulae separated from ribs. Large placental 'space'.



Figure 4. X-ray - severely affected fetus, with straight spine and hydramnios.

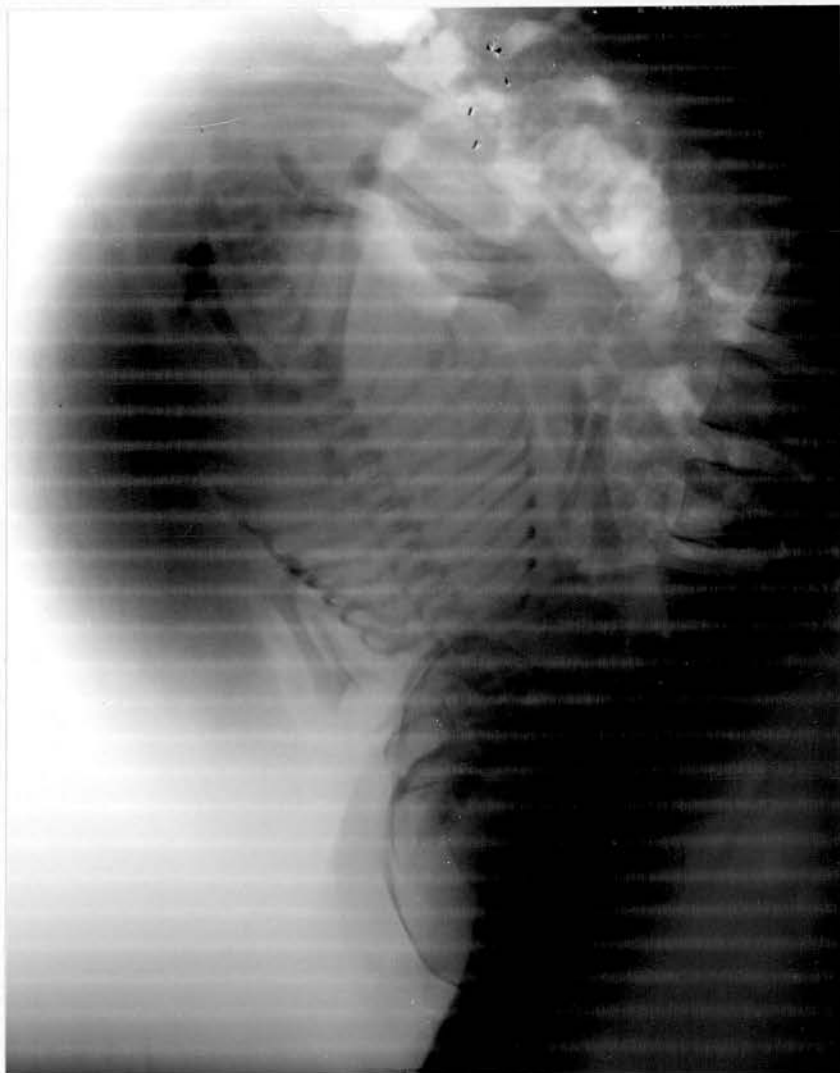


Figure 5. X-ray - severely affected foetus, with elevation of ribs.  
Large placental 'space'.

that died of haemolytic disease during the neonatal period. A large placental 'space' is seen and also a straight spine and prominent abdomen.

In Figure 7, a large placental space is seen and also a protuberant abdomen but the infant is completely unaffected by haemolytic disease. Although 'false positives' may be reported in unaffected infants, an x-ray, taken between 34 and 36 weeks gestation may confirm that the foetus in utero has severe haemolytic disease, and emphasise the need for prompt delivery. In the mildly and moderately affected infants, no change on X-ray is demonstrable.

#### 8. Rhesus Antibodies.

The commonest antibody found in the series was anti-D and this antibody was found in over 75 per cent of patients in the series. Anti C + D was detected in 15 per cent, while other antibodies, singly or in combination, were found in less than 10 per cent of patients who had isoimmunisation. The actual antibodies found are shown in Table XV

In the series 102 patients (18.5 per cent) had received a blood transfusion, and in 13 of these (12.7 per cent of 102) the transfusion was known to be incompatible. The anti-G antibody (Allen and Tippett) 1958) was demonstrated on a number of occasions, and contrary to the findings of Wiener, Wexler and Brancati (1956) anti-E antibodies were found more frequently than anti-c. antibodies.



Figure 6. X-ray - severely affected foetus, with straight spine and protuberant abdomen. Large placental 'space'.

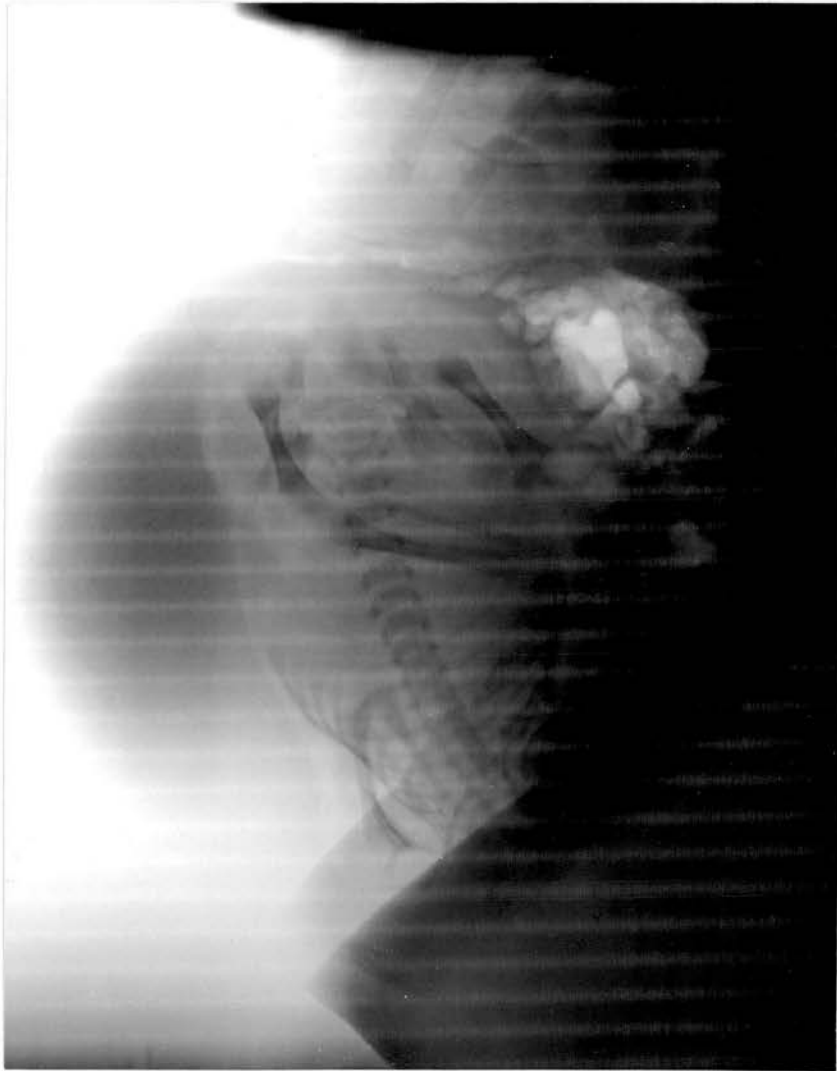


Figure 7. X-ray - unaffected foetus with protuberant abdomen.  
Large placental 'space'.

TABLE XV

Year	Antibodies found								Patients previously Transfused	Transfusion Known to be Incompatible
	Anti-D	Anti C+D including Anti-G	Anti E	Anti-c.	Anti +E.	Anti D+E	Anti C+D+E	Others		
1956	56	-	1	2	0	0	0	2	8	1
1957	60	-	2	1	0	0	0	1	11	1
1958	62	3	1	2	1	0	0	0	14	2
1959	72	8	2	0	0	0	0	0	15	2
1960	60	17	2	1	2	0	0	0	16	2
1961	67	33	6	0	2	1	1	0	24	3
1962	55	22	2	0	0	1	1	1	14	2
<b>Total</b>	<b>432</b>	<b>83</b>	<b>16</b>	<b>6</b>	<b>5</b>	<b>2</b>	<b>2</b>	<b>4</b>	<b>102</b>	<b>13</b>

78.5% 15.1%.

18.5%. 12.7% of transfusions.

TABLE XV Type of antibody found and number of patients transfused.

a. Antiglobulin titres. (A G).

Two hundred and two patients had at least one antiglobulin titre estimated during their pregnancy and the changes in the titre and the height of the titre were correlated with the degree of severity of the infant's haemolytic disease. The findings in 'first immunized' pregnancies and after previously affected infants are tabulated in Table XVI.

In Group I, the titre rose by 2 titres or more on 2 occasions when the infant was unaffected and 2 infants were lost while the titre remained the same or fell. Although, in general, in Group I, if the A.G. titre was high, the infant was more severely affected, there are some exceptions, 4 of the patients with titres of up to 1/10 had severely affected infants. In Groups II and III a rising titre usually indicated an affected infant, but on 1 occasion the titre fell (or remained the same) and the infant was lost, while on 2 other occasions the patients aborted. The height of the highest titre is also of limited value, as although the titre still tends to rise with the more severely affected foetuses, the exceptions are numerous. In this series 9 patients with titres of over 1/40 had unaffected infants, while 7 severely affected infants had titres of below 1/10.

The prediction of foetal severity by means of A.G. titres is possible but there are too many exceptions to allow the prediction to be made with complete confidence. This applies particularly in Groups II and III, but

TABLE XVI

<u>Group I</u>	F o e t a l   R e s u l t					Total
	Unaffected	Mild and Moderate	Severe	Rhesus deaths	Abortions	
Anti-globulin titres						
Titre rising (2 tubes or more)	2	16	1	4	0	23
Titre same or falling	8	10	4	2	0	24
<b>Total</b>	<b>10</b>	<b>26</b>	<b>5</b>	<b>6</b>	<b>0</b>	<b>47</b>
<u>Highest Anti-globulin titres</u>						
1/1 - 1/10	14	32	4	0	0	50
1/20 - 1/40	4	12	3	1	0	20
Over 1/40	0	19	5	6	0	30
<b>Total</b>	<b>18</b>	<b>63</b>	<b>12</b>	<b>7</b>	<b>0</b>	<b>100</b>
<u>Groups II and III</u>						
Anti-globulin titres						
Titres rising (2 tubes or more)	0	13	6	1	0	20
Titres same or falling	4	6	8	1	2	21
<b>Total</b>	<b>4</b>	<b>19</b>	<b>14</b>	<b>2</b>	<b>2</b>	<b>41</b>
<u>Highest Anti-globulin titres</u>						
1/1 - 1/10	8	15	7	0	2	32
1/20 - 1/40	3	21	7	1	0	32
Over 1/40	9	9	11	3	6	38
<b>Total</b>	<b>20</b>	<b>45</b>	<b>25</b>	<b>4</b>	<b>8</b>	<b>102</b>

TABLE XVI Foetal results with changes in anti-globulin titres and with highest titre, by Groups.

also occasionally in Group I.

b. Albumen titres.

In Table XVII the foetal results are presented depending on whether antibodies were detected before 20 weeks gestation or not. It will be seen that in 11 cases in Group I, antibodies were not detected before 20 weeks gestation but developed later, and yet the infant was Rhesus negative and unaffected by haemolytic disease. In 6 second pregnancies, i.e. first immunized pregnancies, no antibodies were detected before the 20th week, but were found later and the infant was Rhesus negative and unaffected. There is little doubt that these patients were sensitized during their first pregnancies and although antibodies developed, the titre was so low in the early weeks of the second pregnancy that no antibodies could be detected. An anamnestic reaction must then have occurred and a recordable titre was later obtained. The serological findings are exactly the same as would be expected in a case of 'primary' immunisation in a second pregnancy, a prior stimulus having been given in the first pregnancy.

In Table XVIII the foetal results are recorded in the 3 groups based on previous history depending on any change which may have occurred in the albumen titre level during the pregnancy. In Group I a rising titre is recorded in 11 patients with unaffected infants and in Group II, 13 deaths and abortions occurred along with a fall in titre. In 7 second

TABLE XVII

Foetal Results	Group I			Group II			Group III		
	Anti- bodies Absent	Anti- bodies Present	Not Known	Anti- bodies Absent	Anti- bodies Present	Not Known	Anti- bodies Absent	Anti- bodies Present	Not Known
Unaffected	11	17	22	1	22	12	0	6	1
Mild and Moderate	80	44	77	15	56	23	0	3	5
Severe	11	5	14	4	17	16	0	8	4
Rhesus deaths and Abortions	4	10	8	1	16	9	0	17	3
Total	106	76	121	21	111	60	0	34	13

TABLE XVII

Foetal results, by Groups, depending on antibody detection test before 20 weeks gestation.

Number of second pregnancies ('first affected') where no antibodies detected before 20 weeks gestation but found later and infant Rhesus negative and unaffected - 6.

Number of apparently first affected pregnancies with rising titre, but infant Rhesus negative and unaffected - 7.

pregnancies (first-immunized) the titre level rose, but the infant was Rhesus negative and unaffected. This again could give rise to confusion, and is another example of the anamnestic reaction reported Levine (1944) and denied by Wiener (1959).

The severity of the haemolytic disease is tabulated with the highest albumen titre obtained during the pregnancy in Table XIX and the results show that the albumen titre is of even less prognostic value than the antiglobulin titre. A number of women (30) who had severely affected infants, had titres which did not rise above 1/16, while a few patients with titres over 1/64 had unaffected infants.

The antibody titre found is of some prognostic value but there are so many individual exceptions that in a particular patient, the information obtained from the titre is unreliable. The accuracy of titres in predicting severity depends very much on the efficacy of the techniques used in their determination and the efficiency of the technicians concerned. In some centres all antibody titre estimations are carried out by one person. It is, however, the routine rather than the research procedures with which we are concerned in practice, and the results obtained by a routine procedure have to be utilised if no other results are available.

#### 9. Examination of the Amniotic Fluid.

When it is necessary to obtain information about the state of the foetus within the uterus, it seems likely that more information will be derived from

TABLE XVIII

Foetal Results	Group I		Group II		Group III	
	Titre Rising	Titre Falling	Titre Rising	Titre Falling	Titre Rising	Titre Falling
Unaffected	11	23	5	20	2	4
Mild and Moderate	93	50	41	36	2	2
Severe	16	4	16	15	6	5
'Rhesus' deaths and abortions	14	1	6	13	8	5
<b>Totals</b>	<b>134</b>	<b>78</b>	<b>68</b>	<b>84</b>	<b>18</b>	<b>16</b>

TABLE XVIII Foetal results, in Groups, with changes in albumen titre.TABLE XIX

Foetal Results	Group I			Group II			Group III		
	Under 1/16	1/16-1/64	Over 1/64	Under 1/16	1/16-1/64	Over 1/64	Under 1/16	1/16-1/64	Over 1/64
Unaffected	31	10	4	18	12	2	3	3	0
Mild and Moderate	96	55	24	47	29	5	3	4	0
Severe	13	7	7	12	16	6	5	6	1
'Rhesus' deaths and abortions	3	6	10	5	13	5	3	10	7
<b>Totals</b>	<b>143</b>	<b>78</b>	<b>45</b>	<b>82</b>	<b>70</b>	<b>18</b>	<b>14</b>	<b>23</b>	<b>8</b>

TABLE XIX Foetal results, in Groups, with highest albumen titre obtained.

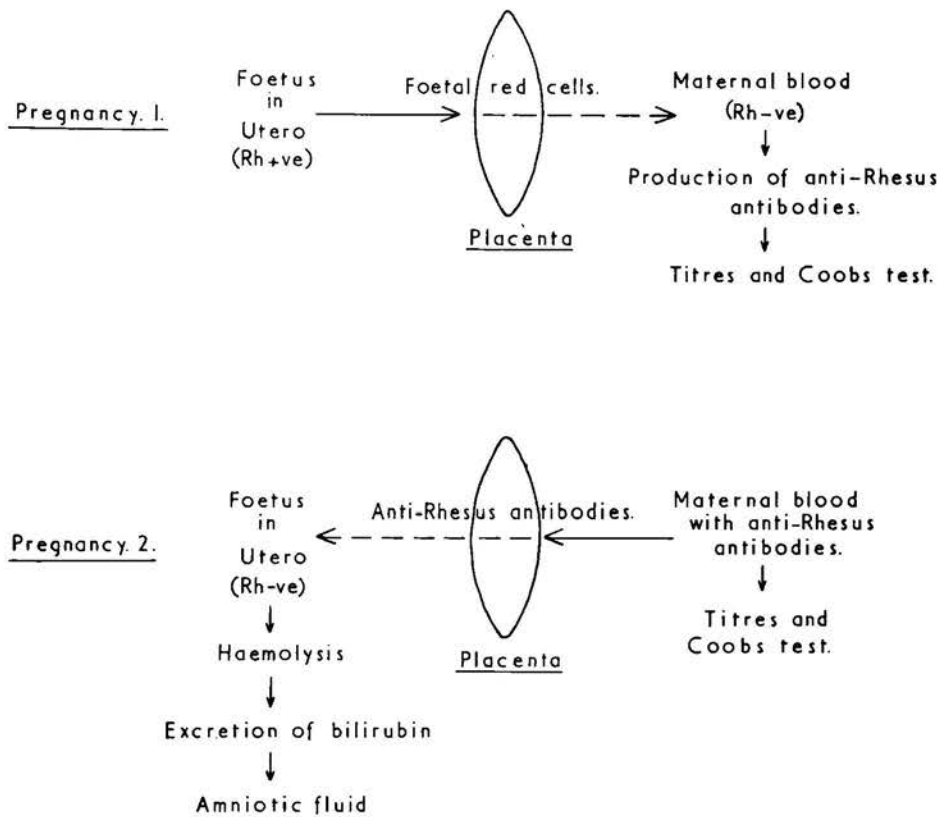
an examination of the 'foetal tissues' rather than an examination of the 'maternal tissues'. As the foetus is bathed in amniotic fluid and the amnion is a foetal membrane, breakdown products from the foetus may well be excreted into this fluid. An examination of the amniotic fluid should reveal the presence of excretion products, and in Rhesus isoimmunisation, we are concerned with the presence of excessive amounts of bilirubin in the liquor.

A simplified diagram (Figure 8) shows the mechanism involved in the light of our present knowledge.

a. Paracentesis uteri or Amnioncentesis.

Liquor amnii is obtained by the insertion of a needle through the anterior abdominal wall. The procedure is carried out with full aseptic precautions and it is explained fully to the patient. She is an out patient and is allowed to return home immediately after the procedure. No sedation is given to the patient, as it has been found that a simple explanation immediately prior to the procedure is quite sufficient.

The instruments used are shown in Figure 9. After the patient has emptied her bladder, the anterior abdominal wall is fully exposed and uterine palpation is performed to ascertain the lie, attitude, presentation and position of the foetus. The foetal heart is auscultated very carefully and the position, and the presence of the foetal heart is confirmed by a second observer. A point is selected at a level just below



**Figure 8. Diagrammatic representation of development of Rhesus Isoimmunisation and Haemolytic Disease of Newborn.**



Figure 9. Instruments required for paracentesis uteri.

the umbilicus on the side opposite to the foetal back on palpation (Figure 10).

The operator then fully 'scrubs up' and, after drying the hands, applies methylated spirit or ether to the anterior abdominal wall. At the proposed site of aspiration, a small skin wheal is raised with 1% xylocaine solution and some more of this local anesthetic is injected into the deeper tissues of the anterior abdominal wall. (Figure 11). A lumbar puncture needle is then inserted through the skin and anterior abdominal wall and can be felt penetrating the parietal peritoneum and uterine wall. Usually penetration of the uterine wall is accompanied by a definite 'giving way' as the needle advances, but occasionally when the placenta is anterior, the needle's advance meets with some resistance. Eventually the resistance lessens as the amniotic cavity is entered. (Figure 12). The stylette of the lumbar puncture needle is then removed and a syringe attached. Two aspirations of 10 ml. of amniotic fluid are sufficient. Sometimes no fluid can be obtained and it is advisable to reinsert the stylette as the needle may be blocked with vernix. At times it is helpful to rotate the needle through 90 or 180 degrees, and if this procedure is not successful it is necessary to either advance or withdraw the needle. This sometimes leads to contamination by blood of the liquor already obtained, so it is advisable to empty the syringe before proceeding with the aspiration. (Figure 13). When sufficient liquor amni has been obtained,



Figure 10. Outline of fetus (cephalic presentation) and point of insertion of needle.



Figure 11. Injection of local anaesthetic.



Figure 12. Insertion of lumbar puncture needle.



Figure 13. Aspiration of liquor amnii.

the syringe is disconnected and the needle withdrawn. Either a dry plaster dressing can be applied or the puncture wound treated with 'Collodion'. The foetal heart is again listened to and checked by another observer.

The patient is allowed to return home and warned that she may have slight pain and tenderness at the site of the paracentesis. The procedure was carried out on 350 occasions between 1958 and 1962 and no complications developed. No death in the series was directly attributed to the paracentesis and no uterine infection could be traced to the insertion of the needle. After the procedure patients who had been apprehensive previously, usually remarked, "Is that all it is?".

During 1958, paracentesis uteri was performed at about 34 weeks gestation as recommended by Walker (1957). A further test was carried out at about 38 weeks if the patient had not been delivered. The second test usually confirmed Walker's observation that the amount of bilirubin in the liquor decreased as term approached. It was eventually decided that one test at 34 weeks was sufficient and the second test was abandoned.

b. Liquor Analysis of Amniotic Fluid.

Initially the amniotic fluid was cooled, filtered and centrifuged as recommended by Walker. As filtering seemed to confuse the analysis, this was dispensed with

and after cooling, the liquor amnii was centrifuged at 3000 r.p.m. for 20 minutes.

After centrifuging, the supernatant fluid was transferred initially to a 10 m.m. cuvette for spectrophotometric analysis. In 1960, because a number of 'false positive' results were being obtained, a diazo test was devised to determine the amount of direct and total bilirubin present in the liquor and, by 'subtraction', the amount of indirect bilirubin.

#### DIAZO TEST.

##### Reagents:

- i. 'Diazo' substance.
  - a. Sulphanilic acid  
1 g./1000 ml.  
Hydrochloric acid  
15 ml/1000 ml.  
Water.
  - b. Sodium nitrite 0.5g./  
100 ml. with water.

The solutions must be prepared freshly each week.

A 'working' solution of 'diazo' is made by adding 0.3 ml. of solution b to 10 ml. of solution a, and this is prepared daily.

- ii. Methyl alcohol.

##### Method:

After the amniotic fluid has been centrifuged, the following solutions are added to a test tube marked "total":-

- 2 ml. amniotic fluid.
- 1 ml. diazo solution.
- 2 ml. methyl alcohol.

After mixing, the solution is allowed to stand for 10 minutes.

To a second tube marked "direct", is added:-

2.6 ml. amniotic fluid.

1.3 ml. diazo solution.

This solution is also allowed to stand for 10 minutes after mixing.

#### Spectrophotometry.

Spectrophotometric analysis is carried out in a Unicam SP 600 Spectrophotometer (Figure 14), the optical density being plotted on semilogarithmic (2 cycles) graph paper at wavelengths from 360 m.u. to 700 m.u. Readings of the optical density are taken at 10 m.u. for almost the whole of the wavelength range from 360 m.u. to 600 m.u. and then at 25 m.u. intervals. A reading at 405 m.u. is also taken. Ten millimetre cuvettes are used with a water blank for comparison. Five curves are plotted (Figures 19 and 20):-

- I Untreated amniotic fluid.
- II Amniotic fluid and diazo solution - direct bilirubin.
- III Amniotic fluid and hydrochloric acid - control.
- IV Amniotic fluid and diazo solution and methyl alcohol - total bilirubin.
- V Amniotic fluid and hydrochloric acid and methyl alcohol - control.

If the untreated liquor graph is nearly a 'straight line curve' (Figure 15), a prediction of an 'unaffected'

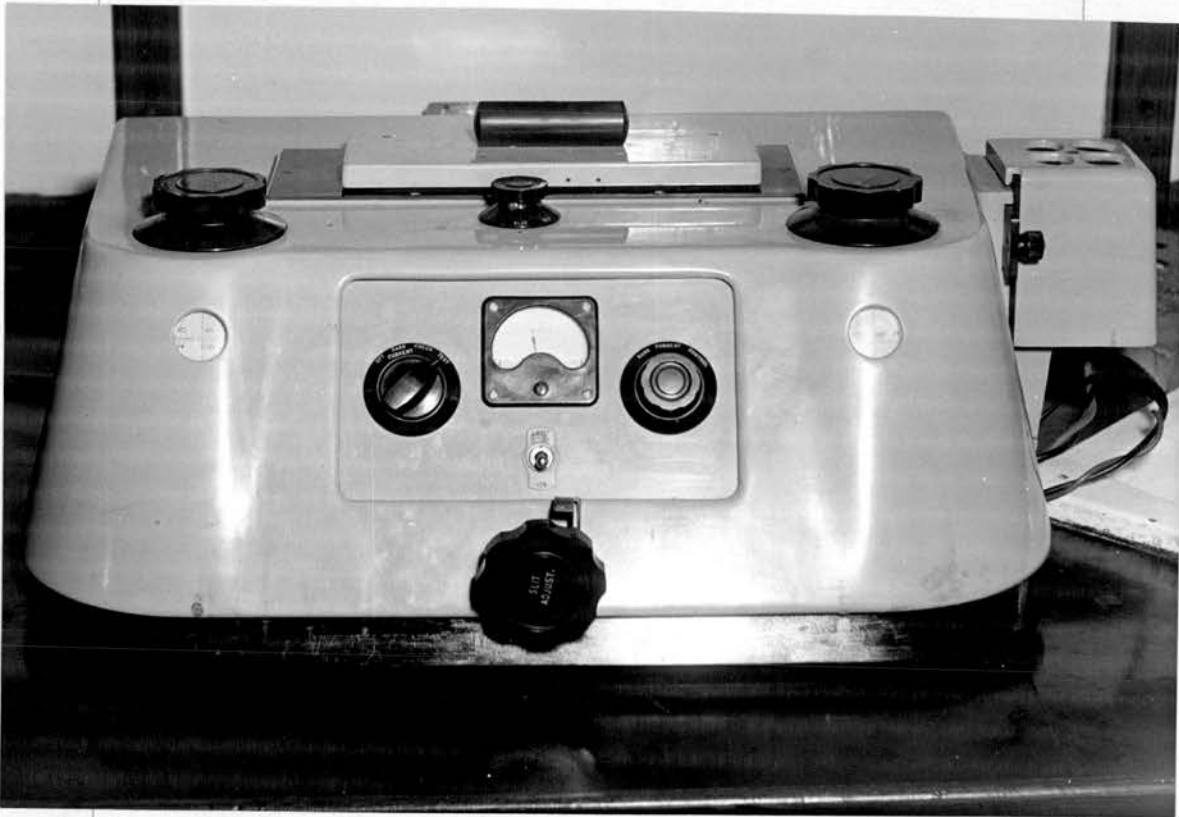


Figure 14. Unicam spectrophotometer SP 500.

Mrs. M. S.

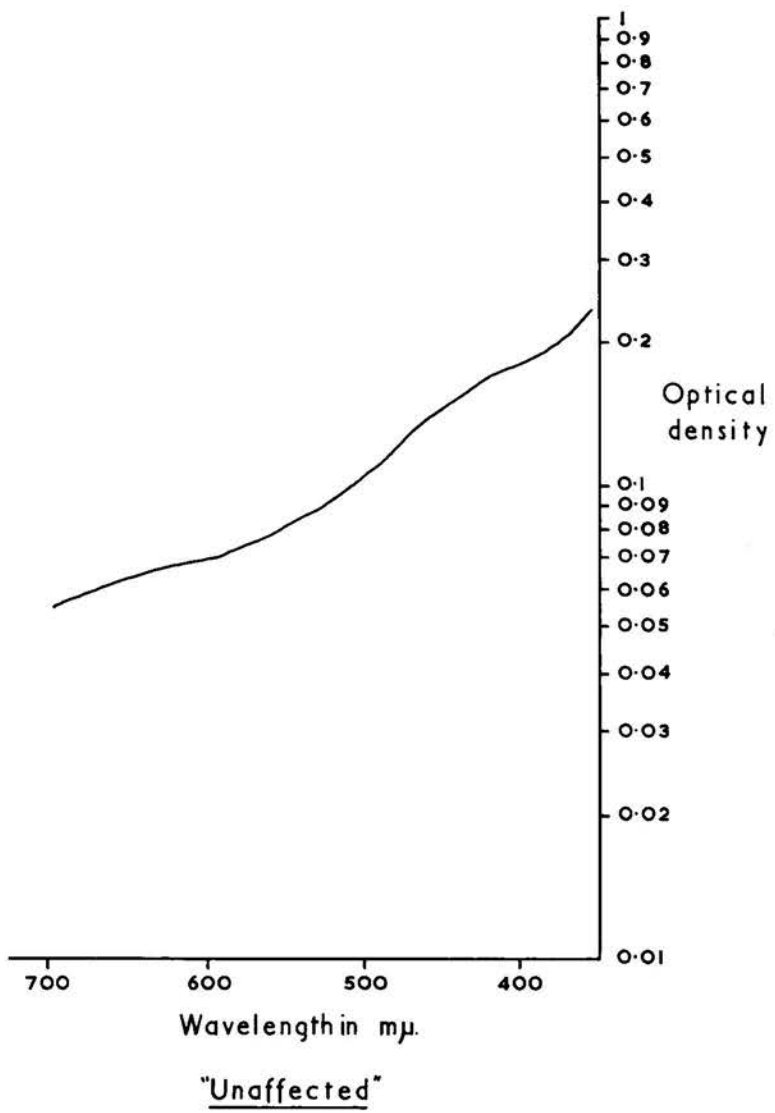


Figure 15. 'Unaffected' or 'mildly affected' liquor curve.

or a 'mildly affected' infant is made. It has been impossible to accurately distinguish between unaffected and mildly affected 'curves' (Liley (1961), Walker and Jennison (1962), and as the management is similar - delivery at or within a few days of the expected date of delivery, - for practical purposes no distinction is required. In a 'moderately affected' foetus, a 'bulge' is seen between 400 and 500 m.u. on the graph, as in Figure 16. The 'bulge' in this region is seen to be greater still in Figure 17, and the foetus is predicted as being severely affected. If the 'bulge' is still further above the base line (Figure 18) then the infant is very severely affected and heroic measures are usually called for to obtain a living infant.

As will be seen in Tables XX and XXI, a number of incorrect predictions have been made, many of these 'false positives'. An infant was predicted as being moderately affected as a result of a liquor examination but, at delivery, was found to be direct Coomb's negative and unaffected by haemolytic disease. The 'diazo' test was then devised, and unless there is a definite bulge between 500 and 600 m.u. in Curve IV (Figure 19) as compared with Curve V, there is no indirect bilirubin present and the infant will be unaffected. On the other hand, the untreated liquor curve may not give a true picture of the severity of the condition and the diazo test may indicate that the amount of indirect bilirubin present is greater than

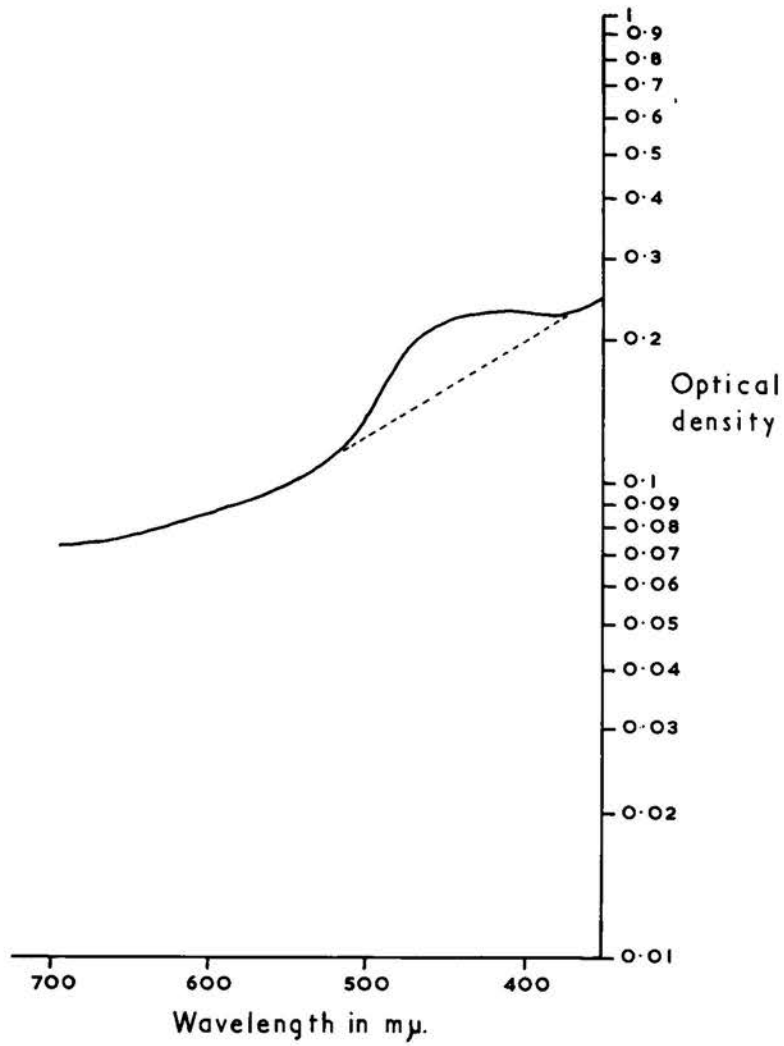
Mrs. A.H.'Moderately affected'

Figure 16. 'Moderately affected' liquor curve.

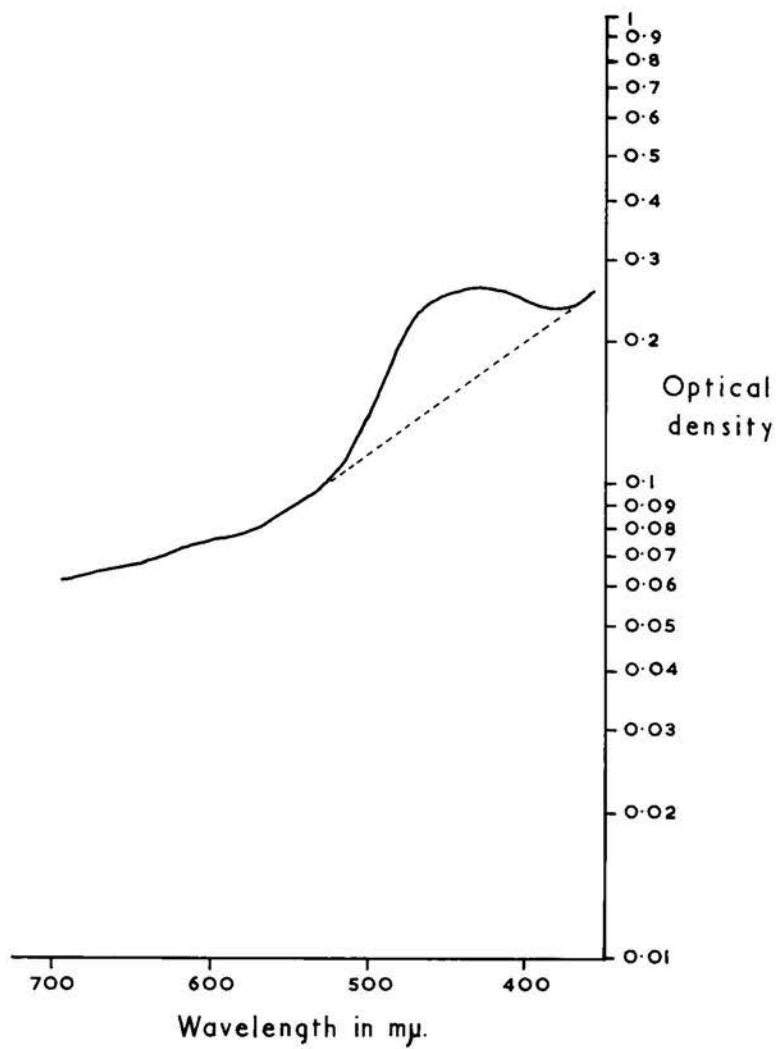
Mrs. K. C.'Severely affected'

Figure 17. 'Severely affected' liquor curve.

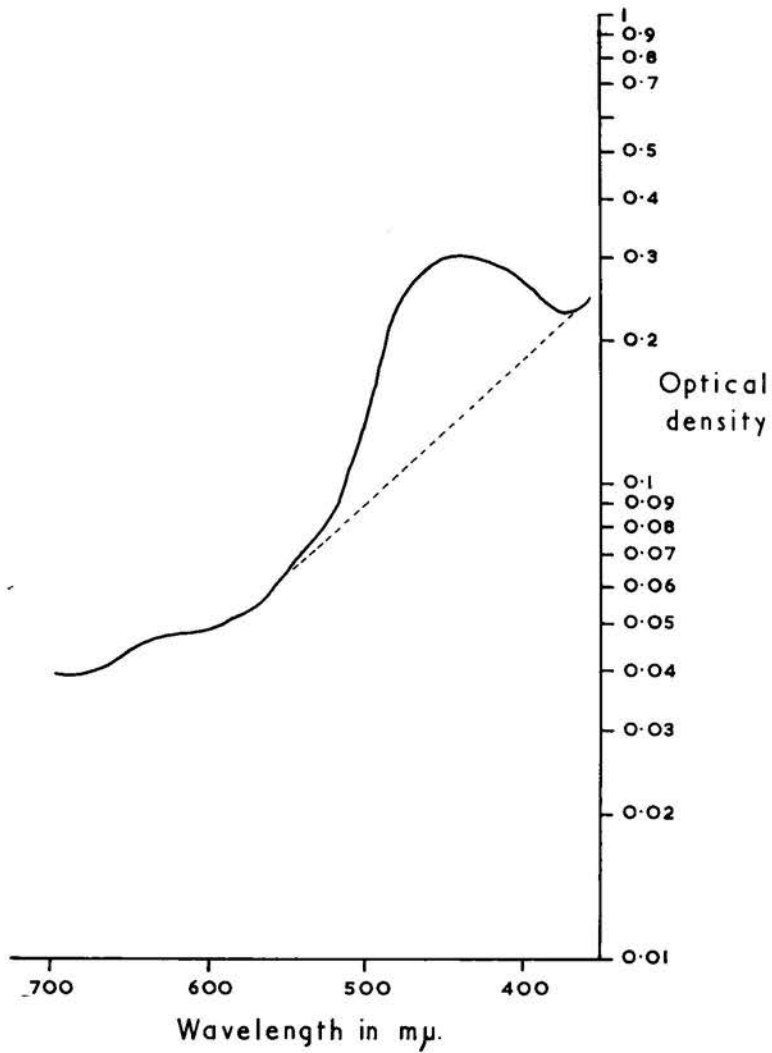
Mrs. A.B.'Very severely affected'

Figure 18. 'Very severely affected' liquor curve.

TABLE XX.

Foetal Results.	Paracentesis before 35 weeks.						Paracentesis after 35 weeks.						Prediction Correct with Diazo.					
	Correct			Almost Correct			Incorrect			Correct & Almost Correct				Incorrect				
	<u>I</u>	<u>II</u>	<u>III</u>	<u>I</u>	<u>II</u>	<u>III</u>	<u>I</u>	<u>II</u>	<u>III</u>	<u>I</u>	<u>II</u>	<u>III</u>		<u>I</u>	<u>II</u>	<u>III</u>		
Unaffected or mildly affected	24	12	1	5	8	0	9	11	5	12	3	0	1	0	0	9	10	2
Moderately affected	29	31	1	4	7	1	5	1	1	7	3	2	3	0	0	2	1	0
Severely affected	5	2	6	8	2	1	1	0	0	1	1	2	0	0	0	1	1	0
Death from haemolytic disease.	2	1	3	2	5	0	1	0	0	1	1	1	0	0	0	0	1	0
Total.	60	66	11	19	22	2	16	12	6	21	8	5	4	0	0	12	13	2
Grand Total.	137			43			34			34			4			27		

TABLE XX. Results of liquor examinations correlated with foetal results, in Groups, and corrected results with Diazo.

TABLE XXI.

Foetal Results.	Almost Correct.			Incorrect.		
	Group I. Number Prediction.	Group II. Number Prediction.	Group III. Number Prediction.	Group I. Number Prediction.	Group II. Number Prediction.	Group III. Number Prediction.
Unaffected or mildly affected.	8 - 8 Moderate	8 - 8 Moderate	0	10 - 8 Moderate 2 Severe	11 - 10 Moderate 1 Severe	5 - 4 Moderate 1 Severe
Moderately affected.	4 - 3 Mild 1 Severe	6 - 5 Mild 1 Severe	2 - 2 Mild	8 - 8 Unaffected	1 - 1 Unaffected	1 - 1 Unaffected
Severely affected.	9 - 9 Moderate	2 - 2 Moderate	1 - 1 Moderate	1 - 1 Unaffected	0	0
Deaths from Haemolytic disease.	2 - 2 Moderate	6 - 3 Moderate 3 Severe	0	1 - 1 Moderate	0	0
Total.	23 - 3 Mild - 19 Moderate 1 Severe	22 - 5 Mild - 13 Moderate 4 Severe	3 - 2 Mild 1 Moderate	20 - 9 Unaffected - 9 Moderate 2 Severe	12 - 1 Unaff. - 10 Moderate 1 Severe	6 - 1 Unaffected - 4 Moderate 1 Severe.

TABLE XXI.

Details of 'Almost correct' and 'Incorrect' predictions following liquor examination.

Mrs. H. F.

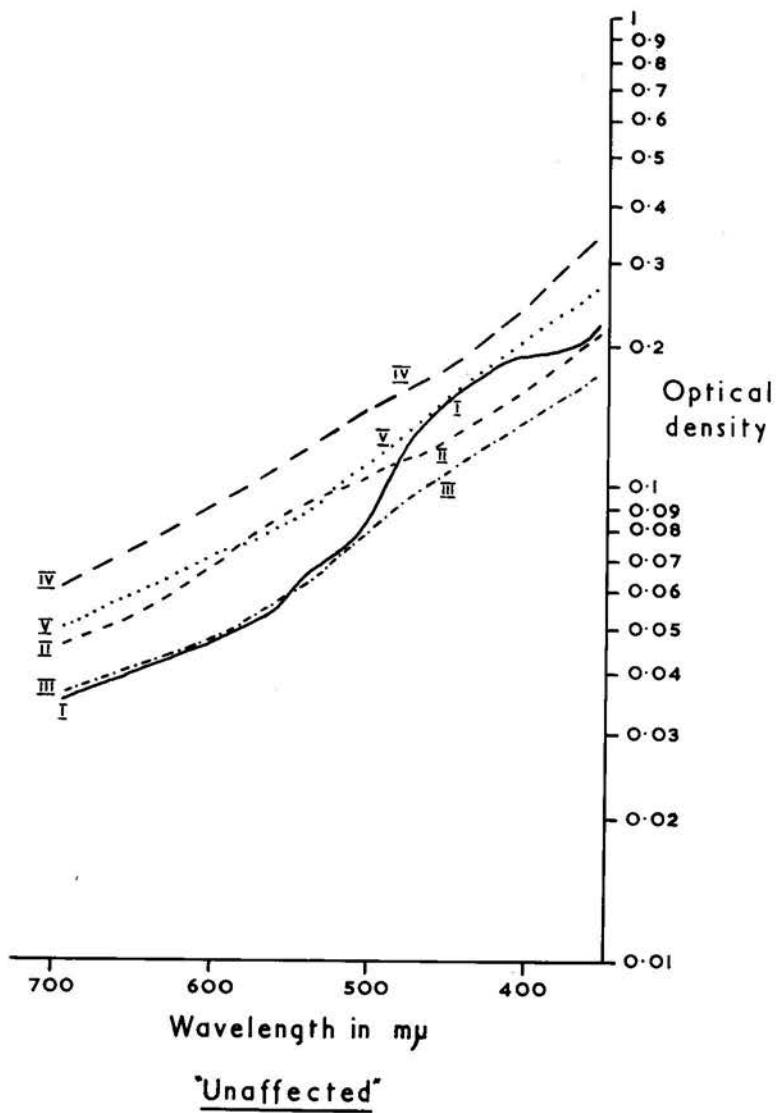


Figure 19. Graph showing curves after treatment of liquor with 'diazo' - 'unaffected'.

is suggested by the untreated liquor curve (Figure 20).

The 'dialzo' test has been of great value in predicting 'unaffected' infants and also in distinguishing mildly affected from moderately affected infants.

The prediction is made on the height of the bulge at 440-450 m.u. above the 'base-line', or the line in continuity with the graph before 400 m.u. and after 500 m.u., but this 'bulge' will appear to be smaller when the 'base line' has a higher optical density. Thus the difference in optical density between the peak and the base-line is measured.

An attempt was made to relate the optical density of the 'peak' at 450 m.u. to the birth haemoglobin but as the time interval between the test and delivery was usually more than 2 weeks, no satisfactory correlation was obtained. The difference in optical density between the peak and base line gives a more satisfactory relationship with the observed severity as previously defined but there are wide variations. Generally in the severely affected groups and 'Rhesus deaths' group, the difference in optical densities is greater than 0.05 but may be as much as 0.2 or 0.3.

Although it is unsatisfactory to compare different sizes of 'bulge', this is necessary when the prediction is based on the size of the 'bulge' and the assessment of severity presents such difficulties. Ultimately foetal survival is the best assessment as to the correctness of the method of prediction.

With the 'dialzo' curves, calculation of the height

Mrs. M.P.

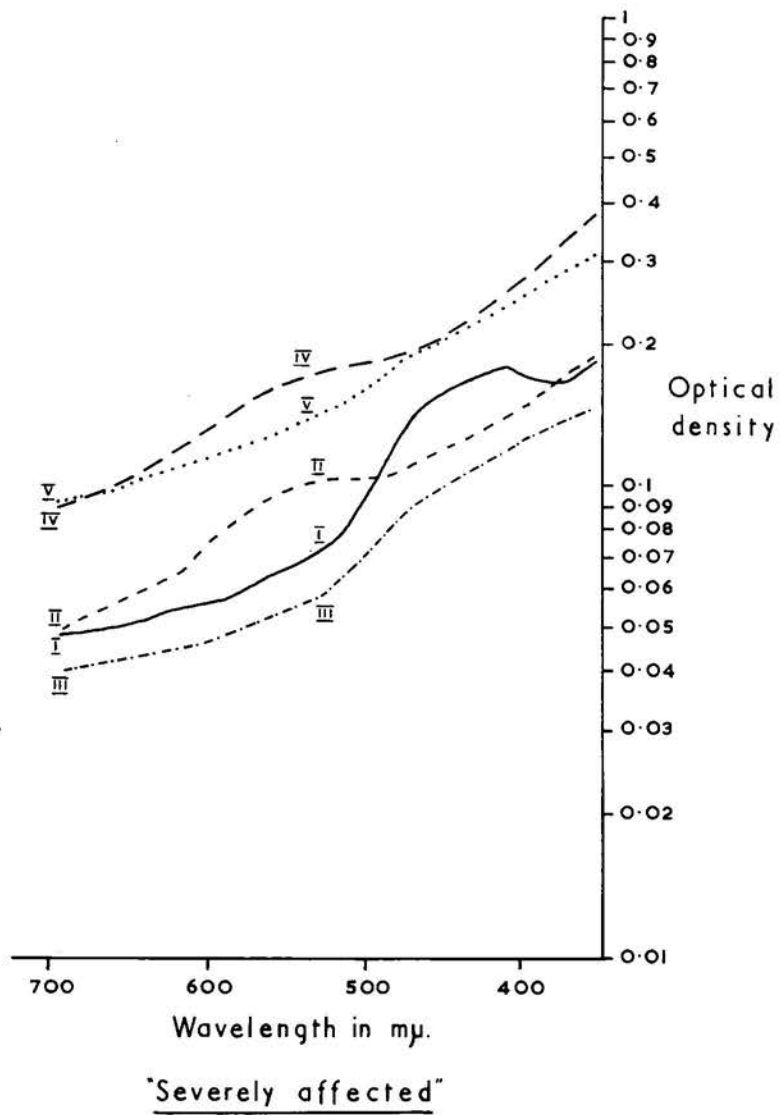


Figure 20. Graph showing curves after treatment of liquor with 'diazo' - 'severely affected'.

of the bulge between 500 m.u. and 600 m.u. above the control at 2 points was originally thought to be of prognostic value, but again no satisfactory numerical correlation could be obtained when delivery took place 4 or 5 weeks after paracentesis.

c. Results obtained.

After a prediction has been made, in terms of severity, it is necessary to decide on the management of the patient. Thus, for practical purposes, the prediction based on the liquor analysis can be related to the gestation which has been recommended for induction, and this offers a practical method for comparing the prediction with the degree of haemolytic disease found in the infant.

In view of the difficulty sometimes found in the interpretation of the liquor graphs when the test is carried out after 35 weeks gestation, because of a possible reduction in the amount of bilirubin in the liquor, the results are in 2 groups. Thirty-eight tests have been carried out after 35 weeks gestation, while 214 tests were performed before 34 weeks. The results in the three groups are recorded as 'correct', 'partially correct' - where the observed severity differed slightly from the predicted severity- and 'incorrect' when they were totally different. The results obtained are shown in Table XX, while details of the 'almost correct' and 'incorrect' predictions and results are given in Table XXI.

In Table XXII are detailed the explanations for

TABLE XXII.False positives 27 -False negatives 11 -TABLE XXII. Reasons forTABLE XXIII.Stillbirths.

Intra-uterine deaths

Deaths during labour

Affected severely and  
breech delivery

Unaffected, 'anoxia'

Total.

TABLE XXIII. Causes of fo

some of the incorrect results found. Eleven, out of 27 who had false positive results, were correctly predicted after the 'diazo' test had been used. One 'false negative' result occurred after the diazo test had predicted an 'unaffected' infant. The infant was 'severely affected'. The usual explanation for 'false negative' predictions was found to be that the period of gestation at the time of the test was in doubt and the test was in fact performed at about 38 weeks gestation.

Twenty-eight infants were lost in the paracentesis series, 19 being stillborn and 9 dying in the neonatal period. This figure includes 1 hydropic foetus who died in utero some weeks following paracentesis during which liquor was not obtained. The causes of death in the paracentesis series are recorded in Table XXIII.

Since the introduction of the diazo test the number of correct predictions has increased as is shown in Table XXIV. During 1958-1960, 85 per cent of the predictions made were correct or almost correct. During 1961 and 1962, 84 per cent were correct, but with the addition of the diazo tests, where these differed from the untreated liquor tests, 92 per cent were correct. The overall percentage of correct predictions was 85 per cent, and with the addition of the diazo corrections, this was increased to 88 per cent.

d. Complications of paracentesis uteri.

It is possible, when carrying out paracentesis uteri, to damage a placental blood vessel or to damage

TABLE XXIV.

TOTAL.				
<u>Foetal results.</u>	Prediction Correct and Most Correct.	Prediction Incorrect.	Total.	% Correct.
Unaffected and mildly affected.	65 (72)	26 (19)	91	71.4 (79.1)
Moderately affected.	85 (86)	10 (9)	95	89.5 (90.5)
Severely affected.	64	2	66	96.9
Rhesus deaths.	16	1	17	
<b>Total.</b>	214 (222)	38 (30)	252	84.9 (88.1)

TABLE XXIV.

: were used frequently.

the umbilical cord. A heavily blood-stained liquor may be impossible to analyse, although a little blood-staining does not seem to make any difference, provided gentle centrifuging is performed initially.

If no liquor amnii can be obtained, another site on the abdominal wall may be used. Sometimes no liquor can be aspirated but blood is withdrawn. Foetal blood can be identified by blood grouping and if the blood were Rhesus positive and direct Coomb's positive, then at least induction can be performed without the fear that the infant will be Rhesus negative. This blood is either obtained from a vessel on the foetal surface of the placenta or by penetration of a vessel in the foetus himself. This has not been carried out as a deliberate procedure but any blood obtained is grouped.

It has been suggested (Moncrieff (1960) that paracentesis of the uterus produces a brisk rise in antibody titre, but others, Kelsall et al (1960) and Walker and Jennison (1962) have denied that this is necessarily due to the paracentesis. During 1956 and 1957 (when no paracentesis uteri was performed in this hospital), 19 patients were found to have significant rises in their antibody titres after the 34th week of pregnancy. In 1958, on 7 occasions the titre rose without paracentesis, while in 4 instances it rose after liquor had been obtained. This would tend to confirm Walker and Jennison's findings.

e. Comparison of antibody titres and amniotic fluid

e. Comparison of antibody titres and amniotic fluid in prediction of the severity of haemolytic disease.

On 60 occasions the severity of the haemolytic disease as predicted by the liquor analysis was found to be correct, and the interpretation of the titre wrong. On 29 occasions the titre was correct and the liquor prediction wrong.

These figures are not of much significance because retrospectively it is difficult to assess the severity which might have been predicted by a study of the antibody titres alone. The previous history would have been taken into account, and earlier induction was not possible in every case.

f. Study of graphs of liquor amnii obtained from patients with severely affected infants.

In an attempt to detect any significant or diagnostic feature in the 'liquor curves' of severely affected infants, the graphs of all the severely affected infants and of those dying of haemolytic disease were scrutinised very carefully. In nearly all cases there was a prominent bulge or the optical density of the liquor at 440-450 m.u. was high, or both. In addition, in many of the curves studied a 'plateau' was seen between 410-420 m.u. and 450-460 m.u.

In the severely affected group where the infant had a rapidly rising bilirubin level and required 2 or 3 exchange transfusions, 19 out of the 25 graphs studied show this plateau, examples of which are shown in Figures 21 and 22. In the first of these (Figure 21) the curve rises from 390 m.u. and reaches the

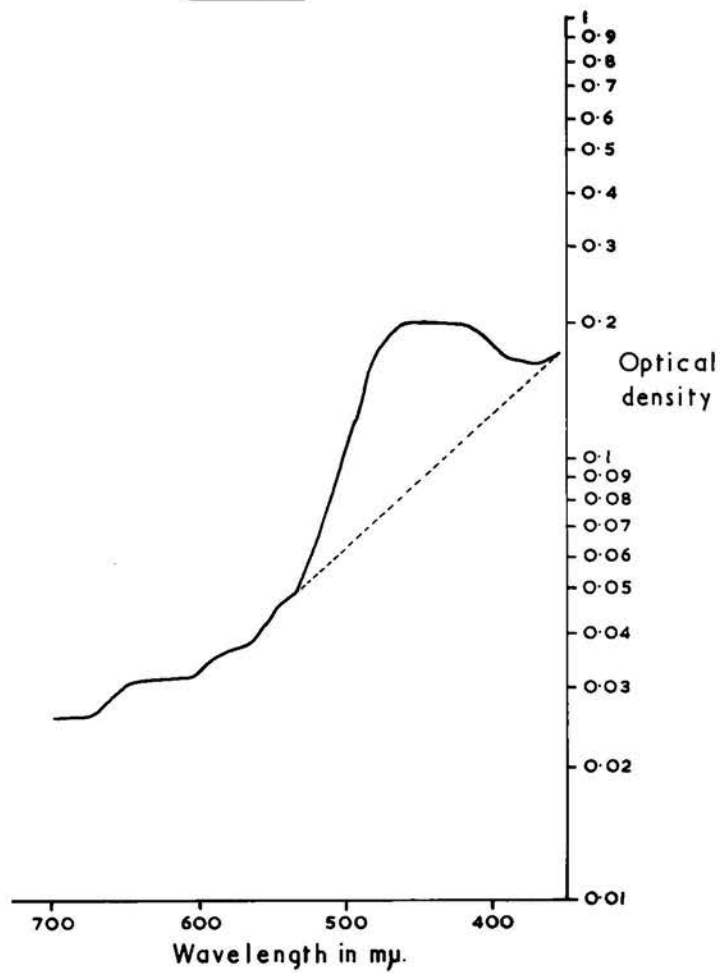
Mrs. A.W.

Figure 21. 'Severely affected' graph showing 'plateau' between 420 and 460 m u. - 'hyperbilirubin-anaemia'.

'plateau' at 420 m.u. After 460 m.u. it falls until at 530 m.u. it has returned to the baseline. This patient, a para 3, with previous neonatal death due to haemolytic disease, was induced at 35 weeks gestation and was delivered of a male infant weighing 5 pounds 8 ounces (2500 g.). The infant required 4 exchange transfusions and one top-up transfusion. The second case illustrated (Figure 22) was that of a primigravida who was Rhesus negative and had previously received a blood transfusion. The liquor graph showed a rise above the base line at 390 m.u. to reach a plateau at 420 m.u. The plateau ceased at 460 m.u. and the curve rejoined the baseline at 530 m.u. again.

In 9 of the graphs studied, a 'hump-peak' at 410 m.u. was found as well as a 'plateau'. This is illustrated in Figure 23. The plateau is less marked and the curve falls to the baseline between 460 m.u. and about 525 m.u. Labour was induced in this patient at 38 weeks and the infant required 3 exchange transfusions.

In the very anaemic group of severely affected infants, 17 graphs out of 22 show the plateau between 410 and 460 m.u. to a varying degree, but there were no 'hump-peaks'. Figure 24 shows a definite plateau, the curve leaving the baseline at 380 m.u. and returning at 525 m.u. When the infant was delivered, at 36 weeks gestation, the haemoglobin was 8.6 g. per cent but only 1 exchange transfusion was required. In figure 25 the curve is seen to rise further from the baseline and the

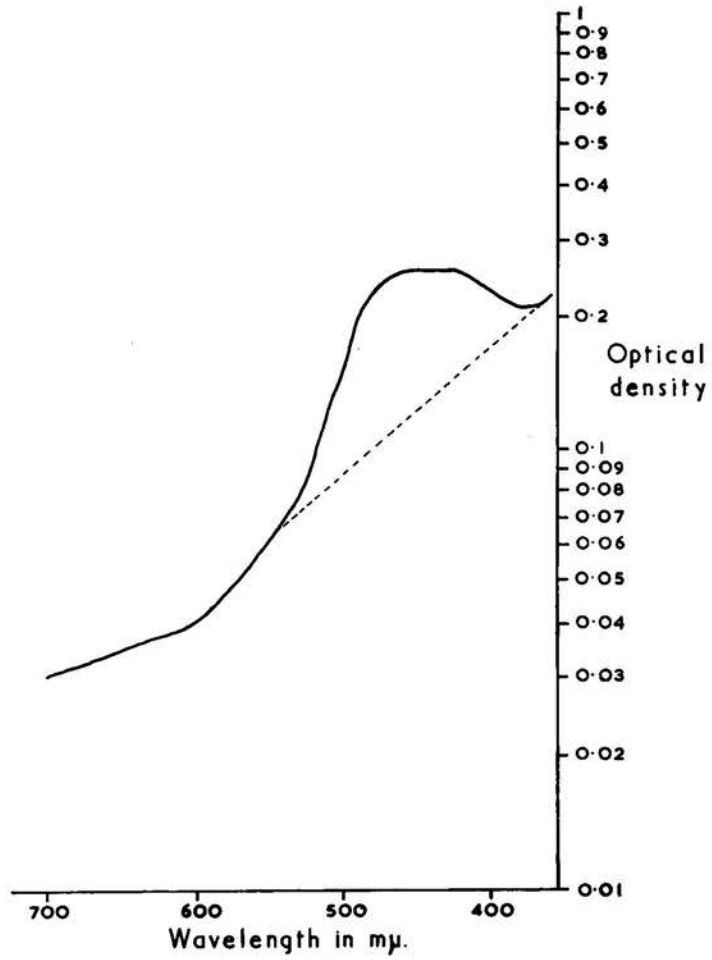
Mrs. C.D.

Figure 22. 'Severely affected' foetus with hyperbilirubinaemia - plateau on graph. (Primigravida.)

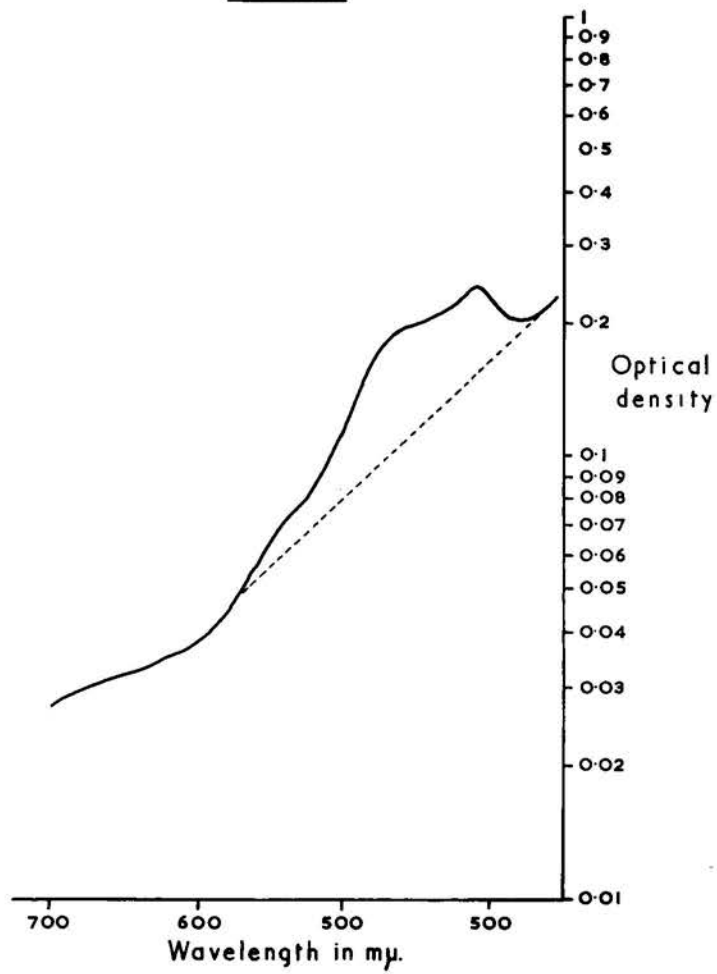
Mrs. K.G.

Figure 23. 'Severely affected' graph - 'hump' at 410 m μ,  
then 'plateau' - hyperbilirubinaemia.

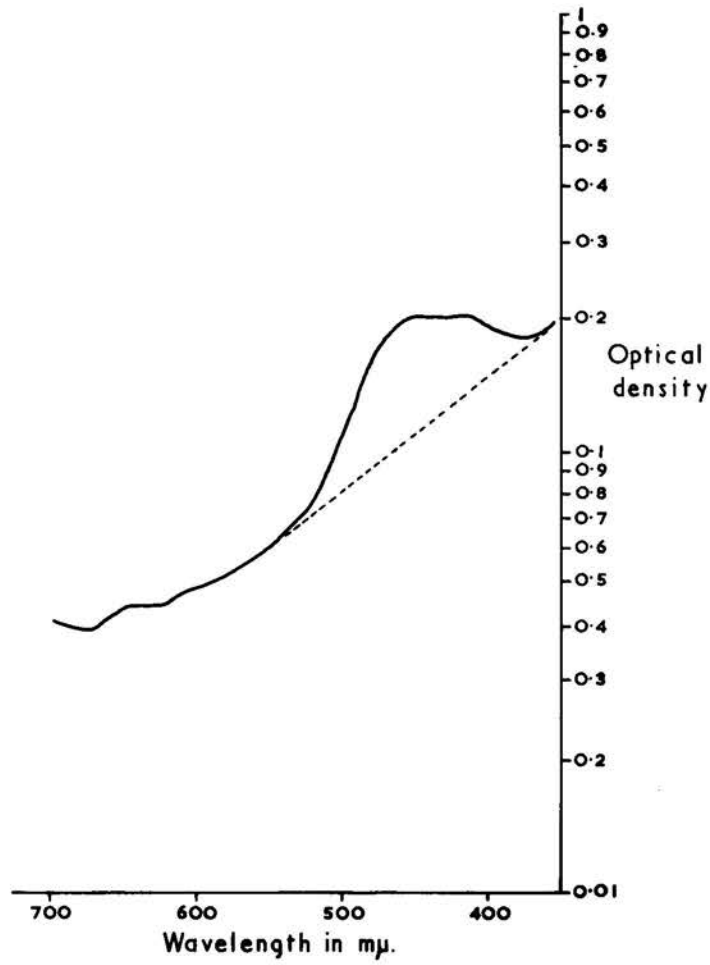
Mrs. I.M.C.K.

Figure 24. 'Severely affected' curve with 'plateau' - anaemic foetus.

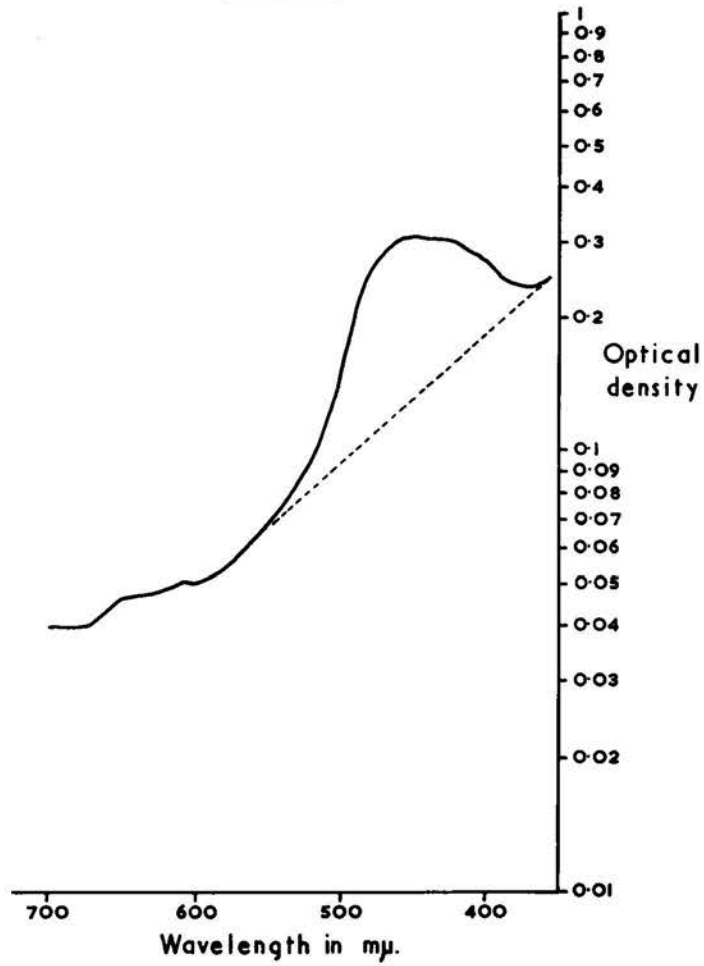
Mrs. A.B.

Figure 25. 'Severely affected' foetus with anaemia - large 'bulge' and high optical density.

optical density at 450 m.u. is higher than in the previous case. Again the bulge is between 370 m.u. and 520 m.u. At birth, the infant's haemoglobin was 6.6 per cent 100 ml. but she made satisfactory progress after 2 exchange transfusions and 1 'top-up' transfusion. In Figure 26, the optical density at 450 m.u. is 0.6 and the bulge is very marked. Again there is a less definite plateau but the curve tends to level off after 420 m.u. This patient had 2 living children after 7 pregnancies. Her third child died soon after delivery, probably as a result of haemolytic disease, while her next 3 children were stillborn and hydropic. The 7th pregnancy ended at 10 weeks with an abortion. The patient's husband was homozygous for the D antigen.

In her 8th pregnancy, a Caesarean section was performed at 34 weeks gestation and a male infant weighing 4 pounds 11 ounces (2125 g.) was delivered. The haemoglobin at birth was 5.6 per cent but the infant survived after 1 exchange and 2 'top-up' transfusions.

The graphs of 13 infants who were lost as a result of haemolytic disease revealed 9 with plateaux, or 'humps'. A typical curve is illustrated in Figure 27 with the plateau between 420 and 460 m.u. No foetal heart was heard when the patient was admitted at 36 weeks and although the foetus had died as a result of haemolytic disease, there was no evidence of hydrops foetalis. This may have been similar to the cases described by Henderson (1942 a. and b.).

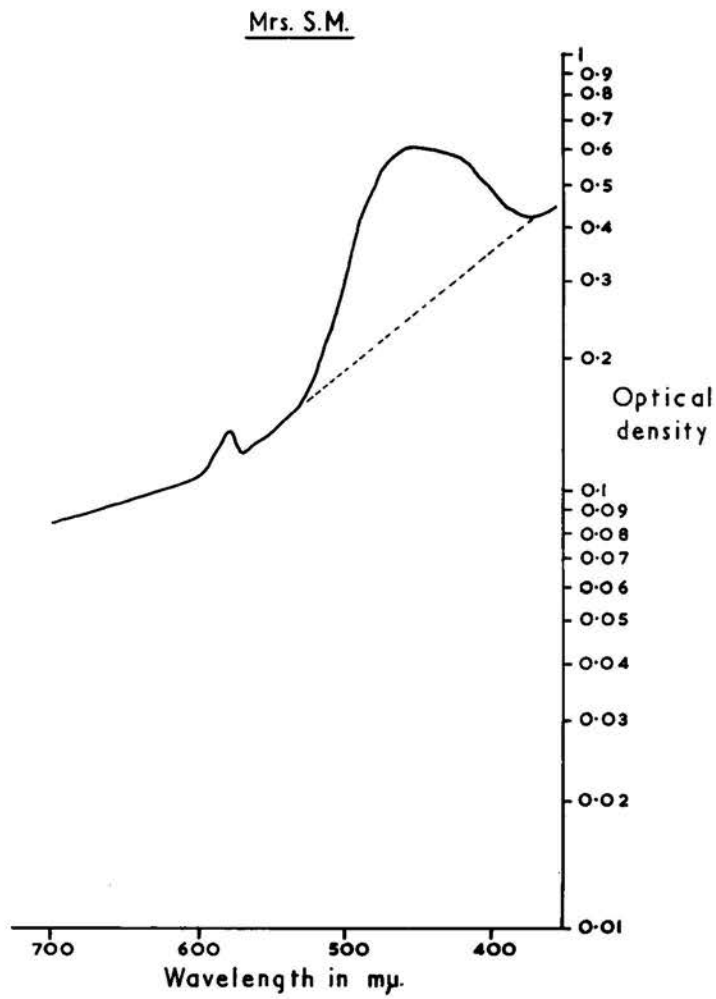


Figure 26. Very severely affected foetus with severe anaemia - pronounced 'bulge'.

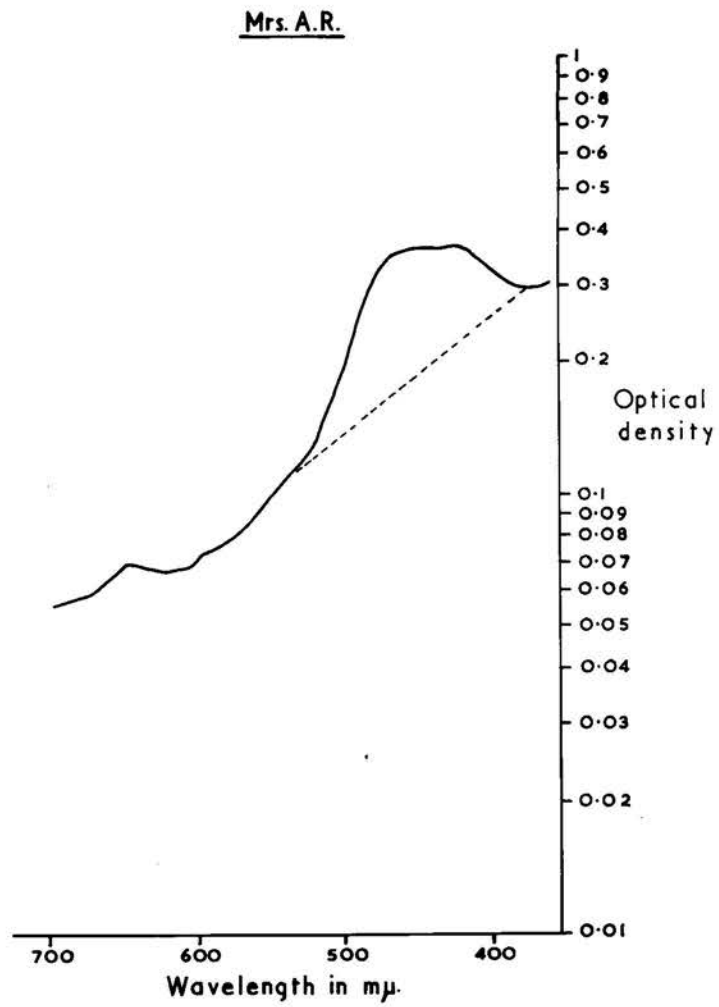


Figure 27. Intra-uterine death - 'plateau' seen on graph.

Figure 28 illustrates the typical curve of an imminent intra-uterine death, the liquor requiring dilution by twice its own volume so that the curve can be plotted. A peak at 405 m.u. was thought by Bevis (1956) and Walker (1957) to be a 'premortal' rise due to the presence of methaemalbumin. When diazo and methyl alcohol are mixed with the liquor, a large 'bulge' is seen between 475 m.u. and 675 m.u., indicating the presence of a very large amount of 'total bilirubin'. The direct bilirubin bulge is much less prominent. When the infant was delivered 10 days later, it was typical of hydrops foetalis.

g. 'False Positive' Results.

If a pregnant woman is Rhesus negative, and has Rhesus antibodies and if her husband is Rhesus positive, it is reasonable to assume that bilirubin in the liquor is the result of haemolytic disease of the newborn. Sometimes after a bilirubin 'bulge' has been found, the infant is reported to be Coomb's negative and Rhesus negative, but develops jaundice and a markedly elevated serum bilirubin. Pre and post natal haemolysis or hyperbilirubinaemia may be due to sulphonamide therapy, salicylates, cytomegalic inclusion disease, toxoplasmosis, and other less common diseases. Examination of the liquor detects the presence of bilirubin only and cannot determine the reasons for excessive haemolysis.

10. Management of Patients with Rhesus Isoimmunisation.

The two alternatives in the management of patients with Rhesus isoimmunisation are to allow labour to

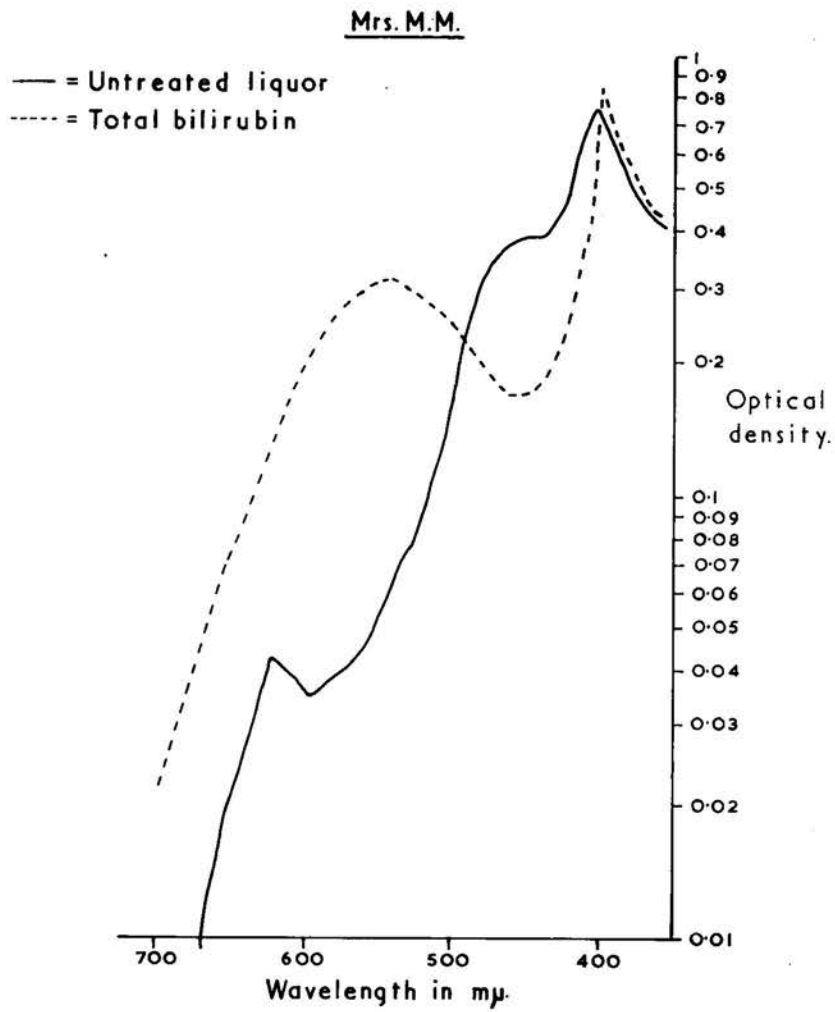


Figure 28. Imminent intra-uterine death - 'premortal' peak at 405 m u.

commence spontaneously or to induce labour artificially either empirically or at a gestation dependant on the predicted severity of the haemolytic disease. Labour may also be induced for some other obstetric or medical reason, e.g. antepartum haemorrhage, pre-eclampsia, or because the foetus has died in utero.

If labour is allowed to commence spontaneously it is necessary to accept a number of stillbirths - from the literature this figure appears to vary between 10 and 20 per cent of the total number of patients with haemolytic disease. On the other hand, infants born alive at or near term would run very little risk of developing kernicterus, if facilities for adequate exchange transfusion were available.

With a policy of empirical induction at 37 or 38 weeks or earlier, a number of stillbirths will still occur but not as many as in the 'spontaneous onset' group. It is, of course, impossible to state the number of deaths which would have been avoided by earlier induction but in every series there will be instances of stillbirths which one knows could probably have been avoided by earlier intervention. One patient who had had 2 children without antibodies being detected was tested at 31 weeks gestation in her third pregnancy and the indirect Coomb's test was found to be negative. At 34 weeks the antiglobulin titre was 1 in 1, and 2 weeks later it was 1 in 20. The patient was seen at 37 weeks and empirical induction suggested at 38 weeks. On admission the patient was found to have a severe degree

of anaemia and a transfusion was given. Before labour could be induced, she developed a deep venous thrombosis and anti-coagulant therapy was commenced. Two days before term labour commenced spontaneously and she was delivered of a stillborn infant with the typical findings of haemolytic disease of the newborn. The foetal heart was definitely heard at 38 weeks gestation and for a week after her admission to hospital.

There are the risks of induction to be considered also both as regards foetal mortality, and foetal and maternal morbidity. It is, therefore, essential that the method of induction used is a satisfactory one and that, should it fail, the obstetrician is prepared to carry out Caesarean section. In this hospital, labour is usually induced by artificial rupture of the membranes followed when necessary by an oxytocic drip. The time interval between rupture of the membranes and the commencement of the oxytocin drip is variable but at present is usually a maximum of 36 hours. In the early years of the series, oxytocin was not always used, or if it was given, administration started after 48 hours. The drip rate and concentration of oxytocin are dependant on the response of the patient. The concentration and rate per minute are increased slowly but steadily until the patient's labour approaches normal labour. The rate is then maintained until delivery unless the contractions become too prolonged, in which case the drip rate is reduced or the drip stopped altogether.

If the time of induction can be determined by the severity of the disease it only remains to decide the earliest gestation at which one is prepared to induce labour (or deliver the patient by Caesarean section). If severity can be predicted accurately, then delivery at or after 36 weeks gestation is a practical proposition. Delivery earlier than this is only likely to be considered in exceptional circumstances. The object of antibody studies and analysis of the liquor amni is to determine the severity of the haemolytic disease.

In the present series, during 1956 and 1958, induction of labour because of Rhesus isoimmunisation was not commonly carried out. It was used more during 1958 to 1960, and very frequently during 1961 and 1962. The results of spontaneous delivery and induction for Rhesus isoimmunisation are compared, in Groups, in these 3 time-intervals, and related to the severity of haemolytic disease in the infant. (Tables XXV, XXVI and XXVII). If the foetus has died in the uterus before 35 weeks gestation, the onset of labour, whether spontaneous or induced, was counted as 'other onset'. If an intra-uterine death occurred after 35 weeks in an 'unbooked' patient, this was usually included under 'other onset', as these deaths were considered to be beyond the control of the hospital. If death occurred after 35 weeks and induction had been considered at a later maturity, this was an 'induction death', otherwise it was a death associated with a spontaneous onset of labour.

TABLE XIV.

GROUP I.

Foetal Result.	1956-57.			1958-60.			1961-62.		
	Spontan- eous Onset.	Rhesus Induction.	Other Onset.	Spontan- eous Onset.	Rhesus Induction.	Other Onset.	Spontan- eous Onset.	Rhesus Induction.	Other Onset.
Unaffected.	9	1	5	4	11	1	1	17	0
Mildly affected.	10	4	2	11	23	2	5	19	1
Moderately affected.	13	8	4	18	40	1	9	27	0
Severely affected - anaemia.	2	0	0	4	2	0	1	4	0
- hyperbilirubinaemia.	3	1	2	2	4	0	0	5	0
Haemolytic disease - Stillbirth.	2	0	1	3	3	1	2	1	4
Neonatal death.	3	0	0	0	0	2	0	0	0
Other deaths (affected or not)	0	1	0	1	1	0	0	0	2
Abortions.		(3)			(1)			(1)	
Not tested.	0	0	0	2	0	0	1	1	1
<b>Total.</b>	<b>42</b>	<b>15</b>	<b>14</b>	<b>45</b>	<b>84</b>	<b>7</b>	<b>19</b>	<b>74</b>	<b>8</b>
Number of Patients in Time Period.		<b>74</b>			<b>137</b>			<b>102</b>	

TABLE XV.

Mode of onset of Labour and Foetal Results in Group I.

TABLE XXVI.

GROUP II.

Foetal Result.	1956-57.			1958-60.			1961-62.		
	Spontan- eous Onset.	Rhesus Induction.	Other Onset.	Spontan- eous Onset.	Rhesus Induction.	Other Onset.	Spontan- eous Onset.	Rhesus Induction.	Other Onset.
Unaffected.	4	1	2	2	10	0	3	9	0
Mildly affected.	0	5	0	5	3	1	0	11	0
Moderately affected.	8	3	1	12	20	0	4	19	0
Severely affected - anaemia.	3	1	0	0	6	0	1	8	0
- hyperbilirubinaemia.	5	1	0	0	4	0	0	8	0
Haemolytic disease - Stillbirth.	1	0	0	2	4	5	0	1	1
Neonatal death.	1	0	0	0	3	1	0	0	1
Other deaths (affected or not).	4	0	1	0	0	0	0	0	2
Abortions.		(2)			(2)			(6)	
Total.	26	11	4	21	50	7	8	56	4
Number of Patients in Time Period.		43			80			74	

TABLE XXVI.

Mode of onset of Labour and Foetal Results in Group II.

TABLE <u>XXVII.</u>	GROUP III.								
	1956-57.			1958-60.			1961-62.		
	Spontan- eous Onset.	Rhesus Induction.	Other Onset.	Spontan- eous Onset.	Rhesus Induction.	Other Onset.	Spontan- eous Onset.	Rhesus Induction.	Other Onset.
Unaffected.	0	1	0	0	2	0	0	3	0
Mildly affected.	0	0	0	0	0	0	0	0	0
Moderately affected.	1	1	0	2	2	1	0	1	0
Severely affected - anaemia	0	0	0	0	5	0	1	3	0
- hyperbilirubinaemia.	0	0	0	0	0	0	0	3	0
Haemolytic disease - Stillbirth.	0	0	5	1	0	1	0	0	1
Neonatal deaths.	1	0	0	0	0	0	0	0	1
Other deaths (affected or not).	0	0	0	0	0	0	0	0	1
Abortions.	(2)			(7)			(6)		
Total.	2	2	5	3	9	2	1	10	3
Number of Patients in Time Period.	11			21			20		

TABLE XXVII.

Mode of onset of Labour and Foetal Results in Group III.

Before attempting any comparison between the 3 time-groups, the percentage of severe cases and Rhesus death was calculated for each group and was found to be almost identical for each group. (Table XXVIII). The 'avoidable foetal mortality in the 3 'time-groups' and the 3 'previous-history' groups is considered with the mode of onset of labour in Table XXIX. It will be seen that in each time-group, the percentage of deaths after 'Rhesus' induction is less than with spontaneous onset, and this also applies to the 3 'previous history' groups. For this selected series, the foetal loss rate from haemolytic disease is 6 per cent, while in labours which commenced spontaneously, 10 per cent were lost. The difference between these proportions is 0.057 and this is  $2\frac{1}{2}$  times the standard error of the difference of the proportions and is therefore significant. The groups also are comparable so that induction of labour would appear to save lives of a number of infants.

As it has been suggested that induction of labour causes a number of immature infants to be delivered, the birth weight under or over 2500 g. has been correlated with the mode of onset of labour and the foetal outcome. (Table XXX). In fact proportionately more small babies were delivered after labour commenced spontaneously (15 per cent) than when labour was induced because of Rhesus isoimmunisation (10 per cent).

Figure 29 shows the percentage of inductions for Rhesus isoimmunisation performed during each year with

TABLE XXVIII

Year groups	Total deliveries in series excluding abortions	Severe and Rhesus deaths	Percentage of 'severe' cases
1956 - 57	121	32	26
1958 - 60	228	53	23
1961 - 62	183	46	25
Total	532	131	25

TABLE XXVIII

Comparison of 'Time Periods' by numbers of  
'severely affected' infants and deaths from  
haemolytic disease.

TABLE XXIX.

Mode of onset  
of labour.

Mode of onset of labour.	1956-57.				1958-60.				1961-62.				Totals.							
	Total No.	Total Deaths.	Rhesus Deaths.	%	Total No.	Total Deaths.	Rhesus Deaths.	%	Total No.	Total Deaths.	Rhesus Deaths.	%	Total No.	Total Deaths.	Rhesus Deaths.	%				
<b>Group I.</b>																				
Spontan. onset.	42	0	5		45	4	2		19	2	2		106	11	10	10	9			
Rhesus induction.	15	1	0		84	4	2		74	1	1		173	6	4	4	2			
<b>Total.</b>	<b>57</b>	<b>6</b>	<b>5</b>		<b>129</b>	<b>8</b>	<b>6</b>		<b>93</b>	<b>3</b>	<b>3</b>		<b>279</b>	<b>17</b>	<b>6</b>	<b>14</b>	<b>5.0</b>			
<b>Group II.</b>																				
Spontan. onset.	26	6	2		21	2	2		8	0	0		55	8	15	4	7			
Rhesus induction.	11	0	0		50	7	7		56	1	1		117	8	7	8	7			
<b>Total.</b>	<b>37</b>	<b>6</b>	<b>2</b>		<b>71</b>	<b>9</b>	<b>9</b>		<b>64</b>	<b>1</b>	<b>1</b>		<b>172</b>	<b>16</b>	<b>9</b>	<b>12</b>	<b>7.0</b>			
<b>Group III.</b>																				
Spontan. onset.	2	1	1		3	1	1		1	0	0		6	2	33	2	33			
Rhesus induction.	2	0	0		9	0	0		10	0	0		21	0	0	0	0			
<b>Total.</b>	<b>4</b>	<b>1</b>	<b>1</b>		<b>12</b>	<b>1</b>	<b>1</b>		<b>11</b>	<b>0</b>	<b>0</b>		<b>27</b>	<b>2</b>	<b>7</b>	<b>2</b>	<b>7</b>			
<b>Totals.</b>																				
Spontan. onset.	70	17	17	25	11	69	7	10	14	9	28	2	7	2	7	167	21	13	16	10
Rhesus induction.	28	1	4	14	0	143	11	8	10	7	140	2	1	2	1	311	14	5	12	4
<b>Grand Total.</b>	<b>98</b>	<b>18</b>	<b>13</b>	<b>13</b>	<b>8</b>	<b>212</b>	<b>18</b>	<b>8</b>	<b>16</b>	<b>8</b>	<b>168</b>	<b>4</b>	<b>2</b>	<b>4</b>	<b>2</b>	<b>478</b>	<b>35</b>	<b>7</b>	<b>28</b>	<b>6</b>

TABLE XXIX.

Selected Foetal mortality, in Groups, depending on mode of onset of labour.

TABLE XXX.

Birth Weight.	Unaffected.	Mildly Affected.	Moderately Affected.	Severely Affected.		Haemolytic Disease.		Other Deaths.	Abortions.	Not Tested.	Total.
				Anaemia	Hyperbilirubinaemia.	Stillbirths	Neonatal Deaths.				
<b>Under 2500 g.</b>											
Spontaneous.	1	0	11	5	2	3	2	1	-	-	25
Rhesus Induction.	5	1	9	10	2	1	1	1	-	-	30
Other.	0	0	2	0	0	11	1	5	30	-	49
<b>Total.</b>	<b>6</b>	<b>1</b>	<b>22</b>	<b>15</b>	<b>4</b>	<b>15</b>	<b>4</b>	<b>7</b>	<b>30</b>	<b>-</b>	<b>104</b>
<b>Over 2500 g.</b>											
Spontaneous.	22	31	56	7	8	8	3	4	-	3	142
Rhesus Induction.	50	64	112	19	24	8	2	1	-	1	281
Other.	8	6	5	0	2	8	4	1	-	1	35
<b>Total.</b>	<b>80</b>	<b>101</b>	<b>173</b>	<b>26</b>	<b>34</b>	<b>24</b>	<b>9</b>	<b>6</b>	<b>-</b>	<b>5</b>	<b>458</b>
<b>Totals.</b>											
Spontaneous.	23	31	67	12	10	11	5	5	-	3	167
Rhesus Induction.	55	65	121	29	26	9	3	2	-	1	311
Other.	8	6	17	0	2	19	5	6	30	1	84
<b>Grand Total.</b>	<b>86</b>	<b>102</b>	<b>195</b>	<b>41</b>	<b>38</b>	<b>39</b>	<b>13</b>	<b>13</b>	<b>30</b>	<b>5</b>	<b>562</b>

TABLE XXX.

Birth weights and severity of haemolytic disease with mode of onset of labour.

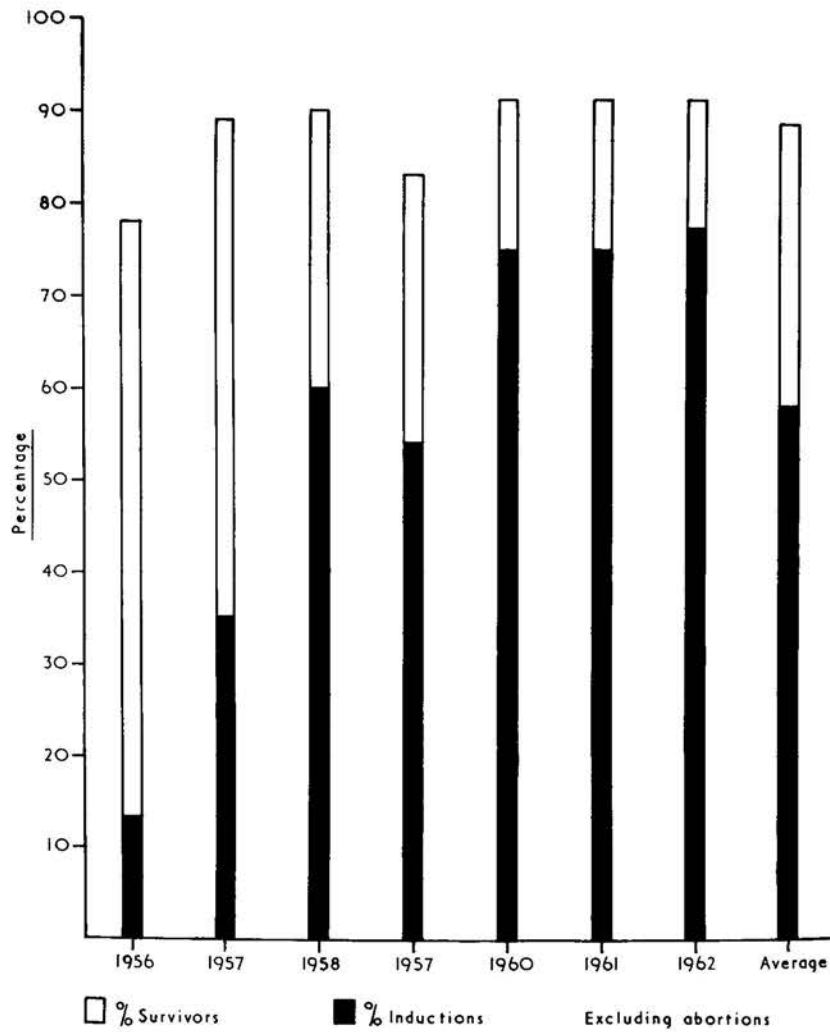


Figure 29. Percentage of inductions of labour and percentage of infants surviving with haemolytic disease, by years.

the percentage of surviving infants. Some of the inductions included were at or about term and cannot be called premature or even 'preterm' inductions. From the graph it appears that a 58 per cent induction rate results in an infant survival rate of 88 per cent. A rise in the induction rate from 10 per cent to 75 per cent, however, only increased the survival rate by 13 per cent from 78 per cent to 91 per cent.

The problem appears to be one of individual patients and not of series of cases. If it is necessary to wait until a stillbirth has occurred in a family before considering active intervention, the chance of further children may have been lost because of the poor prognosis after an infant has been lost from haemolytic disease. A patient who has no living children is quoted later as an example of the importance of considering each patient according to the circumstances of the case, and not by applying strict rules.

Is induction harmful? Provided the infant survives and is healthy, and provided any hyperbilirubinaemia of prematurity is treated by exchange blood transfusion, there should be no harmful effects. If the method of induction is satisfactory and the labour is not prolonged, the maternal morbidity should not be increased. In the series two deaths are definitely attributable to the management of the patient following the induction of labour. In one, labour was not stimulated until 7 days after the membranes had been

artificially ruptured and the infant died of a chest infection probably contracted in utero. A second patient was induced at 38 weeks as the infant was thought to be moderately affected after liquor analysis. Some difficulty was experienced in controlling the labour with an oxytocin drip and after delivery the infant was slow to respond and died within 24 hours from 'anoxia'.

#### 11. Labour and Delivery.

The duration of labour - under or over 24 hours - of patients in the series is recorded in Table XXXI and only 6 per cent were found to have been in labour for longer than 24 hours.

The method of delivery was spontaneous and normal in 78 per cent of the patients (Table XXXII) and 6 per cent of all the inductions performed because of Rhesus isoimmunisation failed and Caesarean section was necessary to effect the delivery of the patient.

#### 12. The Infant.

If the infant is born alive, Walker (1958) considered that with adequate treatment the neonatal mortality should be as low as 6 per 1,000. In order to achieve this he recommended that patients with Rhesus isoimmunisation be delivered in hospitals capable of carrying out prompt investigation of the infant's blood and with staff trained in the technique of exchange transfusion.

The indications for exchange transfusion vary a little from year to year among different paediatricians.

TABLE XXXI.

Duration of Labour (Including Oxytocin Drip)	Number of Patients	Percentage of Total
Under 24 hours	461	84
Over 24 hours	33	6
Not known (including abortions and 'elective' Caesarean Section)	56	-
	550	-

TABLE XXXI

Duration of Labour (including patients  
requiring Caesarean Section).

TABLE XXXII

Method of delivery	Number of infants delivered	Percentage of Total
Abortions (including 2 Hysterotomies)	30	5
Spontaneous	430	78
Forceps or Breech	62	11
Caesarean Section	40	7
Caesarean Section for failed induction	19	6% of 311 Rhesus inductions
Total	562	

TABLE XXXII Methods of delivery.

If the haemoglobin is below 12 g. per cent, exchange transfusion is usually indicated. If the haemoglobin is higher than this, and the serum bilirubin is also high, an exchange transfusion soon after birth is again usually indicated. If the serum bilirubin is low, it is necessary to repeat the estimation frequently and to prevent the level reaching 20 mg. per cent. If the serum bilirubin level is rising at a rate greater than 1 mg. per cent per hour, some paediatricians would perform an exchange transfusion while others are content to wait and to transfuse when the bilirubin is seen to be approaching 20 mg. per cent.

In assessing the results achieved in any series of this kind, it is necessary to consider the infants who were lost and those severely affected. The mildly and moderately affected infants are unlikely to develop complications, and the unaffected infants should behave very normally, provided immaturity and the hazards of induction can be avoided.

a. Foetal Deaths in Series.

In Table XXXIII the deaths occurring in the series as a result of haemolytic disease of the newborn are recorded. It will be seen that 52 affected infants died from haemolytic disease, a mortality rate of 9.8 per cent. This includes 39 stillbirths and of these 13 (33 per cent) occurred before 35 weeks gestation and can be considered "unavoidable" in that the gestation is too early for induction of labour to be successful.

Of the neonatal deaths associated with haemolytic

TABLE XXXIII.

DEATHS IN SERIES FROM HAEMOLYTIC DISEASE.

<u>Total deaths in affected infants</u>	- 52.		
Haemolytic disease - stillbirths	- 39	} ( 20 - 1st pregnancy in which antibodies detected.	
- neonatal deaths	- 8		} ( 21 - Previous infant had haemolytic disease.
Deaths as result of exchange transfusion	- 5		
	Total.	<u>52</u>	
"Exchange transfusion" deaths.			
Moderately affected - 3 (incl. 1 septicæmia)			
Severely affected (anaemia) - 2, (incl. 1 ruptured spleen).		33% of Stillbirths occur before 35/52.	
<u>Abortions with evidence of haemolytic disease</u> - 16.			

TABLE XXXIV.

DEATHS IN SERIES FROM CAUSES OTHER THAN HAEMOLYTIC DISEASE.

Deaths from other causes	- 13.	
Stillbirths from anoxia	- 3	- 3 Unaffected.
Neonatal deaths - congenital abnormalities.	- 4	- 2 Mild.
		1 Moderate.
		1 Severe - anaemia.
Respiratory distress syndrome	- 3	- 1 Unaffected.
		- 1 Mild.
		- 1 Moderate.
Prematurity	- 1	- 1 Unaffected.
Neonatal deaths following induction of labour	- 2	- 1 Unaffected - anoxia and pulm. atelectasis.
		- 1 Moderately affected - empyema and lung abscesses.
	TOTAL.	<u>13</u>

Abortions with no evidence of haemolytic disease = 14.

disease, 5 were the result of exchange transfusion, including 1 infant with septicaemia and 1 with a ruptured spleen.

In Table XXXIV the deaths in the series which were not due to haemolytic disease are shown. (Also Table VII). These number 13, of which 3 were still-births from anoxia in infants found at post mortem to be unaffected by haemolytic disease. The 2 neonatal deaths occurring after labour had been induced were due to 'anoxia' and lung abscesses respectively. These were avoidable but 4 of the others had congenital abnormalities incompatible with life.

In Table XXXV the deaths due to Rhesus isoimmunisation (including two following the induction of labour) are studied in more detail. Seventeen deaths occurred before 35 weeks gestation and of the 37 dying after 35 weeks, 23 were 'booked'. Certainly some of these deaths might have been prevented if an accurate method of predicting the severity of the haemolytic disease had been known.

In 18 patients of the 54, a paracentesis uteri was carried out at the correct time, while in 7 others it was either performed after 35 weeks or before 33 weeks gestation. In the remainder (29) no paracentesis was performed, 14 being delivered during 1956 and 1957.

b. Severely affected Infants in Series.

These infants were all born alive and survived the neonatal period. They have been divided into 2 groups, the first consisting of infants with a haemoglobin at

TABLE XXXV.

Number of haemolytic disease deaths	=	47
Number of deaths following exchange transfusion	=	5
Number of deaths as result of induction because of Rhesus isoimmunisation	=	2
		<u>54</u>

'Haemolytic' deaths before 35 weeks gestation = 17 (3.2% of 532 (excluding abortions)).

'Haemolytic' deaths after 35 weeks gestation = 37 (7.0% of 532 (excluding abortions)).

Booked cases (seen before 34/52) - 23

Unbooked cases - 14

'Haemolytic' deaths after 36 weeks gestation = 34 (6.4% of 532 (excluding abortions)).

Paracentesis at correct time = 18

Paracentesis too late or very early = 7

No paracentesis = 29  
54

TABLE XXXV.

Deaths due to Rhesus isoimmunisation, with possible factors in the prevention of foetal loss.

birth of below 9.6 g. per cent, and the second of infants who had very high serum bilirubin levels during the first 5 days of life. The results are presented in Table XXXVI. Although the number in the first time-period (1956-1957) of those whose labour was induced is less than those whose labour commenced spontaneously, in the other two time-periods many more were induced than allowed to commence labour themselves. This undoubtedly saved lives in the anaemic group but may have contributed to the hazards occurring in the high bilirubin group.

It is of interest and importance to notice that in almost half the pregnancies in which the birth haemoglobin was under 9.6 g. per cent, antibodies had been detected for the first time. Also in 40 per cent of the infants who had high serum bilirubin levels were found to have been delivered from mothers in whom antibodies had not previously been found.

Further analysis of the anaemic group of severely affected infants (Table XXXVII) shows that 13 of the 18 patients in the series with birth cord haemoglobin levels below 7.4 g. per cent had been induced, 5 at under 36 weeks gestation. Nine of the patients had had liquor examination carried out. The lowest haemoglobin recorded in an infant in the series who survived the neonatal period was 2.9 g. per cent.

In Table XXXVIII the number of exchange transfusions given to infants in the hyperbilirubinaemia group and their gestation on admission are recorded. The

TABLE XXXVI.

Year Groups.	Infants with Birth Hb. under 65%.	Booked	Not Booked	Anti-bodies first time.	Prev. affected	Prev. Rh. SB or NND.	Spont. onset.	Induction.
1956-57.	13	8	5	7	5	1	9	4
1958-60.	16	12	4	9	2	5	9	7
1961-62.	21	18	3	7	10	4	4	17
TOTAL.	50	38	12	23	17	10	22	28

Year Groups.	Other severely affected infants. (Hyperbilirubinaemia).	Booked	Not Booked	Anti-bodies for first time.	Prev. affected	Prev. Rh. SB or NND	Spont. onset.	Induction
1956-57.	13	6	7	6	7	0	9	4
1958-60.	14	12	2	6	8	0	2	12
1961-62.	16	14	2	5	8	3	0	16
TOTAL.	43	32	11	17	23	3	11	32

TABLE XXXVI.

Details of patients with Rhesus Isoimmunisation whose infants had a severe degree of haemolytic disease of the newborn.

(Includes some "moderately affected" as well as "severely affected").

TABLE XXXVII.

Number of surviving infants with birth haemoglobin under 50% (7.4 g. %) = 18.  
 Number of these infants delivered after induction of labour = 13.  
 Number of these/induced after paracentesis uteri = 9.

Gestation on induction - 34 weeks - 1  
 35 " - 4  
 36 " - 4  
 37 " - 2  
 38 " - 1  
 39 " - 1  
13

Lowest birth haemoglobin recorded in live infant = 20% (2.9 g. %).

TABLE XXXVII. Results in severely anaemic patients.

TABLE XXXVIII.

<u>Number of repeat exchange transfusions given.</u>	<u>Gestation on induction in hyperbilirubin group.</u>
2 exchanges = 30	35 weeks - 4
3 exchanges = 12	36 weeks - 14
4 exchanges = 3	37 weeks - 6
5 exchanges = 2	38 weeks - 7
6 exchanges = 1	39 weeks - 1
	<u>32</u>

Highest cord bilirubin recorded = 16.6 mg. per cent.

TABLE XXXVIII. Results in patients with hyperbilirubinaemia.

The largest number of exchange transfusions given was 6, with 1 simple transfusion for anaemia 4 weeks after birth. Labour was induced in this patient at 36 weeks gestation, and the bilirubin reached 30.4 mg. per cent. The baby thrived and on discharge was well.

Two infants are known to have developed an 'inspissated bile syndrome' and, with exchange transfusion and conservative treatment, have made satisfactory recoveries.

c. Sex of the Foetus.

Diamond et al (1950) and Armitage and Mollison (1953) found that male infants were more susceptible to haemolytic disease especially if they were immature. This was not confirmed by Knox et al (1961).

The sex distribution by severity is given in Table XXXIX and perhaps the most interesting finding is the large number of unaffected female infants found in the series. There were more severely affected male infants and more deaths from haemolytic disease in male infants, and these differences are of some statistical significance ( $\chi^2 = 9.8$ ,  $n = 3$ ,  $p = 0.02$ ).

If the marked discrepancy between the sexes in the 'unaffected group was found to persist when a very much larger series of cases was studied, it might suggest that in heterozygous fathers, the d antigen was carried on the X-chromosome.

13. The Placenta.

Studies of the placenta are extremely difficult to

TABLE XXXIX.

Sex of foetus.	Unaffected.	Mildly and moderately affected.	Severely affected.	Deaths from haemolytic disease.	Others.	Total.
Male	30	137	43	28	-	238
Female	62	166	37	24	-	289
Not recorded	-	-	-	-	35	35
Total.	92	303	80	52	35	562

TABLE XXXIX.

Sex distribution of infants and severity of haemolytic disease.

undertake because although it can be obtained 'fresh', it cannot readily be studied in situ without jeopardizing the foetus. Studies on placental histology and growth have been carried out by Crawford (1959) and he made a number of very interesting observations on the 'growing ends' at the fringes of the placental cotyledons. He noted that there was a reduction in the number of small and medium-sized cotyledons, but hypertrophy of those which remained increased the size of the placenta.

a. Ratio of Placenta to Foetus by Weight.

Chernyak and Rabtsevich (1959) and Jeffcoate and Scott (1959) studied the relationship of the ratio of the placenta to the foetus by weight to the severity of the haemolytic disease found in the infant.

Goplerud (1961) observed that if the ratio of placenta to foetus was less than 1 in 4, and if the infant survived, the small ratio was the one expected for that gestation.

In Table XI the ratio of the placental weight to the foetal weight at birth is related to the severity of the haemolytic disease which occurred. The number of infants who could be considered immature by gestation or birth weight in each group is also indicated, and in the group where the ratio is 1:1 to 1:3 it will be seen that about half of the affected infants survived.

b. Histochemistry of Placenta.

Twenty placentae have been studied histologically and histochemically by the methods described by

TABLE XL.

Foetal results.	Ratio 1:1-1:3			Ratio 1:4-1:6			Ratio 1:7 and over.			Ratio not Known.	Total.		
	No.	Under 35/52	Under 5lb.	No.	Under 35/52	Under 5lb.	No.	Under 35/52	Under 5lb.		No.	Under 35/52	Under 5lb.
Unaffected	11	1	2	78	2	4	2	1	1	1	92	4	7
Mild	4	0	1	92	0	3	8	0	0	1	105	0	4
Moderate	15	2	2	170	3	11	5	0	0	8	198	5	13
Severe	17	2	4	58	2	6	0	0	0	5	80	4	10
Death from haemolytic disease.	29	15	9	18	0	4	1	0	0	4	52	15	13
Not known	-	-	-	-	-	-	-	-	-	35	35	-	-
TOTAL.	76	20	18	416	7	28	16	1	1	54	562	28	47

TABLE XL.

Ratio of placental weight to foetal birth weight related to severity of haemolytic disease and "immaturity". (5 pounds = 2270 g.)

Ismail (1959). Portions of the placentae delivered from women who were known to have Rhesus isoimmunisation were fixed and stained, and after examination the findings were correlated with the degree of haemolytic disease developed in their infants.

The placental blocks were stained and mounted in the Department of Obstetrics and Gynaecology, University of Edinburgh. In each case 2 slides of placental tissue stained by haematoxylin and eosin were examined to determine the general histological appearance. In addition a number of other stained sections were examined. These were as follows:-

- i. Alkaline Phosphatase - by the modified Calcium Cobalt Method, Gomori (1946), and by the Coupling Azo-Dye Method (Pearse 1953).
- ii. Acid Phosphatase - by the Lead Phosphate Method (Lillie 1952) and the Coupling-Azo Method (Burton 1954).
- iii. Nonspecific Esterase - a - Naphthyl Acetate Azo Coupling Method (Pearse 1953).
- iv. Glycogen - Chromic Acid Schiff Method (Lillie 1952).
- v. Calcium - Von Kossa Method (Pearse 1953).
- vi. Lipoids - Sudan IV Method for neutral fat (Ismail 1959).

Mallory's modification of Schultz Test (Lillie 1952) for cholesterol.

Polarised light (Dempsey and Wislocki 1944), Lillie (1952) for examination of fat.

FINDINGS.Group 1. 'Control' and 'Unaffected'. (4 placentae).

These placentae showed some oedema of the villi, some hyalinisation of villi and also the extravasation of normal and nucleated red blood cells (Figure 30).

Acid and alkaline phosphatase were marked in the syncytium, and sometimes alkaline phosphatase was seen in the stroma.

Non-specific esterase was marked in the syncytium but scanty in the villous stroma. Fat was seen in the stroma, and some cholesterol was present in the syncytium. Glycogen and calcium were only found in the stroma.

Group 2. 'Mild' and 'Moderate'. (4 placentae).

Numerous small (normal) villi were found with hyaline change in some and a few showing slight oedema. Extravasation of nucleated blood cells was quite marked. (Figure 31).

Variable amounts of acid and alkaline phosphatase were seen in the syncytium, and non-specific esterase was found in the syncytium.

The amount of fat present in the villi and stroma was small but rather variable. Cholesterol was also present in varying amounts in the syncytium and stroma. No calcium was seen and glycogen was only found in the stroma.

Group 3. 'Moderate' and 'Severe'. (4 placentae).

Again numerous small villi were found with more

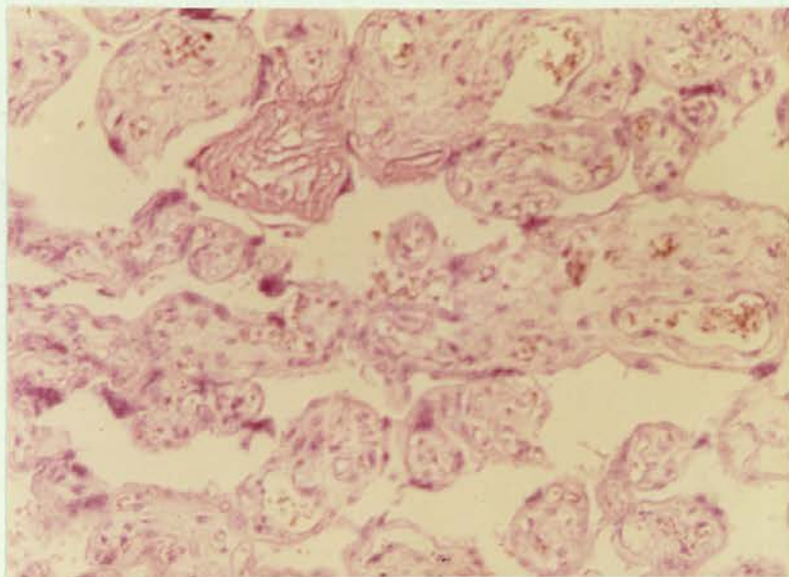


Figure 30. Unaffected infant - oedema of villi and extravasation of red blood cells. H and E X 185.

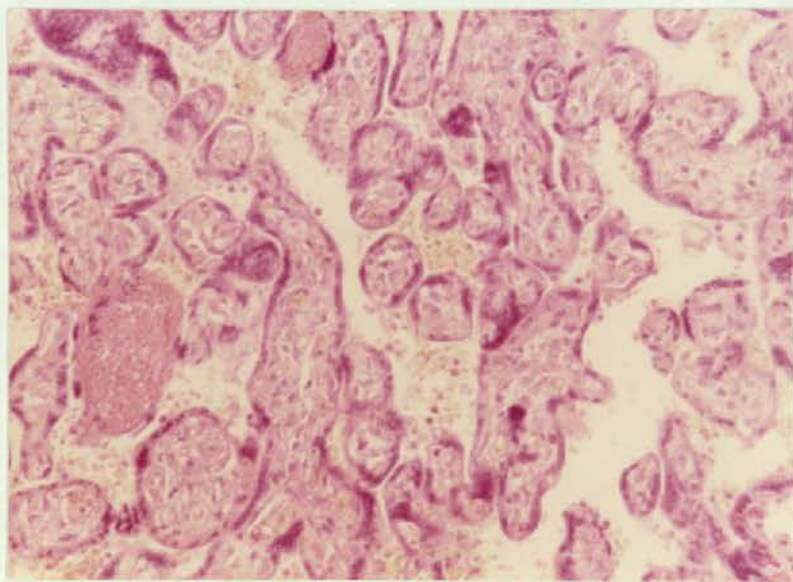


Figure 31. Moderately affected infant - villi small, some oedema and some hyaline change, marked extravasation of red cells. H and E X 185.

marked hyaline change than previously and accompanied by extravasation of nucleated red cells. (Figure 32).

More alkaline phosphatase than acid phosphatase was found, and non-specific esterase was seen in the syncytium. Fat was usually minimal in amount or absent and no calcium was found. The amount of cholesterol found was very variable, but it was never very marked. Some glycogen was seen in the stroma of villi and around blood vessels but the amount present was small.

Group 4. Alive at birth but died in neo-natal period.  
(2 placentae).  
Alive in labour but died before delivery.  
(1 placenta).

Extravasated nucleated cells were prominent, with hyaline change in some villi and a dense stroma in some others. (Figure 33).

Alkaline phosphatase was marked in the syncytium, and some acid phosphatase was also found. No glycogen or calcium were found, and only a little cholesterol was present in the syncytium. An excessive amount of fat was seen in one section in the stroma of the chorionic villi, but may have been due to degeneration. Non-specific esterase was very prominent in the syncytium. (Figure 34).

Group 5. Hydrops foetalis. (5 placentae).

Oedema and hyaline changes in the chorionic villi were marked, and numerous small villi were present with extravasation of nucleated red cells and occasionally a definite Langerhan's layer was seen. (Figures 35 and 36).

Alkaline phosphatase (Figure 37) and to a lesser

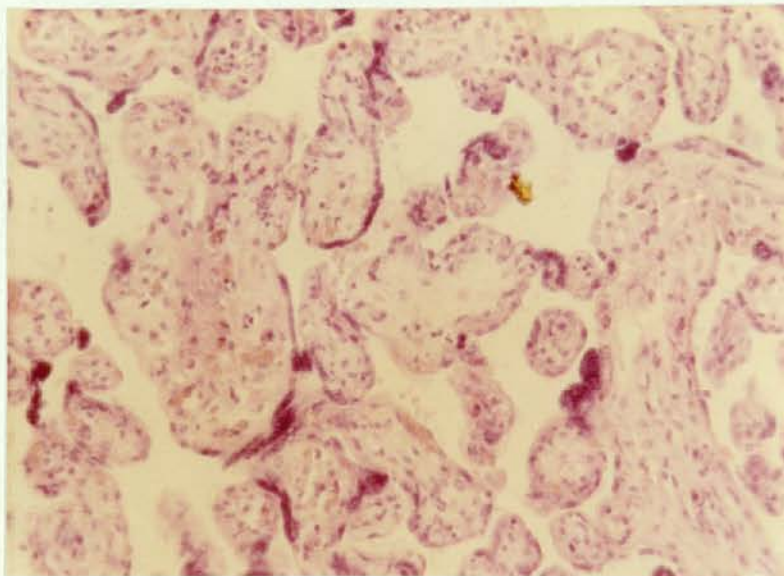


Figure 32. Severely affected infant - small villi, hyaline change and extravasation. H and E X 185.

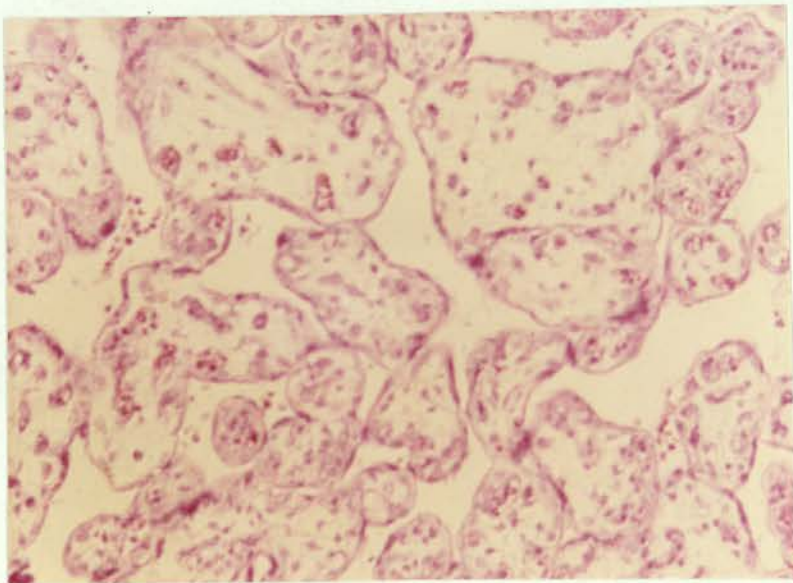


Figure 33. Neonatal death - hyaline change and dense stroma in some villi. H. and E. X 185.

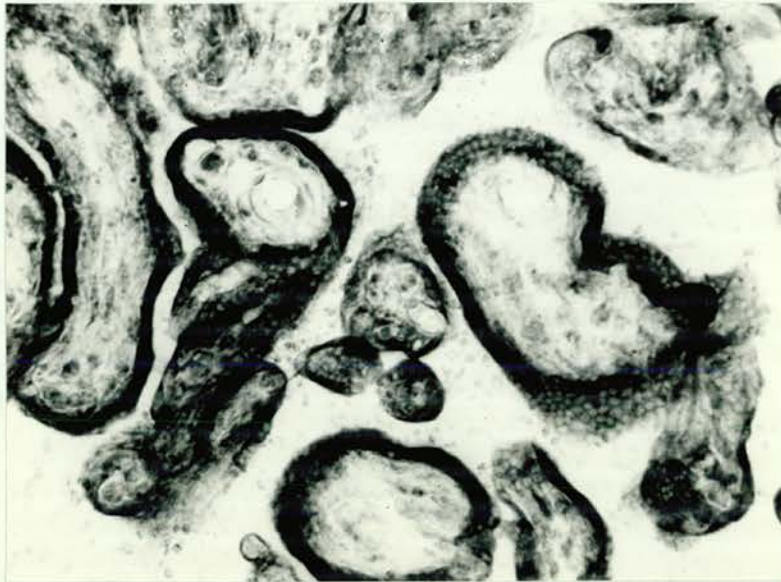


Figure 34. Non-specific esterase - prominent in syncytium. Azo-coupling method X 185.

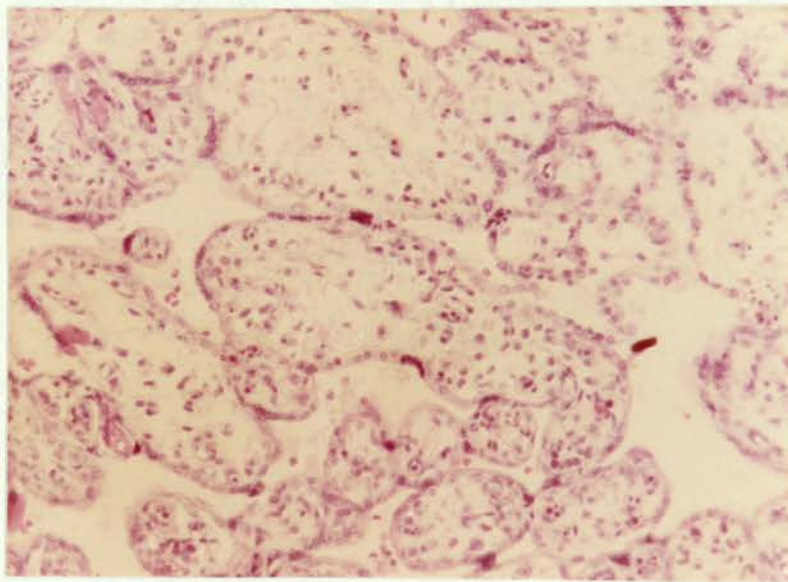


Figure 35. Hydrops foetalis - oedematous villi with persistence of Langerhans' layer of cells.  
H & E X 185.

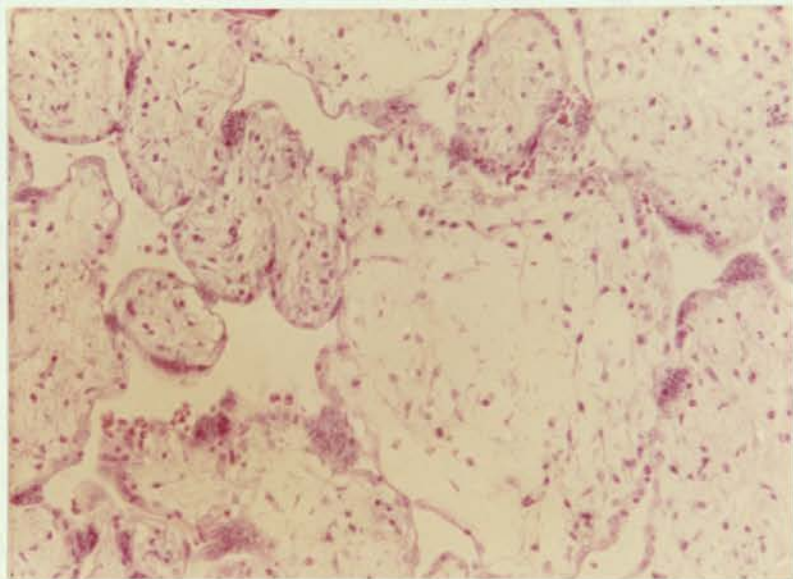


Figure 36. Hydrops foetalis - oedematous villi with extravasation and some hyalinisation. H & E X 185.

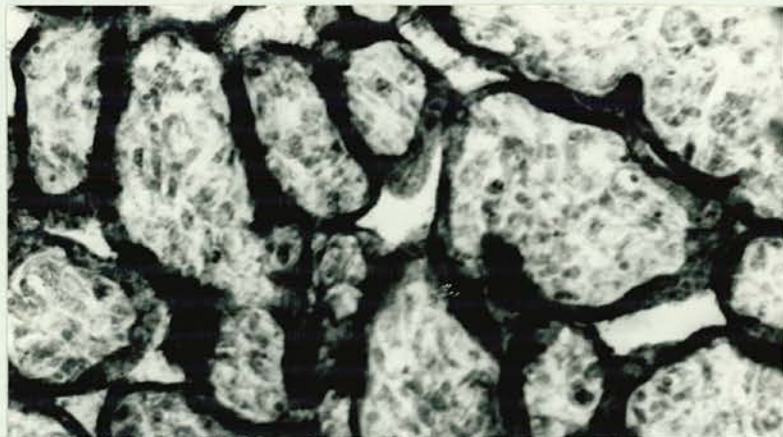


Figure 37. Alkaline phosphatase - present in syncytium. Azo-coupling method. X 185.

extent acid phosphatase were found in the syncytium, and variable amounts of non-specific esterase were present in the syncytium and blood vessel walls. A minimal amount of fat was seen, possibly in Langerhan's layer, (Figure 38), and cholesterol was present (Figure 39). Variable amounts of calcium (Figure 40) were seen and glycogen was found in some villi.

#### Differences in Histology and Histochemistry in the 5 Groups.

In the sections stained with haematoxylin and eosin, there is little difference between the controls and the affected cases, but the hydropic placentae show oedema of the villi and persistence of Langerhan's layer or cytotrophoblast.

Acid and alkaline phosphatases are found in the syncytium in varying amounts and are concerned, as enzymes, in the phosphorylation process necessary for the exchange of nutriment. Their presence in the syncytium of chorionic villi in hydropic placentae suggests that the placenta was functioning actively up to the time of the foetal death.

Glycogen is found in the stroma of the villi but not in the covering layers. The amount of glycogen found in individual placentae is very variable, however. Dempsey and Wislocki (1944) thought that glycogen storage in the placentae might be for the purpose of aiding anaerobic respiration of cells deprived of adequate oxygenation. The glycogen

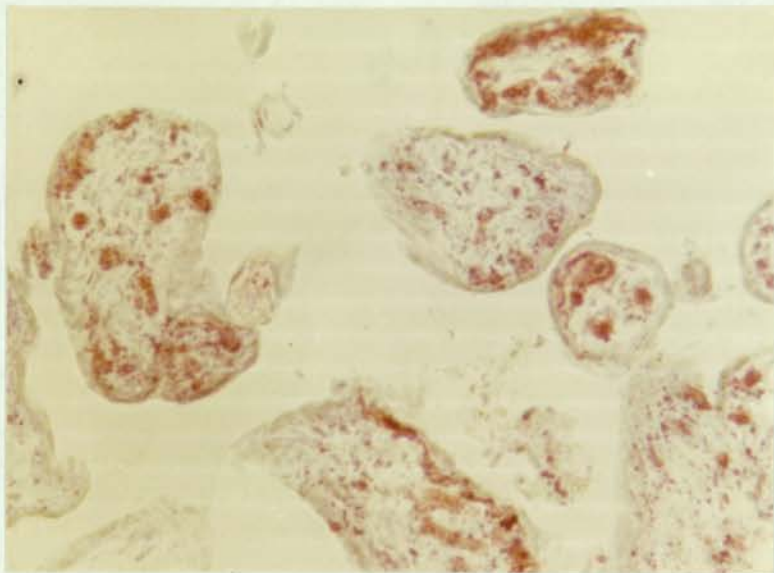


Figure 38. Fat - in Langerhans' layer of trophoblast.  
Sudan IV. X 185.

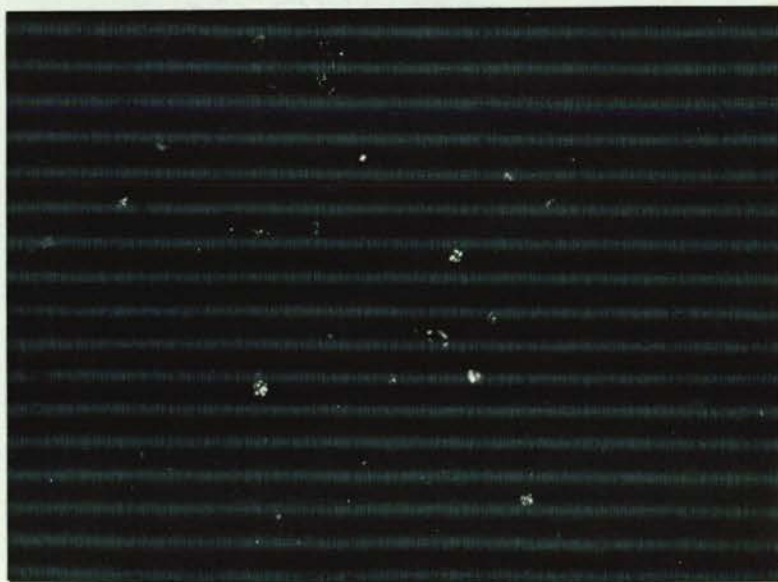


Figure 39. Cholesterol - in stroma of villi.  
Frozen section. Polarised X 185.

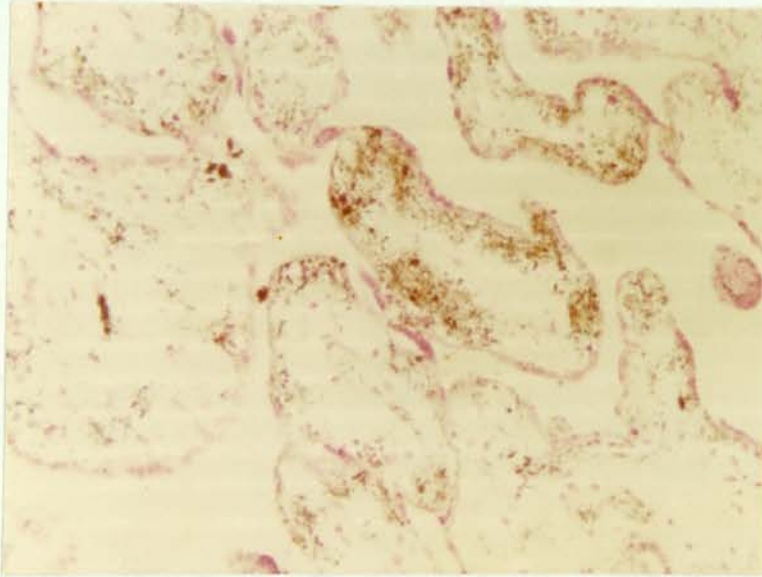


Figure 40. Calcium - in stroma of villi.  
Von Kossa X 185.

found in the third trimester in the placenta is much less than was present in early pregnancy, and it is suggested that the liver is now the principal store of the foetal glycogen reserves.

Fat was found by Ismail (1959) in normal placentae in the following sites:- in the syncytium, in the stroma of some healthy chorionic villi, and in degenerating villi. The syncytial fat decreased during pregnancy, and stromal fat was present in varying amounts. Fat can be seen in some villi near term and is an indication of physiological ageing. Its presence in the stroma and in the trophoblast may be related to the production of steroid hormones and the presence of fat in Langerhan's layer in an hydropic placenta may be related in some way to the increase in chorionic gonadotrophin reported by Scott (1958).

Non-specific esterase was found in the syncytium of the chorionic villi in some placentae, but not in them all. In the normal placenta, Ismail (1959) believed that the amount of non-specific esterase was directly related to the amount of syncytial fat. In this study fat was rarely seen in the syncytium but non-specific esterase was found in the syncytium in nearly every case and also in the stroma. Ismail thought that the enzyme was closely concerned with fat metabolism, but it may have other functions also.

Calcium was found in the stroma of the normal 'control' chorionic villi, and was also present in the

stroma of villi in 2 hydropic placentae. Ismail thought that the fine stromal deposits represented calcium absorbed from the maternal blood stream, on the way to the foetal circulation. In the placentae studied in this series, no calcium deposits in hyalinised areas were seen.

In conclusion, it would appear that while the naked eye appearance of the placenta (Figure 41) resembles 'wool which has poured from the woolsack' (Jakesch 1878) and is pale and friable in appearance, on histological examination, the most striking findings are the oedematous villi and the persistence of Langerhan's layer, which is readily demonstrable. Any hyalinisation is directly related to the period of gestation.

Histochemically there is evidence that the hydropic placenta is still active and functioning, with a good supply of enzymes in the syncytium. The other changes are related to the processes involved in ageing, apart from the presence of a 'fat' in the cytotrophoblastic Langerhan's layer. It is possible that this fat is concerned with the production of the chorionic gonadotrophin which is said to be increased in this condition.

No gradual histological or histochemical change could be detected among the placentae in the different severity groups. By the methods used it is clear that the placenta is functioning normally up until the time the foetus dies or is delivered. If the foetus dies in



Figure 41. Hydrops foetalis - foetus and placenta.

utero, the changes in the placenta are probably secondary to the cardiac failure or anoxia, which are the result of severe haemolytic disease of the newborn.

14. Cases of Particular Interest.

Despite the large number of tables of collected data that have been presented, it is essential that each patient with Rhesus isoimmunisation be treated as an individual. A number of individual cases are briefly presented to illustrate problems in diagnosis or management.

a. Mrs. M.C.

A para 3, with 1 previous infant affected by haemolytic disease and requiring exchange transfusion. Despite this, at 16 weeks gestation the blood was reported as indirect Coomb's test negative although agglutination was seen with enzyme-treated cells. By 35 weeks the antiglobulin titre was 1 in 40, but after induction at 37 weeks, the infant was mildly affected.

b. Mrs. B.G.

This patient had had 1 previous pregnancy and was aged 24. At 8 weeks and again at 35 weeks gestation blood was tested and the indirect Coomb's test was negative on both occasions. The patient was delivered at term, at that time her blood being indirect Coomb's positive and the albumen titre 1 in 256. The birth haemoglobin of the infant was 7.4 g. per 100 ml. and she was severely affected.

c. Mrs. D.F.

A patient of 23, para 1, whose blood at 15 weeks gestation was indirect Coomb's negative. At 34 weeks the albumen titre was 1 in 32 and the antiglobulin titre 1 in 60. At 36 weeks the albumen titre was still 1 in 32 and the antiglobulin titre 1 in 100. By 38 weeks the albumen titre had risen to 1 in 256 and the antiglobulin titre was 1 in 80. The rise between 33 and 38 weeks had occurred without paracentesis uteri being performed.

d. Mrs. C.C.

This patient had had 1 previous pregnancy and her husband was heterozygous positive. At 11 weeks gestation the indirect Coomb's test was positive and the albumen and antiglobulin titres, tested on 3 occasions, rose to albumen 1 in 16 and antiglobulin 1 in 20 at 29 weeks. A liquor test suggested, particularly with the diazo test, that the infant was unaffected. (Fig. 42). Delivery during the 40th week of pregnancy was normal and the infant was unaffected by haemolytic disease, an example of the anamnestic reaction.

e. Mrs. W.B.

A patient, aged 33, with 5 previous viable pregnancies and 2 miscarriages. Her husband was homozygous positive (but ABO compatible - group O - with his wife). No antibodies had been detected in her previous pregnancies and at 11 weeks her blood was Coomb's negative. At 35 weeks, she was Coomb's

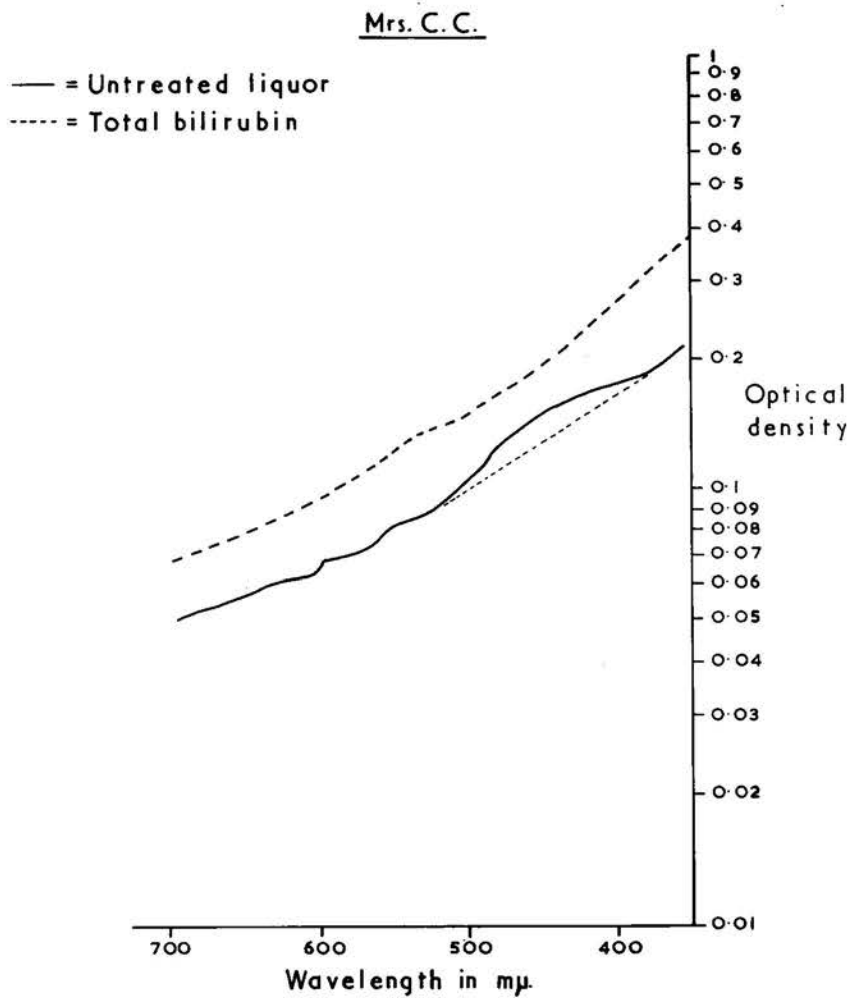


Figure 42. Mrs. C. C. 'Liquor' prediction - unaffected infant.

positive but the infant was unaffected on delivery. It was known that the patient's husband was not the child's father.

f. Mrs. S.M.

The patient's first pregnancy and infant were normal. In the second pregnancy she was Coomb's negative at 10 and 26 weeks gestation, but had developed antibodies by 35 weeks and had an albumen titre of 1/4 at 38 weeks. The child was unaffected and the husband was heterozygous positive. Another anamnestic reaction, but on this occasion the patient's blood early in the pregnancy was Coomb's negative and it seemed as if stimulation and immunisation had occurred during the pregnancy.

g. Mrs. M.S.

This was a similar case to (f) with a patient 'developing' antibodies during a pregnancy but delivering a Rhesus negative infant unaffected by haemolytic disease. On this occasion the liquor test was helpful as both the untreated liquor and the diazo test suggested that the infant was unaffected.

(Figure 43).

h. Mrs. H.F.

This patient had 2 pregnancies in the hospital during the period of the series. Previously she had had 5 pregnancies and from these only 3 infants survived. The other 2 infants had died of haemolytic disease. In her 6th pregnancy she had an intra-

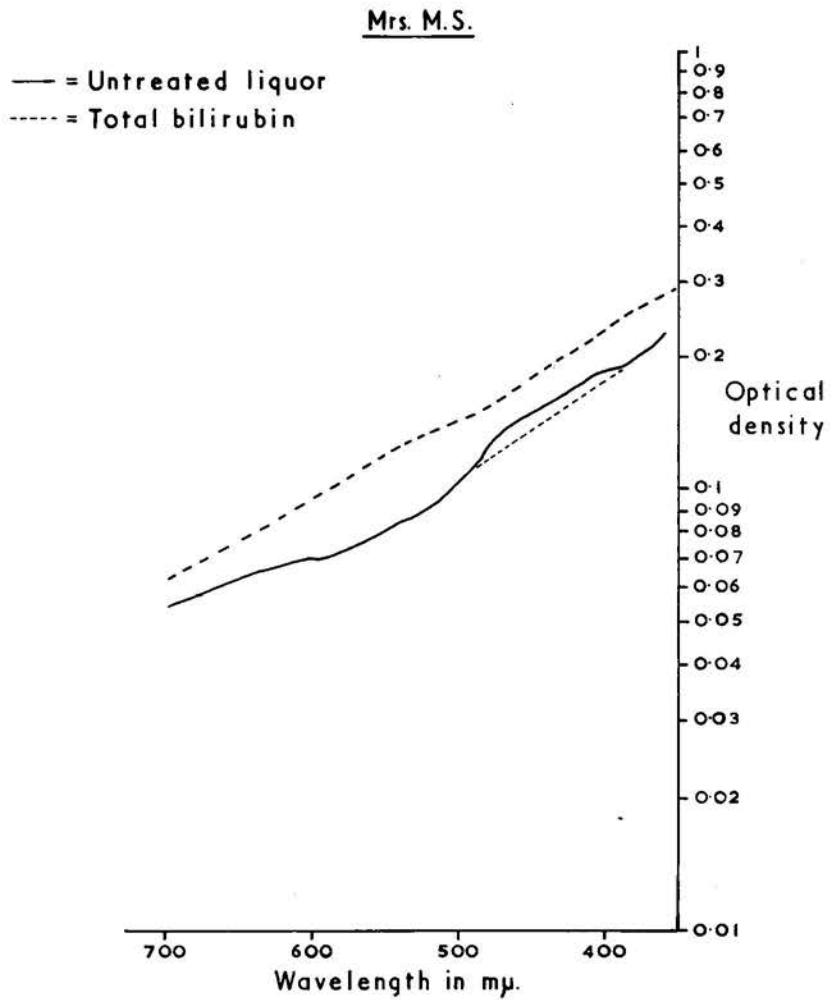


Figure 43. Mrs. M. S. 'Liquor' prediction - unaffected infant.

uterine death at 37 weeks and the infant was hydropic. During this pregnancy she had albumen antibodies of 1 in 1 at 13 weeks and 35 weeks, and at 37 weeks no antibodies were detected.

Two years later, in her 7th pregnancy, she had no albumen antibody titre at 19 weeks, but at 32 weeks, although there was still no albumen titre, the antiglobulin titre was 1 in 80. Liquor examination suggested that the infant was unaffected but this was disregarded and a Caesarean section was performed at 35 weeks, the infant being unaffected.

i. Mrs. C.W.

This parous patient had had 3 affected children previously and at 22 weeks the albumen titre was 1/256. At 30 and 31 weeks gestation, the albumen titre was 1 in 1 and at 33½ weeks the foetus died in utero of hydrops foetalis.

j. Mrs. S.H.

Patient's first baby delivered by Caesarean section. In 2nd pregnancy found to have anti C + D antibodies, but as the husband was homozygous for  $\bar{c}$ , anti C antibody was present, along with D. Agglutination tests were positive at 15 weeks and at 34 weeks the antiglobulin titre was 1 in 80. A macerated foetus was delivered at 35 weeks.

k. Mrs. D. McG.

Para 2, with 1 previously transfused infant. Husband homozygous for D antigen. Liquor examination at 33 weeks showed a 'bulge' (Figure 44) suggesting

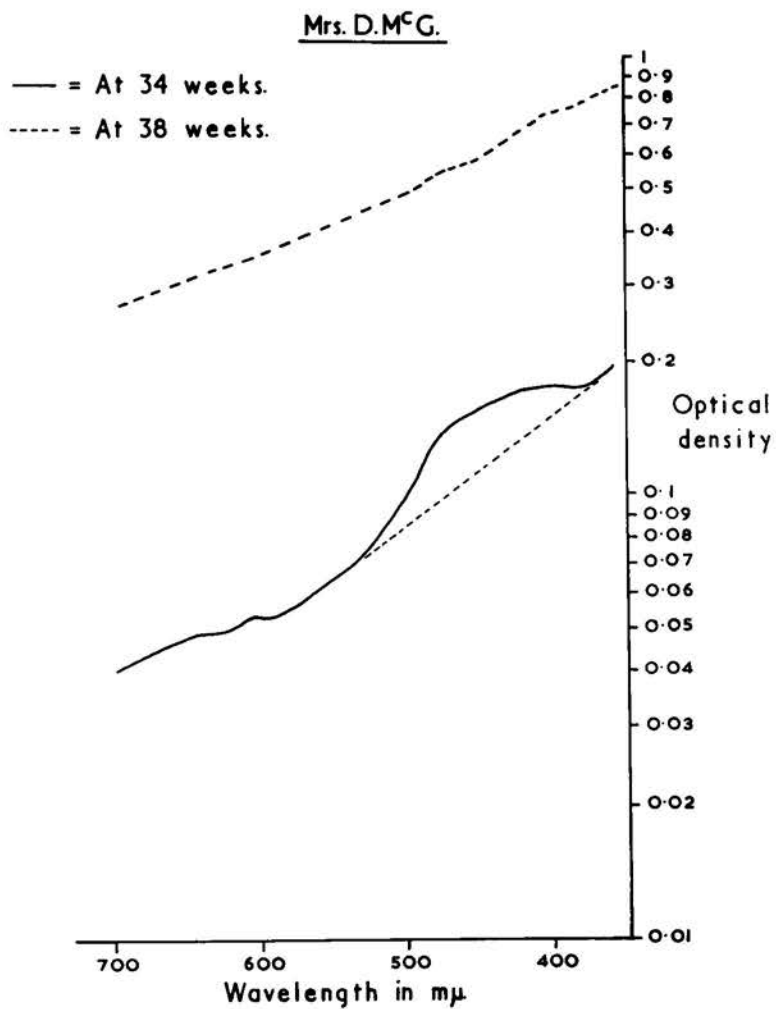


Figure 44. Mrs. D. McG. Liquor curves at 34 and 38 weeks showing disappearance of 'bulge' - moderately affected.

that the infant was moderately affected. At induction, liquor, obtained when the membranes were ruptured, was tested and the bulge had disappeared although the optical density was higher. The infant required an exchange and one 'top-up' transfusion.

l. Mrs. I.B.

This patient had had 1 premature delivery at 34 weeks gestation and then a 12 weeks abortion. The indirect Coomb's test was positive at this time. At the beginning of her third pregnancy, the Coomb's test was negative but by 34 weeks the albumen titre was 1/512 and the antiglobulin titre was 1 in 80. Paracentesis uteri was attempted at 34 weeks but was unsuccessful. Some blood was obtained and found to be group O, Rhesus positive and direct Coomb's positive. As the mother's group was A-ve, this was foetal blood. The antibody titre rose after the test, the albumen titre being 1 in 2048 and the antiglobulin titre 1 in 1000. The infant was delivered at 35½ weeks and required 2 exchange transfusions.

m. Mrs. C.G.

This patient's first pregnancy was uneventful. In the second pregnancy - delivered at home - the infant was found to have haemolytic disease and was transferred to the Royal Hospital for Sick Children, Edinburgh, where exchange transfusion was performed. In her 3rd pregnancy liquor was tested (Figure 45a) and the infant was found to be moderately severely affected. One year later she had her 4th child, the liquor this

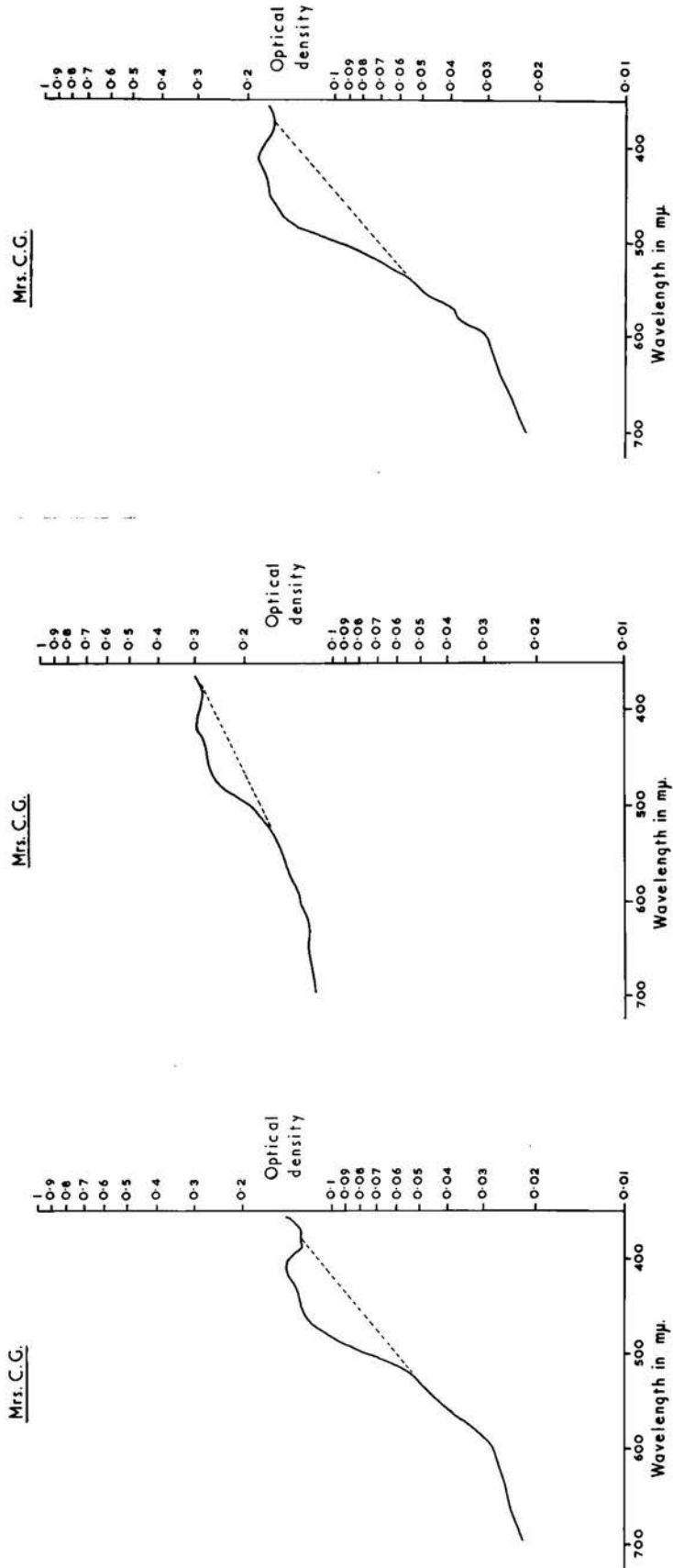


Figure 45. Mrs C.G.  
 a.) 'Liquor' prediction - moderately severely affected infant.  
 b.) 'Liquor' prediction - less severely affected than (a).  
 c.) 'Liquor' prediction - very severely affected infant.

time being less severely affected (Figure 45b). However, 2 exchange transfusions and 1 top-up transfusion were required. In her fifth pregnancy a large bulge with a hump-peak at 410-420 m.u. (Figure 45c) was obtained. The dates were in some doubt, but at 36 weeks labour was induced. The infant required 6 exchange transfusions during the first 4 days of life.

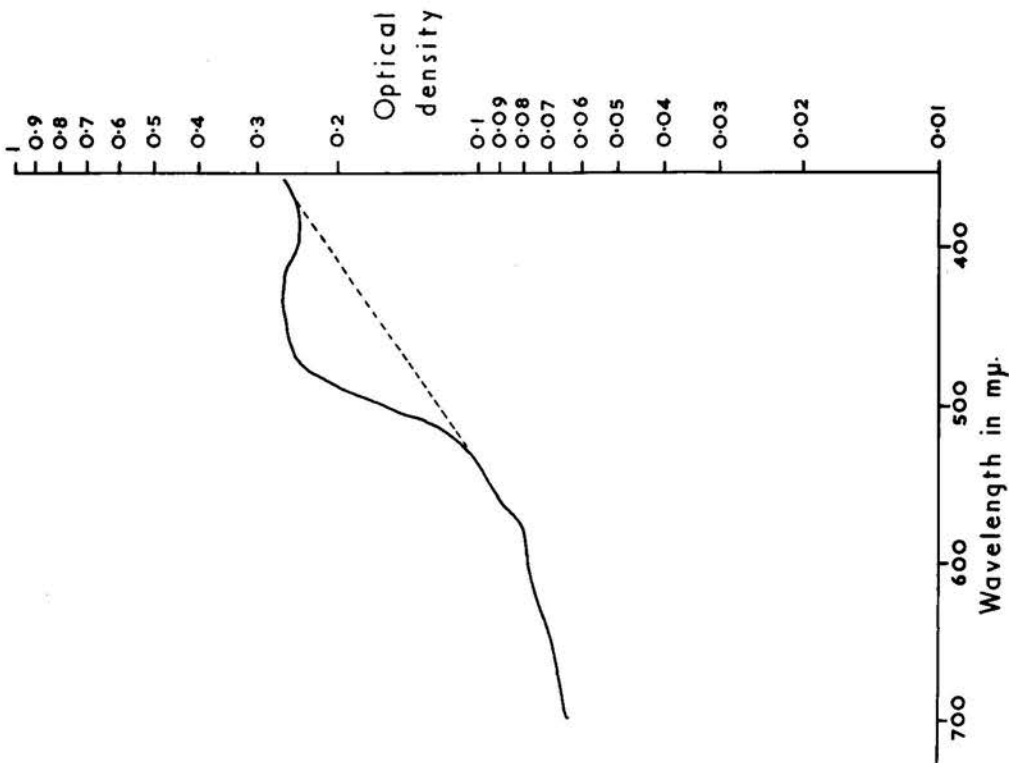
n. Mrs. K.C.

First pregnancy normal. Second pregnancy - infant admitted to hospital at 3 days for exchange transfusion. In 3rd pregnancy, antibody titres - albumen 1 in 32 and antiglobulin 1 in 20. Liquor suggested a severely affected infant (Figure 46a) and the infant was delivered at 36 weeks gestation. He required 1 exchange transfusion and 1 'top-up' transfusion. The following year, she became pregnant again and the highest antibody titre was albumen 1 in 32. Liquor again suggested a severely affected infant (Figure 46b) and the haemoglobin at birth (at 35 weeks) was 2.9 g. per cent. The placenta to foetus ratio in the first pregnancy was under 1 in 4 and in the second under 1 in 3. After 1 exchange transfusion and 2 'top-up' transfusions, the infant made good progress.

o. Mrs. M. McA.

This is a very tragic case. This patient lost her first infant after a spontaneous delivery, as a

Mrs. C.



Mrs. K.C.

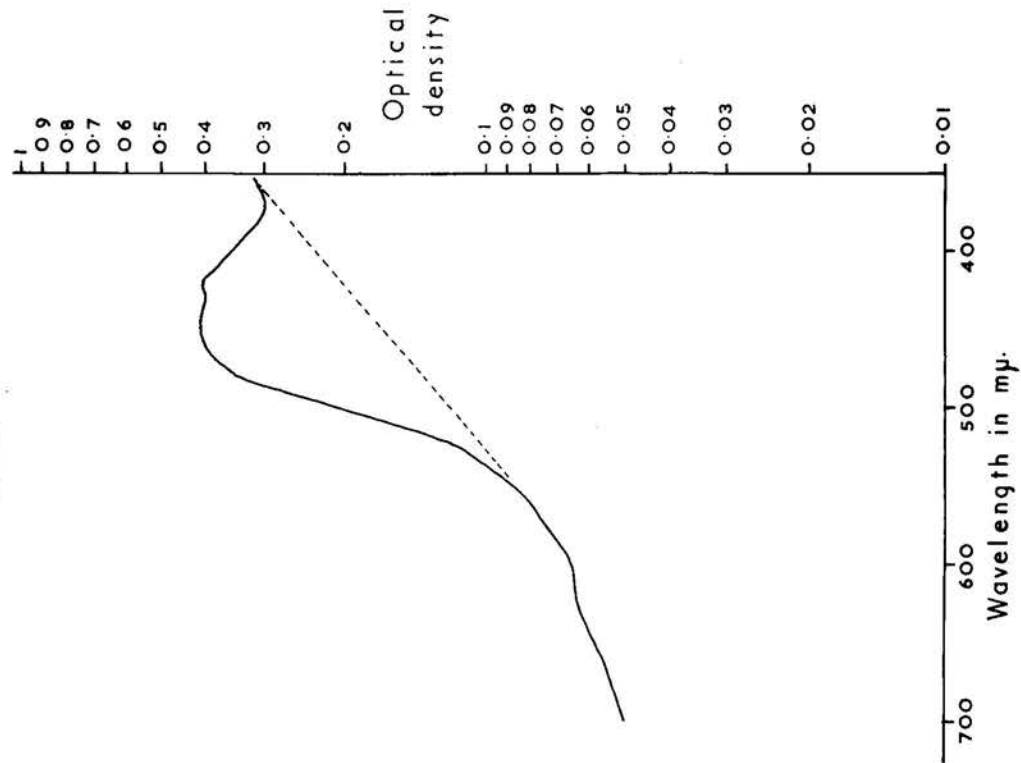


Figure 46.

Mrs K.C.C.

a) 'Liquor' prediction - severely affected infant.

b) 'Liquor' prediction - very severely affected infant - birth haemoglobin 2.9 g per cent.

consequence of pre-eclampsia. In her second pregnancy, when aged 22, antibodies to D and also C were discovered, her husband's genotype being  $R_1R_1$ . By 29 weeks the albumen titre had risen to 1 in 128. A liquor test was performed at 36 weeks and suggested a severely affected infant. During this pregnancy she had essential hypertension and superimposed pre-eclampsia and before labour was induced at 38 weeks, the foetus had died (Figure 47a). This patient had no hydramnios and the foetal weight was just under  $5\frac{1}{2}$  pounds (2500 g.). The placenta to foetus ratio was under 1 in 4. Earlier induction would have given this baby a chance of survival, but as we had waited until the foetus was more mature, this being her first affected pregnancy, the chance was lost. In her third pregnancy the titre of antibodies was albumen 1 in 512 before 34 weeks gestation and when diluted liquor was tested no foetal heart could be heard (Figure 47b) and the curve suggested a very severely affected infant. The placenta to foetus ratio was 1 in 2, the foetal weight (hydropic) being 6 pounds 6 ounces (2890 g.). After three pregnancies this patient has no living children and little prospect of one surviving to a 'viable' gestation.

p. Mrs. C.W.

This patient, aged 38, had a normal first pregnancy, followed by an abortion and an intra-uterine death at 37 weeks gestation. In her fourth pregnancy, she developed albuminuria but not pre-eclampsia and at

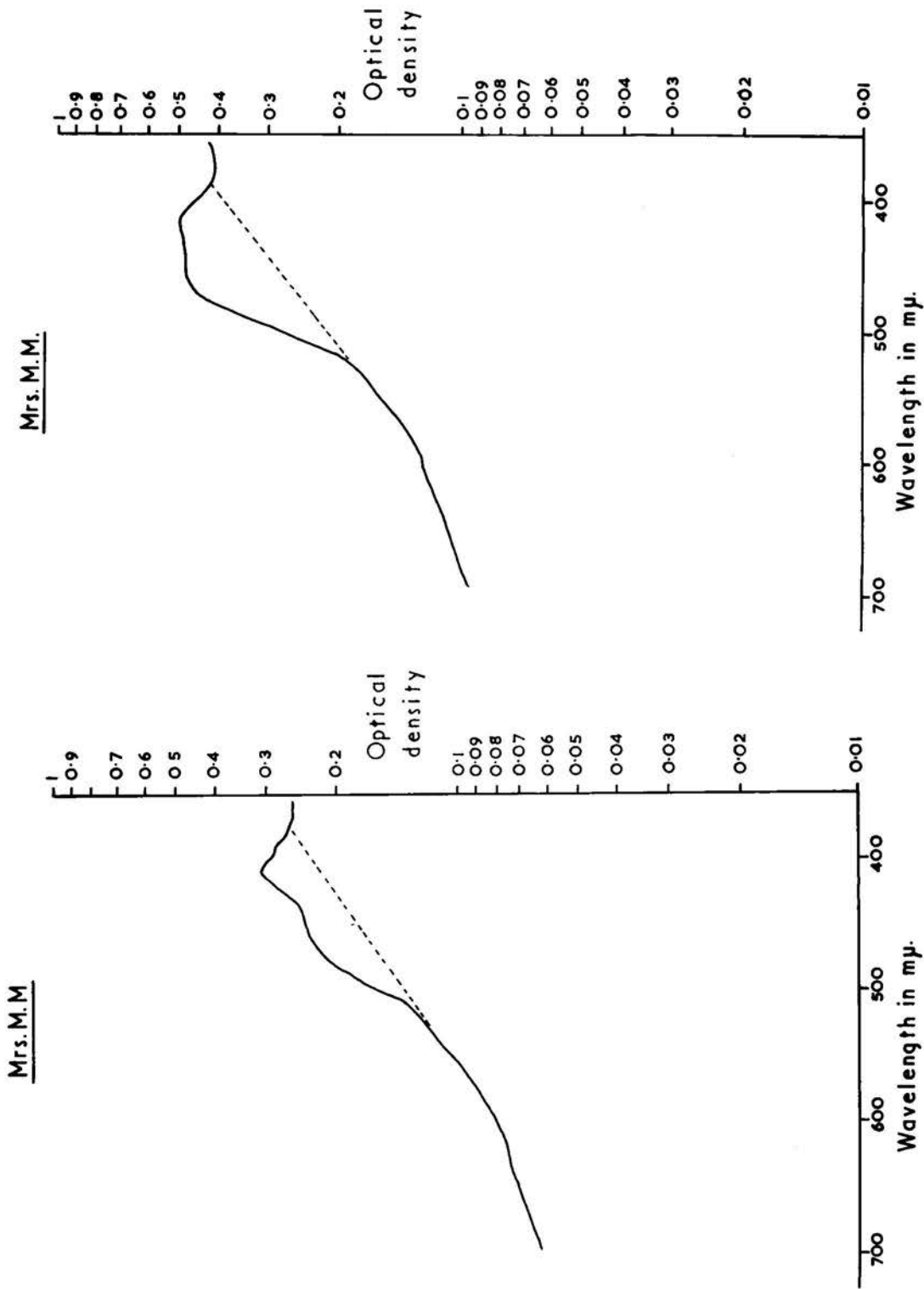


Figure 47.  
Mrs M. McA.  
a) 'Liquor' prediction - severely affected infant.  
b) 'Liquor' prediction - intra-uterine death or very severely affected infant.

30 weeks had a spontaneous delivery of a 'hydropic' living infant weighing 6 pounds 4 ounces (2835 g.) with a placenta weighing 4 pounds 2 ounces (1870 g.). The blood group of the infant was reported as O Rhesus positive but the direct Coombs' test was initially reported as negative, presumably because all the sites for antigen-antibody reaction on the red cells had been blocked and no further agglutination by Coombs' serum was possible. A later specimen was reported as direct Coombs' positive but this was obtained post mortem.

q. Mrs. M.N.

This patient's husband was heterozygous for the D antigen and she already had had four pregnancies, the second infant requiring exchange transfusion. An abortion followed this and then an infant who became jaundiced, but transfusion was not considered necessary. In her fifth pregnancy, the antiglobulin titre was 1 in 20 at 31 weeks and the liquor test suggested that the infant was moderately affected (Figure 48), although the liquor had been contaminated by blood during paracentesis. Labour was induced at 38 weeks, and the infant was unaffected by 'haemolytic disease of the newborn'. However, on the 4th day after delivery, the infant's bilirubin level was 16.4 mg. per cent. No further rise occurred and no definite cause for haemolysis or jaundice was found. The mother and infant were both Group A.

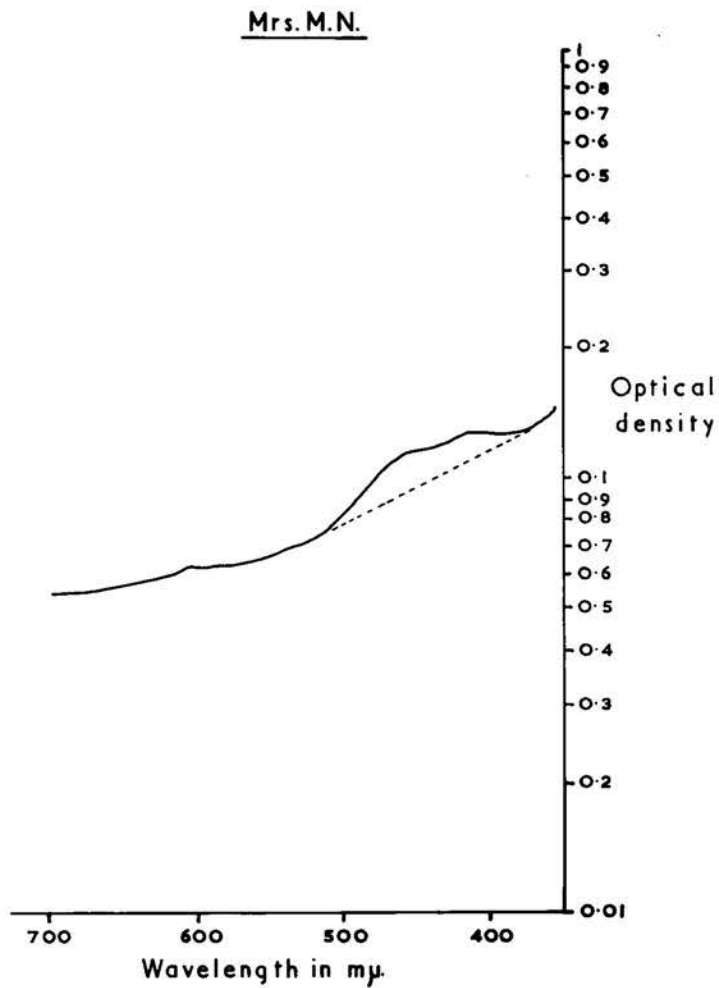


Figure 48. Mrs. M. N. Liquor prediction - moderately affected infant. Infant unaffected, but jaundiced with serum bilirubin of 16.4 m.g. per cent by 4th day.

r. Mrs. J.H.

This patient when aged 36 had her 5th pregnancy. The first infant was normal but the second died as a result of prolapse of the umbilical cord. The 3rd labour was induced at 38 weeks because of haemolytic disease and the infant was affected and required 1 exchange transfusion. Her 4th pregnancy ended in an abortion. In the 5th, the albumen titre was 1 in 16 at 32 weeks, and labour was induced at 39 weeks, but Caesarean section was required because of foetal distress. The foetal haemoglobin at birth was 4.7 g. per cent and immediate exchange transfusion was performed. The serum bilirubin at birth was 7 mg. per cent, but after a week it had risen to 30.6 mg. per cent, 19.8 mg. per cent being 'direct' bilirubin. This infant had the 'inspissated bile syndrome' and after observation and supportive therapy made an uneventful recovery. The birth weight was 6 pounds 9 ounces (2970 g.), the placenta weighing 2 pounds 7 ounces (1105 g.).

s. Mrs. P.C.

This patient's first infant also had the 'inspissated bile syndrome' and required 3 exchange transfusions and 1 top-up. The birth haemoglobin was 5.5 g. per 100 ml. and the bilirubin at birth was 17.4 mg. per cent, half being direct-acting bilirubin. By the 6th day the serum bilirubin had reached 41.8 mg. per cent, again half being direct-acting bilirubin. A Caesarean section had been carried out in labour

because of foetal distress.

Liquor paracentesis was unsuccessful in this patient but mixed foetal and maternal blood was obtained, the foetal blood being Group B, Rhesus negative and direct Coombs' test negative. In this instance the haemolytic process may have been accelerated by the liquor test, although the anti-globulin titre was 1 in 80 before the 'tap' was performed.

The patient had had 2 premarital abortions and was presumably sensitised by a transfusion given with the first of these as antibodies were detected in her second pregnancy.

t. Mrs. P.H.

This patient was group A Rhesus positive, genotype  $CD\bar{e}/CD\bar{e}$ , and her husband's blood group and genotype were O,  $CD\bar{e}/cDE$ . She received a blood transfusion after her first pregnancy which terminated at 12 weeks by an incomplete abortion. She then had 2 normal infants, neither being jaundiced. In her fourth pregnancy she was found to have anti  $\bar{c}$  and anti E antibodies, the husband being heterozygous for these antigens. The dates were uncertain but at 30 weeks the albumen titre was only 1 in 1. A liquor test suggested that the infant was moderately affected and labour was induced at 37 weeks. The infant was group O positive,  $CD\bar{e}/CD\bar{e}$  and Coombs' positive, the haemoglobin at birth being 10.3 g. per cent. The baby

required 1 exchange and 1 'top-up' transfusion and made good progress.

This case illustrates one of the risks of transfusing Rhesus positive women with blood compatible for the D antigen only.

u. Mrs. C.A.

The patient's first pregnancy ended in an abortion at 12 weeks. Some years prior to this she had received a blood transfusion at the time a thoracotomy operation was performed. Her blood group was A negative while that of her husband was A positive,  $C\bar{D}e/c\bar{d}e$ . The indirect Coomb's test was negative at 11 weeks but the albumen titre was 1 in 1 at 31 weeks and 1 in 2 at 38 weeks. On testing at 34 weeks the liquor - untreated with diazo - suggested that the foetus was moderately severely affected by haemolytic disease and induction after 38 weeks was recommended. After artificial rupture of the membranes and an oxytocic intravenous drip, the patient had a spontaneous delivery, the infant weighing 6 pounds 14 ounces (3120 g.) and his blood group being A Rhesus negative. Unfortunately the infant died within 24 hours, anoxia and pulmonary atelectasis being the causes of death. This must be accepted as a death following induction and as the induction was unnecessary, the case is a tragic one. It is to be hoped that the abortion was unrelated to haemolytic disease and that the next pregnancy will be successful in producing a living child.

#### IV. DISCUSSION AND CONCLUSIONS.

The obstetrical problems associated with Rhesus isoimmunisation can be considered in four groups:- causation, prevention, diagnosis and management.

Many aspects of these problems have been investigated during the last 100 years and when Darrow (1938) published her theoretical deductions, it seemed that the only problem remaining was the one concerned with the actual cause of the protein sensitization reaction in the mother. At that time it was known that haemolytic disease of the newborn could present in three way - hydrops foetalis, icterus gravis neonatorum and congenital haemolytic anaemia. Treatment by blood transfusion, either simple or exsanguination, was used in some parts of the world with success. Apart from our knowledge of the Rhesus complex of antigens, we have not progressed very much further in our understanding of the condition or in our management of patients.

With the discovery of the Rhesus antigen by Landsteiner and Wiener (1940) and the association by Levine et al (1941) of haemolytic disease of the newborn and antibody production by the infant's mother, a large new serological field was opened up and also a better understanding of haemolytic disease of the newborn, as many hitherto unexplained findings could now be simply explained. Not all the problems had been solved, however, and further serological research revealed that there were different types of antibodies, each

having different effects, and tests for the detection of these antibodies were devised. The management of the pregnant woman was initially of less importance than the treatment of the infant affected by haemolytic disease of the newborn. When the 'ideal' management had been devised with the neonatal death rate approaching the irreducible minimum - stated by Walker (1958) to be 6 per 1000 live births - more attention was paid to the very large numbers of stillbirths occurring each year. It was reported (Lancet 1954) that 30 per cent of foetuses affected by haemolytic disease were still-born in England and Wales in 1952 and the number of these stillbirths per year in England and Wales was stated to be 500 (Lancet 1958).

Many attempts have been made to reduce the still-birth rate and while some of these are concerned with the obstetrical management of the patient, others are more fundamental and involve immunology, serology, immunochemistry and pharmacology. While attempting to reduce the number of stillbirths, it may be possible to reduce the severity of the disease among live infants also, and, in fact, the condition may be eradicated completely.

The obstetrician firstly requires to know how Rhesus isoimmunisation is caused. It is accepted that foetal tissue, almost certainly foetal red cells, but possibly other tissues, pass into the maternal circulation during pregnancy. This transfer must take place in the uterus and the placenta is the likely

site. Unlike the A and B group antigens, the Rhesus antigens are probably not present in tissue fluids, although Levine and Celano (1961) found the D antigen in spermatozoa from Rhesus positive men. Whether this finding is in any way connected with the problem of immunisation remains to be determined.

When the foetal red blood cells enter the maternal circulation they are either destroyed by antibodies in the blood or, through ageing, by the reticulo-endothelial tissues of the mother. It has been suggested that the cells are only capable of acting as antigens while they are in the circulation, and not after they have been destroyed (Andersen et al 1961). If the cells entering the circulation are Rhesus positive (D) and the mother's group is also Rhesus positive (D), no antibodies are produced. If the mother and foetus are both Rhesus negatives (d), no antibodies are produced. A Rhesus negative foetus will not stimulate antibody production in a Rhesus positive mother - the identification of the anti-d antibody is considered to be most unlikely (Race and Sanger 1962), but a red cell from a Rhesus positive foetus may stimulate antibody production in a Rhesus negative mother. Foetal red blood cells have been demonstrated in the blood of both Rhesus positive and Rhesus negative women during pregnancy. In only a very small percentage of these do the cells stimulate the production of Rhesus antibodies, however, and the reasons for this are not fully understood.

Firstly, it may be that certain types of Rhesus positive foetal cells are more capable of stimulating the production of antibodies than others. The  $R_2r$  was thought by Murray (1957) to be a more powerful antigen than the  $R_1r$  but this was not confirmed by Kirk et al (1959) or in the present series. It may be that  $R_2r$  cells are more sensitive than  $R_1r$  cells to anti-D antibodies but this was not confirmed either.

It has been shown by many workers in this field that when the foetus and mother are incompatible for the ABO groups - the foetal red cells being incompatible with the maternal serum - there is very much less chance of Rhesus isoimmunisation occurring (Levine (1943b), Nevanlinna and Vainia (1956), Levine (1958), Finn et al (1961); Andersen et al (1961) and others.) When the blood groups of the parents are considered alone, incompatibility between the father and mother may not reflect incompatibility between the foetus and mother, because of the possibility of heterozygosity A/O or B/O. However, Rhesus haemolytic disease is virtually unknown in families in which the mother is group O and the father is group AB Levine (1958) and Andersen et al (1961).

Two explanations have been put forward for the finding of Rhesus isoimmunisation so rarely among incompatible ABO matings. The first, by Wiener (1945), suggested that if antibodies to the ABO antigen were being made by the maternal tissues, these tissues would be unable to produce Rhesus antibodies at the same

time, as the A or B antigen was a more powerful stimulus than the Rhesus complex. The second explanation, by Race and Sanger (1950), was that the foetal cells entering the maternal circulation were destroyed by the naturally occurring anti-D antibodies, and so were removed from the circulation before they had a chance to stimulate the production of Rhesus antibodies. This view also assumes that destroyed red cells are incapable of acting as antigens, although presumably the D antigen is still present. Possibly it has been inactivated by the action of Anti-A or anti-B on the A or B antigens of the cell.

If the foetus and mother are compatible for the ABO group system, then the Rhesus positive cell is able to stimulate antibody production provided the mother's antibody-producing tissues respond. It is not known whether this is dependant on a critical amount of foetal red cells entering the maternal circulation or on the duration for which the cells remain in the circulation. The suggestion that a woman may have acquired tolerance for the D antigen if her mother was Rhesus positive was offered by Professor Rogers Bramwell and Mr. N. A. Mitchison (Race and Sanger 1962). Booth and others (1953) found that the number of Rhesus negative grandmothers of Rhesus positive grandchildren with haemolytic disease was exactly the number who were expected to have Rhesus negative daughters. Owen and his co-workers (1954) and Ward et al (1957) agreed with the findings of

Booth et al, but when Owen's group studied the problem in another way, they found that where there had been three Rhesus positive children without evidence of isoimmunisation, there were more Rhesus positive grandmothers than expected whereas if immunisation had occurred there were more Rhesus negative grandmothers. Levine and Sturgeon (in Race and Sanger 1962) found that there had been an excess of ABO compatible matings among the women whose mothers were Rhesus negative, and this accounted for the higher incidence of isoimmunisation, not the lack of tolerance. The original hypothesis was a most attractive one, but the modern view that immunological tolerance is not maintained in extra-uterine life unless the subject continues to be exposed to the antigen, means that it is not now acceptable.

As antibodies are rarely present in the first pregnancy (unless stimulated by a blood transfusion), the mother is apparently sensitized at the time of the first delivery or in a subsequent pregnancy. Attempts to relate the discovery of antibodies to operative obstetrical procedures are not very satisfactory as the procedure itself may not bring about the transfer of foetal cells to the mother but the reason for carrying out the procedure may be the cause of the cell transfer also. This applies to disordered uterine action with foetal distress, leading to either Caesarean section or forceps delivery, or even uterine inertia with consequent manual removal of a retained placenta.

Accidental haemorrhage is a very likely cause of haemorrhage into the mother from the foetus. Pre-eclampsia is not frequently associated with haemolytic disease as seen in this series, but hydrops foetalis may predispose to pre-eclampsia with an increased production of chorionic gonadotrophin (Scott 1958).

It has been calculated that in 15 per cent of marriages a Rhesus positive man marries a Rhesus negative woman. However, only 1 woman in 20 at risk develops isoimmunisation. ABO incompatibility between the mother and foetus reduces the risk but two-thirds of the infants are compatible. If the husband is heterozygous for D and the couple have a small family or no children at all, a further reduction in the number at risk will occur. The number remaining is difficult to assess but some other factors, unknown and unsuspected at the present time must be operating to protect some women from isoimmunisation.

Prevention of Rhesus isoimmunisation is the goal but the solution is not yet apparent. Finn et al (1961) suggested the injection of anti-D antibodies to women who had an excessive number of foetal cells in their circulation after delivery. As only a very few of the patients studied by Finn developed antibodies and were at risk, it would mean the injection of antibody into a large number of women with the risks which this involves, as the critical level of 'cell score' is not certain. One patient developed antibodies

with a very low cell score. Antibody obviously cannot be given during pregnancy to destroy foetal cells in the maternal circulation, otherwise it could cross the placenta and destroy foetal cells. Thus the two patients who were known to have developed antibodies during the pregnancy, because of the 'massive' transference of foetal cells to the mother, could not have benefitted from this procedure.

Although the haptan method of therapy is still used by Carter and her co-workers, the failure of other workers to obtain the haptan or to achieve similar results has caused this treatment to fall into disrepute.

Of more promise is the work of the immunochemists who have found chemical substances which are similar to the Rhesus antigens and which compete for the anti-Rhesus antibodies. Hackel and his co-workers (1958) found that ribonucleic acid derivatives inhibited anti-D, anti-C and anti-E antibodies, and also anti-c and anti-e. They were attempting to identify the chemical composition of the antigens and concluded that ribonucleic acid derivatives were included in the antigen.

Boyd and his associates (1959) concluded from their studies that the D-receptor of Rhesus positive red cells contained 'unnatural' hexoses or their derivatives. Dodd et al (1960) suggested scialic acid as an inhibitor of anti-D and Boyd and Reeves (1961) tried colominic acid. As this was reputed to be non-toxic

and non-antigenic, (Barry (1958), Boyd hoped to attempt to inhibit antibody in vivo with colominic acid, but has not been successful so far. Obviously this line of research is very important and it is necessary to obtain more information about the chemical composition of the Rhesus antigens so that their antibodies may be inhibited more readily in vivo.

It does not seem practical to devise rules of obstetric management for Rhesus negative patients and for these to differ from those applied to Rhesus positive patients. It is to be hoped that all patients will be carefully supervised in labour and that they will not be subjected to unnecessary obstetrical procedures.

From the immediate practical point of view, the diagnosis of Rhesus isoimmunisation is of the greatest importance. Generally speaking blood is taken routinely at hospital ante-natal clinics in every pregnancy and repeated in Rhesus negative patients in the last trimester. Unfortunately this is not the case in general practice where sometimes blood is not taken at all, or a specimen is taken in the first pregnancy and not repeated in subsequent pregnancies, regardless of the reported blood group. As Vaughan (1959) stressed, clerical and technical errors are not uncommon and on each occasion that the group is copied, there is a chance of a mistake being made.

Routine testing in early pregnancy sometimes indicates whether antibodies from the last pregnancy are present, although there are exceptions.

In some of the cases quoted antibodies were absent in early pregnancy but appeared later although the infant was unaffected by haemolytic disease. It is presumed that the initial tests were not sufficiently sensitive to detect the small amount of antibody present. The anamnestic reaction is then quoted as the explanation for the titre rise, but some authorities dispute the occurrence of this (Wiener 1959). There may be some other explanation, but this is not yet apparent.

If Rhesus antibodies are found in early pregnancy, the diagnosis of Rhesus isoimmunisation is made. If no antibodies are detected and the patient is Rhesus negative, tests are repeated at 32 and 38 weeks. Patients with no detectable antibodies at 32 weeks have developed them after this, and it is possible for the foetus to be stillborn at term although a blood test at 36 weeks showed no antibodies. If the patient has been transfused or if there are any occurrences in her history to suggest that a previous infant may have had haemolytic disease, a repeat test is advisable even if the patient is Rhesus positive.

Patients who are Rhesus positive and have developed antibodies require special care because antibody titres can be very unreliable and a low titre can be associated with a severely affected infant. Blood transfusion is nearly always the cause unless anti-D antibody is also present. Some patients with anti-G antibody were encountered in the present series, (Allen and Tippett (1938). If a transfusion of Rhesus positive blood

is given to a Rhesus negative woman and antibodies are produced, a Rhesus positive infant is often severely affected even in the first pregnancy. Here the patient is receiving a large number of incompatible cells in a very short time and the cells are often homozygous for D, differing from the heterozygous positive foetal cells.

Having established the diagnosis of Rhesus isoimmunisation, it is necessary to consider each patient individually. All the available information about the patient should be collected and a decision about her management taken by the obstetrician in consultation with the paediatrician and serologist. The final decision must be made by the obstetrician as he is responsible for any induction of labour or decisions regarding the method of delivery.

The decision regarding the management is difficult because the controversy about induction of labour in Rhesus isoimmunisation still continues. It is not made easier by the generally good results obtained both by induction and by allowing labour to commence spontaneously. The problem is not really concerned with live births. If the baby is mature, he will usually tolerate exchange transfusion. If he is immature the transfusion may give cause for anxiety but it should prevent the development of kernicterus and the baby should survive.

The real problems are associated with stillbirths and with infants who on delivery are found to be

unaffected by haemolytic disease. It is essential in any series of cases of Rhesus isoimmunisation that both these groups are included in the results being reported. The induction of labour is unnecessary and dangerous on occasions when the infant is found at delivery to be unaffected by haemolytic disease. For this reason it is advisable to know the severity of the haemolytic disease while the foetus is still in utero. If unaffected, no induction is theoretically necessary. If a severely affected infant is predicted early intervention may prevent the occurrence of a stillbirth. Although an infant is moderately severely affected at 37 weeks gestation it is difficult to forecast how severely affected he would have been if labour had not occurred until term. Thus it is reasonable to relate the time of induction of labour to the predicted severity of the haemolytic disease. Labour is not induced if the infant is expected to be unaffected, although in practice this group is difficult to distinguish from the mildly affected infants, and might be induced by term. The severely affected infants are induced before 37 weeks and sometimes as early as 34 or 35 weeks, and infants' lives have undoubtedly been saved by this procedure.

In the present series there were 92 unaffected infants, an incidence of 17.5 per cent. Six of these infants died, one probably unnecessarily as a result of induction. In order to predict that a patient with Rhesus isoimmunisation has an unaffected infant in the

uterus, some serologists think it helpful to know the serological pattern of her antibodies during her pregnancy and also her husband's genotype. The genotype is based on probabilities and can be wrong, although it is usually heterozygous positive men who are initially said to be homozygous positive. Low antiglobulin or albumen titres are sometimes associated with unaffected infants but there are numerous exceptions to this. If induction is not being performed, it is not necessary to predict an unaffected infant from the antibody titre reports. Examination of the liquor amni is helpful when an unaffected infant is suspected especially when the diazo test is used, as this confirms the absence of indirect bilirubin. There are no absolute values of antibody titre - either antiglobulin or albumen - for a particular degree of severity, but in general the higher the titre dilutions the more severe the condition. Again there are numerous exceptions. A rising level of antibody titre may also indicate an affected infant but this is certainly not always the case, and sometimes the titre falls before an intra-uterus death occurs.

The maternal hydrops syndrome usually occurs early in the third trimester and the foetus is usually dead before any action can be taken. X-ray examination of the abdomen, however, may be of help in severely affected cases, the diagnostic features being the straight foetal spine, the elevated ribs, the space between the scapula and the rib cage and the size

of the placenta.

The most logical method of obtaining information about the foetus in utero in a current pregnancy is to examine the liquor amnii which is in intimate contact with the foetus during the whole pregnancy. Any 'excretion products' from the foetus are passed into the liquor and by testing the liquor these products can be identified and measured quantitatively.

Obtaining liquor by paracentesis of the uterus is not usually very difficult, although sometimes it may be contaminated by blood. The procedure is certainly a safe one and no infection or haemorrhage has resulted from it in this series. The patients are not unduly disturbed by it, some in the series having had the test repeated in 3 successive pregnancies.

The interpretation of the liquor curves after spectrophotometric analysis is not difficult after a little practice. There seemed to be no obvious correlation between the findings on the graph and the serum bilirubin and birth haemoglobin, but as the liquor is obtained 4-6 weeks before delivery, an accurate prediction of haemoglobin level or bilirubin concentration is hardly likely to be made. The height of the liquor 'bulge' above its 'baseline', the optical density of the liquor and sometimes the shape of the curve are all of prognostic importance.

The bulge occurs between 400 and 500 m.u. and is due to the presence of bilirubin. The diazo test

produces a bulge between 500 and 600 m.u. and is of definite value in distinguishing between unaffected and moderately affected infants. It sometimes indicates that the foetus is more severely affected than the untreated liquor suggests.

Any test which is able to allow satisfactory predictions to be made in about 90 per cent of cases is of value. The reasons for incorrect predictions are interesting, the foetus apparently producing and 'excreting' excessive amounts of bilirubin into the liquor without definite evidence at birth of serious haemolysis. Our knowledge of drugs which can harm the foetus in utero is increasing and the interference with foetal enzyme systems by commonly used drugs is a finding of considerable importance. The uncertainty of women with Rhesus isoimmunisation about the date of their last menstrual period is a complicating factor, also reported by Townsend and his co-workers (1961). The finding that the 'bulge' is reduced when liquor has been examined before and after 36 weeks makes prediction difficult if the patient is uncertain of her menstrual period date, as the absence of a bulge at 34 weeks would indicate an unaffected (or mildly affected) infant, while its absence at 38 weeks might mean that the infant would be unaffected, or more severely affected with a reduction in the bilirubin bulge. This is the usual explanation for an affected infant being delivered after an unaffected infant has been predicted.

Many attempts have been made to justify induction of labour in Rhesus isoimmunisation and it is generally agreed that when a stillbirth is expected, 'preterm' delivery (within the last 3 or 4 weeks of pregnancy) is justifiable. Premature or heroic delivery is advocated by only a few writers in exceptional circumstances. The difficulty met with is that of predicting which pregnancies require early termination. Walker, Murray and Russell (1957a and b) are against the induction of labour because of the dangers of prematurity, but if a stillbirth from haemolytic disease has occurred, they are prepared to induce labour provided the husband is homozygous. This would seem to be a rather negative attitude, particularly as their exchange transfusion technique is obviously very satisfactory and they have a very efficient scheme for diagnosing and detecting patients with Rhesus isoimmunisation in their area. Other writers have stressed the need for considering each immunised patient as an individual problem and although they are against very early induction as a general rule, they are prepared to induce labour if the circumstances suggest that this might be of benefit (Kelsall and Vos 1955), Fisher (1957), Kelsall, Watson and Vos (1957), Tovey and Valaes (1959), Wiener (1959), Aaro (1959), Jacobs (1959b), Ziel and Smith (1960) and Townsend et al (1961). Routine induction empirically has been suggested by Allen (1957) at 37 weeks when the antibody titre was low, in case it rose suddenly. McClure Brown (1960) recommended routine induction at

38 weeks. Dique and Wrench (1959) found that the percentage of surviving infants followed closely the percentage of inductions carried out each year. In the present series the percentage of survivors increased by 13 per cent when the 'induction' rate was increased by 65 per cent. Induction here, however, does not necessarily mean induction before the 39th week of pregnancy, and some inductions in unbooked patients were performed after the expected date of delivery.

Because of the differences in definition and the difficulty of 'matching' cases, the results of two or more series cannot be compared other than in general terms. In the present series the records of patients delivered in 1956 and 1957 have been studied retrospectively and although there was no definite induction-policy during this time, the number of inductions in 1957 was considerable (Figure 29). Since 1958, many of the 'booked' patients have been studied in an attempt to predict the severity of the haemolytic disease, but the management of these patients has sometimes differed from that suggested by the prediction. This has often been of no serious consequence but a small number of foetal deaths in utero have occurred because of the management carried out.

In an attempt to determine the number of infants possibly saved by induction of labour - either 'premature' or 'preterm', those recorded as being severely affected have been studied. Those with low

haemoglobin levels at birth - below 7.4 mg. per cent - were certainly in grave danger of death and have been saved by induction, if the induction was performed before 36 or 37 weeks. Goplerud (1961) considered that the placenta to foetus ratio should be over 1 in 4 if the infant was to survive, apart from very premature infants. Those infants who were 'mature' and had 'placenta to foetus' ratios of less than 1 in 3, associated with a low haemoglobin, might be said to have been saved by induction also. The critical ratio found by Chernyak and Rabtsevich (1959) was 1 in 3.5

From the evidence obtained by histological and histochemical studies of the placenta it would seem that the foetus died before the placenta and that the changes associated with hydrops foetalis are secondary and not a gradual change occurring as the severity of the haemolytic disease increases. Thus, it is likely that the foetus is in a reasonably good general condition until shortly before his death and after removal from the uterus, his prospects are good, provided adequate and skillful exchange transfusions can be given. Here the skill and experience of the paediatrician are of the utmost importance and any successful results obtained in this series are due equally to the efforts of the attendant paediatricians.

Rhesus isoimmunisation is a family problem and as such each family requires special consideration. The finding that 7 to 8 per cent of apparently first affected infants are stillborn as a result of haemolytic

disease draws attention to the importance of preventing Rhesus stillbirth in every pregnancy, and not merely when a previous infant has been lost. If this death should occur in the second pregnancy and the first infant has been lost as a result of pre-eclampsia or congenital abnormalities, the prognosis may be very poor if the husband is homozygous Rhesus positive. Even if he is heterozygous positive, there is no way of knowing how many affected Rhesus positive infants will be delivered before a Rhesus negative child is born. Walker and Murray (1956) did not think that a previous incompatible transfusion increased the risk of a stillbirth occurring in the first affected pregnancy, but it increased the severity of the disease. In the present series, some rather heroic inductions have been carried out in an attempt to prevent a stillbirth after an incompatible blood transfusion. It is, therefore, essential that young girls and women in the child-bearing era receive as few blood transfusions as possible, and if it is necessary for them to be transfused, very careful grouping and cross-matching of the two bloods are necessary.

It is interesting to remember that Rhesus isoimmunisation is a form of natural selection while attempting to delete the Rhesus negative antigen (d). Affected infants are always Rhesus positive and heterozygous, so their survival ensures that there will be a continuation of the d antigen in future generations.

Although the proportion of Rhesus negative persons is comparatively small the number is likely to remain constant or increase as more heterozygous children are saved by treatment. Whether these children are completely normal is also of importance. It is hoped that a follow-up study on the infants in this series will be undertaken soon, in order to determine their intelligence and to detect any sign of neurological damage, including deafness.

As regards the future, the most hopeful research at the present time is concerned with the identification of the structure of the Rhesus antigens. If substances such as colominic acid can inhibit Rhesus antibodies in the maternal blood without harming the patient or foetus, the problem will be solved. The use of marrow transplants from a Rhesus negative foetus to a Rhesus positive foetus in the uterus of a patient with Rhesus antibodies is of interest but is unlikely to be very generally successful. The procedure is quite difficult (Lennon 1960) McClure Brown (1960) and the effect on the infant, should it survive, requires further study. If a 'tolerant' chimera develops, the infant may produce both Rhesus positive and Rhesus negative cells but it is possible that 'auto-antibodies' may develop with a chronic form of haemolytic anaemia persisting throughout life.

A detailed study of Rhesus negative women who have Rhesus positive husbands but do not develop antibodies in pregnancy might help to solve some of the problems

still outstanding, but as this must be prospective and include a number of pregnancies, it is likely to take a long time.

Until a chemical substance is ready for widespread use, we, as obstetricians must continue to diagnose Rhesus isoimmunisation as early as possible, we must predict the severity of haemolytic disease as accurately as possible and we must remove the affected infant from the unhealthy maternal environment before the development of hydrops foetalis whenever practicable. Above all, it is essential that the relevant findings are considered for each patient in every pregnancy and that a decision is made regarding the optimum management. Our aim is to deliver all our patients of live infants and for these to survive as normal and healthy children.

#### V.

#### SUMMARY.

The history of erythroblastosis foetalis and Rhesus isoimmunisation is divided into three periods. The first period is concerned with the syndromes which make up 'erythroblastosis foetalis' or 'haemolytic disease of the newborn', while the second period commences with the identification of the Rhesus factor and includes some of the earlier views on management. The third period commences arbitrarily with the publication of the M.R.C. trial results in Great Britain and continues for 10 years until the end of 1962.

A preliminary report is presented on the search for foetal cells in the maternal circulation during

pregnancy and at the present time it can be stated that small numbers of foetal cells are not infrequently seen in the maternal circulation during pregnancy. Two cases are described in which Rhesus isoimmunisation developed after foetal cells were seen to have entered the maternal blood stream.

The results of a series of 550 women with Rhesus isoimmunisation are presented, there being 532 viable infants in this series. The results are presented in groups relating to the previous obstetrical and serological history of the patient. It is shown that the foetal mortality rate from haemolytic disease is over 8 per cent when antibodies are detected for the first time in a pregnancy, while if there have been previously affected infants the mortality rate is over 13 per cent. If an infant has previously been still-born or died from haemolytic disease, the mortality rate is 33 per cent. The mortality rate from haemolytic disease for the series was 12 per cent while the total mortality rate in the series was 12.2 per cent.

The protection offered by ABO incompatibility between the foetus and his mother is demonstrated as compatibility of blood groups is found 7 times more often than incompatibility in all the groups. There is also a 'shift towards group A from group O' in the mothers of children with haemolytic disease. The Rhesus genotype of the husbands of patients with Rhesus isoimmunisation is correlated with the outcome

of the pregnancy and the severity of haemolytic disease found in the infant, and it is shown that the  $R_2$  genotype is no more liable to produce severely affected infants than the  $R_1$  genotype, and in fact the  $R_1$  is possibly more potent than the  $R_2$ .

The incidence of pre-eclampsia in the series and the number of patients with the maternal hydrops syndrome is reported. The value of diagnostic radiology in the prevention of death of the foetus in utero is considered.

The value of antiglobulin and albumen antibody titres is discussed, the conclusion being that they are unreliable in prognosis because of the numerous exceptions to the general rules established.

The most satisfactory and logical method of predicting the severity of the foetus in utero was found to be by analysing a specimen of amniotic fluid at about 34 weeks gestation. The shape and size of the 'bilirubin bulge' and the height of the optical density were of value in predicting the severity of the condition, and in the whole series, satisfactory predictions were made in 88 per cent of cases, while in the last 2 years of the series, 92 per cent correct predictions were made.

The management of the patient with Rhesus isoimmunisation is concerned with the prevention of stillbirths by the induction of labour before the development of hydrops foetalis. It was considered

that a number of infants had been salvaged by this policy of intervention. Induction of labour when the infant is unaffected by haemolytic disease is unnecessary and may be hazardous, 2 deaths in the series out of 92 unaffected infants resulting.

A study of the placenta to foetus rates and the histology and histochemistry of the placentae of patients with Rhesus isoimmunisation is presented, and also a number of observations have been made on severely affected infants.

Twenty-one cases are briefly reported illustrating certain aspects of diagnosis and management.

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