HAEMOLYTIC DISEASE OF THE NEWBORN

A Clinico-Serological Study

- By -

RONALD R. GORDON, M.C., M.B.,Ch.B.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

C O N T E N T S

INTRODUCTION.

Rapid advances in the scientific field of medicine soon tend to get beyond the understanding of the clinician. From all the vast, experimental work which accumulates daily, he must try to extract that which will be most useful to him in his practice of medicine. Much will be of great use, some will be impracticable.

Recent developments in the subject with which this thesis is concerned have been on a very large scale and have aroused considerable interest throughout the world. A completely different type of disease has been discovered and, in the process, considerable information of a purely genetic interest has been accumulated. Most of the work which has been done has been performed by scientific workers who accepted the clinical material sent to them by various hospitals. Many of them appear to have taken a considerable interest in the clinical condition of their patients. In many ways, perhaps, the clinician has lagged behind in the investigation of the disease - but this is solely because most of the clinical details of the disease were fully worked out prior to 1940. In spite of the vast scientific advances, little has been added to the clinical knowledge of the disease in recent years.

It has, therefore, been felt advisable for someone, whose main interest in the subject is that of a clinician, to review the whole disease process as it stands at present, sift out the information which is useful, study clinical cases in the light of that information, and determine to what extent the treatment of the disease has progressed. These were the main objectives in mind when the work which composes this thesis was first commenced.

PART I.

REVIEW OF THE LITERATURE.

HISTORICAL.

The study of neonatal jaundice was started in earnest by the work of Thomson (1891-92). Although the condition he described was congenital obliteration of the bile ducts, it seems now that it was his attempt to differentiate the various conditions causing neonatal jaundice which led to the isolation of icterus gravis neonatorum as one of them, some seventeen years later. Thomson collected from scattered reports in the literature 49 cases of congenital obliteration, or absence or malformation of the bile ducts, and reported one of malformation himself. He remarks early on in his series of articles that many of the cases showed a familial incidence. In only 24 of his 49 cases had a history of the health of other siblings been obtained but in 8 of these there was a family history of neonatal jaundice.

Thomson also reported a separate group of cases in which no abnormality of the gall bladder or bile ducts had been found, and noted that these infants had died much more quickly than those with a definite abnormality. It seems certain, then, that some of the cases he described were cases of icterus gravis neonatorum, and it is interesting that he suggested that in the cases with no abnormality there had not been time for the obliteration to become complete.

This relationship between congenital obliteration of the bile ducts and icterus gravis neonatorum is still under discussion today (Skelton and Tovey, 1945) and the impression is held by some that the former can, in some cases, result from the latter. Ross (1901) reported a case of congenital obliteration of the bile ducts in which at autopsy the liver was found to contain "small celled infiltration confined to the interlobular spaces" rather than the expected "fibrous tissue between the cells". Rolleston and Hayne (1901) also reported a case and suggested that congenital obliteration of the bile ducts was "primarily started by poisons derived from the mother" which were conveyed to the foetal liver, there setting up a "mixed cirrhosis and cholangitis". They believed that a descending cholangitis could obliterate the bile duct in the same way as appendicitis can obliterate the appendix.

In reply to Rolleston and Hayne's article, Blomfield (1901) reported a family in which a form of neonatal jaundice had abolished any male offspring. This is the first detailed description in the modern literature in which the familial incidence of the disease is strongly indicated. This family also demonstrates the now well known fact that the first child (in his family a female) usually escapes. Three males born later in Blomfield's family all became jaundiced and died, and the last but one was stillborn. The idea of maternal toxaemia as a causative factor was supported by the fact that the mother had excessive vomiting during the pregnancies.

Arkwright (1902) gave further great emphasis to the familial nature of the disease by describing 14 cases in one family with only four survivors: one of the latter was noticed to have "no use in her legs" when seen some years later. There was anaemia in two of his cases and a "dirty, sallow complexion" in two others. He also noted that the motions were not unduly pale in any of them, and used this as support for Rolleston's theory of a descending cholangitis which had not had time to cause complete obstruction. It is obvious that at this time an attempt was being made to correlate all these cases with Thomson's obliteration of the bile ducts, which was the only recognised cause of neonatal jaundice apart from syphilis and sepsis.

Auden (1905) drew attention to the pallor as well as the jaundice in several affected members. In one case sections of the liver showed considerable infiltrations of "small round cells" between the liver cells. In flood films of two cases he reported that the "large mononucleears" appeared to be in excess. Auden also noted that in this family the first child was unaffected. Smith (1902), Busfield (1906) and Duguid (1906) also reported families in which several members developed severe jaundice and died, the first author also reporting bile staining of the liquor amnii, membranes and placenta in all of his three cases.

ICTERUS NEONATORUM GRAVIS.

So far these individual families which had been described had accentuated the familial incidence, the bad prognosis and the presence of bile in the motions. Pfannensteil (1908) collected these cases and others from the literature and reported two families himself. He termed the condition icterus neonatorum gravis in contradistinction to the well-known benign icterus neonatorum or physiological jaundice on the one hand and congenital obliteration of the bile ducts on the other. Although priority for the description of the disease is usually given to Pfannensteil it must be emphasised that he only described part of the disease. Buchan and Comrie (1909) described it in detail as it occurred in 4 cases in two families and their description, both clinical and pathological, included all the important aspects which are known today. Even their title "Four Cases of Congenital Anaemia with Jaundice and Enlargement of the Spleen" shows that they had grasped quite clearly the important and distinguishing features of the disease. They showed that a marked anaemia accompanied the jaundice (all four cases had a red cell count of 2,000,000/c.mm. or less) and they gave accurate counts of the nucleated red cells in the blood films. They also noted bile in the urine and considerable splenic enlargement. In one of their families there was a very marked familial history and three of their four cases were siblings.

At autopsy on two of them and on the single member of the other family, they found, amongst other things,

(1) Normoblasts and megaloblasts in the liver sometimes arranged as islands
(2) Bile thrombi in the hepatic ducts
(3) Increase of fibrin round the interlobular veins
(4) Blockage of the ampulla of Vater, so that bile could not escape, in two cases
(5) Erythroblastic bone marrow.

They came to the conclusion that destruction of red cells had been occurring during the disease because the spleen showed large numbers of phagoeytic cells containing "blood pigment", and also from the fact that phagoeytic cells engulfing red blood corpuscles were found in the circulating blood. This latter condition has but rarely been reported since (Abt 1931). Buchan and Comrie gave four possibilities for the aetiology of the disease:

(1) Haemolytic action of the bile or of its abnormal constituents
(2) General Toxaemia
(3) Imperfection of blood forming organs
(4) Special fragility of the red blood corpuscles.

They realised, therefore, that the disease was vitally concerned with the blood and blood forming organs. This was not generally accepted until many years afterwards, however. Until Diamond Blackfan and Baty's (1932) paper only very occasional reference was made to Buchan and Comrie's work and the former authors paid tribute to the excellence of the latter's description.

McGibbon (1912) also gave a very good description of the disease and stated "the condition can only be recognised by means of blood counts and blood films". He reported a family in which the first child was unaffected, the second, third, fourth and sixth were all jaundiced at birth; the fifth pregnancy terminated in an eight month stillbirth. In Auden's (1905) family also a stillbirth was followed by a live, affected child. Rolleston (1910) described a family in which a normal child was born after the previous three had died of the disease. The mother had been jaundiced herself during all four pregnancies. This occurrence of a normal child in some of the afflicted families tended to upset many of the theories put forward for the causation of the disease, in later years. Rolleston's theory of a maternal toxin was much in favour at this time, but Pfannensteil considered it to be simply an intensification of the physiological jaundice. Nason (1910) supported Rolleston's theory by quoting a family in which there appeared to be a relationship between the jaundice in the mother and her affected infant. When the mother was severely jaundiced the infant also was, and died. When the mother was not at all jaundiced or only slightly so, the child was unaffected. Rolleston (1910) made no reference to the work of Buchan and Comrie and ten years later (Rolleston 1920) in a comprehensive review merely referred to their finding of plugging of the ampulla of Vater. He appears to have looked on the disease as being purely hepatic in

nature, although in both his communications he states that there is no structural derangement of the liver.

The general knowledge of the disease in this country in 1920 is exemplified by a letter (Agnew 1920), written after the appearance of Rolleston's review of the disease, in which the author states that he has never "read or heard of the severe form of congenital jaundice".

In America only one article had appeared, which was at all of value. Abt. I.A. (1917) reviewed the literature and described two families who produced affected children. He thought Knoppelmacher's theory of a septic process being involved in the production of the disease was by no means proved. He stated that the familial form of the disease was not the frequent one. A previous article in the American literature (Pitfield 1912) had described the familial type of the disease under the heading of "Haemophilia Neonatorum in a Family of Four Infants". Pitfield treated one case with the subcutaneous injection of human serum recommended by Welch (1910) in the treatment of "haemophilia neonatorum" and the child recovered.

ERYTHROBLASTOSIS

On the Continent, however, Rehn (1913) and Ylppo (1918) had described enlargement of liver and spleen as well as nucleated red cells in the circulation. They also reported the finding of islets of haemopoietic tissue in the liver at

autopsy. Neither of Ylppo's two cases became markedly anaemic before death: however haemoglobin estimation and red cell counts had been performed. The closer examination of the liver by these workers was probably stimulated by the work of Schridde (1910) who had described the extra-medullary haematopoiesis found in hydrops foetalis. Rautmann (1912) termed this condition "erythroblastosis", and it was quite often described in later years (Capon 1922: Bullard and Plaut 1926) but always in relation to hydrops foetalis. Bullard and Plaut stated "so far no case has been described which showed the erythroblastosis without the oedema". It was still some years before the blood forming islands in the liver of icterus gravis and the erythroblastosis of hydrops foetalis were to be considered associated conditions. The exact nature of the abnormal cells in the liver in icterus gravis neonatorum does not seem to have been generally accepted at this time since Klemperer (1924) described them in one of his cases as "large mononuclears".

JAUNDICE AND ANAEMIA

With the introduction of the Van den Bergh test and the realisation that jaundice could be haemolytic in origin, the haematological side of the disease came more into evidence. (Hoffmann 1924). Fordyce and McAfee (1924) described "erythroblastosis" in the liver of a child dying of icterus gravis neonatorum, and Gregory (1928) reported a case which

showed anaemia and slight jaundice. He stated that his case seemed to fit in best with Buchan and Comrie's cases, except for the fact that the latter were familial and born with jaundice of the obstructive kind. Blood counts in his case showed severe anaemia and an excessive number of nucleated red cells in the blood films.

CONGENITAL HAEMOLYTIC ANAEMIA

Anaemia in the newborn, following jaundice, had been described by Buchan and Comrie. Ecklin (1919) described a case without jaundice and thereafter several more appeared (Donnally, 1924, Sanford 1925; Bonar 1925; Greenthall 1930; Ehrman 1929; Happ 1930; Gelston and Sappington 1930). The cases described by these authors all showed the same clinical features, namely severe anaemia developing within the first few days of life, with splenomegaly and erythroblastaemia. Prognosis in all of them was good, and the infants all recovered some without blood transfusion. No opinion was offered as to the aetiology of the disease, except that it did not appear to be nutritional in origin. Foote (1930), however, stated that it was identical with the "blood depot exhaustion" anaemia seen in premature infants except that it was greater in degree and more violent in its manifestations. A few of the reported cases had shown slight jaundice before the anaemia (Ecklin, 1919; Sanford 1925; Gregory, 1928), but the connecting link with icterus gravis was not yet appreciated.

ICTERUS GRAVIS - A BLOOD DISEASE.

Work on icterus gravis neonatorum proceeded rapidly from 1929 onwards. De Lange and Arntzenius (1929) reported two families and noted the severe anaemia as well as jaundice, and at autopsy the erythroblastosis. These authors strongly advised blood transfusion in the treatment of the disease. Rosenbaum (1928), MacClure (1931), Kramsztyk (1931), Greenwald and Messer (1927), Zahorsky (1928) all reported similar cases, which are now seen to correspond more closely with Buchan and Comrie's description. Hampson (1929), although differentiating icterus gravis from physiological icterus and appreciating that the disease was due to excessive haemolysis, did not lay any stress on the blood picture in the disease. He postulated the presence of an antihaemolytic hormone, and suggested that when this is absent the haemolysing agent which causes physiological icterus is allowed to proceed unchecked and icterus gravis results. However, Goldbloom and Gottlieb (1930) suggested that the cause of the haemolysis which occurs in every newborn infant and is responsible for physiological icterus in some, is due to the altered O_2 tension in the blood. They stated that there existed a state of polycythaemia at birth due to the low O_2 tension of the foetal blood in utero: after birth the O_2 saturation of the blood is more easily accomplished via the lungs than it was via the placenta, so that the now redundant red cells are destroyed by the normal

mechanism of red cell destruction. They supported this contention by keeping guinea pigs under reduced atmospheric pressure, and found that after ten days their red cell count had increased by 30 per cent. After removal from the chamber, they found that the icteric index was increased and the indirect van den Bergh was 1 - 2 units of bilirubin. They concluded that icterus neonatorum is a "haemolytic icterus which is the result of a post natal readjustment from a condition of oxygen unsaturation to a normal oxygen saturation". This strongly suggested that icterus neonatorum (physiological) and icterus gravis neonatorum were unrelated conditions. More recent work (Davidson et al 1941) indicates that hepatic insufficiency plays a part in the production of physiological icterus.

The picture of icterus gravis was by now becoming more clearly defined. Ferguson (1931) described in detail the pathological changes in six cases of erythroblastosis foetalis. Three of the infants were jaundiced, two had oedema and one had neither jaundice nor oedema. He concluded from his study that erythroblastosis foetalis was a clinical entity "in spite of its different clinical manifestations". In the same year Buhrman and Sanford (1931), in a paper entitled "Is Familial Jaundice of Newborn Infants Erythroblastosis?" suggested that the symptoms were caused by destruction of the red cells and reported the presence of 213,000 and 140,000 nucleated red cells per cu. mm. in their

two cases, in support of this. The livers at autopsy showed intense erythroblastosis, which corresponded exactly to the description of erythroblastosis which had previously been reported as occurring only with hydrops foetalis. They also pointed out that there was no reason to differentiate the familial and non-familial types of icterus gravis neonatorum since the familial tendency was the only distinguishing feature. "There is nothing in the clinical picture to warrant the separation and the pathological observations are not different".

Buhrman and Sanford also stressed the anaemia which had occurred in their cases of icterus gravis and called attention to similar reports by Zahorsky (1928) and Gregory (1928). It was possibly this factor which led to the next stage when Diamond Blackfan and Baty (1932) suggested that hydrops foetalis, icterus gravis neonatorum and the anaemia of the newborn previously described were all manifestations of an underlying erythroblastosis. They supported their suggestion by reporting the occurrence of hydrops foetalis and icterus gravis in the same family and the occurrence of icterus gravis and anaemia of the newborn in another family. De Lange (1932) reported the occurrence of hydrops foetalis and icterus gravis in three different families, which strongly supported Buhrmann and Sanford's original contention. No pathological descriptions of the anaemia of the newborn were given at this time, or previously, because

none of the infants suffering from this condition had died. Diamond Blackfan and Baty's reason for including it was due to the blood picture and the familial association. Parsons, Hawkesly and Gittins (1933) and Abbott and Abbott (1935), however, both reported the autopsy findings in cases of congenital haemolytic anaemia and showed that erythroblastosis was present, especially in the liver.

The occurrence of three siblings suffering from this form of anaemia (Seger and Stoeffler 1932) gave further proof of the familial nature of this disease. Two of the infants had moderate jaundice before the development of the anaemia. Pasachoff and Wilson (1935) finally determined the matter by reporting the occurrence of hydrops foetalis and congenital haemolytic anaemia in siblings.

ERYTHROBLASTOSIS OR HAEMOLYSIS?

Diamond Blackfan and Baty's paper excited considerable interest and their thesis that the three conditions were closely related was accepted generally. That paediatricians had known little about erythroblastosis previous to this time is shown by the statement of no less a person than T.B. Cooley (1932) shortly after the publication of the Boston workers paper in which he said he "had heard little until lately of this so called erythroblastosis". Most of the work on erythroblastosis had come from pathologists in maternity hospitals and the work on icterus gravis had come from children's hospitals. It was for this reason that correlation

of findings took so many years. From 1932 it was accepted that the three conditions had a similar basis but the theoretical cause of the erythroblastosis was disputed. Diamond Blackfan and Baty had suggested tentatively that the condition was due to persistence of the foetal type of blood forming organs. They suggested that as a result of this, immature cells were discharged into the circulation (causing erythroblastaemia) and that the non-nucleated erythrocytes resulting from them were also immature and easily destroyed, with a resultant development of anaemia. Parsons Hawkesly and Gittins (1933), on the other hand, believed that the primary defect was haemolysis of the red cells and that the erythroblastosis was a reactive proliferation. Diamond Blackfan and Baty argued that there was no time for the development of this reaction but Parsons Hawkesly and Gittins insisted that, since hydrops foetalis could be present without erythroblastosis, the latter could not be the primary condition. Sobel (1936) thought that neither theory fitted all the facts. He thought that on the one hand no-one knew what caused the "embryonal persistence of haematopoiesis" (Abt. A.F., 1933) and on the other, no-one knew what caused the haemolysis, which was, of course, very true. Ross and Waugh (1936) and Pasachoff and Wilson (1935), however, supported the haemolytic theory and the former stated "one is forced to look on it as a primary haemolytic anaemia of as yet unknown aetiology" (Astrachan (1937) also took this view).

In any event, the accentuation was now on the haematological aspect of the disease and further reports (Hampson 1933: Ross and Waugh 1936: Sobel 1936: Andrews and Miller 1935) stressed this point. The causation of the jaundice was accredited to the haemolysis but when signs of obstructive jaundice were also present this was attributed to the bile thrombi in the bile canaliculi. (Hawkesly and Lightwood 1934, Abt A.F. 1933, McClure 1931). These were said to be caused by inspissated bile due to the excessive secretion of bilirubin and the bile stasis in the disorganised liver. Icterus gravis had now become, primarily, a blood disease, and this conception is still generally held.

The clinical and pathological findings in icterus gravis and haemolytic anaemia of the newborn were fully studied by Diamond et alia (1932) in 12 cases of the former and six of the latter. Abbott and Abbott (1935) gave a further account of congenital haemolytic anaemia and amplified the work of Diamond et alia. Hawkesly and Lightwood (1934) gave a full review of icterus gravis and the disease was put on a very firm basis clinically and pathologically by their work. They described 19 cases of icterus gravis in detail and in addition to the findings, reported by other workers, they found definite evidence of hepatic fibrosis in seven of their cases. They suggested that the "process of recovering from the disease is occasionally accompanied by cirrhosis of the liver". They also stressed the fact that in some of their cases there was

evidence of definite cell necrosis at autopsy. The occurrence of hepatic cirrhosis led these workers to suggest a relationship between icterus gravis and juvenile cirrhosis of the liver on the one hand and "Hepato splenic cirrhosis" (Splenic anaemia: Banti's disease) on the other. This was, however, no more than a suggestion. Finally Hawkesly and Lightwood pointed out that since icterus gravis can occur without erythroblastosis (as shown by the cases of Hart (1925); Greenwald and Messer (1927); McClure 1931) the haemolytic theory of the disease was the more likely one. Their closing sentence on the subject of aetiology is of interest: "The possibility of an hereditarily transmitted factor has never been adequately investigated".

THEORIES OF CAUSATION

Macklin (1937) attempted to show that although the three conditions were pathologically similar, they were different genetically, by statistical analysis only. She concluded that icterus gravis gave evidence of being an hereditary disease due to dominant mutations. Her theory was however strongly opposed by Darrow (1938). Although the disease as a clinical entity was well established by this time, its causation was merely a matter of speculation. The original ideas of excessive normal haemolysis (Ylppo 1918), maternal toxaemia (Rolleston 1920), infection (Knoppelmacher 1910: Dunham 1930) absence of anti-haemolytic factor (Hampson 1929),

defective maternal nutrition (Parsons 1932: Smyth 1931) had all been discarded through lack of proof, and the general impression was that the disease must be associated with some intra-uterine condition which affected the child but not the mother. The presence of bile stained liquor and vernix caseosa in some cases had forced de Lange and Arntzenius (1929) to this conclusion. Ottenburg (1923), when discussing the aetiology of eclampsia and the possible part the foetus might play, also suggested that certain "haemorrhagic" diseases in the foetus might be caused by incompatability of blood group between mother and foetus. This, however, was just a suggestion thrown out at random and attracted no particular attention at the time. Darrow (1938), having reviewed all the suggested theories as to aetiology, decided that although "erythroblastosis" had intrigued those studying the disease "the variability of this factor suggests that its importance as a primary aetiologic influence has been over-emphasised". Darrow, after an intensive study, came out on the side of haemolysis of red cells as being the important factor in its causation, and also stated that "hepatic dysfunction due to injury" also played a part in the production of the clinical and autopsy findings. She concluded her publication with the prophetic words "An antigen-antibody reaction appears to explain best all aspects of these related disorders".

TREATMENT AND PROGNOSIS

Before proceeding with the most modern ideas on the

aetiology it will perhaps be best to consider now the position with regard to treatment and prognosis. About the time Darrow published her theory congenital haemolytic anaemia was regarded as a very benign disease, and few deaths had been reported (Parsons et al. 1933: Abbott and Abbott 1935). Recoveries had been reported with and without blood transfusion, which was, of course, the method of choice. In icterus gravis no treatment had really been considered very effective before the appreciation of the disease as belonging to the blood disorders. Rolleston (1920) had considered that giving intestinal antiseptics to the mother was worth trying: Klemperer (1924) had recommended intravenous glucose saline solution to protect the liver; Pitfield (1912) had given the child human serum intramuscularly in the belief that his cases were haemophilic in nature: Rosenbaum (1928) gave maternal citrated blood intramuscularly "although of doubtful value". Hampson (1929) had recommended the use of human serum given intramuscularly to supply the theoretic anti-haemolytic factor. De Lange and Arntzenius (1929) recommended the intramuscular administration of whole blood. With the full appreciation of the disease as one primarily concerned with the blood, the need for intravenous blood transfusion was felt on all sides. This had previously been used by Opitz (1922), Hart (1925) and Kleinschmidt (1930): Hawkesly and Lightwood (1934) state that in 1933 they introduced blood transfusion, as a treatment for the disease, into this country. Diamond et al (1932) and

Clifford and Hertig (1932) had strongly advocated its use before this, however. Since that time it has been the standard, and sole method of treatment, although Abt. A.F. (1933) did not consider it of much value, and Ross and Waugh (1936) considered it essential in the "haemolytic", but not in the "obstructive" type. This was much the same view as Hampson (1933) came to hold, except that he still held maternal serum to be useful in the "obstructive" cases, which showed little anaemia.

As far as prognosis was concerned, the only data of any size which are available to indicate the mortality in pre-transfusion days are those of Rolleston (1920), Hampson (1929), and Altzitzoglou (1933). Hampson counted in ~~the~~ Rolleston's cases with his own and showed that in 220 cases of untreated familial icterus gravis, the mortality rate was 80 per cent. It should be noted that at the time these figures were published accurate diagnosis of the disease was not yet possible but on the other hand "isolated" (Hawkesly and Lightwood 1934) cases of the disease were not yet recognised. These cases, therefore, are hardly comparable with those met with today since many more less affected children must now be included. Hampson reported recovery in 17 of his 18 cases treated with maternal serum. Parsons et al (1933) also spoke well of the method of treatment, but neither they nor anyone else confirmed the efficacy of the method. Altzitzoglou (1933)

showed that from the literature in pre-transfusion days the mortality rate was 78 per cent, a figure almost identical with Hampson's.

Sobel (1936) stated that "with early and vigorous (transfusion) therapy, the outlook should now be much brighter". Few reports were forthcoming, however, to show what effect blood transfusion was having on the mortality rate in icterus gravis. Its efficacy in congenital haemolytic anaemia has already been noted. Colver (1938), however, reported a general mortality rate of 62 per cent in 200 cases of icterus gravis, but was unable to demonstrate the therapeutic effects of blood transfusion since so many of the cases had been admitted in the late stages of the disease. Javert (1942) reported a 73 per cent mortality in 41 cases of erythroblastosis occurring in the years 1936 - 40. Hellman and Hertig (1938) reported the mortality rate in 20 cases of icterus gravis as 53.8 per cent.

HAEMORRHAGIC DIATHESIS

In many of the reported cases of icterus gravis a haemorrhagic tendency had been noted during the most active phase of the disease. Pitfield (1912) described a family in which all four infants were icteric and had haemorrhages from the gastro-intestinal tract and cord; Hawkesly and Lightwood (1934) stated that purpura is frequently associated with the disease, and found that cerebral haemorrhage had occurred at

autopsy in six out of eighteen cases. Wiener and Wexlar (1943) have recently suggested that this is due to thrombocytopenia and suggest that the administration of Vitamin K would probably have little or no effect. Leonard (1945), however, found only two out of seven cases to have anything approaching a normal prothrombin blood level. In five in whom the prothrombin time was low, this persisted even after the administration of vitamin K. She considered that the haemorrhagic tendency was probably due to Vitamin K deficiency as a major, if not the sole, cause. Javert (1942) found a low prothrombin index in 7 cases of icterus gravis and this author considered that haemolytic disease of the newborn could occur purely as a haemorrhagic disease with no evidence of jaundice or anaemia. It is considered unlikely that his four cases described were really examples of haemolytic disease in spite of the erythroblastaemia in the cord blood. Otherwise there was no other feature of the disease. Potter (1943a) considers Vitamin K to be rarely needed since few of the infants have a prolonged prothrombin time.

KERNICTERUS

Kernicterus was the name given by Schmorl (1904) to the bile staining of the cerebral basal nuclei which occurred in some of his cases of neonatal jaundice. In 120 autopsies on infants dying with neonatal jaundice, he found 6 examples of kernicterus. For some time the condition was considered a

separate entity by the German workers, but it is now considered by most authorities to be associated with icterus gravis. Whether it can occur in other forms of jaundice is disputed (vide infra).

The condition, kernicterus, remained a pathological specimen only until Guthrie (1914) reported a case (with a family history of neonatal jaundice) which he suggested demonstrated the after effects of kernicterus. This child had been jaundiced at birth and this had persisted for six weeks. When seen aged nineteen months she was very mentally backward and had never talked, walked, nor even sat up. Hypotonia of all her musculature was marked, and she had severe choreo-athetoid movements. Tremors and hypertonicity, although expected, were absent. Guthrie suggested that the bile staining of the nuclei which he assumed to have been present, had disappeared, but the degeneration of the nuclei which was the primary cause of their taking up the stain, had persisted, and accounted for the clinical picture which she later presented.

Pitfield (1912) had previously reported mental deficiency in a child who had suffered from jaundice with "haemophilia" but had considered the cause to be a cerebral haemorrhage. Spiller (1915) reported four cases, all with a history of severe jaundice in infancy, who showed in later life choreiform movements, rigidity of muscles and inco-ordination of movements.

He considered that conditions other than haemorrhage might be responsible for the condition. Only occasional cases were reported (Paul, 1924: de Lange 1925: Hoffmann and Houseman 1926: Greenwald and Messer 1927) from this time until Zimmerman and Yannet's classical article in 1933. These last named authors reviewed the literature on kernicterus and noted that in those cases verified at autopsy death had usually occurred on or before the fifth day, and that in about 40 per cent of the cases symptoms suggestive of involvement of the central nervous system (convulsions and spasticity) had been present before death. In contradistinction to this, they noted that in those cases reported as the late effects of kernicterus, the neonatal jaundice had lasted for an unusually long time. In this respect their findings were in accordance with previous reports since in their two cases which survived the jaundice had persisted for two months, and one month, respectively. In their two autopsy cases death had occurred on the eighth and eleventh days of life. Zimmerman and Yannet pointed out that while jaundice is present convulsions and neck retraction may indicate the presence of kernicterus, but on the other hand, kernicterus is most often found at autopsy without previous signs of its presence. Their two children who survived the period of jaundice later showed mental retardation, extrapyramidal spasticity, athetoid movements, and, naturally, difficulty with speech. The triad made up of the first three signs just mentioned is usually considered necessary for the

diagnosis of the disease. As Hawkesly and Lightwood (1934) pointed out, the presence of simple mental deficiency following icterus gravis is hardly sufficient evidence for the diagnosis of kernicterus. Schmorl (1904) described bile staining of the cerebral cortex as distinct from kernicterus itself, and it may be that this may play some part in the production of the mental defectives who show no evidence of basal nuclei involvement (Parsons 1946). Zimmerman and Yannet (1935) completed the picture of the late effects of kernicterus by the autopsy report on a child aged 3 years who was one of the surviving cases reported in their previous paper (1933). As they expected, there was no bile staining of the basal nuclei, but they did find extensive cellular destruction in the caudate nucleus, putamina, cornua ammonis, substantia nigra, dentate nucleus, lateral thalamic nucleus and the red nucleus. From this they assumed that the cellular destruction is the original lesion and that the bile staining is secondary and, in the event of recovery, temporary. These findings were confirmed by de Lange (1934: 36). In both papers, Zimmerman and Yannet conclude, from the absence of hepatic fibrosis in these cases, that there is no connection between kernicterus and Kinnier Wilson's disease. This is also the view of Brouwer (1936). That hepatic fibrosis does occur, however, as a sequel of icterus gravis has been quite clearly shown by Hawkesly and Lightwood (1934) and more recently by Gilmour (1944). Most pathological

descriptions of the liver in icterus gravis neonatorum include a description of patchy necrosis of liver cells in addition to the erythropoietic foci and bile thrombi. Reisner (1943), however, stated that four of his six cases seen at autopsy showed varying degrees of hepatic damage ranging from crowding of the hepatic parenchyma by extra-medullary haemopoietic centres to extensive haemorrhagic necrosis, cellular infiltration, fibrosis and heavy deposits of iron and bile pigment.

The occurrence of hepatic cirrhosis in the disease was stressed by Henderson (1942) when describing his fourth type of erythroblastosis foetalis. He reported three cases of macerated foetus with severe cirrhosis and also noted it to less degree in seven of twenty-three autopsies on cases of icterus gravis. There was a family history of previous icterus gravis in all three of his macerated foetuses.

The question as to whether kernicterus is associated with any conditions other than icterus gravis is not finally settled although the bulk of the evidence seems to point to its occurring exclusively in that disease. Zimmerman and Yannet (1933) stated that kernicterus is "most frequently, if not exclusively, associated with icterus gravis neonatorum" and that "obstructive jaundice has yet to yield an instance of it". Biemond and van Creveld (1937) however, reported two cases of "nuclear jaundice in neonatal (umbilical) sepsis with jaundice". In their first case, however, the previous

pregnancy had resulted in twins, one of whom had died aged three days -- cause unknown, but "it may have been icteric soon after birth". In view of this, it is impossible to accept that their first case was not suffering from icterus gravis as well as umbilical sepsis.

Biemond and van Creveld's second case became jaundiced at 12 hours, and by the thirteenth day the haemoglobin level had fallen to 65 per cent; the jaundice took six weeks to clear. It is unlikely to have lasted this length of time and for the child to recover from it, if umbilical sepsis had been the sole cause (Morrison 1944). Fitzgerald et alia (1939) also considered that factors other than icterus gravis could cause kernicterus but since they quoted Biemond and van Creveld and Pasachoff (1935) in support of this, and did not offer any further clinical proof, they did not seem convinced on the matter. Pasachoff's (1935) case was a freak in which kernicterus was associated with congenital obliteration of the bile ducts and erythroblastosis. Docter (1945) concluded that no proven case of kernicterus due to any cause other than icterus gravis had been reported, but Parsons (1946) seems convinced that sepsis neonatorum may simulate icterus gravis "even to the occurrence of kernicterus".

The incidence of kernicterus in those cases which survive the initial jaundice and anaemia has never properly been investigated in a large enough series of cases. Most quoted figures are based on Schmorl's original six in 120

cases, but these were autopsies and the frequency in the survivors is by no means accurately known. Taylor (1944) stated that 10 per cent of the surviving affected children showed signs of kernicterus. Other sequelae of icterus gravis which have been reported are cirrhosis of the liver (Morris 1911: Braid 1937; Lightwood 1943), generalised osteitis fibrosa (Braid 1932) and pigmentation of the teeth (Parsons 1946).

Before closing this subject of sequelae, it is interesting to note that although the degeneration of the basal nuclei in kernicterus is said to precede the staining by bile pigment I have been unable to trace any report of similar clinical symptoms following on haemolytic anaemia of the newborn.

THE RHESUS FACTOR

It is now possible to turn once more to the work of Darrow (1938) from whose work dates the modern ideas on the aetiology and treatment of icterus gravis neonatorum and congenital haemolytic anaemia. This authoress, as has been stated, propounded the theory of the antigen-antibody reaction as the basic aetiological factor in all three diseases: she came to this conclusion having set out to find an explanation for the disease which accounted for the following generally accepted observations:

"(1) The apparent absence of any hereditary factor

"(2) The fact that the birth of healthy normal children may precede that of the child in whom the condition first manifests itself in a family

"(3) The frequent familial tendency

"(4) The apparent health of the parents

"(5) The apparent absence of significant factors in the prenatal history in the large majority of cases

"(6) The presumptive association of oedema, grave jaundice and anaemia of the newborn.

"(7) The clinical symptoms

"(8) The observations at necropsy

"(9) The erythroblastosis".

Having set these standards she reviewed all the theories which had ever been produced and decided that none fulfilled all the requirements. From her clinico-pathological study of the disease she concluded there were two pathological processes responsible, to varying degrees, for the symptoms and findings in the disease, namely "(1) abnormal destruction of erythrocytes, and (2) injury of the liver".

Since it was known that the disease could occur in one of twins, and could recur in succeeding pregnancies by a different father, the mother seemed to be the one constant factor. Darrow concluded from this that the mother was the primary source of an "influence" which had affected the foetus, and that the "influence" was transferred by the placenta. The "influence" was propounded to be an immune body produced in the maternal serum by the accidental escape of foetal red

cells into her circulation, this immune body then passing back to the foetus to destroy the red cells and damage the liver. The suggestion was also made that the immune body might do further damage after birth by being present in the breast milk. Darrow's final suggestion was that the antigenic stimulus of the foetal red cells might be due to the difference between foetal and adult haemoglobin, a chemical difference in the two having been demonstrated by Trought (1932) and Barcroft (1933).

To follow the next stage in the development one must turn back to Bordley (1931) who collected from literature, reports of the late effects of incompatible blood transfusions. He reported three cases himself, in which patients developed uraemic symptoms following blood transfusion, which in some cases had been given with apparently compatible blood. Following on this original report, several others continued to appear (Parr and Krischner, 1932: Goldring and Graef, 1936: Von Deesten and Cosgrove, 1933: Mandelbaum 1939). In some of these cases a mistake in the original blood grouping was found when rechecked, but in others no incompatability by the usual tests was discovered. The matter remained a mystery until Levine and Stetson (1939) reported a severe transfusion reaction in a woman who developed a post partum haemorrhage after delivering herself of a macerated stillborn foetus. They examined her serum and found that it contained an agglutinin active against 21 out of 104 Group O bloods (including her husband's). The agglutinin was active at 20°C

and 30°C and was independent of the blood factors P, M and N. Although abnormal isoagglutinins had been reported before as a cause of severe transfusion reactions (Neter, 1936) they had been found after repeated blood transfusions. The patient reported by Levine and Stetson had never had a previous blood transfusion. The agglutinin in this patient was found to be inactive a year later and the authors suggested that the dead foetus had caused isoimmunisation of the mother and that the factor responsible for this was inherited by the foetus from the father: since the dominant property was not present in the mother specific isoimmunisation conceivably could occur. An attempt to inject "sensitive" blood into a series of rabbits failed to produce a similar agglutinin in the blood of these animals. Landsteiner and Wiener (1940), however, had more success with rabbits and guinea pigs which received injections of blood from rhesus monkeys. They produced a serum in these animals which agglutinated the red cells of 85 per cent of human bloods and was independent of factors P, M and N. They termed the factor in human blood which reacted with this serum the Rhesus (or Rh) factor. Wiener and Peters (1940) when reporting transfusion reactions following repeated transfusions in three subjects, found that the serum of two of the patients gave similar agglutinating reactions as the guinea pig anti Rh serum. They also remarked at this time that in the cases of transfusion reaction where repeated transfusions were not responsible, the patients were almost

always women who were, or had recently been, pregnant. Levine and Katzin (1940) and Levine et al (1940) showed that these pregnancies were associated with some abnormality either in the mother or foetus - i.e. toxaemic symptoms in former and intra-uterine death of the latter at some stage of pregnancy. They found that of seven sera tested from women having such histories and transfusion reactions, all contained antibodies active against other red cells of the same blood group - all being more active at 37°C than at room temperature. They termed the agglutinins "warm agglutinins" and showed along with Wiener that one of their sera paralleled the reactions of the guinea pig anti-rhesus serum. The fact that repeated transfusions and pregnancy were the two associated factors in the development of the antibodies suggested that it was the foetal blood which was the antigenic factor when pregnancy alone was responsible for Rh sensitisation. Levine, et al (1941a), in a fuller report, showed that transfusion reactions and the anti Rh agglutinin were also associated with infants who had "erythroblastosis foetalis"; they also reported that most of the mothers were Rh -ve and all the fathers and infants Rh + ve. This led them to examine the bloods of the mothers of erythroblastotic infants who had not had transfusions, and they found that most of them were Rh - ve and that their sera contained anti Rh agglutinins. They recalled Darrow's theory of an antigen antibody reaction as a cause of "erythroblastosis". Levine et al (1941b) finally

showed that about 90 per cent of the mothers of "erythroblastotic" infants were Rh - ve and that all the infants and fathers were Rh + ve. Anti Rh agglutinins, however, were only found in about 50 per cent of cases examined within the first two months after delivery. Levine's suggestion that Rh - ve blood only should be used in the treatment of the disease was a natural corollary to his theory of the antigenicity of the Rh factor as a cause of it. The successful use of Rh - ve blood where transfusion reactions had previously occurred was reported by Wiener (1941).

Boorman, Dodd and Mollison (1942) in this country confirmed this work, finding 85.15 per cent of the population to be Rh + ve and 14.85 per cent to be Rh - ve. They found that of 48 mothers of definitely erythroblastotic infants, 46 were Rh - ve and only 2 Rh + ve. In 44 of the 46 Rh - ve mothers they found anti Rh antibodies - most active 7 - 21 days after delivery. They also reported finding the agglutinin in the serum of identical twins both affected by the disease. This is an unusual finding as the agglutinin in most cases is assumed to be adsorbed on to the infant's red cells. Boorman et al. also found that in the 4 cases in which no anti Rh agglutinins were found (two of the mothers being Rh + ve) there was a difference in blood group between mother and child whereby the mother's serum was incompatible with the infant's red cells. This had also been remarked on by Levine et al. (1941b). Boorman, Dodd and Mollison (1944),

continuing their work, found that of 100 mothers of proved cases of haemolytic disease of the newborn, 97 were Rh -ve. Of the 79 affected infants and 45 fathers who were tested, all were Rh +ve. Anti Rh agglutinins were found in 93 of the 97 Rh -ve women and the four in whom they were not found the serum was examined on one occasion only. The 3 Rh +ve mothers all had antibodies active against the infants' red cells - one of these later turned out to be Anti-Rh" (anti-E) and the other two were the Beta agglutinin since the infants were both Group B and the mothers were Group O. The titre of the maternal beta agglutinins was found to be $^{1}/8,000,000$ and $^{1}/32,000$ respectively.

In proving the efficacy of Rh -ve blood transfusion, Mollison (1943) showed very conclusively by means of differential agglutination blood counts that Rh -ve cells persist the normal 80 - 120 days in the affected infant's circulation, whereas Rh +ve cells are usually destroyed completely in a few days.

Race Taylor, Cappell and McFarlane (1943) found that in 50 families in which one or more infants had developed haemolytic disease of the newborn - 44 of the mothers were Rh -ve and 6 were Rh +ve. Anti-Rh agglutinins were found in 38 of the Rh -ve mothers. All of 16 affected infants who were tested were Rh +ve. Polayes (1945) reported 6 infants with the disease, the mothers all being Group O, Rh +ve and the infants all A, Rh+ve. From this report, it seems

that the ABO blood groups may also play a part in the production of the disease.

It was noted by the earlier workers on the subject that all cells did not react equally with the same sera and that sometimes cells would be agglutinated by one serum and not by another. For this reason, testing with at least three powerful sera was recommended (Taylor 1943). Wiener (1941), when reporting the finding of anti-Rh antibodies in 4 of 10 patients who had had transfusion reactions, found that three of them corresponded to the guinea pig serum (85 per cent +ve reactions) but that one gave only 70% reactions with Rh +ve blood. He concluded from this that there existed more than one form of the factor Rh. Landsteiner and Wiener (1941) by this time had shown that the Rh factor was a dominant gene unconnected with the Sex, P, M, or N genes. In 60 families with 237 children, they found that there were no fallacies to this theory. They therefore postulated that three genotypes were possible - Rh Rh, Rh rh (the two making up the phenotype Rh +ve) and rh rh (phenotype Rh -ve). The fact that a person was heterozygous could at this time only be shown by the fact that they were the parent of an Rh -ve child or the offspring of an Rh -ve parent.

Pursuing their studies with the 70 per cent serum, Wiener (1942) and Landsteiner (1943) decided that there was an analogy with the blood groups A_1 and A_2. They termed the bloods reacting with both the 85 per cent and 70 per cent sera,

Rh1 and those only with the 85 per cent serum Rh_2. They therefore now postulated 3 main allelic genes - Rh_1 Rh_2 and rh -, the first two being dominant over the third, and the first over the second. There were, however, about 3 per cent of bloods reacting with the 70 per cent serum and not with the 85 per cent serum. These were termed Rh'. Next Wiener and Sonn (1943) reported a serum reacting with only 30 per cent of human bloods and showed that the blood factor determined by it was also inherited as a Mendelian dominant, and to this factor they gave the name Rh_2. An occasional blood which was thought to be Rh -ve reacted with this 30 per cent serum and the factor determining this reaction was called Rh". Later Wiener (1944a) found that a few bloods reacted with the standard 85 per cent serum only, and not with the other two - the factor determining this reaction was called Rh_0 (later rh_0 - Wiener et al. 1946). Wiener (1943a,b) enlarged on the reactions of the three sera and postulated the presence of six allelic genes - namely Rh_1 Rh_2 Rh (Rh_0) Rh' Rh" and rh. Depending on the reactions a blood gave with the three sera 8 different phenotypes could be recognised (7 of these had already been found) and they further postulated that there were 28 possible genotypes. Wiener Sonn and Belkin (1943) then showed that 95 per cent of the population belonged to the phenotypes Rh_1, Rh_2, Rh_1Rh_2 and Rh -ve -- only 5 per cent belonged to the types Rh', Rh" and Rh'Rh" and Rh (rh_0). In the next year (Wiener et al 1944) they showed that in an examination of 97 families with

275 children the results obtained showed complete agreement with the theory of six allelic genes and pointed out that the 70 per cent serum and the 30 per cent serum stood in a similar relationship to each other as the anti-A and anti-B agglutinin of the ordinary blood groups. It has been Wiener's constant concern to keep the Rh subtypes within the framework of the A B O grouping and his nomenclature has always been based on this idea. He did, however, (1943b) recognise that Rh' was a single allelic gene and that Rh_1 was an agglutinogen "containing two partial antigens inherited as a unit by means of a corresponding gene". For this reason Wiener (1944a) gave the alternative names Rh'_0 and Rh''_0 for the factors Rh_1 and Rh_2, thus showing that they both reacted with the 85 per cent serum, but that they differed in their reactions with the 70 per cent and 30 per cent sera. After some indecision Wiener finally termed his three sera anti-Rh_0 (85%) anti-Rh' (70%) and anti-Rh" (30%). Sera which reacted with Rh' cells as well as giving 85 per cent +ve reactions, he termed anti-Rh_1 (Rho') and correspondingly when two antibodies were present one of which acted on R" cells, he called it anti-Rh_2 (Rho") (Wiener 1945a). Wiener's table finally came to show the blood types according to the reactions with the 3 sera, as follows :-

TABLE I

	Anti-Rh_0 (85% serum)	Anti-Rh' (70% serum)	Anti-Rh" (30% serum)
Rh_1	+	+	-
Rh_2	+	-	+
Rh_1 Rh_2	+	+	+
Rh_0	+	-	-
Rh'	-	+	-
Rh"	-	-	+
Rh' Rh"	-	+	+
rh	-	-	-

+ = agglutination

GENOTYPES

In this country Race and Taylor (1943) and McCall, Race and Taylor (1943) described a serum which reacted with all Rh-ve bloods and all those bloods which were known from family studies to be heterozygous Rh+ve. It failed to react with about 20 per cent of all bloods which, they deduced, must be homozygous Rh+ve (RhRh). The woman from whom the serum was obtained was herself known to be Rh +ve and since, of course, her blood was negative to her own serum she was concluded to be homozygous Rh+ve. Her husband and children were all Rh+ve and their cells were all agglutinated by her serum. Using Wiener's terminology, they suggested that people negative to

this serum might be of the genotype Rh_1Rh_1 and that the factors on which it acts must be Rh_2 and rh. They called the serum anti-rh.

Race, Taylor, Boorman and Dodd (1943) then reported the finding of a 30 per cent serum similar to the anti-Rh" of Wiener. Race, Taylor, Cappell and McFarlane (1944) later found a 70% serum similar to the anti Rh' of Wiener. By the use of these sera, along with the standard 85 per cent serum, it was estimated that the actual genotype of 80 per cent of the population could be estimated according to the following table. (Wiener had claimed recognition of the phenotype only in 95 per cent of the population).

TABLE II

	Percentage	Anti-Rh_0	Anti-Rh'	Anti-Rh"	Anti-rh
rh rh	16.59	-	-	-	+
Rh_1 Rh_1	16.59	+	+	-	-
Rh_1 Rh_2	12.19	+	+	+	+
Rh_1 rh	33.18	+	+	-	+
Rh_2 rh)	12.19				
Rh_2Rh_2)	2.24	+	-	+	+

Race et al (1944) believed that there were seven allelomorphic genes Rh1, Rh_2, rh, Rh', Rh", Rh_0 and Rh_y (the latter being +ve with anti-Rh" but -ve with anti-rh). From

this they concluded there were 28 genotypes and that only 7 per cent of persons belonged to the 22 rare genotypes (Rh'Rh", Rh"Rh", etc.)

Apart from the tremendous genetic interest which this work aroused, the presence of the Rh subtypes (Wiener) and the differences in genotype (Race) explained some of the cases of erythroblastosis in which the mother was Rh+ve and the pregnancy was not heterospecific with regard to the A B O grouping. Instead of it being necessary for a mother to be Rh -ve in order to produce antibodies against the"Rh$_o$ factor" in her infant, it was now considered possible that she could become sensitised to any gene which she herself lacked and which the infant had therefore obtained from the father. Thus, in the case of the Rh_1Rh_1 mother who produced the anti-rh serum previously described, she was able to produce even an "anti-Rh-ve" serum, although it must be strongly emphasised that the children in these cases are <u>not</u> Rh-ve and cannot be since the mother is homozygous Rh+ve. The fact that the gene rh can be recognised serologically shows that it is not a true genetic recessive (Cappell, 1946).

In order to understand the ideas and nomenclature which have been suggested by Fisher (Race, 1944) it is probably best to consider cells which up until now were thought to be of the type Rh_2 (Wiener). These cells had been found both in America and here to react with the 85 per cent serum, the 30 per cent serum and the anti-rh serum. There were, in addition, some

rare cells which gave the last two reactions of the Rh_2 cells but did not react with the standard 85 per cent serum. These cells had been termed Rh". Fisher's hypothesis is based on the fact that each gene has three components, these being allelic in nature, and he designated them as Cc, Dd, and Ee. He suggested that the 85 per cent serum reacted on the component D, the 70 per cent serum on the component C, the 30 per cent serum on the component E and the anti-rh serum on the component c. In order to complete his hypothesis Fisher also postulated that two further sera would be encountered which would enable components e and d to be identified . In the case of the Rh_2 cells, therefore, according to this theory D, E and c are present, and it was therefore suggested that the genotype could be more truly represented as cDE. The Rh" cells had failed to react to the 85 per cent serum and were, therefore, termed cdE. It is still impossible to show the presence of d in the Rh" cells, and its presence is only assumed from the absence of D, but recently there has been mentioned (Cappell, 1946) the possibility of a serum reacting with d having been found by Diamond and Boyd. This serum should agglutinate Rh" cells, but not all Rh_2 cells if Fisher's theory is correct. Mourant (1945) has already found a serum acting on e - in fact it reacted on all but 4 per cent of all human bloods. An important result of this finding is that the homozygous Rh_2 cells (Rh_2 Rh_2 - cDE/cDE) can now be differentiated from the heterozygous Rh_2 cells (Rh_2 rh cDE/cde). As has been seen in connection with Rh_1Rh_1 cells, this is of the greatest

importance in the prognosis of the disease in future infants. Where the father is Rh_2 Rh_2 all further infants will be affected, but where heterozygous (Rh_2 rh) 50 per cent of further children should be Rh-ve (rh rh) when the mother is Rh-ve.

Fisher's theory assumes that the 70 per cent serum reacts with factor C - so that Rh_1 cells since they react with the 85 per cent serum, the 70 per cent serum, but not the 30 per cent serum, are represented by the letters CDe. If cells giving this reaction are negative to the anti-rh sera, they are assumed to be homozygous Rh_1Rh_1 (CDe/CDe). If they are +ve to the anti-rh serum they will be Rh_1 rh. (They could not be Rh_1Rh_2 since they do not react with the 30 per cent serum).

It is important in the understanding of these reactions to realise that the sera may react with either of the genes contained in the red cells, and that the 70 per cent serum and the anti-rh serum are complementary (Levine 1945a) in that when cells are negative to the former they are always positive to the latter and vice versa. In a manner similar to the Rh" cells, Rh' cells may be seen to be represented by Cde.

A serum giving similar reactions to the anti-rh serum of Race and Taylor (1943) had been mentioned by Levine (1941) but it had only given 30 per cent reactions instead of 80 per cent. However, similar sera were later reported (McCall and Holdsworth 1945: Waller and Levine, 1944 and Wiener,

Davidsohn and Potter, 1945) and similar reactions have been noted with it. Levine (1941) had originally suggested that this serum had reacted on another blood property which he termed Hr. Wiener (1945c) took this nomenclature up and, while disputing that genotyping could be performed to the extent claimed in this country, he gradually accepted the ideas of Fisher, Race, Cappell and others, but has tried to fit them once again into the framework of the blood types known before the discovery of Rh. He argues (1945c) that anti-Rh' serum and anti-Hr' serum (his name for anti-rh) are in exactly the same relationship to each other as anti-M and anti-N - i.e., if a blood is negative to one, it must be positive to the other, although it may be positive to both. In accepting Fisher's theory but changing the name, he has made the matter even more complicated than it might have been. For the anti-e serum of Mourant which he has accepted, he suggests the name anti-Hr" and for Fisher's theoretical serum acting on d he has suggested anti-Hr_0. It seems unreasonable, when it is known that each person has genes derived from each parent, that Wiener should suggest that two factors which are obviously related should be kept distinct instead of appreciating that the serum may react with either one or other gene which has been inherited. There is no doubt in my mind that Fisher's theory is correct, although it is agreed that for speech purposes it is somewhat difficult. It certainly makes the whole matter much more understandable. Cappell's (1944)

nomenclature for the sera is the one of choice. The following table represents the reactions found, and predicted, which the sera give with the commoner genotypes. Both Fisher's and Wiener's nomenclature is shown, with Cappell's names for the sera :-

TABLE III

CELLS	SERA					
	anti-Rh_0 anti D	anti-Rh' anti C	anti-Rh" anti-E	anti-Hr' anti-c	anti-Hr" anti-e	anti-Hr_0 anti-d
Rh_1 Rh_1 $\frac{CDe}{CDe}$	+	+	-	-	+	-
Rh_1 Rh_2 $\frac{CDe}{cDE}$	+	+	+	+	+	-
Rh_1 rh $\frac{CDe}{cde}$	+	+	-	+	+	+
Rh_2 Rh_2 $\frac{cDE}{cDE}$	+	-	+	+	-	-
Rh_2 rh $\frac{cDE}{cde}$	+	-	+	+	+	+
rh rh $\frac{cde}{cde}$	-	-	-	+	+	+

As can be seen, none of the reactions are the same, and this is also true when all the rarer genotypes are included, most of which require anti-e and anti-d for their recognition. Fisher thought other two genes would be located since there were other two reactions possible in his table - one of them Rh_z has since been reported by Murray et alia (1945), giving the reactions of CDE. That the matter is even further

complicated is shown by Wiener's (1944a) description of intermediate genes and the discovery by Callender, Race and Paykoc (1945) of the second allelomorph at the Cc locus, termed C^{W}.

Apart from these amazing genetic discoveries, most of the recent work has been serological in nature, in an attempt to find a suitable test to demonstrate that a person has been sensitised to the "Rh factor" or one of its components. Even in Rh-ve women who have infants proved to be suffering from the disease, agglutinating antibodies may be absent from their sera, although the exact percentage in which this has been the case has varied (Boorman et al, 1944 Levine 1945a). Race (1944) and Wiener (1944b) reported the finding of an "incomplete" or "blocking" antibody in some of the sensitised persons who failed to show agglutinins or showed only weak agglutinins. Diamond and Abelson (1945) devised a slide test using undiluted blood, and more recently Diamond and Denton (1945) have had better results using a diluent of serum albumen, either human or bovine. Wiener (1945b) has described a "conglutination" test, the theory of which has been strongly criticised by Coombs, Mourant and Race (1945b). The last named authors have described (1945a;b.1946) a test using anti-human serum prepared from a rabbit which can detect traces of antibody which have been absorbed on to red cells. This test can be used on the red cells of the infant suspected of suffering from the disease

(direct method) or it can be used to demonstrate the presence of antibodies in the maternal serum (indirect method).

The multiplicity of tests which have developed tends to indicate that so far none of them have been found satisfactory in every worker's experience, although experience with the Coomb's test is still limited. All are agreed, however, that the finding of an agglutinating antibody active on Rh+ve cells and inactive on Rh-ve cells is evidence of Rh sensitisation, and that when any woman having such antibodies has an Rh+ve child, the latter is bound to be affected with haemolytic disease of the newborn (Cappell 1946). The latter term is the name now given to the three clinical entities previously described under "erythroblastosis foetalis".

The occurrence of the 10 per cent of cases where the mother is Rh+ve has not yet been fully explained. Levine (1945b) now considers that 92 per cent of the mothers are Rh-ve and that in the other 8 per cent the disease can be attributed to :

(1) Heterospecific ABO pregnancy where the infant is a "non secretor" of the group specific substance.

(2) The"Hr" factor

(3) Finer differences in the Rh factor

Wiener and Unger (1944) have reported a case of isoimmunisation to factor P by blood transfusion. It is possible that this might also occur in pregnancy.

Treatment with Rh-ve blood is the standard treatment for all cases, but there were no authoritative figures published to show to what extent the prognosis had been affected, until Gordon (1947) showed that the mortality rate had dropped from 55.7 per cent to 25 per cent in the transfused cases. This latter figure is in close agreement with the mortality rate of 29.3 per cent given recently by Parsons (1947).

TRANSFUSION.

The question of when the child should be transfused is still under discussion (Parsons, 1946: Cappell, 1946) and attempts to prevent the disease or minimise its effects by Caeserean section before term do not appear to have produced great success. (Broman, 1945; Potter 1943a). Cappell (1946), however, advocates that this should always be done when the mother has antibodies in her serum and the father is homozygous Rh+ve. He recommends immediate transfusion as soon as it is established that the child is affected. This is also the impression of Javert (1942).

BREAST FEEDING

Witebsky et al (1942), Witebsky and Heide (1943) and Langley and Stratton, (1944) have shown conclusively that the antibody is excreted in the maternal milk, although usually in a lower titre than in the serum. These workers recommended that the child should not be given natural

breast milk in case absorption from the bowel might continue the haemolytic process. Langley and Stratton (1944) recommended exhaustion of the breast milk and boiling it before giving it to the child. Taylor (1944) however, seemed to consider that normal breast feeding should be continued.

THEORY

Lightwood (1943) pointed out that Levine's theory did not explain how the liver and basal nuclei came to be damaged if the Rh antigen resided in the red cells only. Boorman and Dodd (1943), however, demonstrated its presence in the tissues in alcohol soluble form. It had previously been shown to be absent from the tissue fluids by Wiener and Forer (1941) and Levine and Katzin (1941). Wiener's (1946a,b,c,d) recent attempt to explain the hepatic and nuclear damage is based on his finding that they occur only when an agglutinating antibody is present in the maternal serum. He considers that both conditions result from intravascular agglutination of the child's red cells with subsequent necrosis of the tissue cells from ischaemia (Wiener and Brody 1946). Wiener considers that the blocking antibodies (glutinins) are responsible for anaemia and hydrops and that "icterus praecox" is due to heterospecific ABO pregnancy. An attempt at statistical proof of his theory has recently been published, (Wiener 1946 d).

INCIDENCE.

Taylor and Race (1944) pointed out that in one mating in eight the mother is Rh-ve and the father Rh+ve and that in one mating in five the pregnancy may be heterospecific with regard to the ABO grouping. Javert (1942) considered the incidence of haemolytic disease of the newborn to be 1.438 whilst Schwartz and Levine (1943) estimated that if based on the results of serological tests the incidence might approach 1:200. This is also the impression of Cole (1947). However, it is obvious that the disease does not occur as often as it should and that there must be something more to it than just a difference in Rh type or ABO grouping. Levine (1945a) still considers that in the ABO grouping it is only the non secretors who become affected, but Wiener (1946b) tends to doubt whether this factor plays any part. Wiener's (1946a) explanation of why some people become easily sensitised is that it depends on further genes which he terms K and k. (The genetic explanation of ease of sensitisation appears to appeal also to Race (Cappell 1946). According to Wiener (1946a) 97 per cent of the population is of the genotype kk (difficult to sensitise), 3 per cent Kk (moderately easy to sensitise) and only rare persons are KK (extremely easy to sensitise). It is the latter type of person who becomes sensitised to one blood transfusion (Dacie and Mollison, 1943) or produces an affected child in the first pregnancy (Race et al 1943). The very great

proportion of fathers have been shown by the last named authors, however, to be homozygous Rh+ve, and since this means that every sibling will be Rh+ve the frequency of stimulation also appears to play a decisive part in the ability of any woman to become sensitised to either the Rh factor or an ABO group factor.

Although, therefore, many great advances have been made in the past few years, many matters remain unsolved or in dispute, especially in relation to pathology and treatment. The clinical manifestations of haemolytic disease of the newborn are many and varied (Potter, 1943a); the pathological manifestations are equally different (Gilmour, 1944) and, as has been seen, the "Rh factor" has now developed into something which only months of study can permit one to understand. To attempt a correlation between these three aspects of the disease has been the main object of the work which composes this thesis.

PART II

PRESENT STUDY

MATERIAL

The basis of this work consists of a study of 160 cases of haemolytic disease of the newborn which were admitted to the Royal Hospital for Sick Children, Glasgow, from January 1934 until November 1946. Twenty eight of these cases were personally observed and the data concerning the remaining one hundred and thirty two were obtained from the case records and from a 'follow up' which was undertaken. The case records were filed under the diagnoses of erythroblastosis foetalis, icterus gravis neonatorum, congenital haemolytic anaemia and (later) haemolytic disease of the newborn. All case sheets so classified were carefully scrutinised and a decision arrived at as to whether (in the light of present knowledge) they were true cases of haemolytic disease of the newborn. In two cases the decision was influenced by blood group testing, but these were doubtful cases clinically and the finding of 'Rh compatibility' was merely sufficient additional evidence to justify a decision against the diagnosis. No true clinical case of haemolytic disease of the newborn was discarded because of evidence obtained from Rh testing

CRITERIA OF DIAGNOSIS

In addition to estimating whether each case was one of haemolytic disease of the newborn an attempt has been made to

classify it as being either of the icterus gravis neonatorum or the congenital haemolytic anaemia type. It is realised that this division is somewhat artificial and that there are some cases which fall between the two. Most cases of icterus gravis will become markedly anaemic if they survive long enough and it is usual to have a history of some jaundice previously in a case of congenital haemolytic anaemia. The decision to divide the two conditions was made because it is believed that clinically there is a considerable difference in the two conditions which is most marked in respect of the

1) Mortality Rate
2) Occurrence of Kernicterus
3) Occurrence of hepatic dysfunction

As far as treatment is concerned congenital haemolytic anaemia should always be curable by means of Rh-ve blood transfusion. This is not always the case with icterus gravis neonatorum as will be seen later. (Table XIV)

THE DIAGNOSIS OF HAEMOLYTIC DISEASE OF THE NEWBORN

The following factors were taken into account - apart from the jaundice and anaemia which will be mentioned later under separate headings.

1. Family history
   a) Pregnancy - if the infant was the result of a first pregnancy this was considered a point

against the diagnosis unless the mother had received a blood transfusion at some time before the birth of the child

b) Previous pregnancies - a history of previously jaundiced or anaemic infant(s) was considered as strongly in favour of the diagnosis.

2. Erythroblastaemia - accurate counts of the nucleated red cells were not always recorded but the description of 'many' or 'numerous' normoblasts and megaloblasts was taken as indicating erythroblastaemia

3. Reticulocytosis - this was used as the index of blood regeneration in practically all cases. A reticulocytic count of 5% or more was taken as abnormal

4. Splenomegaly - enlargement of the spleen to any distance below the left costal margin was taken as being pathological. Its palpable presence above the costal margin was not so interpreted.

5. Urobilinuria - the presence of urobilin in the urine was taken to represent increased blood destruction and was therefore in favour of the diagnosis.

6. Autopsy findings - these were taken as conclusive when intense erythroblastosis was found in the liver. If erythroblastosis was absent but the infant had survived more than two weeks the diagnosis was made in spite of this, if the clinical picture was such as to warrant it.

7. Rh testing - on those families who reported in the

follow up was taken into consideration, but no combination of Rh types was taken as conclusive evidence in favour of the diagnosis nor against it

Not all of these criteria were observed in every case but the majority were present in all. A careful assessment was made and if any reasonable doubt still remained the case was discarded.

ICTERUS GRAVIS NEONATORUM

1. Jaundice - this was (naturally) essential to the diagnosis. The time of onset of this sign is, however, important and no case was included in this group which did not become jaundiced within 48 hours of birth. Further, if the child was admitted late in the disease and untreated, a history of persistent jaundice lasting at least 7 days must have been obtained. If admitted within the seven days assessment was based on clinical condition on admission and the course of the disease.

2. Biluria - the presence of bile in the urine was taken as conclusive evidence of the case being icterus gravis as opposed to congenital haemolytic anaemia

3. 'Cerebral Symptoms' - the occurrence of spasticity, ocular movements, convulsions, tremors, and nuchal rigidity in a jaundiced infant was not taken as evidence of kernicterus and therefore did not influence the diagnosis in any way

4. Anaemia - the presence of anaemia in a jaundiced infant

was, in the presence of erythroblastaemia and/or reticulocytosis taken as definite evidence in favour of the diagnosis, if the infant was apyrexial. The absence of anaemia did not preclude the diagnosis.

CONGENITAL HAEMOLYTIC ANAEMIA

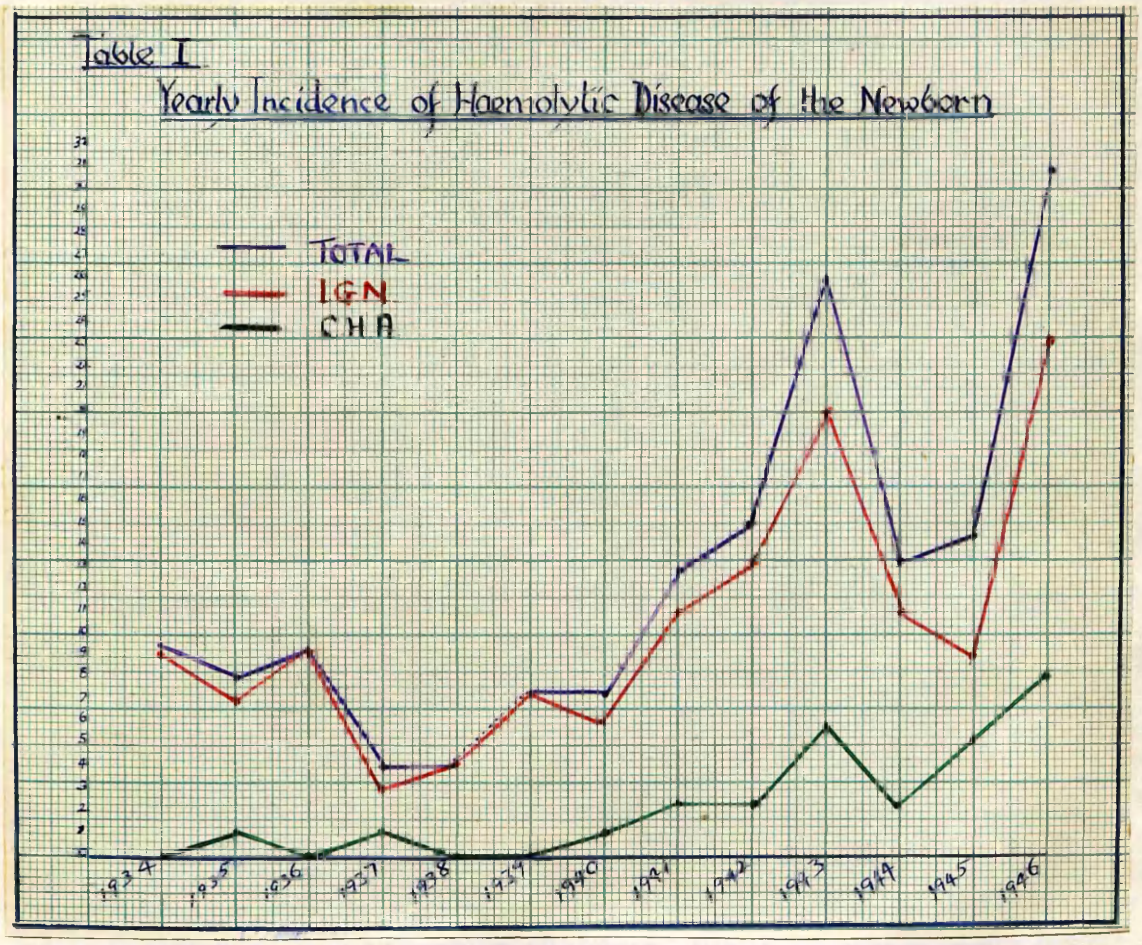
1. The only essential sign apart from those of haemolytic disease of the newborn (see above) was the onset of a severe anaemia within two weeks of birth. No case in which pallor had been noted for the first time more than two weeks after birth has been included in the series. The presence of previous jaundice was accepted as part of the picture - but not an essential part. The jaundice usually appeared after the second day and faded quickly giving place to a marked pallor.

On analysing the 160 cases of haemolytic disease of the newborn along the lines just described it was found that 132 fell into the category of icterus gravis neonatorum and 28 into the congenital haemolytic anaemia group.

YEARLY INCIDENCE

It is seen from Table I that the discovery of the "Rhesus factor" in 1940 and its association with haemolytic disease of the newborn in 1941 had a great effect on the "incidence" of that disease.

TABLE I. YEARLY INCIDENCE OF HAEMOLYTIC DISEASE OF THE NEWBORN



Until 1941 the disease was quite infrequent and was represented almost entirely by icterus gravis. There were only six cases of congenital haemolytic anaemia before 1942. This is not very surprising since Abt. A.F. (1932) was able to collect only 12 cases from the whole of the

world's literature and it is well known that the disease is usually benign. That it was only the most severe who were admitted to hospital at this time is supported by the fact that two out of these six cases died - the only deaths from congenital haemolytic anaemia in the whole series although one further case did die under circumstances which will be mentioned later (p. 79)

Of the 31 cases which were admitted from January to November 1946, 28 were seen personally: one occurred whilst the writer was on holiday and the other two were admitted during the night and died almost immediately. Three of the cases were treated in the Sick Nursery of the Glasgow Royal Maternity Hospital instead of transferring them to the Sick Children's Hospital. This was as a result of the discovery during this work that many of the infants who die have evidence of terminal infection at autopsy (p. 87)

SEX INCIDENCE

Table II shows the incidence of haemolytic disease of the newborn in the sexes. There is an overall preponderance of males which is greatest in the icterus gravis group where the incidence is almost 2 males to 1 female. In the congenital haemolytic anaemia group however there is almost a 4 to 1 preponderance of females.

TABLE II. SEX INCIDENCE IN 160 CASES OF HAEMOLYTIC DISEASE OF THE NEWBORN.

Type	No.of Cases	Males	Females	/ Ratio
I.G.N.	132	86	46	1.87:1
C.H.A.	28	6	22	0.27:1
H.D.N.	160	92	68	1.35:1

Halpern et al. (1945) found an incidence to 2 males to 1 female in their series of 40 cases of 'erythroblastosis foetalis' and suggested that there may be some connection between the sex genes and the Rh genes to explain it. Although this increased incidence is confirmed by the figures shown in Table II it is felt to be unlikely that they are of any significance. Certainly even with 160 cases it is impossible to come to any definite conclusion and a series of some hundreds taken from the literature would be required before anything conclusive could be arrived at.

BIRTH WEIGHT

It has for long been recognised that haemolytic disease of the newborn occurs mainly in mature well developed infants. It is one of the tragedies of the disease that frequently a healthy lusty infant of normal appearance may become acutely ill and die within a matter of hours after delivery.

The premature infant is more liable to develop a deep 'physiological icterus' than the full term child and the

percentage of immature red cells in its peripheral blood is also higher than is found in infants of normal weight. It is, therefore, advisable to set a 'higher' standard for the blood picture before diagnosing icterus gravis in a premature infant. However even in a premature infant if deep jaundice is accompanied by severe anaemia (RBC's were 1,200,000/cu mm in one case), severe erythroblastaemia (100 nucleated red cells per 100 RBC in another case) or extreme reticulocytosis (48% in a further case) accompanied by enlargement of spleen it is impossible to consider this physiological and these cases must be considered as haemolytic disease.

The birth weight as recorded in the history was noted in each case and this was confirmed by the weight on admission to hospital. When this was within a few days of birth the check weighing in hospital were a useful guide to the accuracy of the history but later on it was of little value. The weights therefore are not strictly accurate but they do demonstrate (Table III) that 88.08% of cases of haemolytic disease of the newborn occur in mature infants. Although the international standard of maturity is adopted here (over 2.5K.) the majority of the infants were well over this weight and usually weighed between 7 and 8 lbs. at birth

TABLE III. RECORDED BIRTH WEIGHTS IN 160 CASES OF HAEMOLYTIC DISEASE OF THE NEWBORN

BIRTH WEIGHT	NUMBER	PERCENTAGE OF TOTAL NO. RECORDED
Over 5½ lbs.	133	88.08
Under 5½ lbs.	18	11.92
Not recorded	9	

Almost 12% of the present series therefore consists of premature infants which is a surprisingly high figure. In a few of them the prematurity was due to artificial induction of labour for disease in the mother but in most the labour came on spontaneously sometimes because of twin pregnancy but often for no known reason. It is commonly believed that the premature deliveries which occur in relation to haemolytic disease are usually associated with hydropic infants but it seems quite clear that premature delivery can be associated with true icterus gravis although it is impossible to state that it is the diseased condition of the foetus which is the cause of the onset of early labour. The conclusion is reached therefore that because a severely jaundiced infant is premature it does not exclude the diagnosis of icterus gravis

ABORTIONS AND STILLBIRTHS IN PREVIOUS PREGNANCIES

It will be convenient at this stage to consider the question of the incidence of abortions and stillbirths in

families known to have produced at least one infant suffering from haemolytic disease of the newborn. The number of families from whom this and other familial data were obtained was only 146 since 12 families had two siblings included in the series and one had three. As far as families were concerned therefore 14 infants are 'redundant': this figure subtracted from 160 gives the number of families actually involved.

In a previous report (Gordon 1947) it was shown that the incidence of abortions in these families was not higher than in the population as a whole but that the incidence of stillbirths was twice the figure given by the Medical Officer of Health for Glasgow (1943). Since the families have now increased in number Table IV shows the incidence of previous abortions and stillbirths in families which have produced at least one child suffering from haemolytic disease of the newborn.

TABLE IV. INCIDENCE OF ABORTIONS AND STILLBIRTHS IN 146 FAMILIES

Number of Pregnancies	Number of Abortions	Number of Stillbirths	Number of Livebirths
520	19	30	471

ABORTION RATE	$\frac{\text{ABORTIONS}}{\text{PREGNANCIES}}$	3.6%
STILLBIRTH RATE	$\frac{\text{STILLBIRTHS}}{\text{LIVE BIRTHS}}$	6.4%

No official figures for the abortion rate in the general population but obstetric textbooks (Eden & Holland 1937: Munro Kerr et al. 1933) give anything up to 20%. There is certainly no increased incidence in these 'affected' families.

The Medical Officer of Health's report for Glasgow in 1943 gave the incidence of stillbirths as 36 per 1000 live births. In Table IV it is seen to be almost double this figure in the families under consideration

It is seen therefore that a history of previous stillbirths may be of some importance in the clinical diagnosis of haemolytic disease of the newborn but that a history of previous abortions is of none

It will be seen later (Table VI) that in the pregnancies which <u>follow</u> an affected infant the incidence of stillbirths is even greater. Eleven stillbirths occurred as compared to 31 live births - a proportion of almost 33%. There were only 4 abortions out of 46 pregnancies which is still not unduly high.

PARITY OF THE MOTHER (Partition of first affected infant)

It is proposed in this, and further paragraphs, to coin a general phrase for the products of conception whatever their nature. It has been found difficult to express this satisfactorily with the present nomenclature. If, for example, one wishes to make reference to the result of the sixth pregnancy in a woman who has had two previous abortions it is impossible to refer to it as the sixth child: it is impossible to refer to it as the sixth pregnancy since the latter terminated when the infant was born: one is forced therefore to refer to it as "the result of the sixth pregnancy" which is very clumsy. It is suggested, therefore, that the "result" of any pregnancy should be referred to as a "partition". This is an expressive word indicating the inevitable separation of mother and foetus and also has the advantage of being associated phonetically with the obstetric word parturition. In the example quoted above it is possible by the use of this word in the manner indicated to refer to the infant concerned as being the "sixth partition" and if for any reason it is considered necessary the fact that two of the partitions were abortions can be added "The infant was her sixth partition, two others having been abortions". If all the infants had been born alive the sixth could be referred to as the "sixth live partition" and if one had been prematurely delivered the words "one of which occurred at

the seventh month of gestation" could be added.

The heading of this paragraph should now read "Partition of the first affected infant", and the facts on this point as they occurred in the 146 families, are set out in Table V.

TABLE V. PARTITION OF FIRST AFFECTED INFANT IN 146 FAMILIES

PARTITION	NO. OF FAMILIES	PARTITION	NO. OF FAMILIES
1st	8	8th	3
2nd	46	9th	1
3rd	33	10th	1
4th	24	11th	1
5th	24	12th	NIL
6th	2	13th	1
7th	2		

When compiling this table the 'first affected infant' was not always the one studied in the series of 160 cases. If a mother of a definitely affected child gave a history which was strongly suggestive of the disease in previous infants the first of these to have presented these symptoms was counted as the "first affected child". The table is not therefore based on strictly controlled facts but on the other hand two of the most important points which it stresses are unaffected by this qualification. The first of these is the occurrence of the condition in the first child in 8 of the families. In none of them is there a definite history of blood transfusion prior to the birth of the infant but since only two of them reported at the

follow-up this is the only number in which blood transfusion can be definitely ruled out by the mother's own statement. Of the mothers who reported one was Rh-ve and one Rh+ve. Although rare, therefore, it does occasionally happen that a first child is affected without previous sensitisation. The accuracy of the previous obstetric history in some of these cases may, however, be in doubt. If a woman had a previous illegitimate child it is unlikely that she would reveal the fact. The variety which characterises the whole disease process is well demonstrated by the fact that one case was the thirteenth partition in the family, all of the previous ones being full term healthy infants except the 12th which was a stillbirth. In other families the 9th, 10th and 11th partitions were the first to be affected. It is clear therefore that variability in the number of pregnancies required to produce sensitisation is considerable. Whether this is due to the genetic factor K (Wiener 1946) or to the fact that the husband is heterozygous is not finally decided. Evidence from one family will be presented later (page 102) to show that in some cases at least the number of pregnancies required to produce Rh sensitisation depends on the husband's genotype, and also on the A.B.O. blood group of the foetus.

PARTITIONS FOLLOWING KNOWN CASES OF HAEMOLYTIC DISEASE OF THE NEWBORN.

In the follow-up of the families concerned in the investigation details of further pregnancies were obtained in thirty-five. In these there had been 46 further partitions and the results are shown in Table VI.

TABLE VI. FURTHER PARTITIONS IN 35 FAMILIES

NATURE OF PARTITION	NUMBER
Normal Infant	11
"Affected Infant"	20
Stillbirths	11
Abortions	4

The eleven normal infants occurred in nine families so that on these figures it may be said that only one quarter of the further partitions are normal - and only about one quarter of the families concerned produced a normal child.

The usual explanation of the occurrence of a normal child after an affected one is that the father is heterozygous Rh+ve and that the infant is Rh-ve. This was so in the six cases tested in this series. On the other hand two of the children in one family were Rh+ve and were born after a very definite case of icterus gravis with severe anaemia. The mother was O Rh-ve (rr:cde/cde) and her serum contained anti Rh agglutinins. All three infants - including the affected one - were Rh+ve (R,r:CDe/cde). The mother was Gp O - the affected infant was of the same

group but the unaffected ones were both Gp A. The significence of this family will be discussed more fully later (p.107).

PREVIOUS BLOOD TRANSFUSION AS A CAUSE OF MATERNAL SENSITISATION

It has been recommended (Levine 1945c) that no Rh-ve female should ever receive a blood transfusion of Rh+ ve blood because of the inherent danger of her becoming sensitised to the 'Rh factor' and producing infants suffering from haemolytic disease of the newborn later on in life. Levine (1945c) states that the most severe forms of the disease tend to occur when sensitisation has been produced by previous blood transfusion. The truest evidence that this can occur is given by the occurrence of haemolytic disease in a firstborn infant after the mother has, at one time, received a blood transfusion. If transfusion of all Rh-ve females with only Rh-ve blood is adopted as a general practice it will involve the use of very much larger amount of this type of blood - which is by no means plentiful. It is felt therefore that however correct this may be as an ideal measure - the problem must be considered as a whole and the incidence of the disease following blood transfusion should be estimated. This has been studied in this series during the course of the personally studied cases and during the follow-up on previous cases. Of 83 mothers to whom the definite question

"Have you ever had a blood transfusion?" was put - only five gave a positive reply. In all five, at least one previous pregnancy had also occurred. Details of the five cases are given in Table VII.

TABLE VII. PARTITIONS OF 5 MOTHERS POSSIBLY SENSITISED BY BLOOD TRANSFUSION

1st PARTITION	2nd PARTITION	3rd PARTITION	4th PARTITION	5th PARTITION	REMARKS
AW:NJ	AW:NJ	AW:NJ	AW:NJ	Patient	PPH after 4th: Blood transfusions given at that time
AW:NJ	AW:NJ	Twin Misc. at 6/12	AW - J at birth-recovered	Patient	Two blood transfusions after 3rd partition
AW:NJ	AW:NJ	AW:NJ	Patient	Abortion at 3/12	Had blood transfusions after 2nd & 5th partitions
AW:NJ	Died 2 days after birth -? cause.	Patient			Mother anaemic - had two transfusions 3/12 before birth of patient
AW:NJ	AW:NJ	Died 1 yr. - BR.PN.	S.B.	Patient	Transfusion after 3rd partition

N.J. = No jaundice

All 5 mothers had at least two pregnancies before the birth of the affected child and before the blood transfusion. It is very possible therefore that these pregnancies had acted as sensitising agents rather than the blood transfusion. In the case of Mrs. S. the history of her second partition is very suggestive that this child also had been affected and this was before she had ever received a blood transfusion. In the two families tested the infant was Rh+ve and the mother was Rh-ve with weak anti Rh agglutinins.

In no case therefore can blood transfusion be definitely incriminated and although from the published reports such sensitisation does definitely occur - it is suggested that the numbers of cases in which this occurs makes it unnecessary to Rh test all females before they are given a blood transfusion. This statement does not apply of course to women who are to be transfused during a pregnancy and it is considered that in order to prevent transfusion accidents and anticipate the occurrence of haemolytic disease all women should have their Rh testing done at antenatal clinics. Before the childbearing period however it is considered that routine testing, although an excellent procedure is not yet a practical one, in view of the difficulties of staff, supplies of serum, etc. It should, however, be generally adopted as soon as these difficulties have been overcome.

BLOOD LEVELS ON ADMISSION

Haemoglobin levels were for the most part estimated in a Sahli haemoglobinometer. A slightly high reading is liable to be obtained by this method when the serum is jaundiced. Although this difference is slight in the majority of cases it is felt that the red cell count gives a more accurate idea of the extent of red cell destruction. The average red cell counts in the icterus gravis and congenital haemolytic anaemia groups on admission are shown in Table VIII.

TABLE VIII. AVERAGE RED CELL COUNT ON ADMISSION

TYPE	NO. OF CASES	AVERAGE RBC COUNT	RANGE
Icterus Gravis	126	2,350,000/ cu mm.	6,400,000 - 500,000
Haemolytic Anaemia	28	1,800,000/ cu mm.	3,600,000 - 500,000

It is rather surprising to find that the average counts only differ by 500,000/cu mm since it is a clinical "impression" that the anaemic type of case has usually got a considerably lower blood level than the jaundiced type. However cases were not always admitted so early in the disease as they are now and this is a probable explanation of the low counts obtained in the icterus gravis group. Of the 31 cases admitted in 1946 the average red cell count in the 23 cases of icterus gravis was 3,700,000/ cu mm whilst in the 8 of haemolytic anaemia it was 1,700,000/ cumm.

The difference is thus now more marked and is probably associated with the earlier admission of the cases (p. 84)

SPLENOMEGALY, BILURIA AND UROBILINURIA

These three clinical signs will be considered together although only two of them are in any way connected. It was considered easier to record them in tabular form together since in each case it is the frequency of the sign which is of interest. The results are shown in Table IX.

TABLE IX. SPLENOMEGALY, BILURIA AND UROBILINURIA IN HAEMOLYTIC DISEASE OF THE NEWBORN

	ICTERUS GRAVIS			HAEMOLYTIC ANAEMIA		
SIGN	RECORDED IN	PRESENT IN	% PRESENT	RECORDED IN	PRESENT IN	% PRESENT
Splenomegaly	132 Cases	103 Cases	78.0	23 Cases	16	69.5
Biluria	80 Cases	46 Cases	57.5	12 Cases	Nil	0
Urobilinuria	49 Cases	30 Cases	61.2	9 Cases	3	33.3

Splenomegaly is seen to have been present in 78 per cent of the cases of icterus gravis. The splenic size was recorded in all the case records of this condition. Enlargement was taken to be present when the spleen was felt at any distance below the left costal margin. When it was noted to be palpable but not below the costal margin this was not taken as enlargement. Potter (1943a) stressed the great importance of the splenic size in the clinical

diagnosis and these figures bear out her statements. This is especially so when it is realised that palpability of the spleen is more likely to pass unnoticed than to be noted when absent. The records were of course compiled by many different observers and it is possible that the figures may be even a little higher than this - they certainly will not be lower. It is in keeping with the generally milder course of the disease that splenomegaly was present in a smaller percentage (69.5) of the cases of haemolytic anaemia although even in that condition it is a sign of the greatest importance.

Biluria was present only in the icterus gravis type as would be expected. That it was only present in 57.5 per cent of cases is due to the fact that many of the older infants had passed the "obstructive" stage of the disease before admission. Most cases of icterus gravis will have biluria in the early stages of the disease - an important clinical point which immediately differentiates the condition from severe physiological jaundice.

Urobilinuria was present in about two thirds of the cases of icterus gravis and one third of the cases of haemolytic anaemia. This is also therefore a sign of some importance in the diagnosis of excessive blood destruction in these conditions.

Reverting for a moment to splenomegaly it is a point of interest that of the 29 cases of icterus gravis in which no splenic enlargement was found, 11 died. It is not therefore a sign of good prognosis.

HAEMORRHAGIC DIATHESIS

Fifteen of the 160 cases of haemolytic disease of the newborn showed some form of visible haemorrhages. One of these occurred in a haemolytic anaemia where two siblings (also included in the series) had similar manifestations but came into the icterus gravis category. The cases in the latter group showed the following haemorrhagic manifestations :-

Purpuric eruption	- 11 cases
Subconjunctival haemorrhages	- 3 cases
Melaena	- 2 cases
Haematemesis	- 1 case

Autopsy reports showed the presence of cerebral haemorrhage in 6 of 52 autopsies performed. Three of the six had shown some form of visible haemorrhage during life.

The prothrombin time was estimated in 14 cases of haemolytic disease of the newborn - the results are shown in Table X. The prothrombin index is arrived at by comparison of the infant's prothrombin time with that of a control and expressing it as a percentage of normal. Thus if the control prothrombin time is 12 secs and the infant's is 24 secs - the prothrombin index is 50% (of normal). The 'control' however is always an older child whose veins are reasonably accessible. In view of this difference in age, cases other than the ones with a haemorrhagic tendency are included in Table X and it is seen that many of the infants have perfectly 'normal' times. Others however, e.g. No. 5,

had prolonged prothrombin times without any evidence of external or internal haemorrhages.

TABLE X. PROTHROMBIN INDICES IN HAEMOLYTIC DISEASE OF THE NEWBORN.

NO.	TYPE	P.I.	HAEMORRHAGIC SYMPTOMS	REMARKS
1	IGN	70%	Purpura	Recovered
2	IGN	60%	Melaena	Recovered
3	IGN	45.4%	Purpura	Died aged 2 days
4	IGN	100%	Nil	Received Vit K before admission. Recovered
5	IGN	50%	"	Prolonged jaundice Recovered.
6	CHA	100%	"	Recovered
7	IGN	125%	"	Died aged 2 days
8	IGN	107%	"	Recovered
9	CHA	88.9%	"	Recovered
10	IGN	31.0%	"	Died aged 6/12 ? Cirrhosis of liver
11	IGN	96%	"	Died aged 3/12 - jaundice clear
12	IGN	92%	"	Recovered: mentally defective
13	IGN	50%	"	Died aged 3 days
14	IGN	70%	"	Died aged 3 days

It is seen from the table however that prothrombin times of 70% or under occurred exclusively in those cases showing :

1) Haemorrhagic symptoms
or 2) Prolonged jaundice
or 3) Death within 3 days

One case however which died on the second day of life had a prothrombin index of 125%.

No definite conclusions can therefore be drawn from these figures: low prothrombin times can however occur in haemolytic disease of the newborn and <u>may</u> be associated with

any of the three factors mentioned in the previous paragraph

NON PROTEIN NITROGEN.

There is some similarity between icterus gravis and an incompatible blood transfusion, in which condition jaundice does not appear for some time, haemorrhagic symptoms may occur and finally death may result (after an interval of some 7-10 days) from uraemia. The haemolytic theory of the causation of icterus gravis would fit in quite well with the idea of it being a disease similar in nature to an incompatible transfusion. It was decided therefore to have the non protein nitrogen estimated in haemolytic disease of the newborn and in eleven cases it was considered possible to remove sufficient blood without detriment to the child. The results are summarised in Table XI.

TABLE XI. NON PROTEIN NITROGEN IN HAEMOLYTIC DISEASE OF THE NEWBORN

No.	TYPE	NPN - mg.%	REMARKS
1	IGN	22.9	Lived
2	CHA	36.2	Lived
3	IGN	33.3	Lived
4	IGN	33.3	Died
5	IGN	24.5	Died
6	IGN	32.1	Lived
7	CHA	68.4	Lived
8	IGN	24.1	Lived
9	IGN	38.5	Died
10	CHA	32.1	Lived
11	IGN	83.3	Lived

Only two cases had abnormally high figures - both recovered completely. The three cases which died all had blood N.P.N.'s within normal limits. There is no evidence therefore that uraemia (renal or extrarenal) occurs in haemolytic disease of the newborn.

SERUM PROTEINS.

Serum proteins have been estimated in ten cases one of which is not included in the series. This case was a sibling of one already included. It was delivered before term by caeserean section since it was known (Prof. D.F. Cappell) that the father was homozygous R1R1. The infant was very nearly a hydrops foetalis and only lived 10 hours in spite of immediate blood transfusion. The diagnosis was confirmed at autopsy. The result of the serum proteins, in the 10 cases are shown in Table XII.

TABLE XII. SERUM PROTEINS IN HAEMOLYTIC DISEASE OF THE NEWBORN.

TYPE	T.S.P. gms per cent	ALB gms per cent	GLOB gms per cent	ALB/GLOB RATIO	REMARKS
IGN	9.01	-	-	-	Died
IGN	4.54	2.47	2.07	1.14	Lived
CHA	6.98	4.05	2.93	1.38	Lived
IGN	5.15	3.17	2.50	1.27	Lived
HF	4.13	2.21	1.92	1.15	Died
IGN	5.11	3.94	1.17	3.36	Died
IGN	4.56	-	-	-	Lived
CHA	5.09	3.72	1.37	2.71	Lived
IGN	4.28	3.04	1.24	2.45	Lived
IGN	4.10	-	-	-	Died

Total serum proteins are normally lower in the newborn than in later life but readings of 4.1 gms. per cent are below the lowest limit of normality (4.5 gms. per cent). The two cases here where this occurred (5 & 10) were both seriously ill infants and both died. One was the hydropic infant just referred to and the other was a very severely affected twin - its sibling also dying. Unfortunately differential albumen and globulin readings could not be done in case 10 but in the hydrops the albumen-globulin ratio was only 1.15-1. A similar ratio was also obtained in case 2 where there was no oedema but evidence of liver dysfunction was indicated by a persistent jaundice - (This is the case also shown as Case 5 in Table X with a prothrombin index of 50%).

There is therefore a <u>suggestion</u> that low serum proteins with reduction in the albumen globulin ratio may be responsible for the oedema in some severe cases of haemolytic disease. In others it may indicate disturbed liver function not sufficient to cause clinical evidence of oedema. The fact that this combination can occur suggests that it is <u>not</u> the oedema which causes the low serum proteins but rather the low serum proteins which are responsible for the oedema. The whole matter however requires much fuller investigation in obstetric units where true cases of hydrops foetalis are encountered.

MORTALITY RATE

In estimation of mortality rate it is not only necessary to discover the death of the child but often the decision has to be made as to whether the child died from the disease process itself or from intercurrent disease. With the cases which are seen personally this is usually easy but when only the case record is available it is sometimes more difficult. Thus in one case included in this series the infant while still jaundice developed diarrhoea and B. Dysenteriae (Flexner) was cultured from the stools. The infant was transferred to an infectious diseases hospital and died there one week later. Dysentery is not a usual complication of haemolytic disease and it might be quite fair to exclude such cases as a death from icterus gravis. On the other hand some cases have been included as recovered who were well on dismissal from hospital, but never reported as out patients and could not be traced in the "follow up". It is possible that some of these may have died. It has therefore been decided that any case dying with some manifestation of haemolytic disease still present will be included as a death. It is felt by this means that a balancing effect will be obtained and a truer estimate of the actual mortality rate can be made.

The mortality rate since the use of Rh-ve blood was instituted in the hospital has been estimated and this has

been compared with the mortality rate when ordinary 'bank' or paternal blood was used. The rates in icterus gravis and in haemolytic anaemia have been kept separate owing to the considerable difference between them. The total number of cases, deaths and mortality rate in each condition is shown in Table XIII.

TABLE XIII. MORTALITY RATE IN 160 CASES OF HAEMOLYTIC DISEASE OF THE NEWBORN

	1 ICTERUS GRAVIS			2 HAEMOLYTIC ANAEMIA			1 & 2 HAEMOLYTIC DISEASE OF NEWBORN		
	CASES	DEATHS	M.R.%	CASES	DEATHS	M.R.%	CASES	DEATHS	M.R.%
Before Rh	71	43	60.5	8	2	25	79	45	56.9
After Rh	61	26	42.6	20	1	5	81	27	33.3

A considerable reduction in mortality has seen to have occurred in association with the use of Rh-ve blood. These figures however include cases which died before blood transfusion could be performed (one was actually dead on admission the diagnosis being made at autopsy) so that in order to get a clearer view only those cases which received at least one blood transfusion should be considered. There were twelve cases 'before Rh' and nine 'after' who died without being transfused. When these figures have been deducted the mortality rates in the transfused cases only are as shown in Table XIV.

TABLE XIV. MORTALITY RATE IN HAEMOLYTIC DISEASE OF THE NEWBORN IN 193 TRANSFUSED CASES

	1 ICTERUS GRAVIS			2 HAEMOLYTIC ANAEMIA			1 & 2 HAEMOLYTIC DISEASE OF THE NEWBORN		
	CASES	DEATHS	M.R.%	CASES	DEATHS	M.R.%	CASES	DEATHS	M.R.%
"Before Rh"	59	31	52.5	8	2	25	67	33	49.2
"After Rh"	52	17	32.7	20	1	5	72	18	25.0

The overall mortality is seen to have been halved but much of this is due to the very low mortality in haemolytic anaemia. A drop of 20 per cent has occurred in the icterus gravis group but from these figures almost one third of the transfused cases are still dying. These figures are slightly different to those reported elsewhere (Gordon 1947). This is due to the fact that in the present series some of the previous cases had to be discarded since they did not satisfactorily fulfil the necessary criteria although they had been diagnosed as 'erythroblastosis' at the time. The mortality rate given in my previous communication in the icterus gravis group was 25 per cent so it may safely be said that the correct figure lies somewhere between 25 and 33 per cent. There are still a considerable number of these cases dying therefore and it is certain that the use of Rh-ve blood has not revolutionised the treatment of these cases in the way in which it was at first expected.

The one case of haemolytic anaemia which has died since Rh-ve blood was used (p. 56) did so under peculiar circumstances. The infant was very anaemic (Hb 46 per cent)

but not very ill. She received 70 c.c. of Rh-ve blood slowly into a scalp vein and collapsed at the end of the transfusion, dying a short time later. At autopsy a dilated right ventricle was found and the liver microscopically showed intense congestion of the blood vessels with many haemorrhages. Although this is usually considered to be caused by too rapid transfusion it is unlikely to be the cause here since it is not an easy matter to put blood into a scalp vein rapidly. Also, in another autopsy this intense hepatic congestion with haemorrhages was seen, in an infant suffering from icterus gravis who died before blood transfusion could be performed. In the case of haemolytic anaemia which died no further investigations of the donor's blood had been recorded.

The drop in mortality rate has so far been shown to have been associated with the use of Rh-ve blood. This is not necessarily to say that it has been caused by it. For example the great publicity which the 'Rh factor' has received has brought the disease process very much to the fore in the minds of all practitioners and as a result cases may be being seen now which previously remained at home and recovered. That this may be so is shown from the considerably larger number of patients admitted in more recent years (Table I). It may be also that transfusion therapy is more actively carried out nowadays or that 'secondary' infections have become a less frequent accompaniment of

death owing to the use of sulphonamides and penicillin. In an effort to evaluate the other factors, besides the use of Rh-ve blood, which may have influenced the mortality rate the following factors were therefore more fully investigated.

1. Amount of blood transfused
2. Age of patient on admission and at death
3. Autopsy findings

In addition the variation in mortality in the familial cases (i.e. where a previous sibling had also been affected) and the mortality between the sexes have also been estimated. In all these matters the conditions obtaining in the icterus gravis cases only have been studied. The mortality rate in haemolytic anaemia should be nil.

1. AMOUNT OF BLOOD TRANSFUSED

Table XV clearly demonstrates that the number of transfusions and the total amount of blood given, per patient was actually more 'before Rh' that it was 'after'.

TABLE XV. AMOUNT OF BLOOD TRANSFUSED TO CASES OF ICTERUS GRAVIS

	BEFORE Rh	AFTER Rh
Average Number of Transfusions per Case	2.3	1.4
Average Volume of Blood Transfused per Case	195 c.c.	154 c.c.

There is no evidence, therefore, that the reduction in mortality has been connected with the more 'energetic' use of blood transfusion. The fact that more blood was

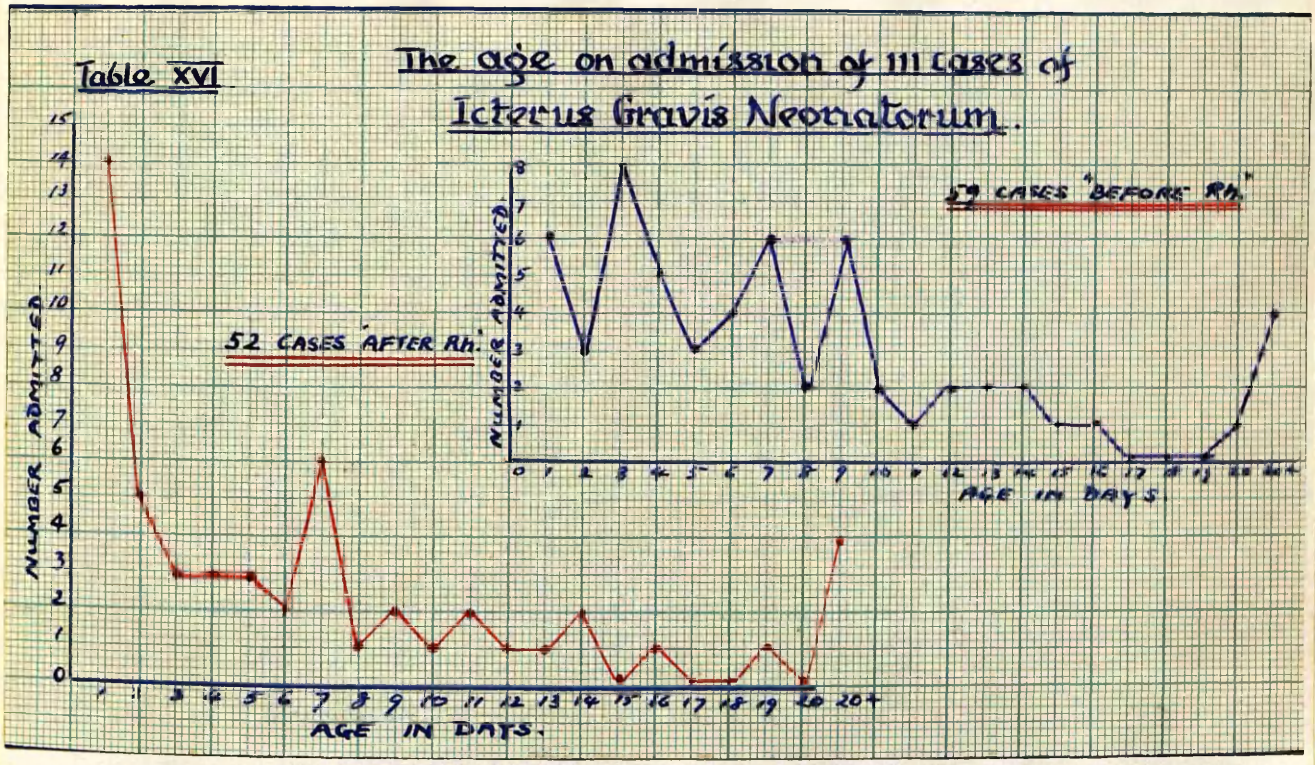
given 'before Rh' is probably due to the more protracted course which was taken by many of the cases at that time. Much of the blood given then was quite quickly destroyed so that more frequent transfusions were required. Although the cases 'after Rh' received less blood than before none appear to have died as the direct result of anaemia. The 17 deaths which occurred 'after Rh' received between them 22 blood transfusions totalling 2,105 c.c. of blood. This constitutes an average blood transfusion of 95 c.c. which is usually considered quite adequate if the standard amounts of 15 c.c. per pound of body weight is taken as a guide. Each case received on an average 123.8 c.c. of blood before death which is certainly sufficient to keep alive an infant with a blood volume of probably less than three times this amount. That these cases did not die of severe anaemia is shown by the fact that in eight of the cases which died 'after Rh' the last haemoglobin estimations before death average 97 per cent: the average red cell count in the remaining nine was 2.8 million per cu. mm: the three lowest levels in this group had received a further transfusion before death. The deaths therefore are not due to the direct results of anaemia and the reduction in mortality rate is not due to more and bigger transfusions.

2. AGE OF PATIENT ON ADMISSION AND AT DEATH

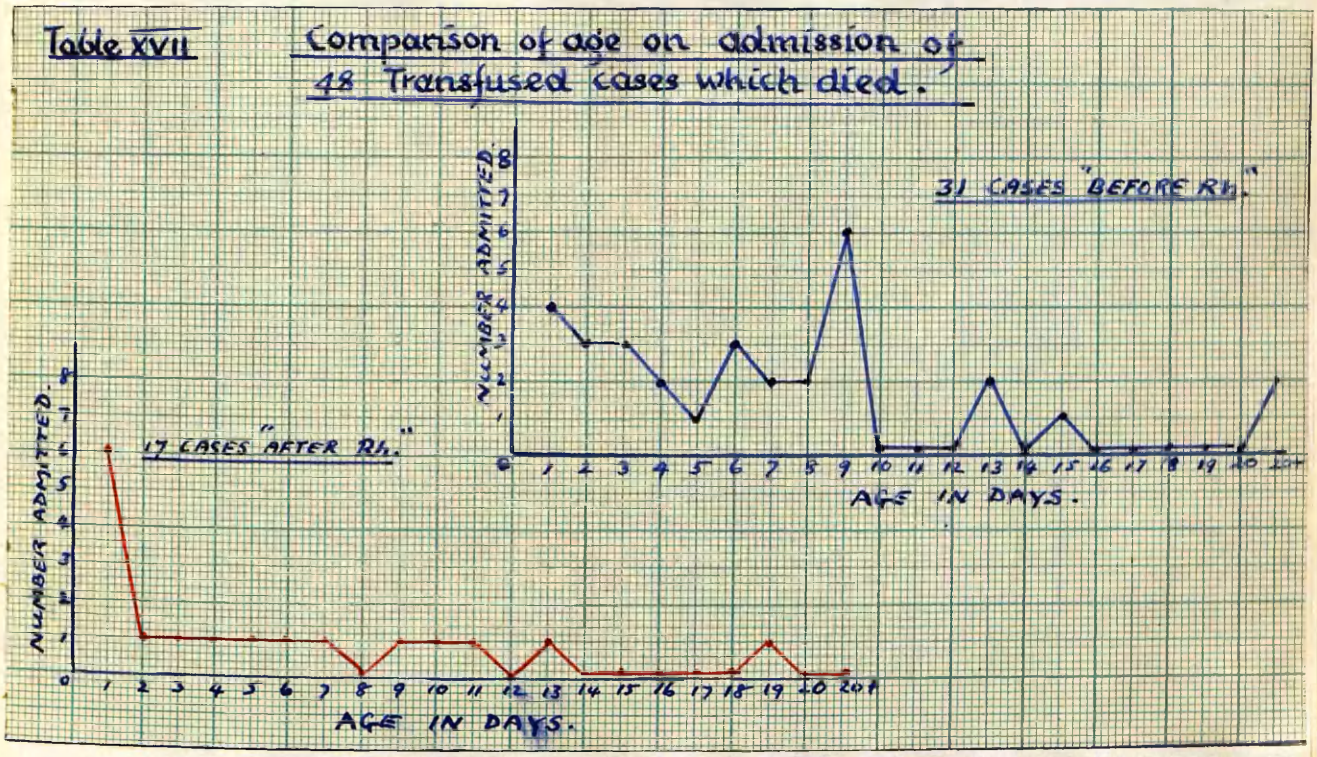
This is a matter of considerable importance because it is held by some writers that prognosis is considerably

improved the earlier the case is diagnosed and treated. It is probable that, with the generally wide interest now being taken in the disease, cases are being admitted earlier. If this is so it may well have had a considerable effect on the mortality rate, and might even be responsible for the entire lowering in the rate which has already been recorded.

The fact that cases are being admitted to hospital earlier is seen from Table XVI. 'Before' Rh the peak day for admission was the third whereas 'after Rh' the 'peak'-day is the first. (Any case admitted within 24 hours of delivery is included as having been admitted on the first day). It is seen also from this chart that whereas 'before Rh' 17 out of a total of 59 cases (less than one third) were admitted on the third day or earlier, 'after Rh' 23 out of 52 (nearly one half) were admitted within that period. After the third day the charts follow a very similar pattern if allowances be made for the differences in numbers. It is surprising that even in recent years four cases were admitted when aged 20 days or more.



There is no doubt therefore that the cases are arriving in hospital considerably earlier now than previously, but one cannot conclude from this alone that the earlier admission has affected the mortality rate to such a considerable extent. It is possible that more severe cases are reaching hospital now which previously would have died outside. To estimate the effect of early admission on the numbers dying Table XVII has been constructed. This table shows the age on admission of each infant who died 'before' and 'after' Rh.



The chart in Table XVII must be considered always in relation to that in Table XVI. If this is done it is seen that a decrease in mortality has occurred at all stages but if anything it is greater in the 'older' ages. For instance 'before' Rh 29 cases were admitted in the first 6 days of life with 16 deaths (mortality rate 55.1 per cent) whilst 'after Rh' 31 cases were admitted within this age period with 11 deaths (mortality rate 38.7 per cent). For those admitted aged more than 6 days however the figures are,

30 cases 'before' with 15 deaths (M.R. 50 per cent) and 21 cases 'after' with 6 deaths (M.R. 28.6 per cent). The main decrease in mortality has therefore been in the 'older' age group in which it has been almost halved. The numbers involved are, however, very small but it does seem clear that not all of the reduction in mortality is due to earlier admission to hospital although this may have played some part. If the cases admitted 'after Rh' on the first day of life which died before transfusion could be given, are included it is found that the mortality rate is 50% (nine deaths out of 18 admissions). This confirms the fact that severe cases are now reaching hospital who previously would have died outside and it may be concluded therefore that most of the reduction is due to the use of Rh-ve blood especially in the more prolonged case. Many of the latter have probably reached the severely anaemic stage of the disease and it is highly probable that it is this phase which reacts so well to the use of Rh-ve blood as compared to Rh+ve blood. It is almost certain that blood transfusion is only effective in the restoration of the anaemic state to normal and in those early cases which die deeply jaundiced, but not severely anaemic, blood transfusion has little or no effect even when Rh-ve blood is used. The question of exsanguination transfusion as a method of treatment will be discussed later (p. 129)

3. AUTOPSY FINDINGS

The importance of this factor is to discover whether any other condition has become less prevalent since the introduction of Rh-ve blood which might explain the reduction in mortality.

Records are available of 52 autopsies (including the ones personally witnessed). Permission for autopsy was not permitted in nine cases "before Rh" and 8 "after". The 52 autopsies therefore consist of 34 "before Rh" and 18 from "after".

Apart from the jaundice, hepatomegaly and splenomegaly the macroscopic pathological lesions which were found are shown in Table XVIII.

TABLE XVIII. MACROSCOPIC PATHOLOGICAL LESIONS IN 52 AUTOPSIES

LESION	CASES BEFORE RH	CASES AFTER RH
Broncho Pneumonia	5	11
Haemorrhage		
Cerebral	6	0
Splenic	1	0
Gastro Enteritis	1	2
Kernicterus	9	6
No additional lesions	17	3

The most striking conclusion to be drawn from the table is that the use of Rh-ve blood has not prevented the occurrence of kernicterus. Three of the 6 cases of kernicterus, however, had died before transfusion could be given but the three others were all transfused early (two

within 24 hours of birth and the others on the third day of life). The diagnosis of kernicterus at autopsy in these cases was based on bile staining of the basal nuclei obvious to the naked eye. The incidence of the condition pathologically is 15 cases out of 52 autopsies, i.e. 28.8%. This is a higher incidence than occurs in the cases which recover (p. 93) so it is concluded that either the presence of kernicterus predisposes to death or that kernicterus can occur clinically without permanent sequelae. The former possibility is more likely.

In eleven of the 18 autopsies performed 'after Rh' broncho pneumonia was found pathologically. These figures do not include conditions referred to in the report as "terminal broncho pneumonia" or "basal congestion with some consolidation". In all eleven the pathologist considered there was a definite and often widespread broncho pneumonic process present. In spite of this it is not considered that this condition plays a very major part in the deaths of these infants but it does draw attention to their susceptibility to infection. For example, in twin affected infants who were personally seen, and who died, the infection may easily have been aided by an excessive amount of travelling and waiting in the cold which was imposed upon them before their admission to hospital. Clinically it was surprising that they died (at the age of 2 and 3 days respectively) - at autopsy

however both had a massive bilateral broncho pneumonia. It is considered that if conditions like this can be prevented the mortality rate can be reduced further.

The presence of 7 cases of internal haemorrhage before Rh and none after is striking: it is considered to be associated with the prevention of the prolonged type of the disease in which haemorrhage of this type was liable to be the final outcome.

It will be convenient at this point to mention briefly the question of 'liver damage' in icterus gravis. Sections of liver, stained by haemotoxylin and eosin were available for examination from twelve of the autopsies. In none of these was any evidence of hepatic cell necrosis or even cloudy swelling detected. Occasionally some vacuolation was seen but this was the only degenerative process observed. "Erythroblastosis" was variable - mainly dependant on the age of the patient, being more marked in the younger infants. Bile staining of the liver cells and bile thrombi in the hepatic ducts were seen in 4 cases but in none was there any evidence of hepatic fibrosis to be seen. The picture was much more one of an obstructive jaundice than of an hepatitis. In all the 'severe' cases there was great separation of the columns of liver cells and in some the liver lobule appeared quite disintegrated. It was in these that 'erythroblastosis' was most marked but even in them the liver cells themselves seemed quite normal in appearance.

The significance of the hepatic function in icterus gravis will be discussed later (p. 138)

4. PREVIOUSLY AFFECTED SIBLINGS

In 19 families 'before Rh' and 20 'after' - a previously affected infant was known to have occurred or there was a very strongly suggestive history of its occurrence. Of the 19 cases 'before', 13 died and of the 20 cases 'after', 10 died. The difference in mortality rate is not very striking. It is well recognised that in many families successive partitions are more severely affected and this may explain the still very high death rate in those cases with a familial history. As far as the comparison of before and after Rh is concerned, however, there is almost an equal number in each group who had a familial history and the severity of the disease was probably equal in the two groups.

5. MORTALITY RATE IN THE SEXES

This paragraph is only inserted to show that the figures in this series disagree with those of Halperin et al (1945) who found that the mortality rate in males was 5:1 as compared to females. My figures are shown in Table XIX.

TABLE XIX. MORTALITY RATE AMONGST SEXES IN HAEMOLYTIC DISEASE OF THE NEWBORN

SEX	NO.	DEATHS	MORT. RATE
Males	86	50	58.1%
Females	46	20	43.4%

Although the deaths in males as compared to females is 2.5:1 the incidence is also almost 2:1 and as was seen earlier this is thought unlikely to be of great importance.

FOLLOW UP

A follow up on all the cases of haemolytic disease of the newborn which were admitted from 1934 to 1945 was attempted. A considerable number of the families could not be traced and others lived too far away to make the special journey. One mother is known to have died in the intervening years and no doubt this had also occurred in other families. However 54 of the 118 mothers concerned were contacted but two of these refused to give blood for examination. In each family either the affected child, another child, or the husband was also asked to come for blood examination and one or other was forthcoming in almost all of them.

When the mother was seen the family history recorded on the infant's case sheet was confirmed and further partitions recorded (the information collected on this point has already been noted Table VI). The mothers were all specifically asked whether they had ever had a blood transfusion (Table VII). If the child was the affected child a full history was taken of its condition since last seen with special reference to mental development. From the history and examination of the child a decision was

made as to whether it was mentally defective or not. This was by no means a difficult decision to arrive at since no attempt was made to distinguish minor grades of backwardness. Only frank mental deficiency was looked for, with or without choreoathetoid movements of the arms and face. So that the picture could be as clear as possible no infant under 1 year of age has been included in the figures for the follow up of kernicterus.

In all cases the teeth were examined and the size of the liver and spleen determined. The child's general condition was noted.

The family history, and the examination of the child having been completed, 10 c.c. of venous blood were removed from the mother with a dry sterile syringe and put into a dry sterile test tube. From this serum was obtained and from the remaining clot and serum saline suspensions of cells were made. From the children and the fathers cell suspensions in saline only were obtained. Blood was obtained in exactly the same way from the mother and child in the cases which occurred during the period of study. The results of the serological examinations will be presented shortly - meanwhile the results of the physical and mental condition of the child will be considered.

KERNICTERUS

In the 54 families interviewed, there were 38 infants still living at the age of one year or more. 31 of these

had been classified as icterus gravis and 7 as congenital haemolytic anaemia. The latter can be easily and quickly considered since all seven were perfectly normal healthy children without any suggestion of mental deficiency or cirrhoses of the liver. Of the 31 who had suffered from icterus gravis however four were mentally defective. Two of the mentally defective children were not actually seen by me. One of these was in a home for mental defectives and the other had been seen by Prof. G.B. Fleming when aged 2½ years and had been noted at that time to be mentally deficient with choreoathetoid movements. Of the two seen by me neither had choreoathetoid movements but both had been very late in all their 'developmental milestones' and although they could both walk neither could say any more than an occasional word and both behaved in an exceedingly bizarre manner. Both mothers appreciated only too well that their children were 'mentally backward'.

GREEN TEETH

Ellis (1938) reported the occurrence of bile stained teeth in a child who had suffered from icterus gravis in infancy. This occurred in 2 of the 31 cases reported here. One of the children was mentally defective but the other was not. It is concluded therefore that, although the original staining originates from the neonatal jaundice, damage to the basal nuclei and undeveloped teeth do not necessarily occur together. The teeth of the other children seen were perfectly normal.

HEPATOMEGALY AND SPLENOMEGALY

None of the 36 infants seen showed any enlargement of liver or spleen.

GENERAL CONDITION

The general physical condition of all the children seen was excellent. There was no undue incidence of disease of any kind amongst them and the only abnormalities found were mental deficiency and green teeth.

SEROLOGY

At the beginning of the study sera were given to me by the kindness of Prof. D.F. Cappell and Prof. Todd of the Blood Transfusion service. This enabled me to carry on until suitable test sera were obtained from my own resources. As these were forthcoming they were used at the same time as the previous, trusted, ones and an estimate of the strength and uniformity of their reactions was thereby obtained. All the sera used contained anti-D (Rh_o) and all reactions which will be reported on are simply Rh+ve or Rh-ve depending on whether the red cells reacted with these sera or not. It was impossible to genotype owing to absence of the suitable sera and the only success in the search for the rarer sera was to find two containing anti-E (anti Rh") as well as the anti-D (anti Rh_o)

METHODS

Rh testing of cells - the method used was that described by

Taylor (1943) which is the standard method used by all workers in this country. The cell suspensions were made in saline and one drop only of serum and cell suspension were used. The tubes were placed in a wooden rack and when ready the rack was incubated in dry heat ($37^{\circ}C$). for at least one hour. The deposits were read macroscopically and any showing a doubtful or negative reaction were examined microscopically as well. This latter reading was taken as the final decision. A separate pipette was kept for each test serum and washing was carried out in frequently changed distilled water. Three test sera were used in every estimation and a control of + ve and -ve cells was included in each series of tests performed.

TEST FOR AGGLUTINATING ANTIBODY

In this the method described by Taylor (1943) was also used. The standard cells against which every serum was tested were my own - Gp. O R_1R_1: the sera were also tested for activity against all other Rh+ ve and Rh-ve cells of suitable blood group available at the time. Whenever possible the serum was tested against the cells of the child or husband.

The titre of the serum against R_1R_1 cells was also determined by making serial dilutions with normal saline - to a titre of $1/64$ as a routine. When necessary further dilutions were made to determine the exact titre of the antibodies.

The reactions obtained in this test are usually, of course, considerably weaker than in the testing of the red cells. In general most of the reactions required microscopic confirmation and in some of them agglutination was feeble. No positive reaction was however ever recorded unless some areas of distinct 'clumping' were seen. In many, however, the whole area of the sediment under review did not show uniform clumping.

TEST FOR BLOCKING (INCOMPLETE) ANTIBODY

The method of Wiener (1944b) was used routinely. The serum was put up with R_1R_1 cells in the usual way for one hour and then there was added a drop of a sufficiently powerful serum which would normally produce agglutination of the cells (the original drop of the serum being tested having been taken into account). The tube was re-shaken and set up again for another hour. A negative result at the end of this time was taken as indicating a positive reaction for blocking antibody. The titre of blocking antibody was estimated by making serial dilutions of the test serum at the beginning of the test and the last tube in which no agglutination had occurred at the end of the test represented the titre of the blocking antibody.

"CONGLUTINATION" TEST

This test was described by Wiener (1945b). It is exactly the same as the test for agglutinating antibody

except that the cells are suspended in plasma or serum instead of saline. In the tests performed here Group AB plasma was used as the diluent.

The difficulty with the 'conglutination' test is the occurrence of intense rouleaux formation. For this reason microscopic readings were not used since it was found impossible in many cases to determine whether 'clumping' was or was not present. For the test to be considered positive frank macroscopic clumping had to be present. Any result which was doubtful was considered to be negative.

RESULTS

Twelve of the families concerned had already had their Rh examinations done by Prof. D.F. Cappell before my work started. The results had been recorded in the case histories and I have included them in my figures. The results which will be recorded include the families traced in the follow up as well as those involved during the period of study.

MOTHER'S RH TESTING

Blood was obtained for testing from 81 mothers in all. The results are summarised in Table XX.

TABLE XX. BLOOD TYPES OF 81 MOTHERS OF AFFECTED INFANTS

	O	A	B	AB	Rh+ve	Rh-ve
Number	42	27	10	2	4	77
Percentage	51.9	33.3	12.3	2.5	4.9	95.1

In the Landsteiner blood groups there appears to be some excess of Group O mothers over Group A but since the numbers involved are small this is unlikely to be of importance. The percentage of Rh+ve mothers is somewhere in the region of the presently accepted figures (3-8%). The presence of this little group in every series of cases published has always been a considerable obstacle to the full appreciation of the aetiology of the disease. Only a very small proportion of them seem to be associated with a difference in genotype between mother and infant and even fewer of them show any maternal antibody. Some are possibly associated with an ABO group difference whereby the maternal serum agglutinates the infant's red cells. On the whole however their elucidation is somewhat obscure. In two of the four cases in this series in which the mother was Rh+ve, she was group O and the infant group A. In the other two however there was no such incompatibility. In none of the four was any agglutinating or blocking antibody detectable in the maternal serum. It is likely that with the fuller use of genotyping and more delicate tests for Rh sensitisation that the explanation of these cases will become clearer. It may be that the clinical findings in these cases will be found to differ from the recognised description of the disease but this was not so in any of the four cases in this investigation and they, by themselves, did not form a separate clinical group. One of the cases

was of the haemolytic anaemia type and was never at any time jaundiced: the other three were all cases of icterus gravis.

MATERNAL ANTIBODIES

Agglutinating or blocking antibodies were found in 64 of the 77 Rh-ve mothers. These were all active against R_1R_1 cells and also against other Rh+ve cells and were, therefore, probably of the type anti D (anti Rh_0). In thirteen sera no agglutinating antibodies were detected.

In September 1946 it was decided to determine the relative values of the agglutinating, 'conglutination' and 'blocking' test in the detection of anti Rh antibodies. At that time 93 sera were available, twenty one of which came from the mothers of infants suffering from a variety of conditions apart from haemolytic disease of the newborn. These were used as controls. The remaining 72 sera consisted of various test sera given to me and some of the sera obtained from the mothers in the present investigation. The cells against which all the sera were tested were group O R_1R_1 and the tests were all put up on the same day in the manner described under the three tests. All the sera had previously been tested for the presence of agglutinating antibodies and the results obtained when the sera were originally obtained have been included in Table XXI along with the results obtained in September 1946.

TABLE XXI. AGGLUTINATING, CONGLUTININ & BLOCKING REACTIONS OF 72 SERA

Source	No.	Agglutination Test +ve Before	Agglutination Test +ve Now	Conglutination Test +ve	Blocking Test +ve
Sera from mothers of affected infants	72	56	37	53	15
Controls	21	NIL	NIL	NIL	NIL

Before = when first obtained Now = September 1946.

None of the controls gave a positive reaction in any of the tests.

The figures given here bear no close relationship to the figures for +ve agglutinating reactions given at the beginning of this paragraph since not all of the latter sera were available for the present investigation. It is seen from Table XXI however that of the seventy two sera which might have contained agglutinating antibody, 56 had at one time done so and 37 still did. There were therefore 19 which had lost the power of agglutinating. Sixteen of this 19 appear to have given a positive conglutination test but this was not really the case. The actual number of "inactivated" sera giving a +ve conglutination test was thirteen and the other three giving a +ve reaction had never given a +ve agglutination reaction previously. In no case did a positive agglutination reaction occur without

there being also a positive 'conglutination' reaction. The 'conglutination' test does therefore appear to remain positive in a considerable proportion of sera in which the agglutination test has become negative. It was also frequently noted that when an agglutination test was weak the conglutination reaction was considerably stronger. On no occasion did the reverse occur. As has been noted the 'conglutination' test revealed sensitisation in three mothers in whom it had not previously been possible to demonstrate it.

The importance of the blocking test is not truly reflected in Table XXI since this test was only performed when a negative agglutination reaction had been obtained. The blocking test was really therefore positive in 15 out of 19 sera which at one time had contained agglutinating or blocking antibody. On only one occasion was a negative conglutination reaction obtained with a positive "blocking" test.

The conclusion drawn from the above investigation is that in the absence of agglutination the conglutination test is likely to demonstrate the presence of antibody in a few but by no means all cases. In this respect it is slightly better than the blocking test. It is considered likely however that the Coombs' indirect test (Coombs et al 1945a) will replace both 'conglutination' and 'blocking' tests since it is apparently (Mollison 1947) much more accurate and sensitive than either of the other two.

FATHERS

Blood from 22 fathers was obtained for testing. Eleven of them were group O and eleven group A. All were Rh+ ve. Only two fathers were genotyped and since they were of special interest the results are given in Table XXII along with the rest of each family. The genotyping was very kindly done for me by Prof. D.F. Cappell.

The interest of the families whose genotypes are given in Table XXII lies in the number of pregnancies which are required to produce iso-immunisation of the mother. In the families recorded here Mrs. D and Mrs. M were sisters and their husbands were uncle and nephew respectively. Mrs. D had six partitions (the second being twins) before producing her first affected child (included in the series). Mrs. M however had only two partitions before her affected child (also included in the series). Since then Mrs. M has had two further children and one stillbirth. Both of these children died shortly after birth the exact cause being unknown. It appears on the surface therefore that one sister was more easily sensitised than the other. The original genotyping however showed that Mr. D must be heterozygous Rh+ve (R2 r) since three of his children were Rh-ve (rr). If it is assumed that Mrs. M's first child was also of the type R_2 r it is seen that two Rh+ve children were required to produce sensitisation in each family. The assumption that the first child in family M must have

been R2 r was proved by Prof. Cappell by testing Mr. M's cells with some anti e (anti Hr") serum (Mourant 1945) This reaction was negative showing that Mr. M was of the genotype R_2R_2 (cDE/cDE) and therefore all his children must have been R2 r (cDE/cde) including the known affected one (3rd) which died.

TABLE XXII. GENOTYPES OF FAMILY D AND FAMILY M

FAMILY D		FAMILY M	
Mrs. D	Group O rr(cde/cde)	Mrs. M	Group O rr(cde/cde)
Mr. D	" " R_2r(cDE/cde)	Mr. M	" " R_2R_2/cDE/cDE)
1st child	" " rr(cde/cde)	1st child	- Dead
2nd "	" " rr(cde/cde)	2nd child	Group O R_2r(cDE/cde)
3rd "	" " R_2r(cDE/cde)	3rd "	(Patient) Died
4th "	" " R_2r(cDE/cde)	4th "	- Dead
5th "	" " rr(cde/cde)	5th "	- Dead
6th "	" " rr(cde/cde)	6th "	- Stillbirth
7th " (Patient)	" " R_2r(cDE/cde)		

Variations in ease of sensitisation are in some cases at least only superficial as the conditions in these two families clearly demonstrate. Although there was an apparent difference, exactly the same number of Rh+ ve pregnancies was required to sensitise both women.

INFANTS

64 affected infants were tested and all were found to be Rh+ ve. This number includes those cases seen personally as well as those traced in the follow-up. The result is in agreement with all other figures published on the subject.

MOTHER-INFANT COMBINATIONS

Cappell (1946) suggested that hetero-specific pregnancies might act as a deterrent to the Rh sensitisation of an Rh-ve mother. Wiener (1945 d) has shown that there was an increased incidence of 'compatible' matings in the parents of affected infants as compared to the population as a whole. Cappell's (1946) figures are even more striking, however, since in 90 pregnancies, only 8 were hetero-specific with regard to the ABO blood groups, whereas the expected number is about 18. The results obtained in the present investigation on this subject are shown in Table XXIII :-

TABLE XXIII. MOTHER-INFANT BLOOD GROUP COMBINATIONS IN 64 FAMILIES

		No. of families	Remark
Mother Group O -	Infant Group O	34	Mother Rh+ ve in both
" " O -	" " A	2	
" " O -	" " B	0	
" " A -	" " O	7	
" " A -	" " A	15	
" " B -	" " O	2	
" " B -	" " B	3	
" " AB -	" " AB	1	
		64	

In only two of the 64 families was there incompatibility of blood group between the mother and her affected infant. In both of these, the mother was Rh+ ve. In the 62 Rh-ve mothers, therefore, ABO compatibility was present in all.

In an attempt to determine the accuracy of this finding on a larger series of cases, a search of the literature has revealed four fairly full investigations in which blood groups of the affected families have been given (Race et al 1943: Boorman et al 1944: Broman 1944: Plaut et al 1945). In these papers also, an attempt has been made to indicate the accuracy of the diagnosis in each case and only those families, in whom it is considered likely that the infant definitely was affected, and in which the mother was Rh-ve, have been included in Table XXIV, where the results are summarised. Cappell's (1946) figures have not been included since it was thought likely that some of his cases had been previously included in the paper of Race et al (1943).

TABLE XXIV. BLOOD GROUPS IN 254 AFFECTED FAMILIES WITH RH-VE MOTHERS

	Race et al	Boorman et al	Broman	Plaut et al	Gordon	Total
Mother/Infant compatible	15	18	25	50	52	160
Mother/Father compatible	8	4	9	26	4	51
Mother Group AB	2	-	-	3	-	5
Mother Group A	7	3	-	9	8	27
						243
Mother/Infant incompatible	2	4	1	4	-	11
						254

In Table XXIV, the 'mother/father compatible' combination was recorded when, by the blood groups, it was impossible for any pregnancy to be hetero-specific, e.g. both A or both O. The Group A mothers have been included in the compatible group since it is only very rarely that a Group B or AB infant will be born to such a woman. In addition to the figures shown in the table, there were 34 families in which compatibility could not be ascertained since the only information available was that the mother was Group B or Group O, or that the mother/father combination could have produced a heterospecific pregnancy.

It can be concluded from the table therefore that, of 254 mother/infant combinations, only 11 were hetero-specific in regard to the ABO blood groups. This is an incidence of 1:23 as compared to the expected 1:5. There seems no doubt therefore that incompatibility in this direction between mother and infant is nearly five times less common in affected families in which the mother is Rh-ve than in the population as a whole. It can be concluded from this that when an Rh-ve woman has hetero-specific pregnancies, she is considerably less liable to become sensitized to her Rh+ ve foetus than if the pregnancy is homospecific.

In the Rh+ ve mothers from the same series of cases as those in Table XXIV, there were seven 'incompatibles' in 22 pregnancies; this is in keeping with the expected incidence.

A further step in the understanding of Rh sensitisation in general has been taken if these findings are confirmed by other workers since it explains to a certain extent why all Rh-ve women with Rh+ve husbands do not become sensitised. One family, which was discovered during the present investigation, tends to indicate that even once the mother has been sensitised, a further Rh+ve partition may remain unaffected if it is of an incompatible blood group to that of the mother. In this family (Family D) there were three full-time partitions from 1934 to 1936. All were apparently normal in the neonatal period. In 1938, a male child (J.D.) was delivered who was a very definite clinical case of haemolytic disease of the anaemic type (details are given in the Appendix, case 3). In 1940 and 1942, two further children were born who were perfectly normal and are now alive and very healthy. There have been no further pregnancies since - Mr. D having died in 1943. The Rh testing performed by myself showed Mrs. D to be Group O Rh-ve and her serum contained (in 1946) agglutinating antibody to a titre of 1/64. J.D., the affected child, was Group O Rh+ve and the two succeeding children were found to be Group A Rh+ve. In view of this surprising finding, fresh supplies of blood were obtained and submitted to Professor Cappell for genotyping. Mrs. D was found to be rr (cde/cde) and all three children were R_1r (CDe/cde). The mother was questioned still further about the neonatal

period of her last two children but denied that anything abnormal had occurred. It is indeed unlikely that both could have been affected and for the condition to have passed unnoticed. It must be concluded therefore that, in this case at least, the fact that the affected child was 'compatible' with the mother, and the unaffected were 'incompatible', indicates that this in some way prevented her last two children from developing the disease. In fact, the incompatibility acted as a protection to the child. The exact mechanism by which this might occur is not quite clear, since because the foetal red cells are agglutinated as soon as they cross the placenta, it does not necessarily mean that the antibody which is already in the maternal serum is prevented from passing to the foetus. The suggestion does remain however that a protective mechanism is at work.

DIFFERENTIAL DIAGNOSIS OF NEONATAL JAUNDICE

The accepted diseases which are said to produce jaundice shortly after birth and which therefore might be confused with icterus gravis are

1. Physiological jaundice
2. Congenital obliteration of the bile ducts
3. Congenital Syphilis
4. "Infection" - especially umbilical

During the period of study and during the investigation of the case records examples of the above conditions were encountered and Rh testing of the families concerned was carried out in some cases.

1. PHYSIOLOGICAL JAUNDICE

Three severe cases of physiological jaundice which might have been confused with icterus gravis were among the cases seen personally. The diagnosis in all three was based on the absence of anaemia and erythroblastaemia, the benign course, and the Rh testing. In two of the cases the jaundice appeared at one and two days respectively: in both it was quite deep enough to be confused with icterus gravis. In neither was the spleen palpable nor was there anaemia or erythroblastaemia. The jaundice cleared within one week in both cases. Both mothers were group O Rh+ ve; both children were group A Rh+ ve. These cases correspond to the icterus praecox of Halbrecht (1944) and Wiener (1946a). The third case which was seen personally became jaundiced at the third day and this persisted until his admission to hospital aged seven weeks. At this time he was 63 per cent of his expected weight and his haemoglobin level was 80% with no increase in reticulocytes or nucleated red cells. The child was the first partition in the family. Wassermann reaction was negative and bile was present in the faeces (Schmidt Test). There was no bile in the urine and the spleen was not palpable. The child was dismissed and when last seen aged eleven weeks the jaundice had disappeared and he had gained weight. In the absence of any signs to the contrary this case must be considered as a prolonged type of physiological icterus. The mother was A Rh+ ve as

was the child so that heterospecific pregnancy was not concerned in this case.

It is especially true that in premature infants Rh testing of mother and child may be of great value in the differential diagnosis of neonatal jaundice. These infants frequently show a deep early jaundice often with some degree of anaemia and an increased number of nucleated red cells in the blood as compared to a mature infant. Although the three infants referred to here were not premature the Rh testing was a useful confirmatory sign.

2. CONGENITAL OBLITERATION OF THE BILE DUCTS

One case of this condition was personally observed during the period of study and followed to autopsy. Case records of a further thirty four cases were studied but in only seventeen of them had the diagnosis been confirmed at post mortem. Most of the cases on which autopsy had not been performed had been taken home by the parents and presumably died there. In view of the possible relationship between icterus gravis and congenital obliteration of the bile ducts (page 2) it was thought to be of interest to investigate the Rh type of the mothers of the infants who had died from congenital obliteration of the bile ducts. Of the 18 mothers concerned (including the mother of the case seen personally) seven were traced: six of them were Rh+ve and one Rh-ve: in none were any complete or incomplete antibodies detected. The one mother who was

Rh-ve had three further children following the jaundiced one - all perfectly normal. In none of the seven families was there a history of similarly affected infants either before or after the one suffering from congenital obliteration of the bile ducts: nor was there any evidence of haemolysis in the haematological examination carried out when the infants were first seen. However, in six of the eighteen cases the jaundice had appeared within 48 hours of birth so that there is some initial danger of confusion between the two conditions. However, neither clinically nor serologically is there any apparent relationship between congenital obliteration of the bile ducts and icterus gravis neonatorum.

3. CONGENITAL SYPHILIS

One case of this condition occurred during the period of study. The infant was, however, three months old and had not become jaundiced until he was seven weeks old. No difficulty in diagnosis from icterus gravis could therefore arise in this case. The Wassermann reaction was positive.

4. INFECTIVE JAUNDICE

To what extent neonatal infection can cause jaundice is not quite clear. It is quite understandable that if the umbilicus becomes septic and the septic process extends into the liver setting up liver abscesses, that jaundice will be an inevitable accompaniment of this condition. Short of

this rare and drastic occurrence it is difficult to estimate, in the case of an infant with an infection and jaundice, whether the two conditions are associated or not.

In the Appendix (Case I) is recorded in detail the case history of one infant, seen personally, who demonstrates that infection can be associated with a haemolytic anaemia and jaundice. It is safe to assume that in this case the infection was the cause of the jaundice since the infant was well outside the age period at which jaundice might occur as a 'normal' phenomenon. The child was admitted aged seven weeks having been jaundiced for two weeks. Spleen was enlarged but the kidneys were not palpable. There were many pus cells in the urine and B Coli was cultured from it. As the jaundice cleared the level of the infant's haemoglobin fell from 108 to 65 per cent in six days and a reticulocytosis of 11 per cent developed. The pyuria was controlled with sodium bicarbonate and sulphathiazole but the general condition deteriorated and he died with high fever and vomiting. During the terminal phase the haemoglobin level was around 80 per cent but by this time haemoconcentration no doubt played a considerable part. (The infant was 93 per cent of his expected weight on admission and presumably was not dehydrated then). At autopsy a healing pyelonephritis was the only lesion found.

Although the differentiation of this case from icterus

gravis never really arose it was interesting to find that the mother, father, brother and patient were all group O Rh-ve and the maternal serum did not agglutinate the cells of any of the others, and did not contain any "incomplete" antibody.

Although this infant was considerably older than the age period for icterus gravis the case does demonstrate that infection with B. Coli can be the cause of haemolysis and jaundice. Infections with the same bacillus can occur in the first few days of life and it seems possible that this may sometimes be the cause of jaundice at that time.

A further case from the case records is recorded in the Appendix (Case 2). This infant was a boy and the first partition in the family: there have been none since. Jaundice appeared at six days and there had been frank melaena before admission to hospital on the fifteenth day. At first there was only haematuria microscopically, but as this cleared pus was found and B. Coli were grown. (Pyuria is just as common in boys as girls in this age group). At this time also the jaundice started to fade and there was a marked drop in the haemoglobin level from 90 to 50 per cent with a reticulocytosis of 19%. The melaena had cleared by this time so it is very unlikely to have been the cause of this drop. The blood level returned to normal after two blood transfusions. The child was seen after dismissal and at five months of age he was considered to be mentally

defective and blind. He is now (aged 3 yrs) awaiting admission to a colony for mental deficients. The mother, father and child are all Group O Rh+ve and the mother's serum contains no demonstrable anti Rh antibody.

In this case also, therefore, there was the similar picture of jaundice and haemolytic anaemia being associated with a urinary infection. The occurrence of mental deficiency following this cannot be attributed to kernicterus on the evidence available but it is a reminder that mental deficiency can follow a neonatal jaundice whose aetiology is not that of haemolytic disease.

The conclusion reached from the study of these two cases is that infection with B. Coli can cause haemolysis and jaundice in the neonatal period but that the question as to whether kernicterus may result from this (or any other form of neonatal jaundice) must still remain open.

APPENDIX.

CASE I

A.W. Male. Born 6.5.46 Admitted 25.6.46 Aged 7 weeks

Family History: 1) Male - alive & well; never jaundiced or pale
2) Patient

Mother has never had a blood transfusion

History: Birth weight 9 lbs. Became jaundiced at five weeks - has been drowsy for 5 days before admission

Examination: Icterus +++. 93% of expected weight. Neck retraction but no rigidity. Spleen 1 fb. Liver 3 fb's.

Urine albumen ++. Pus cells +++ Bile ++

Blood Hb 108% RBC 4.6 million. Retics. 1.2%

Film - no nucleated red cells seen.

Differential W.B.C. Count normal

Urine Culture - coliform bacilli abundant in film and on culture

Lumbar Puncture - xanthochronic fluid - Pandy -ve

Cells - 12 per cu. mm. - all polymorphs

Faeces - Schmidt test + ve

Treatment with Soda Bicarb. and sulphadiazine was started

Progress:	25.6.46	Hb 80% Urobilinuria
	27.6.46	" 75% RBC 3.3 million
	28.6.46	" 65% " 2.7 " Retics 6.8%
	30.6.46	" 68% " 3.1 " " 11%
	1.7.46	Jaundice fading rapidly
	10.7.46	Has been making good progress in general condition - but worse today
	11.7.46	Appears to have a terminal infection. Urine culture scanty coliform bacilli Hb 82%
	12.7.46	Very ill. Temp. 105 Vomiting ++
	13.7.46	Died

AUTOPSY The relevent findings were :-

Macroscopically - a healing pyelo - nephritis in the pole of the left kidney No kernicterus

Microscopically Liver - no evidence of 'erythroblastosis' or fibrosis

Left Kidney - healing pyelo - nephritis with extensive scarring

APPENDIX

CASE 2

J.W. female. Born 23.12.43. Admitted 7.1.44 aged 15 days 1st Partition: no succeeding pregnancies. Full time spontaneous delivery. Jaundice noticed aged six days At 13 days had 'twitching' of eyes for 'several' hours Jaundice has become deeper. On day of admission passed frank blood per rectum

On examination

65 per cent of expected weight. Jaundice

Spleen and liver not palpable

Urine - albumen +. Blood ++. Bile -

Hb 92% RBC 3.5 M WBC 11,400 R 1%

Progress

Treated as a haemorrhagic disease of the newborn and given Vitamin K intra-muscularly

8.1.44 Hb 90% Prothrombin index 125% Still melaena

9.1.44 Hb 92% Jaundice less marked

11.1.44 Hb 90% Has had fever since admission which has now settled

13.1.44 Jaundice fading: looks pale

Hb 50% Reticulocytes 19%

Film - numerous immature red cells

Urine culture - growth of B. Coli on culture

Blood transfusion 120 c.c. group O (Bank) blood - (not Rh-ve).

14.1.44 Hb 98% Reticulocytes 8.3% Looks better

Urine 20 pus cells per high power field

R, Soda Bicarb. Gr X 4 hourly

19.1.44 Very pale - jaundice completely cleared

Hb 61%

Blood transfusion 115 c.c. Group O Rh-ve blood

20.1.44 Hb 97%

29.1.44 Dismissed. HC 86%

31.1.44 Readmitted with diarrhoea which cleared quickly

25.5.44 Having 'salaam' fits: cannot see. Constant jerky movements of arms and legs

1.6.44 - aged 5½ months - mentally defective

March 1946 - at home awaiting admission to a mental defective colony

APPENDIX

CASE 3

J.D.

Born 2.4.38 Admitted 9.4.38 aged 7 days

Sibship 1. 1934 F. - a.w.

2. 1935 M. - a.w.

3. 1936 M. - died 3 months gastroenteritis

4. 1938 Patient

5. 1940 F. - a.w., never jaundiced or pale

6. 1942 F. - a.w. " " " "

Mother never had blood transfusion

History: Jaundiced at birth: birth weight 8 lbs. Jaundice deepened for 24 hours then started to fade: breast fed since birth: colour has become "lemon yellow"

Examination: In extremis: air hunger — : very feeble cry on stimulation: jaundice —

Liver 4 fb. Spleen 2 fb.

Hb. 12% R.B.C. 520,000 Retics 14%

Urine - No bile. Urobilin —

Blood transfusion: 90 c.c. of paternal blood

Progress

10.4.38 Hb 36%. Much improved: crying vigorously

B.T. 90 c.c. paternal blood

12.4.38 Hb 56%. Retics 2.6% Looks well.
Liver 4 fb. Spleen 3 fb.

13.4.38 Dismissed for breast feeding

15.4.38 Hb 55%. Well

20.4.38 Hb 42%. Gaining weight

21.4.38 B.T. 100 c.c. paternal blood

24.4.38 Hb 60%. Liver and spleen impalpable.

15.5.38 Aged 6 weeks. Hb 55%.

12.6.48 Hb 80%.

25.6.38 Aged 11 weeks. Colour good
No need to return.

PART III

D I S C U S S I O N

CLINICAL FEATURES

These have already been reviewed and criteria for diagnosis defined (p. 51). Apart from jaundice and/or anaemia, there is no single clinical component which must be present. The most useful additional factors in aiding the diagnosis are :-

1. Erythroblastaemia:
2. Splenomegaly:
3. Hepatomegaly.

Each case must of course be considered as a whole and the family history, sibship, etc. taken into account. The importance of serological investigation as an aid to diagnosis will be discussed later (p. 140).

Three conditions enter into differential diagnosis of icterus gravis neonatorum. These are "physiological" jaundice, congenital obliteration of the bile ducts, and neonatal infection.

PHYSIOLOGICAL JAUNDICE:

There is no doubt that physiological jaundice can occur within the first forty-eight hours of life and I have even seen it within the first twenty-four. Davidson et al (1941) found that 25 per cent of their cases showed some degree of bile staining of the skin within twenty-four hours of birth. This is probably a considerably higher percentage than is found on routine clinical examinations

but it does show that many cases of physiological icterus are very close to clinical jaundice within the first day of life. This is confirmed by the blood bilirubin levels (Davidson et al, 1941). Although many cases of physiological icterus show only a slight degree of staining, some may become quite deeply affected. Halbrecht (1944) has tried to split this earlier, and more severely affected group, off from the remainder and to call it "Icterus praecox". This worker claims that icterus praecox occurs in hetero-specific pregnancies and this contention has been supported by Wiener (1946). It is stated that these cases are more deeply jaundiced and more anaemic than the ordinary examples of physiological icterus but that they are not so severe as icterus gravis.

The presence of this separate group, however, has not yet been generally accepted. It does, however, emphasise once again that "physiological jaundice" can occur (and may be quite marked) within forty-eight hours of birth. When this occurs in a premature infant in whom some degree of anaemia and erythroblastaemia may also be present, a definite problem in diagnosis occurs. In this type of case, serological investigations will be of the greatest possible value. Normally however, in the mature infant, blood examination and the absence of splenomegaly will be sufficient to differentiate physiological jaundice from icterus gravis.

CONGENITAL OBLITERATION OF BILE DUCTS

It has been shown (p. 111) that this congenital deformity can also produce jaundice on the first day of life. Since icterus gravis can also have an obstructive phase, the absence of bile from the motions (after meconium has been passed) is of little importance in the differentiation. Blood examination will of course be the deciding factor in most cases although serological tests will also be of very great help. No confirmation of the suggestion (Shelton & Tovey 1945) that some cases of icterus gravis may progress to congenital obliteration of the bile ducts, has been found (p. 111).

NEONATAL INFECTION

At one time, "infection" (Knopfelmacher 1910) was thought to be a possible cause of icterus gravis. Dunham (1930) reported positive blood cultures in her cases and throughout the literature, "infection" has always been one of the conditions to be considered in differential diagnosis. "Infection" in the newborn, is, in most cases, rather a vague condition and it is certain that some clinicians rather doubted whether it should be considered seriously as a cause of jaundice. There seems no doubt however (p. 112) that infection, with B. Coli at least, <u>can</u> be responsible for haemolysis and jaundice. Any pyrexial icterus must therefore be viewed with great suspicion before icterus

gravis is diagnosed. Serological investigations will be required in most cases to help in the diagnosis.

The other two types of haemolytic disease of the newborn - hydrops foetalis and congenital haemolytic anaemia do not usually offer much difficulty in diagnosis. Potter (1943b,) however, has reported a series of cases of foetal hydrops which showed no erythroblastaemia and whose mothers were Rh+ve. (No further serological data given). The fact that foetal hydrops could occur without erythroblastaemia was, of course, one of the points put forward by Parsons et al (1933) against the theory that erythroblastaemia was the primary defect in the disease. Because of these reports therefore, in any case of foetal hydrops not showing erythroblastaemia, the serological investigations will be of use in diagnosis, although this can always be confirmed at autopsy in both types, in any case.

Congenital haemolytic anaemia requires to be diagnosed from any of the other causes of anaemia within the first two weeks of life. The chief amongst these is "infection" (p. 111) although peculiar aplastic anaemias also start to develop round about this time. The idea of this being a true nutritional iron deficiency anaemia in newborn infants (Parsons 1931) is now discarded. When considering congenital haemolytic anaemia, it must be remembered that although the haemoglobin level may be very low, the marrow response with young red cells may be very poor. Regeneration becomes more active with the increase in severity of the disease process (p. 138)

SEQUELAE

The incidence of mental deficiency following icterus gravis has been found to be just over 12 per cent (p. 93). This figure is closely in agreement with those previously published (Taylor 1944: Wallerstein 1947) although these authors did not report the number of cases on which their results were based. It is realised that, owing to the relatively small number of cases investigated, this figure of 12 per cent is only approximate. It does, however, show that the active treatment of icterus gravis is well worth while and that the vast majority of infants who recover are perfectly normal mentally. The term 'kernicterus' has been used to describe those cases of mental deficiency although in only one of the four cases were choreoathetoid movements known to have been present. It is possible that some of the others might have been mentally defective in any case but no definite statement can be made on this point except that the incidence is certainly not greater than 13 per cent. Yannet & Liebermann (1946) have attempted to show that there is a significantly greater number of mothers of infants showing primary amentia who are Rh-negative as compared to the incidence of Rh-negative persons in the population as a whole. From this, they suggest that some cases of primary amentia may be due to mild or undiagnosed cases of icterus gravis. Three of their "undifferentiated" cases were, however, definitely "kernicteric". Their contention has

recently been criticised by Wiener & Brody (1946). Yannet & Liebermann's suggestion was based on purely statistical evidence on a relatively small number of cases but, apart from any criticism of that fact, it is felt to be unlikely that any considerable number of cases of icterus gravis remain undiagnosed and recover. It is doubtful too whether all their cases could be fairly classified as "primary amentia".

Pathologically, kernicterus was found in 28 per cent of 52 autopsies. It is probable that if sections were taken from the basal nuclei in all cases, and examined microscopically for cellular destruction, the incidence would be considerably more. It is generally agreed (Guthrie 1914; Parsons 1946) that cellular damage precedes the bile staining and that when the latter is absorbed, the cellular destruction persists (Zimmermann & Yannet 1935). It is possible therefore to have the essential damage present without the bile staining and that it would be found more frequently if microscopic sections were examined in all cases. The considerably greater incidence of 'pathological' kernicterus, as compared to 'clinical', indicates that this condition may predispose to the death of the infant. Wiener (1946 c) however takes the other view by stating that kernicterus occurs only in the cases of "erythroblastosis proper" (the most severe form of haemolytic disease of the newborn apart from hydrops foetalis). Wiener's theory is

that when an agglutinating antibody is present, "true erythroblastosis" with kernicterus and liver damage results. His statistical evidence in support of his theory is unconvincing (p.135).

The occurrence of kernicterus in any condition other than icterus gravis is still subjudice (Docter 1945: Parsons 1946). In my own series, autopsy reports on the 18 cases of congenital obliteration of the bile ducts (p.110), and on many others dying from 'infective' jaundice, failed to show a single example of kernicterus. Clinically, however, one case (p. 113) appears to be mentally defective following a B. Coli urinary infection with jaundice. It can be stated however that, in this series of cases, definite kernicterus was associated only with icterus gravis.

Cirrhosis of the liver (Hawkesly & Lightwood 1934: Braid 1937: Braid & Ebbs 1937: Gilmour 1944: Drummond & Watkins 1946:) was not encountered in any of the 31 cases 'followed up'.

Bile staining of the teeth occurred in two cases (p. 93). One of the children so affected was mentally defective but the other was perfectly normal so that in the latter case, associated damage to the basal nuclei could not be postulated.

TREATMENT.

The use of Rh -ve blood in the transfusion therapy of haemolytic disease of the newborn is now a standard procedure. Ideally, of course, blood of the same genotype of the mother should always be used but this is not a generally practicable procedure and, since more than 90 per cent of mothers are Rh -ve, this type of blood is correct in the vast majority of cases. Darrow (1945) however has suggested that Rh +ve blood should be used so as to absorb the antibody from the infant's blood stream as quickly as possible. She states that equally good results are obtainable by this method but has received no support from other workers. There may however be something in Darrow's idea since it is becoming more and more apparent that the simple destruction of red cells is of little importance (p. 82). The difficulty with Rh+ ve (and unselected) blood has always been that no great rise in haemoglobin blood level could be obtained so that even in the relatively mild form of the disease, the infant succumbed after a week or so to intercurrent infection. If a considerable amount of Rh+ ve blood was given, and this then followed by Rh-ve blood, the end result might be quite good. It is likely, however, that most of the damage is already done before any type of blood transfusion can be given. The giving of Rh— ve blood to these infants does not seem to have the same effect as it would if given to the

mother for, on looking through the case records of the 'pre Rh' period, it was found that the frequency of severe post transfusion reactions was no greater than in the last three years. This is of interest in view of the fact that no definitely authenticated claim of the finding of an agglutinating anti Rh antibody in the infant's serum has yet been made although Baar (1945) has found the incomplete antibody.

The question of when to give the blood transfusion has been a matter of some discussion. Cappell (1946) suggests that as soon as it is established clinically and/or serologically that the infant is affected, blood transfusion should be performed. Parsons (1946) states that it is his practice to wait until the haemoglobin level indicates that the infant requires more blood. The decision as to which is correct depends on one's appreciation of the pathology of the disease. If it is considered that the destruction of red cells is the main cause of mortality, then immediate transfusion to depress the erythropoietic tissue is the rational treatment. Likewise, if it is thought that the effect of the hepatic erythropoiesis (p. 138) is the main cause of liver dysfunction and death, immediate transfusion is indicated. If, however, it is thought that it is the direct effect of the circulating antibody on the hepatic cells which is the main trouble, then immediate transfusion would have no effect. The position is, however, likely to

become clearer in the near future since a method of removing both the cells and the antibody is now coming into prominence (Wallerstein 1947). The attempt to do an exsanguination transfusion was first reported in 1925 (Hart 1925) but the difficulties in technique were considerable. There are few routes by which blood can be removed from an infant. Wallerstein (1946: 1947) used the longitudinal sinus and then the radial artery. Wiener & Wexlar (1946) also used the latter method. It is very understandable that the longitudinal sinus route would be difficult and having taken part in the radial artery method, I know it can be difficult and tedious. However, an excellent method has now been devised by Diamond (Mollison 1947) whereby a catheter is passed up the umbilical vein into the inferior vena cava. The removal of blood is done through this catheter and the transfusion is given by the same route. In this way, as much as 85 per cent of the infant's blood can be exchanged in about 1½ hours with no risk to the child. I have seen two of these performed and technically, the procedure is very easy. If the infant is anaemic, an additional amount of blood can be put in at the end of the procedure but up till that time, alternate amounts are removed and replaced. It is likely that this will become the method of treatment in the future - and it must of course be done as soon as the diagnosis is made. It will be of great interest to see whether this method will improve

the prognosis. Wallerstein (1947) reports recovery in seven severe cases of icterus gravis on whom the exsanguination transfusion was performed within 24 hours of birth and the death of two on whom it was not performed until the infants were more than 48 hours old. The method will of course only be applied to those infants who are most severely affected, but as routine Rh testing of mothers becomes more generalised, more and more affected infants are going to be diagnosed before assessment of severity can be made so that the procedure may be adopted ultimately in all cases, even when the infant is the first affected partition in the family.

Apart from this radical procedure, I am in favour of immediate transfusion as soon as the case is diagnosed in an effort to depress the erythropoiesis and prevent further hepatic damage. The amount of blood to be given is another variable factor. The standard amount is 15 c.c. per lb of body weight when the infant is anaemic. Gimson (1943) recommends that the amount given depends on the haemoglobin level and gives a formula based on the haemoglobin increase required, If, however, the infant is not (or only slightly) anaemic, Cappell (1946) still recommends that a relatively large transfusion of 120-150 c.c. should be given slowly. The work of Delmarsh et al (1942) is of interest in this connection, these workers showing that an average of 107 c.c. of blood is retained in the placenta if the umbilical cord is clamped immediately, but when all this blood was allowed

to re-enter the infant's circulation, the increase in blood volume affected only the red cells. They concluded that the plasma was eliminated and stated that this concentration occurred in 15 - 180 minutes. They hold that this explained the rise in haemoglobin and red cells which occurs shortly after birth. The point of interest in connection with transfusion is that it may take up to three hours for the infant to reduce his blood volume to normal after 107 c.c. of blood. Accidents can - and do - happen when a large amount of blood is given too quickly. It is strongly recommended therefore that blood transfusion should be given by slow, continuous drip of not more than 8 drops per minute. This can be maintained quite easily through a Bateman needle and the drip kept running for several hours. If the infant is anaemic, the blood can be run in faster at the start, or injected by means of a syringe. As long as the slow drip is used, amounts up to 200 c.c. can safely be given (Gimson 1943). This takes about 5-6 hours to run in. The giving of large amounts of blood is of considerable use in an anaemic patient since a small transfusion of about 90 c.c. rarely raises the haemoglobin level far enough. Large transfusion by slow drip is therefore the best treatment if exsanguination transfusion is contraindicated or impracticable.

One other aspect of the treatment which must be decided is whether to continue with breast feeding or not. There is

no doubt that the anti Rh antibodies are present in the maternal milk (Witebsky & Heide 1943; Langley & Stratton 1944) and it has been recommended that the milk should be exhausted and heated or even that breast feeding should be abandoned. In this country, certainly, no infant should ever be deprived of breast feeding without due cause (and manual exhaustion soon leads to this) and there is absolutely no direct evidence that the maternal milk has any ill effects on the infant. In the first place, it should be noted that the infants who are most severely affected and die, do so before there is anything but a little colostrum in the mother's breasts. Secondly, many cases who are bottle fed continue to haemolyse for as long as 6-8 weeks but their recovery is always complete so long as an adequate transfusion therapy is employed and the jaundice has cleared. Thirdly, there is no evidence that breast feeding is associated with prolonged jaundice. In the personally observed series of cases, it was rare for the jaundice to last more than 10-14 days, and in the few cases in which this occurred, there were just as many babies bottle-fed (for other reasons) as breast-fed. The only possibility which is acceptable therefore is that breast-feeding <u>might</u> cause some continued haemolysis of red cells. This can easily be replaced by blood transfusion but there is no such effective remedy for infantile diarrhoea, which occurs almost solely in bottle-fed babies. It is strongly

recommended therefore that for the present, breast feeding should be continued in all possible cases. From this point of view, it would be ideal to have a room in a children's ward to which the mother might be brought. If the delivery occurs in hospital, treatment should be carried out there instead of moving the infant to a children's hospital.

OEDEMA, JAUNDICE AND ANAEMIA

Oedema, jaundice and anaemia are the three outstanding features of the different types of haemolytic disease of the newborn. The one clinical feature common to all three types of the disease is anaemia. It may be that an occasional case of icterus gravis will die before marked anaemia has developed but any case which survives a few days will become anaemic if untreated. Destruction of red cells with inadequate replacement, is, therefore, common to them all and it must be that this is, in some way, connected with their very different clinical appearance. In congenital haemolytic anaemia, there is purely destruction of red cells - and when these are adequately replaced, recovery will follow with no sequelae. Icterus gravis is however complicated by severe jaundice and a considerable mortality, and hydrops foetalis by extreme oedema and an extreme mortality. There is therefore some pathological change which occurs in addition to the red cell destruction which causes jaundice and it seems fair to assume that, if this change progresses even

further, it will lead to oedema (without any severe degree of jaundice). An attempt will be made to show what this additional pathological condition may be.

When the haemolytic nature of the disease was first propounded and confirmed, it was thought that the jaundice of icterus gravis might be haemolytic in origin and that as long as sufficient blood were given to prevent the infant dying of anaemia, recovery would occur once the haemolysis had stopped. Mainly owing to the fact that the mortality rate is still about 33 per cent, this concept has had to be abandoned and the idea of 'liver damage' as the cause of the jaundice and mortality has been revived again. (It must be remembered that Darrow (1938) included this as one of the factors which must be explained in the aetiology of the disease). The nature of this 'liver damage' is not, however, quite clear (p. 138). Pathologically, the lesion is much more one of 'obstructive jaundice' than of 'hepatitis'. Clinically, it could be either. Any conception of the liver in icterus gravis must however be considered in relation to the assumption that an extension of the lesion will result in oedema and a further extension will result in a macerated foetus with hepatic fibrosis (Henderson 1942). It must also be thought of in relation to the fact that it can often clear completely, leaving no sequelae, but that rarely, fibrosis may result in the living child (Lightwood 1943). There can be no

doubt therefore that destruction of liver cells can occur and Gilmour (1944) has shown that there is some degree of hepatic fibrosis in sections from most cases of icterus gravis although this required special staining for its demonstration.

If it be agreed that some form of cellular destruction is in progress in all types of the disease other than haemolytic anaemia, some cause of this must be sought. Apart from the anaemia, there are two other constant factors in the disease - the presence of some degree of "erythroblastosis", and the occurrence of isoimmunisation to the 'Rh' or other factor with the production of antibody, in the mother. Wiener (1946 c) has postulated that the latter is responsible for the different forms of the disease and attributes the occurrence of jaundice to the presence of an agglutinating antibody and the anaemia (with or without oedema) to the "blocking" antibody. Wiener seems to consider that with the agglutinating antibody, intravascular clumping occurs which blocks the small arterioles of the liver and basal nuclei (but nowhere else) and are thereby responsible for liver cell damage and kernicterus. He has recently (Wiener 1946 d) produced very unconvincing statistical evidence in favour of his theory. In this report on the statistical evidence, the clinical diagnoses were made by different clinicians and no criteria are laid down as to what differentiates icterus gravis from congenital

haemolytic anaemia. He reports his findings in ten cases of icterus gravis and twenty-four of haemolytic anaemia. This is, of course, quite an abnormal proportion since icterus gravis is 3-4 times commoner than haemolytic anaemia (Javert 1942: Henderson 1942). In my own personally observed cases, there were 22 cases of icterus gravis and 7 of haemolytic anaemia. In the former group, 18 mothers had anti Rh antibody - 11 being agglutinins and 7 being "glutinins". In the anaemic group, 6 mothers had anti Rh antibody - 5 being agglutinins and 1 only being a "glutinin". There does not, therefore, seem to be much to Wiener's theory although it may be that his clinical classification of the disease will contain differential points which have not been included by other workers. His original amended classification (1946 a) appears to be more pathological and serological than clinical.

There is no evidence therefore that a difference in antibody is responsible for the difference between icterus gravis and haemolytic anaemia. The suggestion has been made however (Cappell 1946) that the blocking antibody is usually associated with hydrops foetalis. There is also evidence that in succeeding pregnancies, agglutinin is liable to change to glutinin with the occurrence of subsequent stillbirths (Cappell 1946). This does not however help very much in assessing the difference between all three types of the disease.

"Erythroblastosis" is the other factor common to all three conditions. It was first recognised historically, and is most severe, in hydrops foetalis: still marked but less severe in icterus gravis, and only slight in haemolytic anaemia. It is considered very likely that as Davidsohn (1945) suggested, hepatic cellular damage is due to the pressure atrophy caused by the hepatic "erythroblastosis". In less severe cases, only the bile channels are obstructed and, as the erythroblastic tissue disappears, these channels open up again and the infant may recover, although rarely. sufficient damage may have been done to cause subsequent fibrosis. This theory also fits in well with the suggestion that the oedema of hydrops is due to defective liver synthesis of protein so that low serum proteins, with reduction of the albumin/globulin ratio, occur. This is supported to a small degree by my figures (p. 76). In the most severe degree of all, 'liver damage' in utero has been so excessive that fibrosis occurs before delivery, leading to intra-uterine death and a macerated foetus. As a final link in the chain of explanation, the fact that hydrops cases are not jaundiced, and that some cases of icterus gravis do not become jaundiced for some hours, is explained by assuming that the bile pigments are disposed of through the placenta (Hampson 1928) and take some time to accumulate in sufficient amount to cause clinical jaundice. Were any

case of hydrops foetalis to live long enough, it is highly probable that it would become severely jaundiced.
It is possible that the exact form the disease takes depends on at what time, and in what amount, the antibody passes from mother to foetus.

It is suggested therefore that the 'hepatic' aspect of haemolytic disease of the newborn and foetus is due to "erythroblastosis". The argument put forward by Abt. A.F. (1933) that this is unlikely since jaundice does not occur in other hepatic infiltrations, such as leukaemia, is untenable, since in these other conditions, there is no excessive haemolysis in progress and there is no immaturity of liver function as there is in the newborn (Hampson 1933: Davidson et al 1941: Larsen & With 1943: Mollison 1947).

A further argument in favour of the supposition is that Leonard (1945) found that a persistently high nucleated red cell count was a sign of poor prognosis. She found, in those cases that recovered, that this count was normal at the end of one week.

This explanation does not, however, cover the occurrence of kernicterus nor of bile stained teeth.

My final conclusion on this point, therefore, is that oedema, jaundice and hepatic fibrosis are all related to the occurrence of the excessive erythropoietic tissue in the liver and that the latter exerts its effect through pressure on the bile canaliculi and pressure atrophy of the liver parenchyma.

SEROLOGY.

Although the serological studies and investigations of the past six years have contributed greatly to our understanding of the aetology of haemolytic disease of the newborn their exact place in diagnosis is not, as yet, clearly defined. It is however generally agreed that when an Rh-ve mother, with anti Rh antibodies in her serum, has an Rh+ ve child whose red cells are agglutinated, or 'blocked' by the material serum, that infant is almost certain to be suffering from the disease. If the child also shows some clinical evidence that it is affected, the diagnosis is complete. Final judgement does, of course, still rest on the clinical condition of the child. The fact that it is jaundiced is by no means sufficient clinical evidence and there has been a tendency in the more recent literature (Wallerstein 1947) to present cases without full clinical details. This can lead to nothing but misconceptions because, even if the serological evidence is in favour of the diagnosis, slight doubt must always remain as to its correctness. Most cases of haemolytic disease are quite easily diagnosed clinically but when the issue is in doubt, serological investigations are of the greatest help (p.121).

Until recently, there have been two great difficulties in the presentation of serological evidence. The first is the difficulty in demonstrating the maternal anti Rh antibody: the second is the occurrence of 5-10 per cent of

Rh+ ve mothers in most of whom no evidence of iso-immunisation could be detected. These two factors will now be considered.

MATERNAL ANTIBODY

American workers (Levine et al 1941) have always claimed that agglutinins were found in the maternal sera in only about 50 per cent of cases. In this country, Boorman et al (1944) claimed to have found them in over 90 per cent of their cases. There is reason to believe however that these figures were somewhat enthusiastic. The discovery of the 'blocking' (Wiener 1944 b), or 'incomplete' (Race 1944) antibody was a considerable advance in the demonstration of immunisation. Recently, the Coombs' Test (Coombs et al 1945 a,b: 1946) has been introduced and appears to have met with considerable approval (Levine 1946). It is likely to supersede Wiener's (1945 b) conglutination test. If the Coombs' test is as accurate and sensitive as it promises to be, the difficulties over the detection of maternal antibodies should largely disappear. The greatest advantage of all is that it can detect the presence of the antibody on the infant's own red cells (direct method) and is therefore a very rapid method of detecting an affected child.

THE RH+VE MOTHER

Ever since the original work of Levine and his

co-workers, the presence of some 10 per cent of Rh+ve mothers in every series of cases published has been a considerable obstacle to complete understanding of the disease process. The discovery of the anti rh (Hr; c) serum has explained a very small number of these, but since these sera are very rare, there must be still further explanations. Coombs et al (1946) have quoted a case in which an antibody was present in the maternal sera which agglutinated her affected infant's red cells but was quite unconnected with the Rh system as it is known today. This fact, coupled with the variety of agglutinogens recently described in connection with transfusion reactions (Callender et al 1945), including the variety at the Cc locus (Race et al 1946), and at the Dd locus (Stratton 1946), makes it clear that there is still much more to be discovered about agglutinogens in general and even about the Rh agglutinogens themselves. Difficulty has always been experienced in the discovery of the antibody and the Coomb's test may make it possible to demonstrate this in some of these cases. It is probable also that some of the cases have been misdiagnosed and were not true clinical cases of the disease in the first place. Finally, some of them are explainable on Levine's original idea that iso-immunisation of a Gp. O mother may occur if her foetus is Gp A, B, or AB, and that if the foetus is a non-secretor of the group specific substance, it will become damaged in

exactly the same way as occurs with the Rh antibodies. The suggestion that all infants in this group are non-secretors has been denied by Wiener (1946b) and also by Austin & Smith (1946). In many of the cases, an increase in the titre of the natural agglutinin, corresponding to the foetal blood group, has been found (Boorman et al 1942: Polayes & Ohlbaum 1945: Austin & Smith 1946). This is not so in the two cases reported here (p. 98). The great difficulty in appreciating that this type of heterospecific pregnancy is responsible for haemolytic disease is that, up to date, no family has been reported in which a series of infants have all suffered from the disease and have all had a blood group incompatible with the mother, such as happens with the Rh isoimmunisation. For this reason, Halbrecht (1944) suggested, and offered some proof, that these constituted a minor condition resembling icterus gravis, but with a very much better prognosis. He termed the condition 'icterus praecox' and his views have been adopted by Wiener (1946 e). In one of the cases in the present series, the haemoglobin level was not low but otherwise the case appeared clinically to be affected and transfusion was performed immediately, with recovery. In the second case, the haemoglobin had fallen to 78 per cent when the infant was 24 hours old. In both cases, erythroblastaemia was marked. This type of heterospecific pregnancy does not explain all the cases which are reported as occurring in

Rh+ ve mothers, and in truth, no very convincing evidence has been brought forward to prove that it can cause haemolytic disease at all. It may well be that any explanation for the other cases occurring in Rh+ ve mothers may also apply to this 'incompatible group'. It is considered that the explanation will be found most likely to come from wrong diagnosis and some rarer agglutinogens.

The Landsteiner blood group of mother and infant is however of the greatest importance in Rh iso-immunisation (p. 106). Levine (1943) noted that in only 25 per cent of his families, the mother's serum was compatible with the infant's cells instead of the expected 35 per cent (Wiener 1945 d) showed by a study of four series of cases that there was a significant increase in compatible matings in the affected families. Cappell (1946) reported that in 90 pregnancies, only 8 cases were heterospecific. These results are fully supported by the figures collected in this thesis (p.105).

The fact that a heterospecific pregnancy protects the mother against immunisation appears to be definitely established by these figures but (as with the Rh+ ve mothers) there is no definite rule on the subject and cases do occur in which the diagnosis cannot be doubted (Cappell 1946). The figures quoted showing the low incidence of Group A infants from Group O mothers are also rendered somewhat less significant by the work of Levine (1943) and Waterhouse

& Hogben (1947) in demonstrating that there is a statistical deficiency of A infants from O mothers in the population as a whole. However, since in one pregnancy in five (Race et al 1943) the mother's blood is incompatible with her infant's red cells, in the general population, it is easily seen that 1 pregnancy in 23 is a significant decrease in incidence, even if the reduced incidence reported by those other workers is taken into account.

The suggestion which results from the findings in Family D (p. 107), that heterospecific pregnancy may also protect the infant after immunisation of the mother has occurred, has, as far as I am aware, never been made before. It is very unlikely that the two succeeding unaffected infants were so mildly affected that the disease passed unnoticed in both. Professor Capbell could show that all three infants were of the same genotype. There is no doubt of the diagnosis and no doubt of the presence of maternal agglutin. There seems to be no explanation therefore, other than that the agglutination of the Group A children's cells was a protection against their developing the disease.

The protection afforded to the Rh-ve mother by a heterospecific pregnancy (1 in 5 of all pregnancies), combined with the occurrence of heterozygous fathers (p.103) and the small number of children in modern families, almost certainly explains why all Rh-ve mothers, with Rh+ ve husbands, do not become immunised and produce affected

children. This then solves one of the problems which arose immediately after the original theory of iso-immunisation has been propounded. Many more await solution.

To conclude this thesis, it may be said that some improvement in the treatment of icterus gravis neonatorum has resulted from present methods, but that the incidence of sequelae remains much the same. No effect on hydrops foetalis is obtainable, even when the infant is born alive, but, with the use of Rh-ve blood, cases of haemolytic anaemia should now never die. With newer methods of diagnosis, and the more recent appreciation of the need for radical treatment by means of exsanguination blood transfusions, the outlook, as far as mortality (and perhaps sequelae is concerned), may be considerably improved. It is, however, possible that in many cases, the damage is done before the child is born and it must be towards some method of desensitising the mother that we must eventually look for the real prevention of this disease.

SUMMARY AND CONCLUSIONS

1. The literature on the subject of haemolytic disease of the newborn from 1891 to the present day has been reviewed.

2. A study has been made of 160 cases of this condition admitted to the Royal Hospital for Sick Children, Glasgow from 1934 to November 1946. Twenty-eight of these cases were personally observed during the period of study.

3. One hundred and thirty-two of the cases fell into the category of icterus gravis neonatorum as defined by

certain criteria: the remaining 28 belonged to the congenital haemolytic anaemia type of the disease.

4. The clinical features of the disease in the 160 cases have been reviewed and the incidence of Splenomegaly, Biluria and Uribilinuria determined. All cases were jaundiced and/or anaemic.

5. A history of previous stillbirths has been shown to be of some importance in the family history of neonatal jaundice but a history of previous abortions is of none.

6. It has been suggested that the word 'partition' might be adopted to mean the products of conception, whatever their period of gestation.

7. In succeeding pregnancies following a child suffering from haemolytic disease of the newborn, about one quarter of the partitions are unaffected infants and these are produced in about one quarter of the families concerned.

8. Previous blood transfusion was found to be an infrequent cause of Rh sensitisation in mothers of 'affected' infants.

9. There was no increase in the blood non-protein nitrogen in 11 cases; it is concluded that the cause of death in haemolytic disease of the newborn is not the same as in incompatible blood transfusion.

10. The haemorrhagic diathesis in icterus gravis neonatorum and the oedema of hydrops foetalis may be associated with liver dysfunction causing decreased plasma prothrombin and serum proteins respectively.

11. Since the use of Rh-ve blood, the mortality rate in icterus gravis neonatorum has fallen from 52.5 per cent to 32.7 per cent. Some of this drop may have been associated with earlier treatment.

12. The mortality rate in congenital haemolytic anaemia should be nil.

13. The mortality rate in "familial" cases of icterus gravis has not been appreciably lowered by the use of Rh-ve blood.

14. Mental deficiency following icterus gravis neonatorum (kernicterus) was found in four of 31 (12.3 per cent) infants over one year who reported for examination. "Green teeth" were found in 2 of the 31 cases.

15. Kernicterus occurred pathologically in 15 of 52 autopsies (28.8 per cent) performed on cases of icterus gravis neonatorum.

16. Of 81 mothers of 'affected' infants, 77 were found to be Rh-ve and 4 Rh+ve. Antibodies - agglutinating or "incomplete" - were found in the sera of 64 of the 77 Rh-ve mothers.

17. The "conglutination" test of Wiener was found to give a higher number of positive reactions than the ordinary method of detecting agglutinating antibodies.

18. 22 fathers and 64 affected infants were all found to be Rh+ve.

19. Evidence has been offered to show that incompatibility of blood group between mother and infant protects the mother against Rh sensitisation and may even protect the infant once sensitisation has developed.

20. It is suggested that the jaundice of icterus gravis and the oedema of hydrops foetalis are due to hepatic cell damage caused by pressure atrophy from the excessive erythropoiesis present in the liver.

21. The reason why all Rh-ve women with Rh+ ve husbands do not produce affected infants is because :-
    a) There are insufficient pregnancies in some modern families.
    or b) The father is heterozygous Rh+ve.

    or c) The infants are of incompatible blood group to the maternal serum.

22. Modern methods of treatment have been discussed and the conclusion has been reached that they are not as satisfactory as was at once hoped they might be. Some form of maternal desensitisation will be the ideal to be aimed at. In the meantime, further experience with exsanguination transfusion is indicated.

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