

VOLUME 2.

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has certain characteristics but the individual case may show all of these, many of them, or frequently only a few. Furthermore there is no single pathological lesion which is constantly present in all cases. As Potter (1943) has remarked, this inconstancy has led to many errors in diagnosis at post-mortem examination.

1. Hydrops Foetalis (36 cases)

A. Morbid Anatomy.

It is a common misconception that all hydrops cases are macerated at birth or at least are still-born. Although these comprise the majority, there are a few exceptions who survive birth. In this series of 36 cases, 10 were macerated, 21 were still-born and 5 were live-born. Of these five, only one lived for 24 hours.

State of Maturity : Only two of Gilmour's cases were full-term and the average foetal life was thirty three weeks. In this series there were 8 full term and 28 premature cases. As Gilmour (1944) found, the average period of gestation was thirty-three weeks. Maturity, in cases of hydrops foetalis, was judged on the duration of pregnancy and on the length of the foetus and not on weight alone as the latter is obviously rendered inaccurate owing to the presence of oedema.

Sex : Seven of Gilmour's cases were males and seventeen were females. He also found twenty-seven males and forty female cases reported in the literature and suggested a predominance of females. In this series/

series of 36 cases there were 12 males and 19 females. In 5 cases the sex was not stipulated. It is doubtful if the apparently greater incidence of this form of haemolytic disease in females is of any significance.

Oedema : (Fig.1.) Generalised subcutaneous oedema is one of the most constant features in hydrops cases as the name implies. It is less constant in the macerated foetus as the tendency is for the foetus to dry in utero if birth is delayed. Gilmour reported one case of foetus papyraceus in his series. There was no such instance in this series. Hydropericardium, hydrothorax and ascites are common. The fluid is generally clear and straw-coloured, or slightly jaundiced, in the still-born and live-born cases, and blood-stained in those which are macerated.

Petechial Haemorrhages : Petechial haemorrhages may be found in the skin over the abdomen, limbs or face, subpleurally over the lungs, subepicardially and over the thymus gland. Occasionally subependymal petechiae lead to intraventricular haemorrhage.

Jaundice : This is not usually present but two cases showed a slight yellow tint of the skin and mucous membranes. One case (Series I No. 32) was covered by bright orange-yellow vernix at birth and the skin and mucous membranes were deeply jaundiced. The fluid in the chest and abdomen was dark yellow in colour. In one case there was pronounced jaundice of the umbilical cord. Such infants would have exhibited icterus gravis had they survived. As was stated above the/

the dividing line between icterus gravis and hydrops foetalis is not pronounced.

Organs :

(I) Liver : The liver is usually enlarged and reddish-brown in colour, or brown in colour if maceration has occurred. Its consistence is variable. In some cases the consistence was normal. In a few it was rather tough to cut and in the severely macerated cases, the liver was soft and diffluent. The Prussian Blue reaction is usually positive.

(II) Spleen : The spleen is almost always enlarged and may measure 9 cm. in length with corresponding increase in the other dimensions. It is usually rather soft and dark red in colour. The Prussian Blue reaction is usually positive.

(III) Kidneys : The kidneys may be rather pale but no gross change is found on macroscopic examination.

(IV) Suprarenal Glands : Gilmour (1944) reported a "yellow radial streaking of the inner part of the foetal cortex " in eleven cases. He ascribed it to lipoid infiltration. It was present in some of the cases in this series but was not such a pronounced feature. It was also described by Yasukawa (1934) Liebegott (1938) and O'Sullivan and Gilmour (1940).

(V) Lungs : The lungs were usually collapsed as a result of the hydrothorax. Subpleural petechiae are not uncommon. Pneumonia is seldom present.

(VI) Heart : The heart was enlarged as a result of hypertrophy and dilatation in twelve cases. The myocardium/



Fig. 1.

Hydrops Foetalis twins. One jaundiced and one macerated. Note large friable placenta whose weight equalled that of one twin. The mother's serum contained saline agglutinins in a titre of 1/4000 and albumin agglutinins in a titre of 1/8000.

myocardium is often pale and oedematous.

(VII) Brain : Intracerebral lesions are uncommon. In no case were the basal ganglia affected by selective degenerative change. Occasionally subependymal haemorrhages may rupture into the ventricular system and cause widespread cerebral damage. This occurrence is, of course, not confined to haemolytic disease.

Bones : Capon (1922), O'Sullivan and Gilmour (1940) and Gilmour (1944) have described changes in the epiphyseal lines of long bones. They found that the ossifying zone was deeper than usual and rather yellowish in colour. It was present in 12 cases of Gilmour's series (1944). Unfortunately in this series the bones were not examined in every case but this change was found in two instances.

Placenta : The placenta is enlarged, pale and oedematous. Its weight is greatly above the normal and the placenta may equal half the body-weight of the affected foetus (Fig.1.)

B. Morbid Histology.

Extensive extramedullary haemopoiesis is one of the commoner histological features of hydrops foetalis. (Schridde , 1910). This is of importance since nucleated red cells resist maceration long after the other cells of the organs have disintegrated. An increase of nucleated red cells in the peripheral blood can sometimes be detected even in the macerated foetus. All types of nucleated red cell may be found from/

from the haemocyto blast to the late normoblast. Early and late erythroblasts are especially numerous in hydrops. The liver and spleen show the greatest amount of haemopoiesis and lesser degrees are found in the kidney, pancreas, lungs, suprarenal glands, thymus gland and lymph nodes.

(1) Liver : (Figs. 2, 3, 4.5).

Marked extramedullary haemopoiesis is almost invariably present. The parenchyma may be greatly obscured by large foci of erythropoiesis composed of normoblasts and erythroblasts. The liver cells show varying degrees of degeneration depending on whether maceration has occurred. In a few cases the liver cells in the central zone of the lobule show necrosis. There is frequently a fine intercellular fibrosis. (Fischer 1912, Sauvage 1913, Gilmour 1944) and the reticulin is increased. Henderson (1942) has described cirrhosis of the liver in the macerated foetus resulting from haemolytic disease. He believes this to be a separate condition and the most severe type of haemolytic disease. It is more probable that it is merely a variation of the changes to be found in the hydrops foetalis group (Fig. 5 a). The fibrosis is much less than is often found in congenital syphilis although it is by no means a constant feature of the latter disease either (Fig. 51). Fischer 1912, de Lange and Arntzenius (1929), Salomonsen (1931) and Gilmour (1944) have found bile plugs in the intercellular canaliculi. These are more commonly found in/



Fig.2. LIVER. H. & E. x 80. Portal tract showing infiltration with nucleated cells. Case of Hydrops Foetalis.

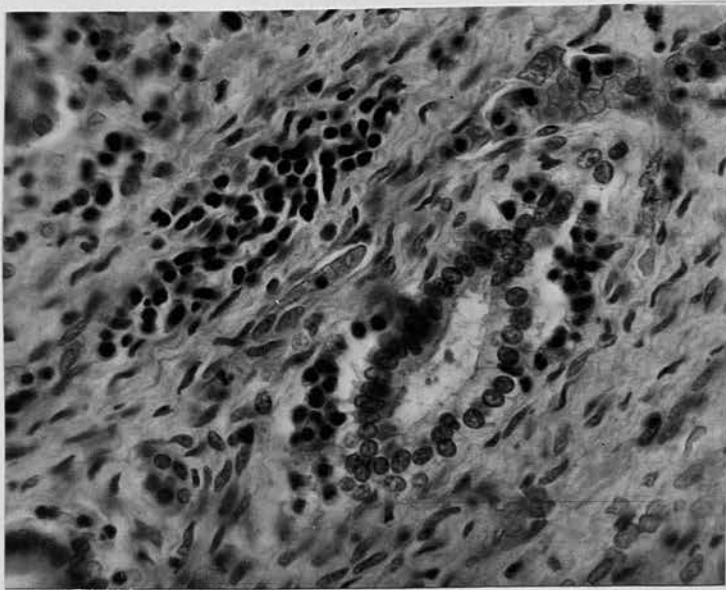


Fig.3. LIVER x 375. H.& E. Portal tract showing nest of primitive red cells. Case of Hydrops Foetalis.

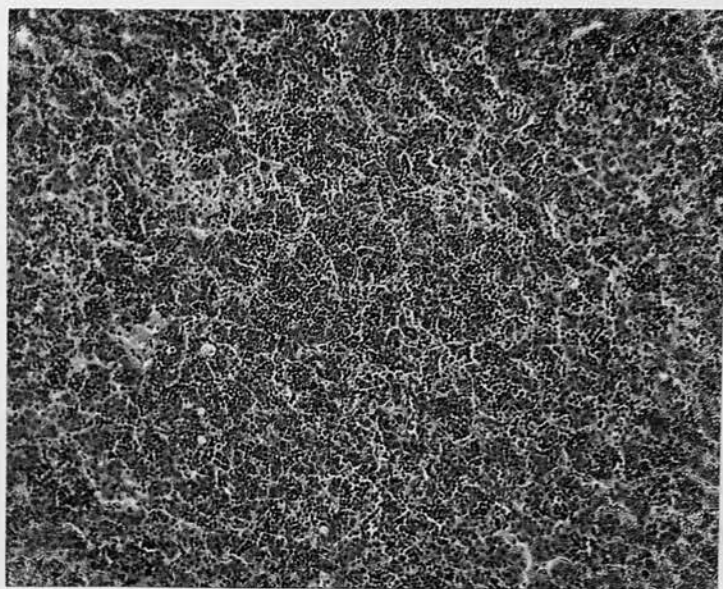


Fig. 4. LIVER x 80. Haematoxylin and Eosin.
Extensive haemopoiesis throughout liver
parenchyma.
Case of Hydrops Foetalis.

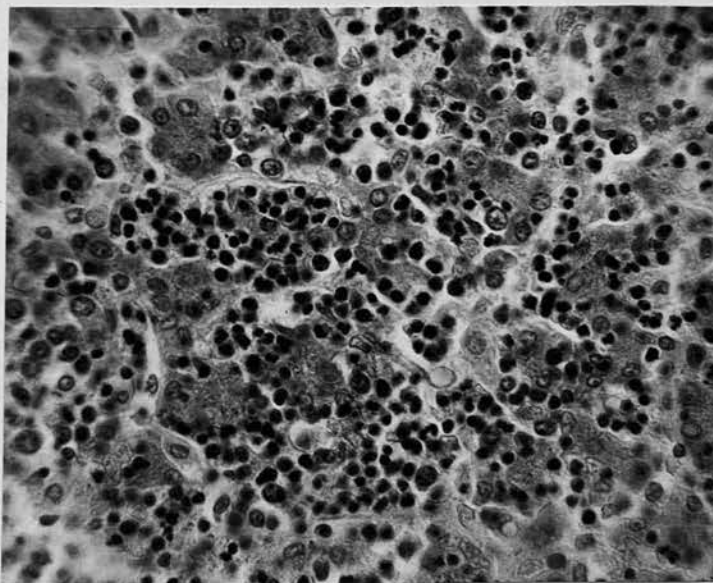


Fig. 5. LIVER x 375. Haematoxylin and Eosin.
Numerous foci of normoblasts and erythroblasts.
Case of Hydrops Foetalis.

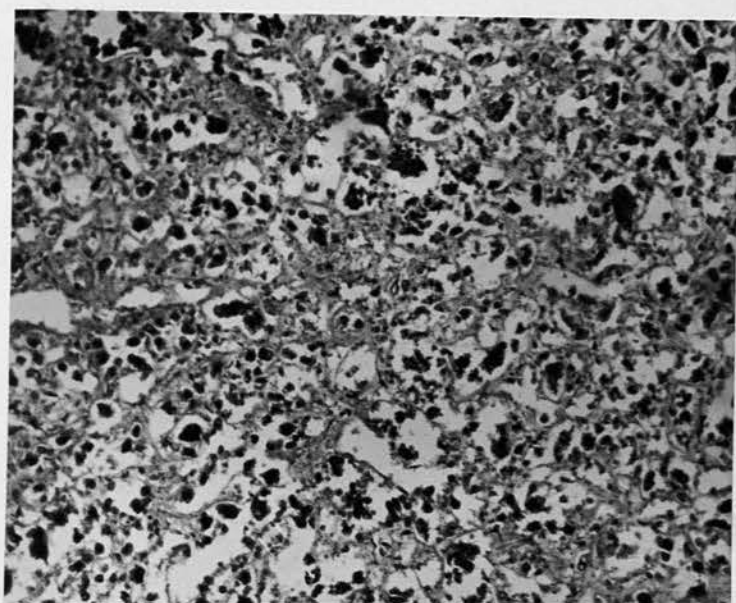


Fig. 5 a. LIVER x 200. Azan. To show fine pericellular fibrosis. Case of hydrops foetalis (macerated).

in icterus gravis and were not observed in this series, in the cases of hydrops foetalis.

Haemosiderosis is a very common finding and was present in nearly every case (Fig.18). The liver cells and Kupffer cells contained the characteristic brown granules of haemosiderin and the Prussian Blue reaction was positive. The cells at the periphery of the lobule are chiefly affected.

The portal tracts show infiltration of haemopoietic cells. Normoblasts, erythroblasts, myelocytes, lymphocytes and eosinophils are commonly present. Some increase in the fibrous tissue is not uncommon. The bile ducts show little change.

(11) Spleen : (Figs. 6, 7, 8) The capsule and trabeculae show little change. The Malpighian bodies tend to be absent (Fig.6.). This feature is stressed by Potter (1943). In one case (Series I No. 18) the Malpighian bodies were fibrosed. A diffuse fibrosis of the spleen is not uncommon. The pulp is generally congested and numerous foci of haemopoiesis are present. Haemosiderosis is less common than in the liver (Rautmann 1912, Salmonson 1931). It is found characteristically lying free in the subcapsular region and contained by histiocytes throughout the pulp (Fig.19). De Lange and Arntzenius (1929) and de Lange (1932) have found a non-iron-containing pigment in the spleen in a few cases.

(111) Kidneys (Fig.9): In a few cases in this series the lining epithelium of the convoluted tubules contained haemosiderin and the organ gave a positive/

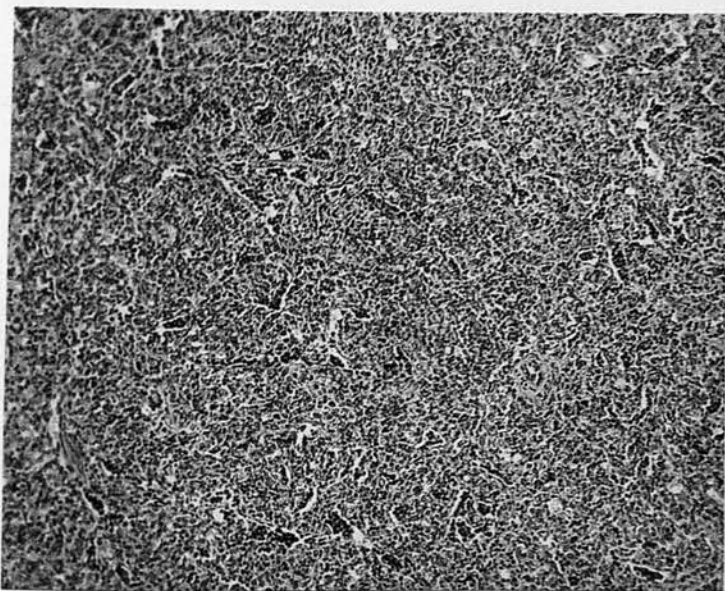


Fig. 6. SPLEEN x 85. Haematoxylin and Eosin.
Numerous haemopoietic foci in splenic pulp.
Note absence of Malpighian bodies.
Case of Hydrops Foetalis.

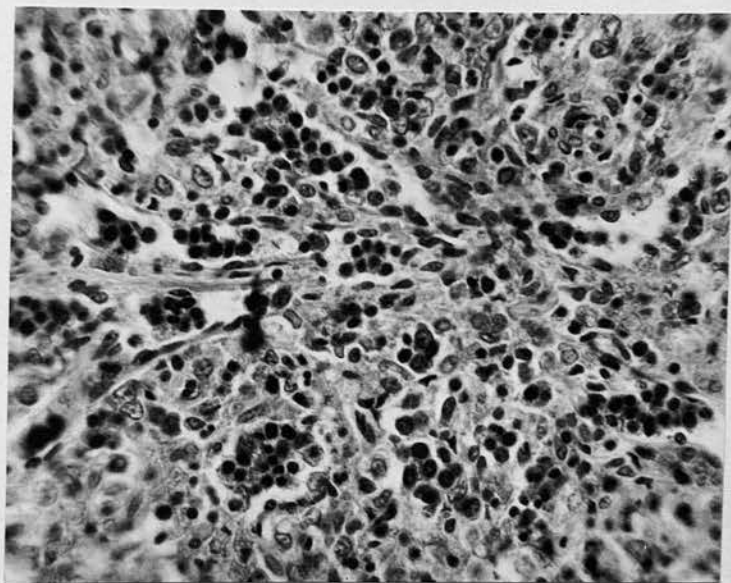


Fig.7. SPLEEN x 375. Haematoxylin and Eosin.
Foci of normoblasts and erythroblasts.
Case of Hydrops Foetalis.

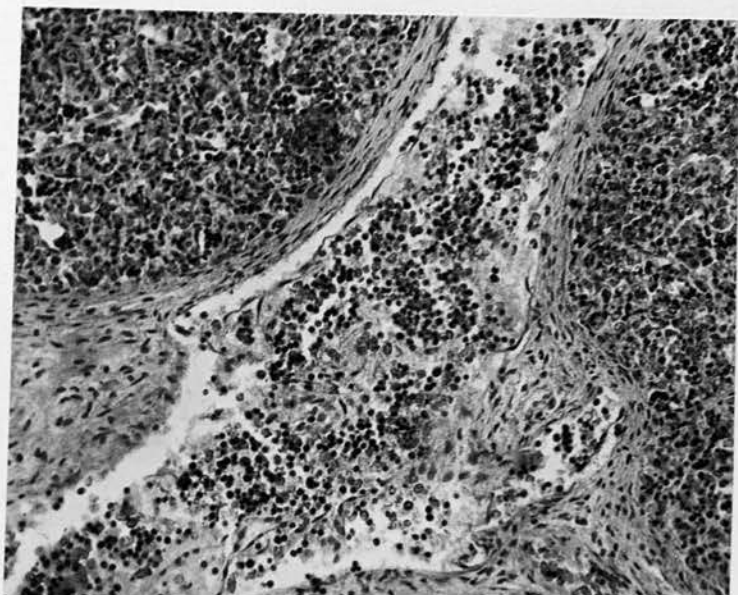


Fig.8. SPLEEN x 150. Haematoxylin and Eosin.
Large vessel containing many nucleated red
cells. Case of Hydrops Foetalis.



Fig.9. KIDNEY x 80. Haematoxylin and Eosin.
Numerous erythropoietic foci in juxta-
medullary zone of the cortex.
Case of Hydrops Foetalis.

positive Prussian blue reaction. This was also found by Schridde (1910), de Lange and Arntzenius (1929) and Gilmour (1944). Extra-medullary haemopoiesis was scanty in the kidney and occurred in two areas - firstly in the boundary zone of the cortex and secondly in the perivascular connective tissue in the renal pelvis (Fig.10).

(IV) Suprarenal Glands : (Fig.11 & 12).

A few foci of haemopoiesis may be found in the cortex. Gilmour (1944) found excessive vacuolation of the inner half of the cortex in sixteen out of eighteen cases. He showed that this was due to lipoid. In this series the finding has not been so constant but occurred in 60% of the cases where the suprarenal was examined. (Figs. 13, 14, 15.)

(V) Pancreas : (Fig.16) Potter, Seckel and Stryker (1941) and Gilmour (1944) have described an increase in the number and size of the islets of Langerhans. This is a very variable finding and large islets are not infrequently present in normal premature infants and in infants whose mother's are diabetic. As hydrops foetalis tends to result in premature births the finding of large islets is of doubtful value. Erythropoietic foci are found in the peri-lobular connective tissue and are seldom very numerous. In one case (Series I, No. 51), however, the whole of the pancreatic stroma was densely infiltrated with nucleated red cells and eosinophil leucocytes. (Fig.17).

(VI)

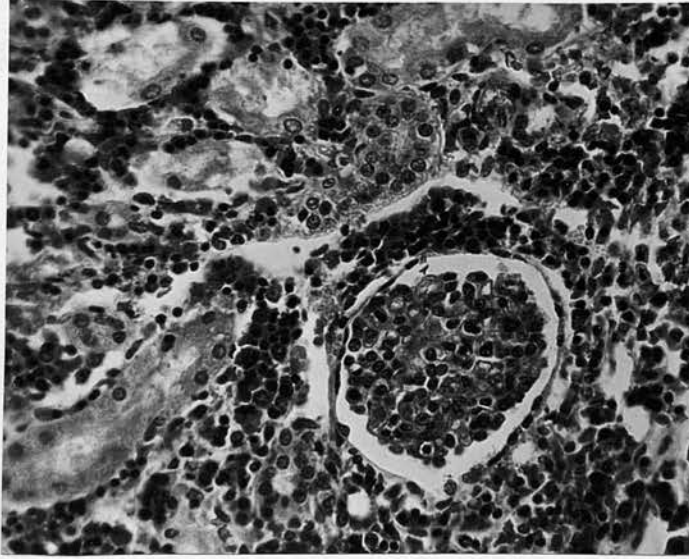


Fig. 10. KIDNEY x 275. Haematoxylin and Eosin. Foci of nucleated red cells lying between the tubules. Case of Hydrops Foetalis.

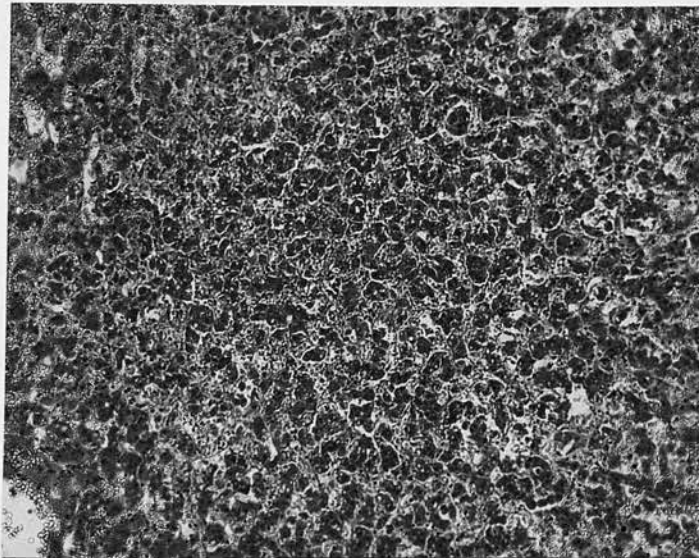


Fig. 11. SUPRARENAL GLAND x 80. Haematoxylin and Eosin. Deep layer of the cortex showing absence of vacuoles. Case of Hydrops Foetalis.

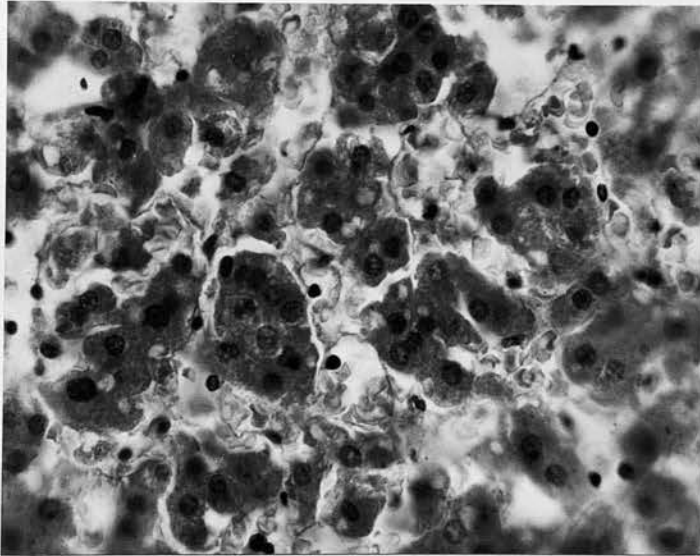


Fig.12. SUPRARENAL GLAND x 375. Haematoxylin and Eosin. Deep layer of the cortex showing absence of cell vacuolation. Case of Hydrops foetalis.

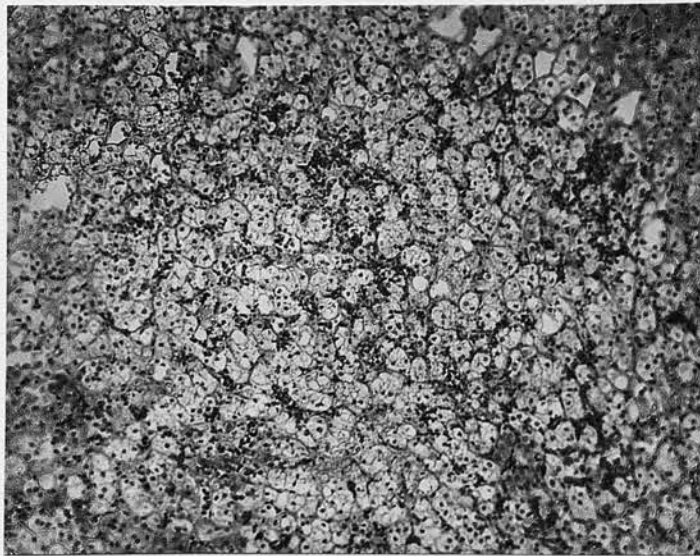


Fig. 13. SUPRARENAL GLAND x 80. Haematoxylin and Eosin. Deep layer of cortex showing marked vacuolation of the cells. Case of Hydrops foetalis.

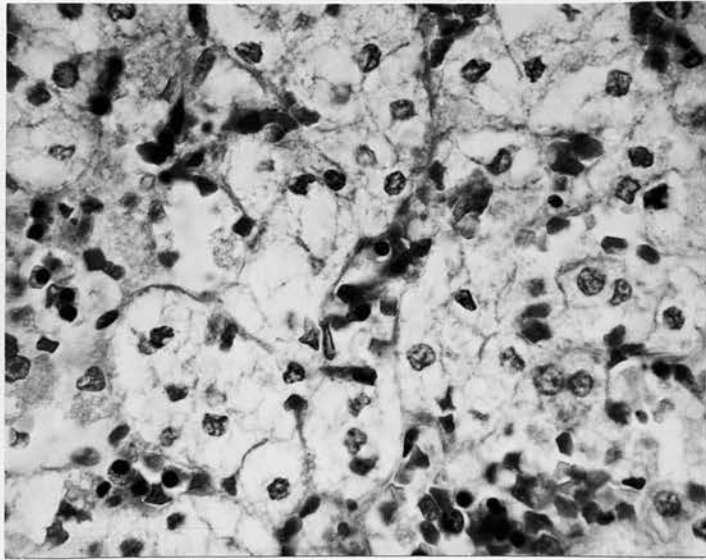


Fig. 14. SUPRARENAL GLAND X 375. Haematoxylin and Eosin. Deep layer of cortex showing large vacuoles. Case of Hydrops Foetalis.

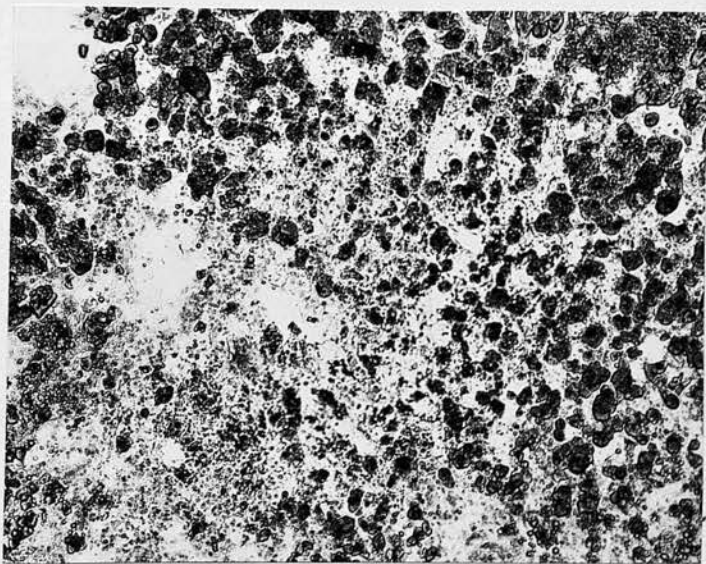


Fig. 15. SUPRARENAL GLAND x 70. Scharlach R. Heavy deposit of lipid in cortical cells. Case of Hydrops foetalis.

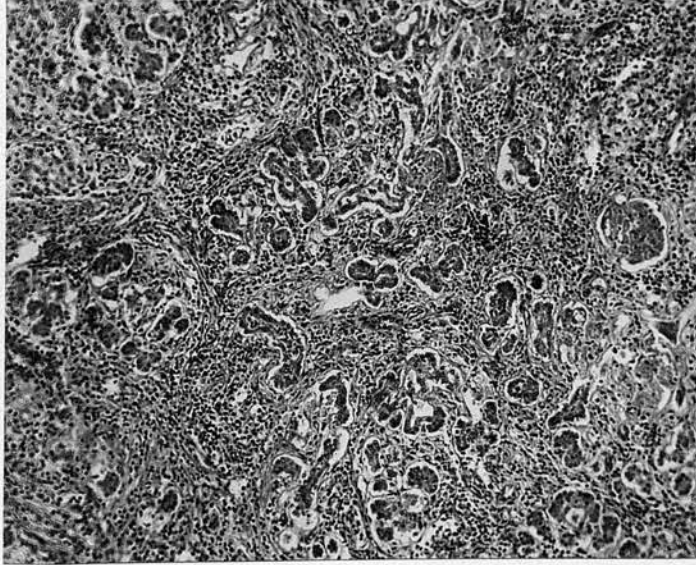


Fig. 16. PANCREAS x 80. Haematoxylin and Eosin. Heavy haemopoietic infiltration of the periacinar and perilobular connective tissue. The islets are of normal size. Case of Hydrops foetalis.

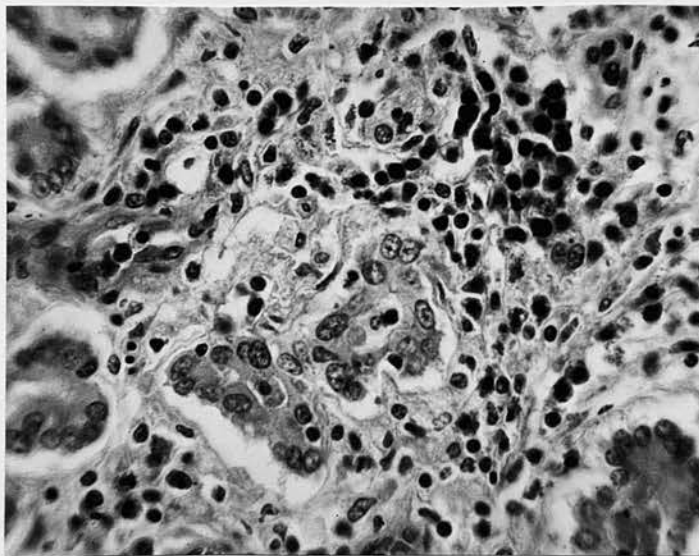


Fig.17. PANCREAS x 375. Haematoxylin and Eosin. Large focus of erythropoietic cells in the periacinar connective tissue. Case of Hydrops foetalis.

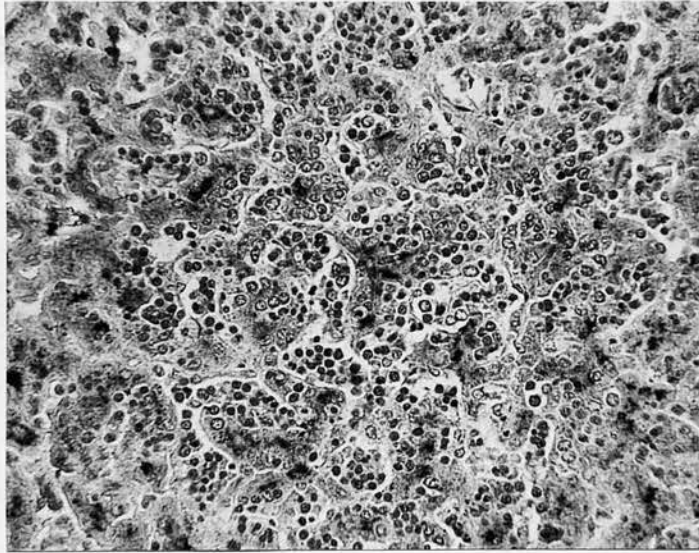


Fig. 18. LIVER x 250. Prussian Blue.
Dark staining deposits of haemosiderin in the
liver cells. Case of Hydrops foetalis.

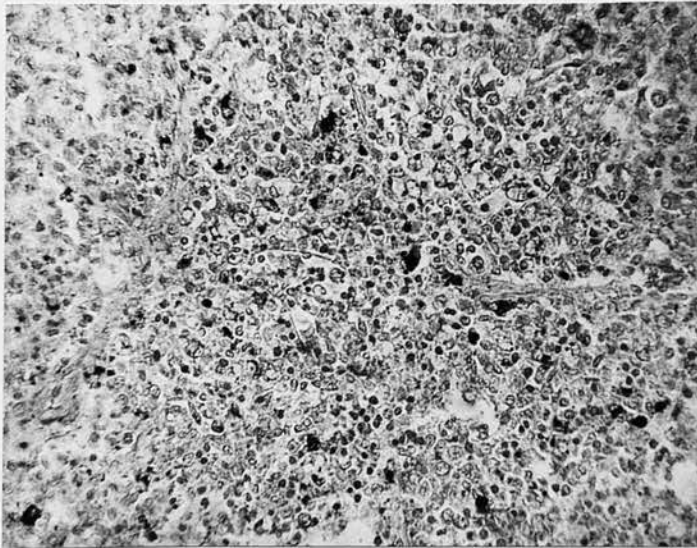


Fig. 19. SPLEEN x 250. Prussian Blue.
Dark staining deposits of haemosiderin in the
splenic pulp. Case of Hydrops foetalis.

(VI) Lungs : (Fig. 21) The lungs are usually unexpanded, airless and congested. Pulmonary oedema is frequently present and may be accompanied by evidence of inhalation of liquor amnii. A few foci of haemopoiesis may be found (Figs. 22 & 23). These are rare but, as Potter (1946) remarked, erythropoietic cells may be present in the capillaries. Their staining persists long after that of the surrounding cells has been lost.

(VII) Heart : The myocardial fibres are frequently separated by oedema and this accounts for the apparent hypertrophy found on macroscopic examination (Fig. 20).

(VIII) Brain : In this series no case showed specific areas of necrosis in the basal ganglia or other cerebral nuclei. In one case there was necrosis of the cortex but this was secondary to haemorrhage.

Placenta (Fig. 27) The villi are large, swollen and oedematous and the intervillous spaces are reduced in size. Foci of haemopoiesis are not common but many of the foetal capillaries may contain large numbers of nucleated red cells. The stroma cells are large, vacuolated but not increased in number. Occasionally the syncytial cells are prominent and hyperplastic. Langhan's cells are generally not recognisable. (Fig. 28)

Bones (Figs. 24, 25, 26) : Gilmour (1944) found abnormal endochondral ossification in fourteen of nineteen cases. He found a deepened zone of trabeculae/

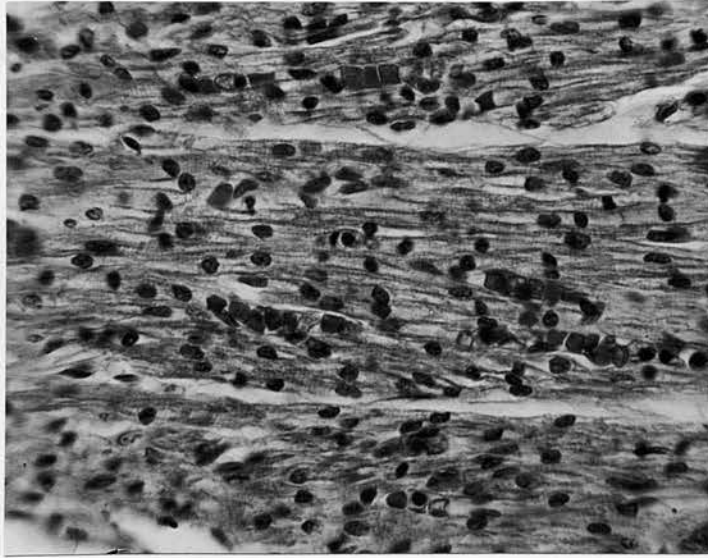


Fig. 20. HEART x 400. Haematoxylin and Eosin.
Note oedematous swelling of the myocardial
fibres. Case of Hydrops foetalis.



Fig.21. LUNG x 90. Haematoxylin and Eosin.
Large vessel showing "ghost" red cells and
well stained nucleated red cells. Case of
Hydrops foetalis (macerated foetus).

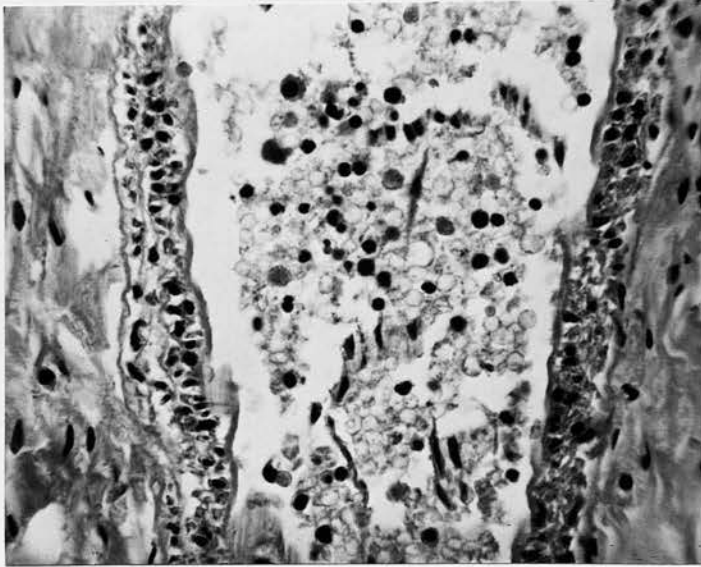


Fig. 22 LUNG x 375. Haematoxylin and Eosin.
Large vessel showing nucleated red cells.
Case of Hydrops foetalis (macerated foetus).

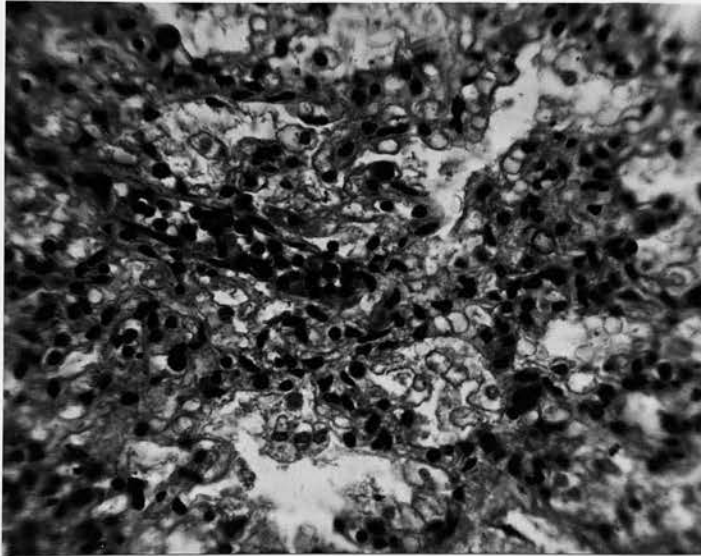


Fig. 23. LUNG x 375. Haematoxylin and Eosin.
Nucleated red cells in pulmonary capillaries.
Case of Hydrops foetalis (macerated foetus).

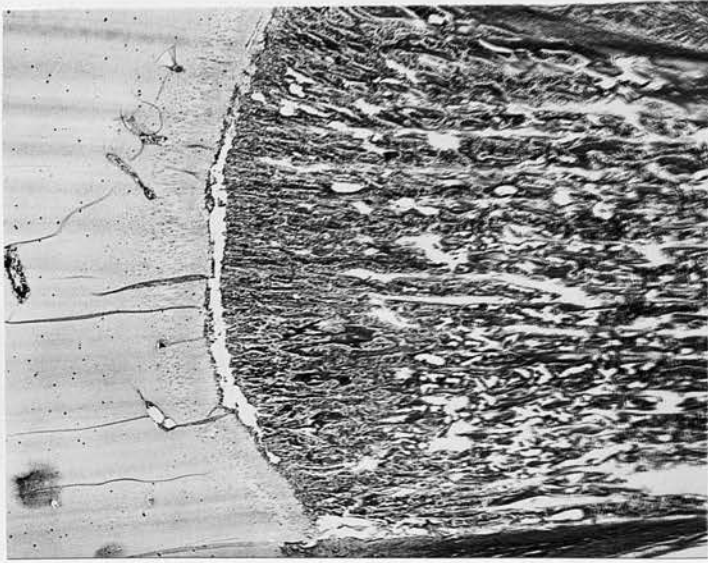


Fig. 24 BONE x 10. Haematoxylin and Eosin.
Costo-chondral junction showing trabeculae
and marrow spaces of shaft. Normal control.

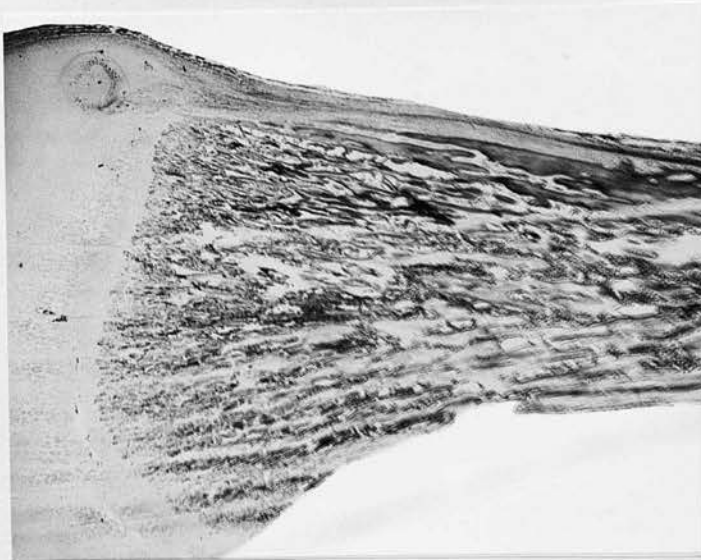


Fig. 25. BONE x 10. Haematoxylin and Eosin.
Costo-chondral junction showing little
difference from Fig. 24. Case of Hydrops
foetalis.

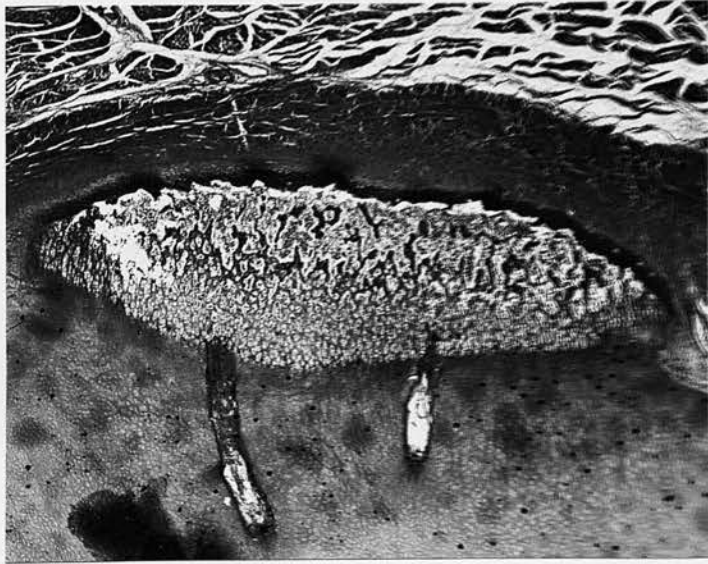


Fig. 26. BONE x 18. Haematoxylin and Eosin.
note normal line of ossification and only
very slight broadening of the trabeculae.
Case of Hydrops foetalis.

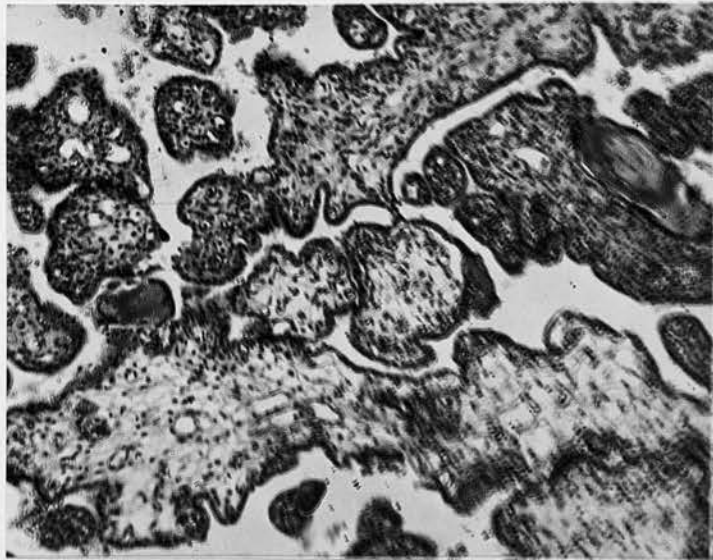


Fig. 27. PLACENTA x 80. Haematoxylin and Eosin.
Note large, swollen, oedematous villi. The
foetus was hydropic.

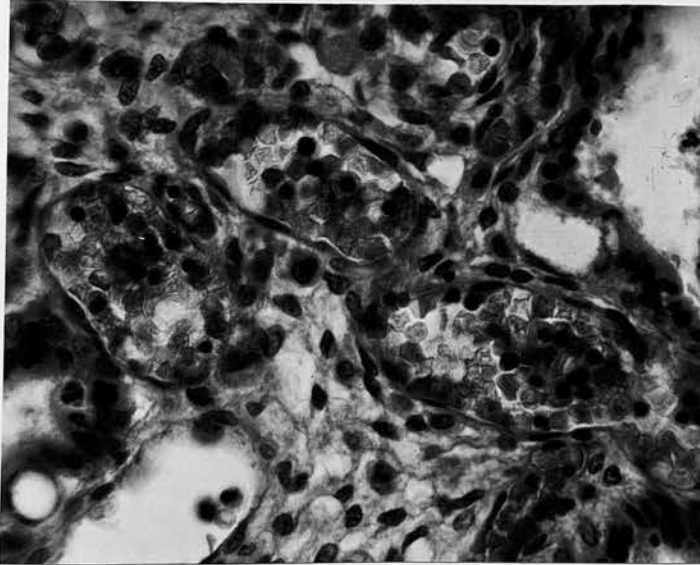


Fig. 28. PLACENTA x 400. Haematoxylin and Eosin.
Oedematous villi and nucleated red cells in
foetal capillaries.

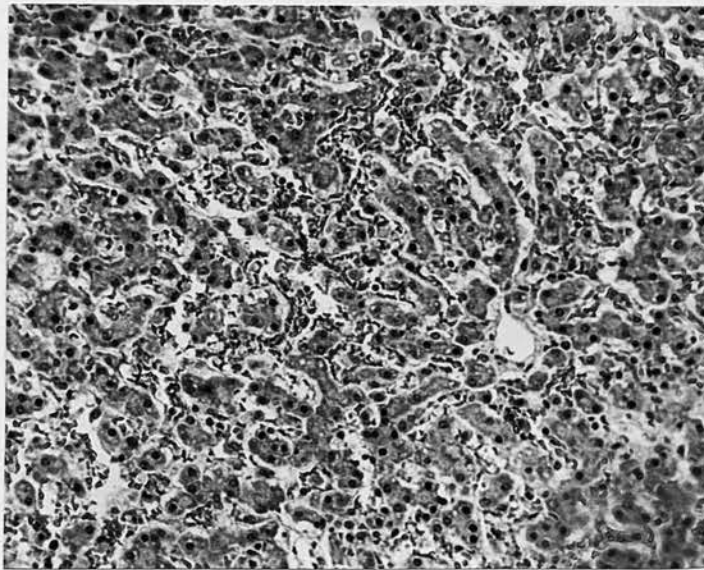


Fig. 29. LIVER x 150. Haematoxylin and Eosin.
Normal full term infant. Note absence of
haemopoiesis.

trabeculae of calcified cartilage " and narrow medullary spaces. These trabeculae were covered with bone and osteoid tissue and the author ascribed the condition to deficient osteoblastic activity and a decrease in osteoclastic resorption. This change was not obvious in the bones examined in the present series. One case examined later (macerated foetus in Fig.1.) showed an abnormally broad layer of unossified cartilage in the epiphyseal region.

2. Icterus Gravis (77 fatal cases).

A. Morbid Anatomy.

Included in this total of seventy seven fatal cases are thirty-five cases which showed kernicterus. This cerebral manifestation is of some interest and will be dealt with in a later chapter (Chapter 14).

Sex : Gilmour (1944) in his series had sixteen males and ten females in a total of twenty-six cases. An examination of cases reported in the literature revealed a predominance of four to three in favour of male infants. In this series there were 48 males and 29 females.

Maturity : The proportion of pregnancies reaching term is greater in cases of icterus gravis than in hydrops foetalis. In this series 59 of the infants were mature and 18 premature.

Jaundice : As the name implies, jaundice is one of the main features of the disease. The skin and mucous membranes are generally a bright golden-yellow colour and not infrequently the infant is covered with/

with golden vernix at birth. The jaundice varied in intensity from case to case and there was no direct correlation between the depth of the jaundice and the severity of the pathological lesions. All the cases which showed kernicterus were deeply jaundiced but many cases which were equally jaundiced had not suffered from intracerebral lesions.

Petechial Haemorrhages : Petechial haemorrhages into the skin, under the pleura and pericardium are commonly found. They are presumably related to a low prothrombin level in the blood as a result of hepatic damage (Dam et al 1939).

Pallor : This is more frequently observed in cases older than five days as the jaundice tends to mask the underlying pallor in earlier cases.

Oedema : Subcutaneous oedema is not common unless the accompanying anaemia is severe or the liver damage is very extensive. The serous sacs frequently contain some bile-stained fluid but this never approaches the quantities found in foetal hydrops.

Organs :

(1) Liver : The liver is unusually enlarged and may extend several fingers-breadth below the costal margin. It is unusually reddish-brown in colour but may show greenish-discoloration as a result of bile staining. This latter is usually more obvious on the cut surface. The liver is firm in consistence but occasionally may be rather tough to cut. De Lange (1918) reported a case of fatal cirrhosis of the liver/

liver following an icterus gravis but no true cirrhosis was present in this series. In a few cases yellow areas indicating fatty degeneration were present.

The reddish-brown discoloration of the liver is due to haemosiderin and in many cases the Prussian Blue reaction was positive.

(11) Spleen : The spleen is usually enlarged and may measure from $2\frac{1}{2}$ " to 4" in length, with a corresponding increase in its other dimensions. An enlarged spleen of this size is an important necropsy finding but unfortunately it is not invariable and many cases in this series had a spleen of normal size. The capsule is smooth and the cut surface is dark red in colour. The Prussian blue reaction is frequently positive. In one case (Case 25, Series II), the spleen had ruptured and lead to a massive intraperitoneal haemorrhage. This finding has also been reported by Capon (1922), von Gierke (1930) and Rhamy (1938).

(111) Kidneys : The kidneys are of normal size and shape. They may be pale and are frequently bile-stained. In one case (Case 22, Series II) death resulted from pyelonephritis.

(1V) Lungs : The most important lesion in the lungs in icterus gravis is massive intrapulmonary haemorrhage. All the lobes may be firmly consolidated and dark red in colour. Large quantities of blood may be expressed from the cut surface. Since the lesion/

lesion is presumably associated with a low prothrombin level in the blood as a consequence of liver damage, it is more frequently found when the latter is great.

Bronchopneumonia, either primary or secondary to intrapulmonary haemorrhage is a common complication.

(V) Brain : Nuclear staining or kernicterus was commonly present and is fully described in a later chapter. A large number of cases, however, showed a diffuse yellow coloration of the leptomeninges and the brain tissue. This has also been found by Capon (1922) and Hawksley and Lightwood (1934).

Subdural haemorrhage was occasionally present and may be associated partly with birth trauma and partly with the haemorrhagic diathesis common to icterus gravis.

B. Morbid Histology.

(1) Liver (Fig. 31.) : The capsule is normal. The liver parenchyma is frequently almost obscured by large foci of extramedullary haemopoiesis. These are composed of normoblasts and primitive erythroblasts. Some leucopoietic cells, myelocytes and lymphocytes, may also be present. These primitive cells also invade the portal tracts.

The liver cells frequently show degeneration and necrosis chiefly affecting the cells in the portal zone of the lobule (Fig. 34, 35). In many cases the cells in the outer zones of the lobules and the Kupffer cells contained haemosiderin and gave a positive/

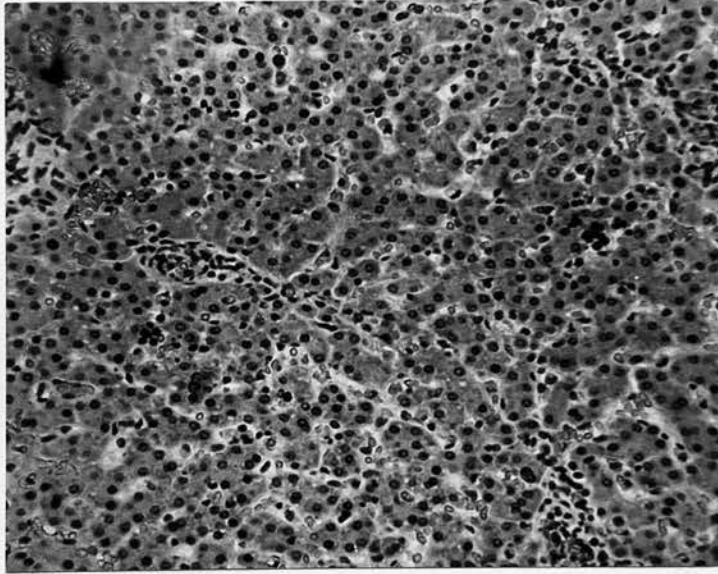


Fig. 30. LIVER x 150. Haematoxylin and Eosin.
Normal premature infant (36 weeks). Note
scanty foci of normoblasts.

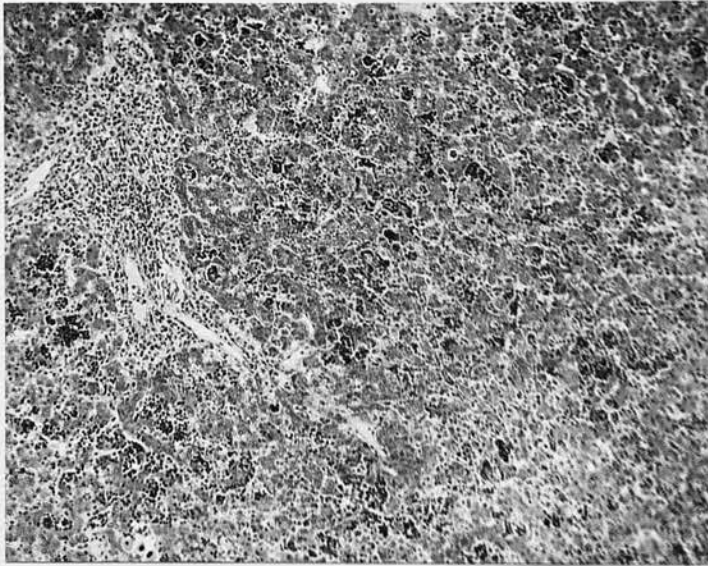


Fig. 31. LIVER x 80. Haematoxylin and Eosin.
Numerous foci of extramedullary haemopoiesis.
Case of icterus gravis.

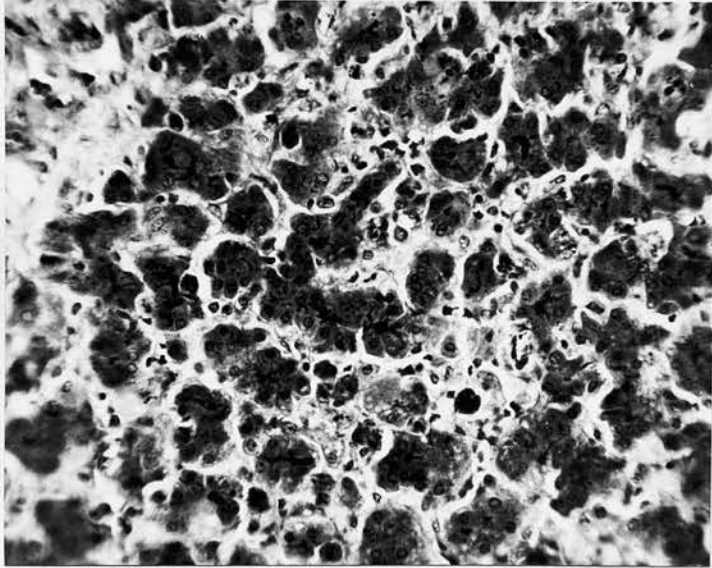


Fig. 32. LIVER x240. Haematoxylin and Eosin.
Note bile plugs in intercellular canaliculi.
Case of icterus gravis.

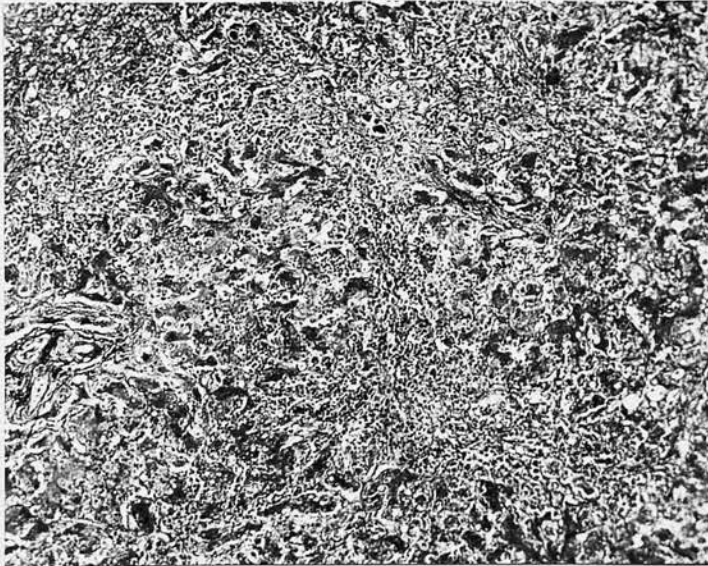


Fig. 33. LIVER x 80. Azan. Increased
fibrous tissue in portal zones. Case of
icterus gravis.

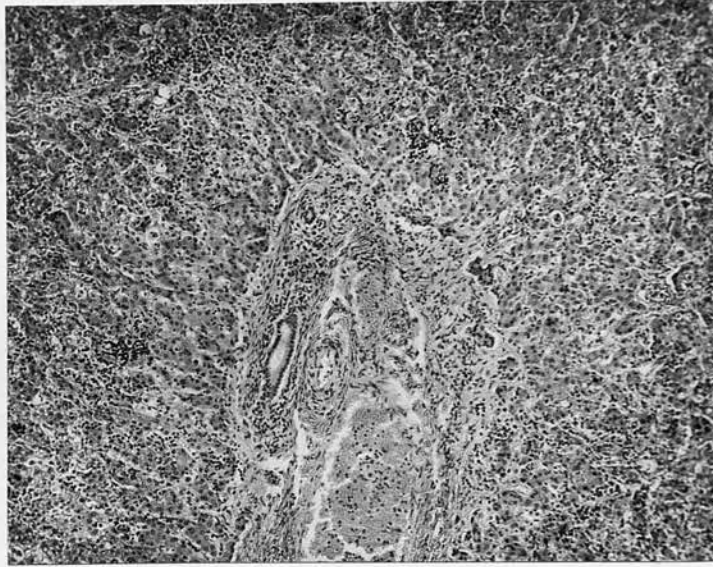


Fig. 34. LIVER x 80. Haematoxylin and Eosin.
Note damage to parenchymal cells in the
portal zones of the lobules. Case of
Icterus gravis.

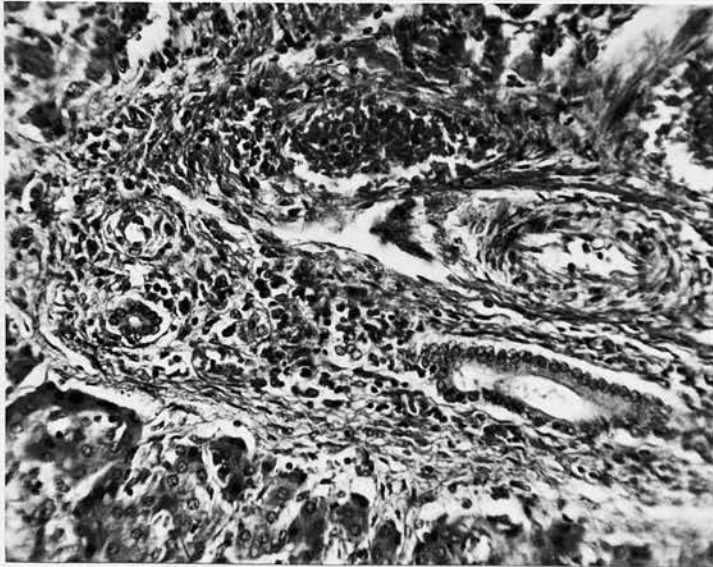


Fig. 35. LIVER x 250. Haematoxylin and Eosin.
Damage to liver cells in portal zones.
Case of Icterus gravis.

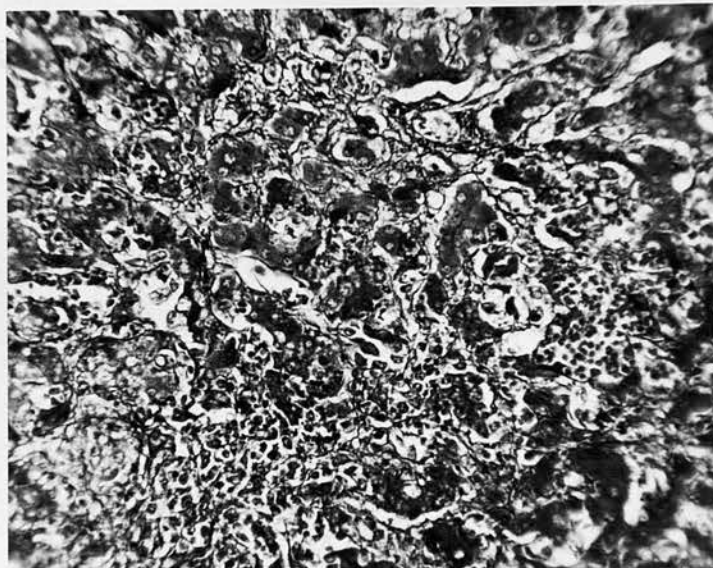


Fig. 36. LIVER x 250 Azan. Increase in fibrous tissue in portal zones. Case of Icterus gravis.

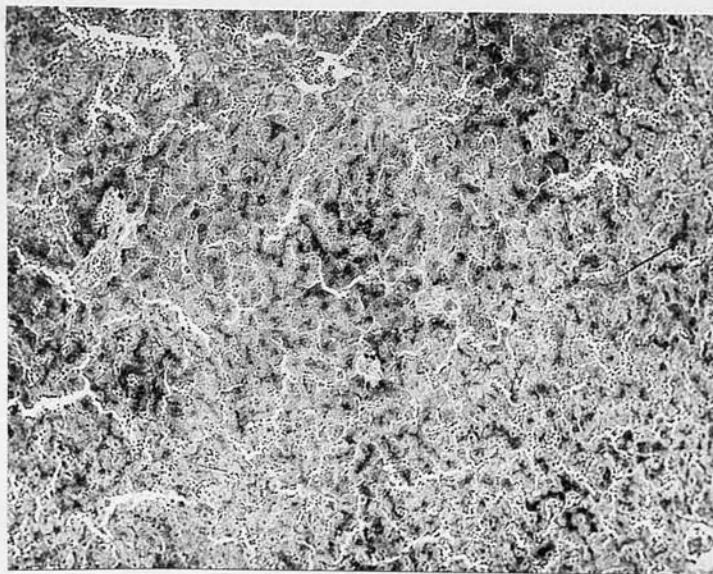


Fig. 37. LIVER x 80. Prussian Blue. Deposits of haemosiderin in parenchymal and Kupffer cells. Case of Icterus gravis.

positive Prussian blue reaction (Fig. 37). Central zone necrosis was present in a few cases, but was seldom severe.

The intra and intercellular canaliculi are plugged with inspissated bile in many cases (Fig. 32).

If death is delayed for a week or longer a fine diffuse fibrosis may be found. In one case (Case 4, Series II) which died at the end of seven weeks there was considerable periportal fibrosis with numerous new bile-ducts in the fibrous stroma (Fig. 33 & 36).

Diffuse fibrosis has been reported by Buchan and Comrie (1909), Pfannenstiel (1908) Hawksley and Lightwood (1934) and Hoffman and Hausmann (1926).

Fibrosis of the periportal type was described by Diamond, Blackfan and Baty (1932), Hawksley and Lightwood (1934) and Bernheim-Karrer (1936).

Regeneration of the liver cells was not present in the cases in this series.

(11) Spleen : (Fig. 38) The capsule and trabeculae show little change. There may occasionally be a diffuse fibrosis with decreased cellularity of the pulp. More often the pulp is very cellular, deeply congested and the sinusoids are packed with erythropoietic cells. Many are normoblasts but erythroblasts are also common. The Malpighian bodies are often small and poorly developed but are seldom absent as in Hydrops foetalis.

Haemosiderosis is frequent. The pigment has a predilection for the subcapsular area where it may/

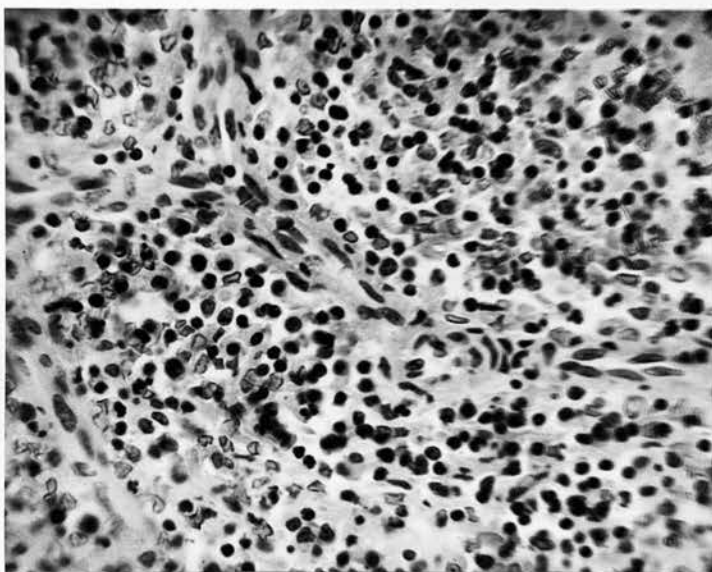


Fig. 38. SPLEEN x 350. Haematoxylin and Eosin.
Numerous foci of haemopoiesis in splenic
pulp. Case of icterus gravis.

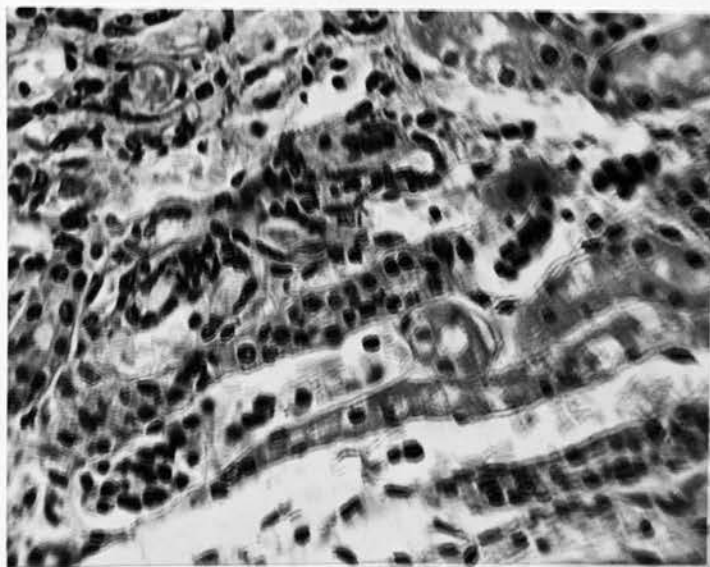


Fig. 39 a. KIDNEY x 375. Haematoxylin and
Eosin. Haemopoietic foci in boundary zone
of renal cortex. Case of icterus gravis.

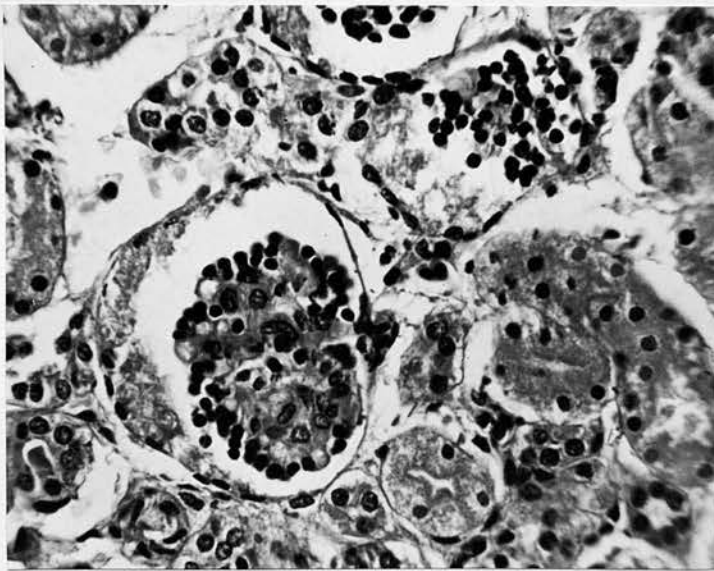


Fig. 39 b. KIDNEY x 375. Haematoxylin and Eosin. Foci of haemopoiesis in boundary zone of renal cortex. Case of icterus gravis.

may be found lying free or contained by histiocytes. The Prussian blue reaction is strongest in this zone. The pigment may also be found in histiocytes or endothelioid cells throughout the pulp.

(111) Kidney : (Fig 39a & b). Haemopoiesis is rarely well-marked and is chiefly confined to the boundary zone of the cortex and to the perivascular connective tissue in the renal pelvis. The cells lining the convoluted tubules may contain haemosiderin and give a positive Prussian blue reaction. More rarely they contain bile. The kidneys tend to be pale and the tubules may show early fatty degeneration.

(1V) Brain : The changes following on kernicterus are described elsewhere (Chapter 14). The cases in which the brain was diffusely bile-stained showed no abnormality of the cerebral tissue on microscopic examination.

(V) Lungs : The lungs frequently show foci of haemopoiesis in the alveolar walls or in the peribronchial connective tissue. Intrapulmonary haemorrhage may be found in severe cases of icterus gravis (Fig. 40). The entire lung may be filled with blood and the alveoli and bronchi are obscured by masses of erythrocytes, many of which are nucleated. Bronchopneumonia is a common complication.

(VI) Other Organs : Potter, Seckel and Stryker (1941) have found the islets of the pancreas enlarged in two cases. This was not found in the present series. Foci of erythropoiesis may be found/

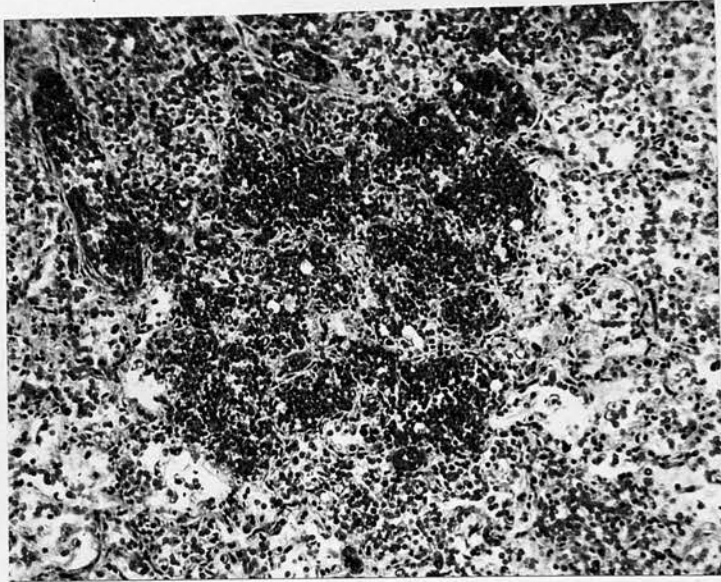


Fig. 40. LUNG x 120. Haematoxylin and Eosin.
Area of intrapulmonary haemorrhage.
Case of icterus gravis.

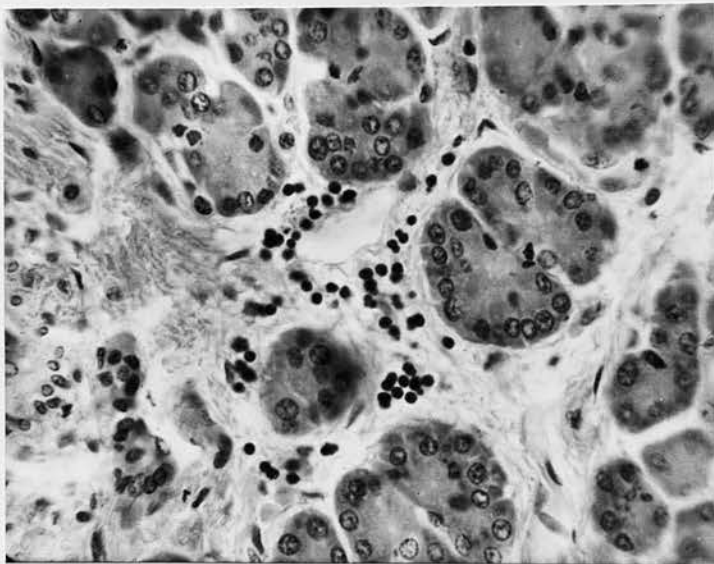


Fig. 41. PANCREAS x 350. Foci of haemopoiesis
in periacinar tissue.
Case of icterus gravis.

found in the interstitial tissue of the pancreas (Fig. 41), the suprarenal cortex, the thyroid gland, the thymus gland and occasionally in lymphatic glands.

3. Congenital Haemolytic Anaemia.

A. Morbid Anatomy : (3 fatal cases)

Sex : Two were males and the third was a female.

Maturity : All three were born at term.

Pallor : This is the most obvious feature in cases of congenital haemolytic anaemia. It affects both the skin and mucous membranes and the internal organs.

Jaundice : One of the cases (Case 42, Series II) showed slight jaundice. The other two were not icteric.

Oedema : Oedema of the feet and ankles is quite common and ascites or hydrothorax may also be present.

Petechial Haemorrhages : These were not present in the three cases but were found in two cases reported by Gilmour (1944).

Organs :

(1) Liver : The liver is enlarged, firm in consistence and reddish brown in colour. The Prussian blue reaction is strongly positive.

(11) Spleen : The spleen is enlarged and in one case (Series II, Case 8) measured $4\frac{1}{2}$ " in length. It is dark red in colour and the Prussian blue reaction/

reaction is strongly positive. In one case (Case 12, Series II) there was a small subcapsular rupture of the spleen with extravasation of blood into the abdominal cavity.

(111) Kidneys : The kidneys are pale and the Prussian blue reaction may be positive (Case 8, Series II).

(1V) Lungs : The lungs are pale and oedematous. Bronchopneumonia is commonly present.

(V) : Heart : The heart may be dilated and the myocardium is usually pale.

B. Morbid Histology :

(1) Liver (Fig. 43) There are numerous foci of erythropoiesis and both normoblasts and erythroblasts are numerous. The parenchymal and Kupffer cells are loaded with haemosiderin and the Prussian blue reaction is positive (Fig. 44, 47). One case (Case 42, Series II) showed fatty degeneration of the cells in the central zone of the lobules.

(11) Spleen (Fig. 45) The capsule and trabeculae are normal. The Malpighian bodies may be small and poorly developed. The pulp is highly cellular and numerous erythropoietic cells are found. The endothelial cells and histiocytes are laden with haemosiderin and the Prussian blue reaction is positive (Fig. 46 & 47 b).

(111) Kidneys : The cells lining the convoluted tubules may contain haemosiderin and give a positive Prussian blue reaction (Fig. 47).

(1V) /

(IV) Suprarenal Gland (Fig. 48) In one case (Case 8, Series II) there were numerous erythropoietic foci in the suprarenal cortex.

(V) Peripheral Blood (Fig. 42) Nucleated red cells are numerous and may number as many as 200,000/cu.mm. All types are seen from the occasional haemocytoblast to the late normoblast. Numerous erythroblasts are present in the severe cases. The red cells which are not nucleated show anisocytosis, poikilocytosis and polychromasia. The colour index is usually over unity and numerous reticulocytes (up to 60%) are present.

(VI) Bone Marrow : Satisfactory specimens of marrow are difficult to obtain from children of this age period. There is a tremendous reaction in the haemopoietic and to a lesser extent in the leucopoietic tissue. Nucleated red cells are abundant and all stages from the haemocytoblast to the erythrocyte are seen. The eosinophil leucocyte is found in larger numbers than usual.

A final remark must be made about cases of hydrops in the foetus which are not the result of congenital haemolytic disease. Potter (1946) very correctly pointed out that the diagnosis of hydrops resulting from haemolytic disease must be made after very careful histological examination of the tissues. She points out the value of examination of the lungs. These organs resist maceration for a much longer period than the liver or spleen and primitive cells of the erythrocytic series retain their staining potential/

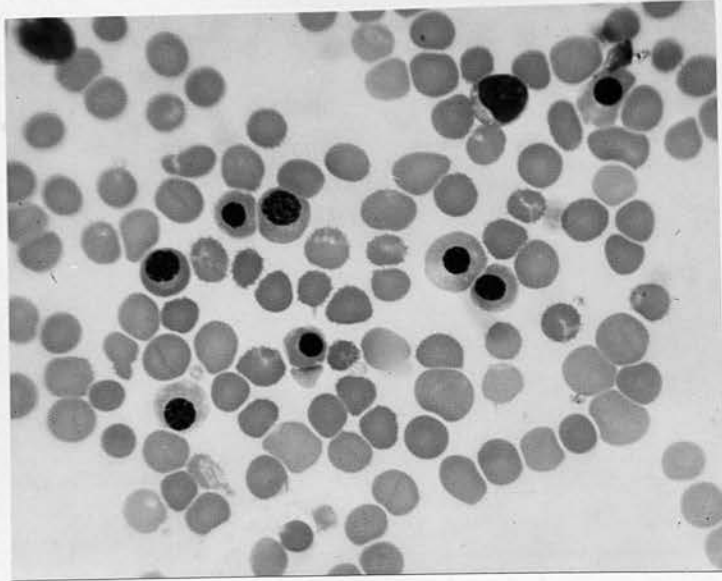


Fig. 42. BLOOD FILM x 600. Leishman.
Numerous normoblasts and erythroblasts are
present. Case of haemolytic anaemia.

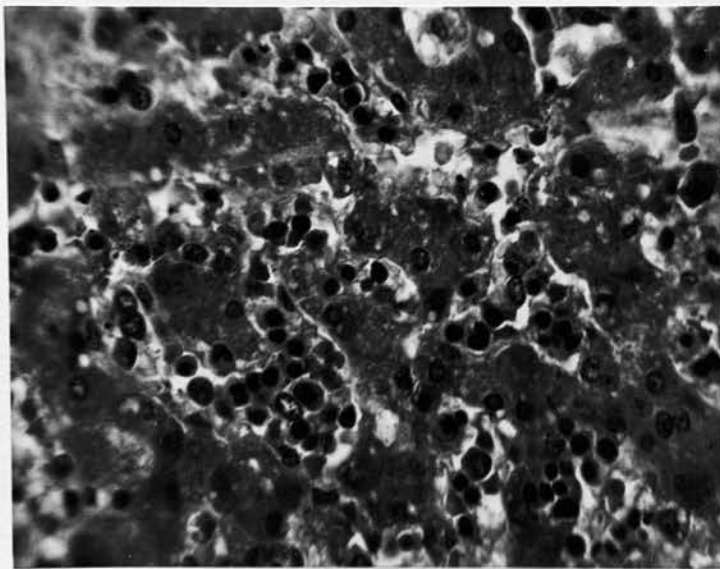


Fig. 43. LIVER x 350. Haematoxylin and Eosin.
Numerous haemopoietic foci. Case of
congenital anaemia.

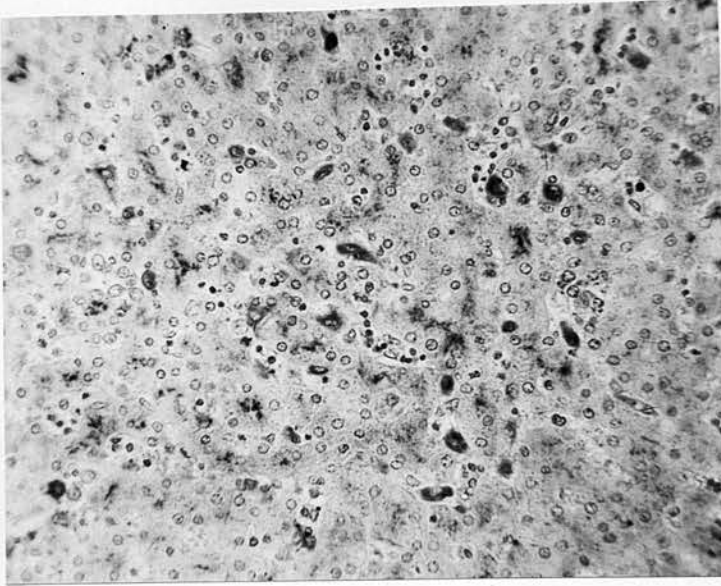


Fig. 44. LIVER x 230. Prussian Blue.
Deposits of haemosiderin in parenchymal
and Kupffer cells. Case of haemolytic
anaemia.

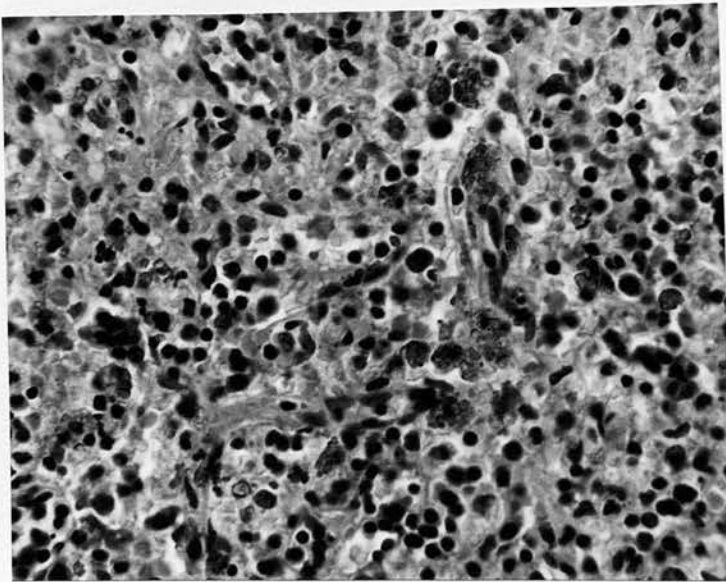


Fig. 45. SPLEEN x 350. Haematoxylin and Eosin.
Foci of haemopoiesis and haemosiderosis in
splenic pulp. Case of haemolytic anaemia.

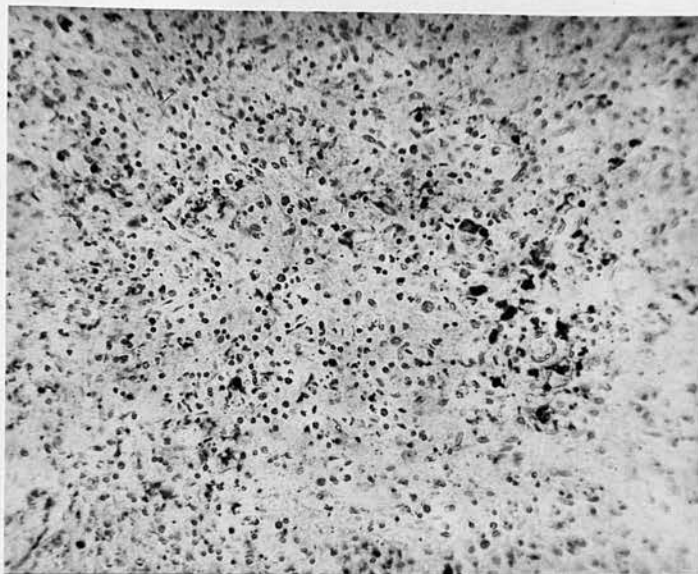


Fig. 46. SPLEEN x 230. Prussian Blue.
Widespread haemosiderin deposits in splenic
pulp. Case of haemolytic anaemia.

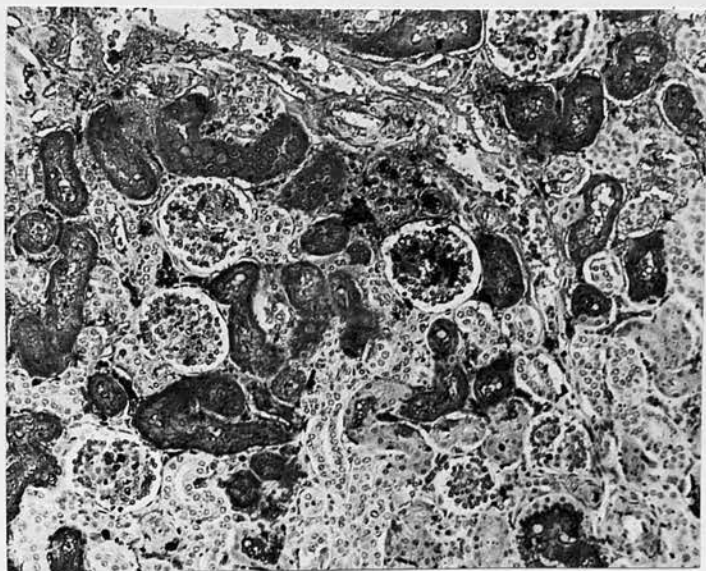


Fig. 47. KIDNEY x 110. Prussian blue.
Haemosiderin in renal tubular cells.

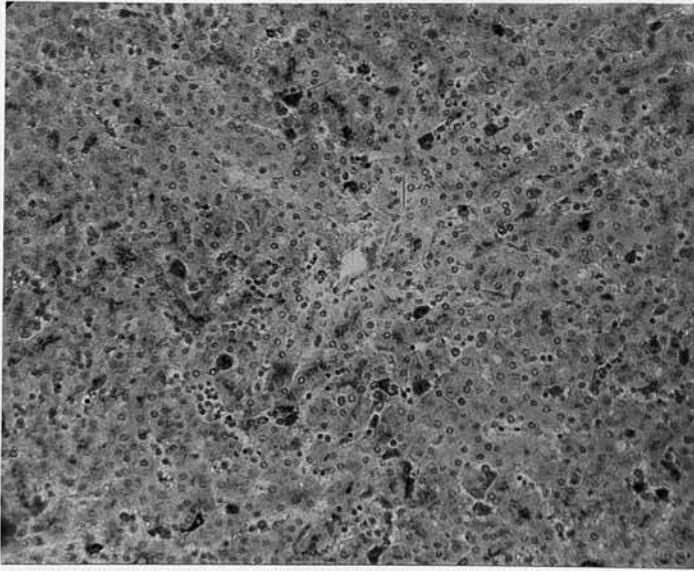


Fig. 47 a. LIVER x 110. Prussian blue.
Deposits of haemosiderin in liver cells.
Case of haemolytic anaemia.

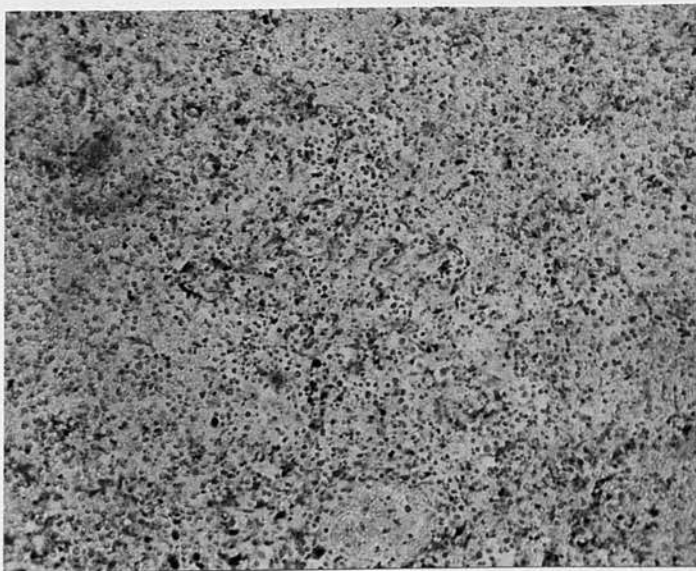


Fig. 47 b. SPLEEN x 110 . Prussian blue.
Deposits of haemosiderin in splenic pulp.
Case of haemolytic anaemia.

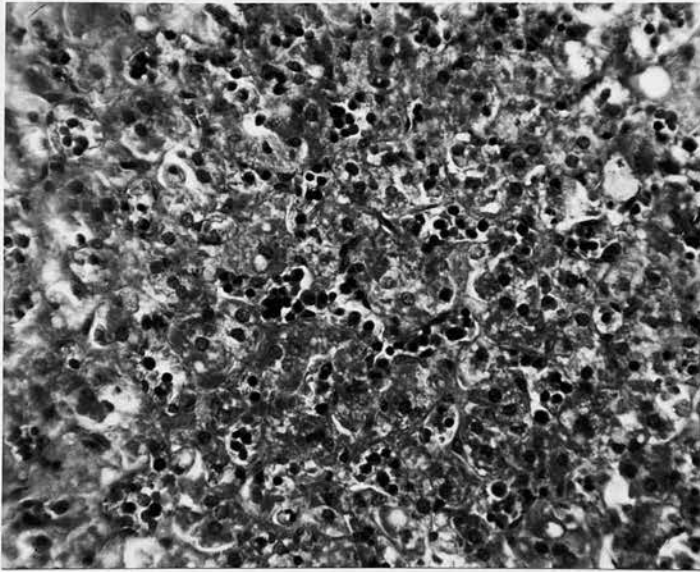


Fig. 48. SUPRARENAL GLAND x 250.
Haematoxylin and Eosin. Foci of
haemopoiesis in the cortex. Case of
congenital haemolytic anaemia.

potential long after other cells have lost theirs. Thus the discovery of primitive erythroblasts in the pulmonary capillaries of a macerated foetus is of some diagnostic significance. The placenta is also a valuable aid in arriving at a correct diagnosis, but only too often it is not sent with ^{the} foetus for examination.

These cases of hydrops sine erythroblastosis are of obscure origin. They are not due to congenital syphilis. They frequently occur in first pregnancies and may be associated with congenital malformations of the foetus such as spina bifida and diaphragmatic hernia (Macgregor, 1949). They are nearly always followed by normal children (Potter, 1946). Thus, if the mother is Rh. negative and is delivered of a still-born foetus, it is of prime prognostic importance to ascertain whether the case is one of congenital haemolytic disease or not. An entirely erroneous prognosis with regard to the outcome of further pregnancies in the mother may be given, if such cases are incorrectly diagnosed as examples of haemolytic disease. Much needless anxiety on the part of parents can be avoided if a full post-mortem examination is performed.

Series I. S.M.M.P.

AUTOPSY FINDINGS.

Table 34a.

Case.	Age at DEATH.	Wt. (lbs).	Liver.	Spleen.	Kidneys.
1	6 weeks.	5 $\frac{3}{4}$	Normal size. Moderate erythropoiesis.	Twice normal. No haemopoiesis.	Normal.
2a	S.B.	3	Normal size. Marked haemopoiesis.	Normal size. Marked haemopoiesis.	Marked haemopoiesis
2b	S.B.	3	Normal size. Marked haemopoiesis.	Normal size. Marked haemopoiesis.	Normal.
3	4 days	9	Very enlarged. P. Blue positive. Haemopoiesis	V. enlarged. P. blue pos. Haemopoiesis.	Normal.
4	7 days	4 $\frac{1}{4}$	Normal size	Normal size.	"
5	S.B.	6 $\frac{1}{2}$	Enlarged. Haemopoiesis.	V. enlarged. Haemopoiesis.	"
7	S.B.	5 $\frac{1}{2}$	Normal size. Haemopoiesis.	Normal size. Haemopoiesis.	Haemopoiesis.
8	S.B.	5	Enlarged. Haemopoiesis.	Enlarged. Haemopoiesis.	"
9	2 days	5 $\frac{3}{4}$	Enlarged.	V. enlarged. Haemopoiesis.	Nil.
10	10	6	"	V. enlarged. Haemopoiesis.	"
11	S.B.	5	" Macerated	V. enlarged. Haemopoiesis.	"
12	S.B.	5 $\frac{1}{4}$	"	V. enlarged. Macerated	Macerated.
13	S.B.	1 $\frac{1}{4}$	"	Enlarged.	"

contd.

Case.	Age at DEATH.	Wt. (lbs.)	Liver.	Spleen.	Kidneys.
15	4 days	5½	Enlarged. Haemopoiesis.	Enlarged. Haemopoiesis.	Nil.
16	S.B.	2¾	V. "	"	"
17	S.B.	3¾	S. "	"	"
18	1 day	4	"	V. "	Haemopoiesis.
19	S.B.	4¾	"	Enlarged.	S.
20	19 days	8	V. "	V. "	Nil.
21	S.B.	5½	"	"	Haemopoiesis.
22.	S.B.	6½	S. " Macerated.	"	"
			S. " Fibrosis.	Enlarged	Nil.
			S. " Macerated.	V. " Haemopoiesis.	Macerated.
23	S.B.	1	Macerated.	V. "	Nil.
24	2 hrs.	Small	Enlarged. Haemopoiesis.	V. "	Nil.
25	S.B.	5	" P. Blue +ve. Marked haemopoiesis.	V "	Haemopoiesis.
26a	3 days	4½	Enlarged. Haemopoiesis	Enlarged.	Nil.
26b	3 days	2¾	" "	"	"
27	6 hrs.	3	Normal size.	Normal size.	"
28	1 hr.	6¼	Enlarged.	V. enlarged.	Haemopoiesis.
29a	3 days	5	S. "	V. "	Nil.
29b	3 days	4¾	S. "	"	"
30	9 days	3	Enlarged.	V. "	"
31	7 days	4¼	S. " S.	Enlarged	Nil.

Case.	Age at DEATH.	Wt. (lbs.)	Liver.	Spleen.	Kidneys.
32	S. B.	7 $\frac{3}{4}$	Enlarged. Haemopoiesis. Prussian Blue positive.	V. enlarged. Haemopoiesis.	Nil
33	S. B.	6 $\frac{1}{2}$	Enlarged. Haemopoiesis.	V. " Macerated.	Haemopoiesis.
34	9 days	5	" S. "	V. " S. Haemopoiesis.	Nil.
35	S. B.	3 $\frac{3}{4}$	" Macerated.	Enlarged. Macerated.	Macerated.
36	3 days	4 $\frac{3}{4}$	S. " Haemopoiesis.	" Haemopoiesis.	Haemopoiesis.
38	12 days	7 $\frac{1}{4}$	S. " "	S. " "	Nil.
39	6 days	4 $\frac{1}{4}$	Normal size.	Normal size.	Nil.
40	1 hour	5 $\frac{3}{4}$	" Haemopoiesis.	Enlarged. Haemopoiesis.	Nil.
41	1 hr. 40min.	3 $\frac{1}{4}$	Enlarged.	" "	Nil.
42	5 days	6 $\frac{1}{4}$	" "	V. " "	Bile-stained.
43	6 days	6 $\frac{1}{4}$	" "	V. " "	Haemopoiesis.
44	S. B.	7	S. " Macerated.	Normal size. Macerated.	Macerated.
45	S. B.	2 $\frac{1}{2}$	Enlarged. Haemopoiesis.	V. Enlarged. Haemopoiesis.	Nil.
46	9 hours	4 $\frac{1}{2}$	Normal size.	Enlarged. "	Nil.
47	7 days	4 $\frac{3}{4}$	" " "	Congested. Haemosiderin.	Nil.
50	3 days	9 $\frac{1}{4}$	Enlarged. S. "	Enlarged. Haemopoiesis.	Nil.
51	15 mins.	3 $\frac{1}{4}$	V. enlarged. M. "	V. " "	Haemopoiesis.
53	48 hours	4 $\frac{1}{2}$	Enlarged. P. blue positive. Haemopoiesis.	V. " P. Blue positive. Haemopoiesis.	Haemopoiesis. P. blue positive.
54	Few hours	7 $\frac{1}{2}$	Enlarged. Haemopoiesis. P. blue positive.	Enlarged. Haemopoiesis. Prussian blue positive.	Pale.

Case.	Age at Death.	Wt. (lbs.)	Liver.	Spleen.	Kidney.
55	S. B.	3	Enlarged. Haemorrhages. Prussian Blue positive.	Enlarged. Haemopoiesis.	Nil.
56	2 hours	6½	Enlarged. Haemorrhages. P. blue positive.	" " " " " " " "	Haemopoiesis.
57	5 days	5½	Enlarged.	P. blue positive.	Nil.
60	S. B.	5¾	Enlarged. Haemorrhages. P. blue positive.	Enlarged. Haemopoiesis. P. blue positive.	Haemopoiesis.
61	S. B.	2	Enlarged.	Enlarged.	Nil.
64	2 days	2½	Normal size.	P. blue positive.	Nil.
65	12 hours	8½	Enlarged. Haemorrhages.	Enlarged. Haemopoiesis.	Nil.
70	2 weeks	3¾	Normal size.	V. " " " " " "	Haemopoiesis.
71	2 days	6¼	" " "	V. " " " " " "	Nil.
73	38 hours	8¼	Enlarged. Haemorrhages	V. " " " " " "	Nil.
76	2½ days .	7	" " "	V. " " " " " "	Nil.
77	8 days	9	" " "	V. " " " " " "	Congested.
79	15 mins.	6½	" " "	V. " " " " " "	Haemopoiesis.

AUTOPSY FINDINGS.

Series I. S.M.M.P.

Table 34 a. contd.

Case.	Lungs.	Brain.	Other.
1	Normal	Normal	Nil
2a	"	"	"
2b	"	"	Haemopoiesis in suprarenal
3	Oedematous	Diffuse yellow	Nil
4	Nil	Kernicterus	"
5	Oedematous	Nil	"
7	Nil	"	"
8	Oedematous	"	"
9	Nil	Kernicterus	Haemopoiesis in suprarenals
10	Nil	Nil	
11	Macerated	Macerated	Macerated
12	Macerated	Macerated	Macerated
13	"	"	"
15	Oedematous	Kernicterus	
16	Nil	Nil	Nil
17	"	"	Nil
18	"	"	"
19	"	"	"
20	"	"	Placental villi large and oedematous.
21	"	"	Placental villi large and oedematous.
22	Macerated	Macerated	Nil
23	"	"	Placental villi large and oedematous.
24	Nil	Nil	Macerated.
25	Nil	Nil	"
26a	Nil	Kernicterus	Nil
26 b	Nil	"	Placental villi large and oedematous. Nil.

AUTOPSY FINDINGS.

Table 34 a contd.

Series I. S.M.M.P.

Case.	Lungs.	Brain.	Other.
27	Nil	Nil	Nil
28	Oedematous	Nil	Haemopoiesis in suprarenal
29a	Nil	Kernicterus	Nil
29b	Nil	Kernicterus	Nil
30	Nil	Kernicterus	Nil
31	Nil	Kernicterus	Nil
32	Nil	Nil	Placenta large and oedematous.
33	Nil	Nil	Haemopoiesis in suprarenal Placental villi enlarged and oedematous.
34	Nil	Kernicterus	Nil
35	Macerated	Macerated	Macerated
36	Nil	Kernicterus	Haemopoiesis in suprarenal
38	Nil	Kernicterus and meningitis.	Nil
39	Nil	Kernicterus	Nil
40	Nil	Nil	Nil
41	Nil	Oedematous	Placenta enlarged and oedematous.
42	Nil	Kernicterus	Nil
43	Nil	Nil	Haemopoiesis in suprarenal gland.
44	Macerated	Macerated	Macerated

contd.

Case.	Lungs.	Brain.	Other.
45	Nil	Nil	Nil
46	Nil	Nil	Nil
47	Congested.	Kernicterus.	Nil
50	Pneumonia	Kernicterus	Nil
51	Oedematous	Vascular necrosis.	Haemopoiesis in suprarenal and pancreas.
53	Haemorrhage	Kernicterus	Nil.
54	Oedematous	Nil.	Nil.
55	Nil	Nil	Nil
56	Oedematous	Nil	Nil
57	Oedematous	Kernicterus	Nil
60	Pale	Nil	Haemopoiesis in pancreas and suprarenal.
61	Pale	Nil	Nil
64	Haemorrhage	Nil	Nil
65	Haemorrhage	Nil	Ruptured spleen.
70	Bronchopneumonia	Kernicterus	Nil.
71	Nil	Kernicterus	Nil
73	Oedema and haemorrhage	Kernicterus	Nil
76	Nil	Nil	Nil
77	Nil	Nil	Nil
79	Oedema	Nil	Heart enlarged.

AUTOPSY FINDINGS.

Series II. R.H.S.C.

Table 34 b.

Case.	Age at Death.	Wt. (lbs.)	Liver.	Spleen.	Kidneys.
1	10 days	-	Enlarged. Haemopoiesis.	V. enlarged. Haemopoiesis.	Nil.
3	3½ weeks	-	V. " "	V. enlarged. Haemopoiesis.	Bile stained. Haemopoiesis.
4	7 weeks	-	S. " Portal fibrosis.	S. enlarged. " "	Nil
5	3 weeks	-	" " Haemopoiesis.	Enlarged. " "	Nil
6	1 day	-	" " "	" " "	Haemopoiesis.
8	3 weeks	-	" " "	" " "	Haemosiderin
9	4 weeks	-	" P. blue positive.	Enlarged. P. Blue positive.	Nil.
10	5 weeks	-	Enlarged. Fibrosed.	Enlarged. Haemopoiesis.	Nil
12	17 days	-	" Haemopoiesis.	V. Enlarged. " "	Nil
13	3 months	6½	P. blue positive.	S " "	Nil
15	3 days	-	Normal size.	Enlarged.	Nil
16	1 day	7	S. enlarged. Haemop.	V. " "	Haemopoiesis.
17	4 months	-	V. " "	Normal size.	Nil.
20	1 week	6½	Normal size. Portal fibrosis.	Haemosiderosis.	Bile stained.
21	1 week	6	Enlarged. Haemopoiesis.	V. Enlarged.	" "
22	3½ weeks	6	" " "	Normal size.	Pyelonephritis.
22	2 months	6½	Normal size. Fatty degeneration.	Congested.	
23	11 days	7	Enlarged. Haemopoiesis.	Normal size.	Bile-stained.
24	6 days	7	P. blue positive.	Haemopoiesis.	
25	4 days	6	Enlarged. Haemopoiesis.	Enlarged.	Pale.
			P. blue positive.	Enlarged. Haemop. P. blue positive.	Haemopoiesis.

AUTOPSY FINDINGS.

Table 34 b. contd.

Series II. R.H.S.C.

Case.	Age at Death.	Wt. (lbs.)	Liver	Spleen	Kidneys.
26	2 weeks	7½	Enlarged.	Enlarged. S. Haemopoiesis.	Haemosiderosis.
27	5½ weeks	7	" Haemopoiesis.	"	
29	3 days	7¼	Pruss. Blue positive.	P. blue positive.	Nil.
31	1 week	-	Enlarged.	V. " Haemopoiesis.	
			" Haemopoiesis.	P. blue positive.	P. blue positive.
32	13 days	6½	Enlarged. P. blue pos.	Enlarged. P. blue pos.	Nil.
34	23 days	-	Enlarged. S. haemopoiesis.	V. enlarged. Haemopoiesis.	Pale and bile-stained.
37	2 days	-	P. blue positive.	P. blue positive.	
			Enlarged. Haemopoiesis.	V. enlarged. Haemopoiesis.	
38	26 days	-	P. blue positive.	P. blue positive.	Haemopoiesis.
			Normal size.	Enlarged. Fibrosis.	
39	12 days	7¾	Enlarged. P. blue strongly positive.	P. blue positive.	Uric acid deposits.
41	5 days	9¼	Enlarged. Haemopoiesis.	Enlarged. P. blue strongly positive.	Nil.
42	1 week	6¼	"	Enlarged. Haemopoiesis.	Bilateral congenital hydronephrosis.
			" P. blue positive.	V. " "	
44	2 days	6	Enlarged. Haemopoiesis.	P. blue positive.	Nil.
46	2 days	-	P. blue positive.	V. enlarged. Haemopoiesis.	Haemopoiesis.
			Enlarged. Haemopoiesis.	P. blue positive.	Haemopoiesis.
47	16 days	8	Enlarged.	V. enlarged. Haemopoiesis.	Nil.
			P. blue positive.	P. blue positive.	Nil.
48	17 months	-	No change.	No change.	Nil.

AUTOPSY FINDINGS.

Table 34 b. Contd.

Series II. R.H.S.C.

Case.	Lungs.	Brain.	Other.
1	Nil	Nil	Nil
3	"	"	"
4	"	"	"
5	"	Subdural haemorrhage.	"
6	"	Kernicterus	"
8	Oedematous	Nil	Haemopoiesis in suprarenal.
9	Nil	"	"
10	Oedematous	"	"
12	"	"	Thrush oesophagitis
13	Nil	Acute leptomeningitis	Ruptured spleen
15	Oedematous	Bile staining (diffuse)	Nil
16	"	Kernicterus	Nil.
17	Nil	" (late)	"
20	Bile-stained	"	"
21	Oedematous	Diffusely bile-stained	Subdural haemorrhage.
22	Nil	Nil	Nil
23		Diffuse bile-staining	Subdural haemorrhage.
24	Subpleural petechiae	"	"
25	Oedema. Haemorrhage	"	Nil
26	Nil	"	Early fibrocystic disease of pancreas. Peritonitis.
27	Diffuse broncho-pneumonia	Nil	Early fibrocystic disease of pancreas.

Case.	Lungs.	Brain.	Other.
29	Nil	Kernicterus	Nil
31	Oedematous	Diffuse bile-staining	Subarachnoid haemorrhage.
32	"	" "	Nil
34	"	Nil	Terminal peritonitis and abscess.formation.
37	Nil	Kernicterus	Nil
38	Nil	Nil	Nil
39	Pneumonia	Kernicterus	Subarachnoid haemorrhage
41	Pneumonia	Kernicterus	Haemorrhage into left suprarenal.
42	Oedema. Haemorrhage.	Nil	Nil
44	Oedema.	Kernicterus	Nil
46	Inhalation of liquor.	Kernicterus	Haemopoiesis in pancreas.
47	Nil	Diffuse bile-staining.	Nil.
48	Nil	Destruction of cornu ammonis	Nil.

Case.	Age at DEATH.	Wt. (lbs.)	Liver	Spleen.	Kidneys.
8a	5 days	5 $\frac{3}{4}$	Enlarged. Haemopoiesis.	V. enlarged. Haemopoiesis.	Nil
8b	S. B.		Macerated	Macerated	Macerated
13	2 days	5 $\frac{3}{4}$	Enlarged. Haemopoiesis.	Very enlarged. Haemopoiesis.	Nil.
14	18 hours	6 $\frac{1}{2}$	Enlarged. "	Very enlarged. Haemopoiesis.	Haemosiderin.
15	5 hours	5 $\frac{3}{4}$	P. blue positive. Enlarged. Haemopoiesis.	V. enlarged. Haemopoiesis. P. blue positive.	Haemopoiesis.
21	S. B.	6 $\frac{1}{2}$	Enlarged. P. blue positive. Macerated.	Very enlarged. P. blue positive. Macerated.	Macerated.

AUTOPSY FINDINGS.

Series III. E.I.M.H.

Table 34 c. contd.

Case.	Lungs.	Brain.	Other.
8 a	Oedematous	Nil	Nil
8 b	Macerated	Macerated	Macerated
13	Oedematous Early pneumonia	Kernicterus	Nil
14	Oedematous	Kernicterus	Nil
15	Oedematous	Nil	Oedema of myocardium.
21	Collapsed.	Macerated.	Macerated.

Chapter 7.

The Rhesus Blood Groups.

The earlier discovery by Landsteiner (1901) of four main blood groups : AB, B, A and O (or I, II III and IV according to the Moss symbols) was a piece of brilliant research which paved the way for the recent advances in this field of medical enquiry. It was followed in 1928 by the discovery of the factors M, N and P by Landsteiner and Levine. These factors are of medico-legal rather than of clinical interest. In the years following these major discoveries, numerous reports of intragroup transfusion reactions and possible new blood factors appeared in the literature. Some of these, designated as the G, H, X and Q factors etc., were probably related to the P factor (Andersen 1935).

In 1939 Levine and Stetson reported a case in which unusual agglutinins were present in the serum of a pregnant woman. These agglutinins did not seem to fit the A-B-O or M-N series. They were subsequently proved to be anti-Rh agglutinins.

A new impetus to medical research was given by Landsteiner and Wiener (1940) when they discovered that by injecting the red cells of a Rhesus macacus monkey into a rabbit, an agglutinin could be obtained from the serum of the latter which would agglutinate the red cells of approximately 85% of the white population/

population (Caucasian). They designated this new agglutinin " Rh " in view of its origin and it became known as the Rhesus (Rh) factor. Later work has shown that numerous variations of Rh exist, so it has been decided to classify them under the comprehensive term - " Rhesus blood groups. "

The discovery of Landsteiner and Wiener (1940) was important in itself, but the discovery of the clinical application of their work aroused intense interest throughout the medical world. It has led to the publication of an immense number of articles on the subject. These are too numerous to mention individually and this account will be strictly confined to those which have an important bearing on the subject under consideration.

1. Clinical Application of Rh.

In a review of the pathogenesis of congenital haemolytic disease (erythroblastosis foetalis) Darrow (1938) had postulated the isoimmunisation of the mother to foetal haemoglobin and thought that this led to the passage of the resulting antibodies across the placental barrier into the blood stream of the foetus. The effect on the latter was to cause haemolysis of its red cells and the development of erythroblastosis. This is very near to the truth if " Rh factor " is substituted for " foetal haemoglobin. " This theory was varied slightly by Levine and Stetson (1939). They suggested that the foetus possessed an antigen derived from the father which/

which passed to the mother and immunised the latter. Thereafter antibodies developed in the maternal blood which had a deleterious effect on the foetus. Wiener and Peters (1940), applying the new discovery of Rh, showed that intragroup transfusion reactions could be explained by the development of anti-Rh antibodies in an Rh-negative recipient, following a transfusion of the latter with Rhesus positive blood. Levine and Katzin (1940) were then able to show that the case of Levine and Stetson (1939) was the result of Rh isoimmunisation. Levine, Katzin and Burnham (1941) and Levine, Katzin, Burnham and Vogel (1941) were then able to show that Rh isoimmunisation was of importance in the etiology of congenital haemolytic disease. They demonstrated that in the vast majority of cases of congenital haemolytic disease, the father is Rh positive and the mother Rh negative while the child is Rh positive. They believed that the foetus inherited the Rh characteristic from the father and that when some of its blood escapes into the maternal circulation it sensitises the mother and agglutinins developed by the latter pass back through the placental barrier, combine with the foetal red cells and institute the disease process. They pointed out that whereas only 15% of the general population were Rh negative (i.e. did not possess the " Rh factor " in their red cells) 90% of mothers of children showing evidence of congenital haemolytic disease were Rh negative.

These/

These facts were soon confirmed by Wiener (1942) and Levine (1942) in America and by Boorman, Dodd and Mollison (1942 and 1943) in this country.

It would seem at first sight that the presence of these newly discovered agglutinins in human sera should not have passed unnoticed for such a long time, but Mollison (1944) pointed out that these agglutinins give a relatively poor reaction in vitro compared with the anti-A and anti-B agglutinins.

It was soon found that the incidence of congenital haemolytic disease in incompatible pregnancies was only $1/200 - 1/300$ births (Javert, 1942) although the theoretically possible incidence was $1/10$. The reason for this discrepancy is still rather obscure. Haldane (1942) suggested that a hereditary difference in placental permeability may be responsible. Wiener and Wexler (1943) believed that pre sensitisation of the mother and her capacity to be sensitised were most important. Developing the latter part of this theory, Wiener (1946 a) has postulated the existence of a constitutional factor K which confers on the patient the ability to respond readily to an exposure to specific antigens by the production of specific agglutinins.

Levine (1944) was able to show that only small doses of red cells are sufficient to cause production of specific antibodies, so that there are some grounds for the belief in the existence of a constitutional factor in women who develop such antibodies.

In/

In addition to the importance of the Rh factor in the serological diagnosis of congenital haemolytic disease and of hitherto obscure intra-group transfusion reactions, Wiener, Wexler and Gamrin (1944) showed the value of transfusion with Rhesus negative blood in the treatment of haemolytic disease. These authors, and more recently Wiener and Sonn (1946), stressed the desirability of using only Rhesus negative blood. Darrow and Chapin (1947) on the other hand believe in the use of Rh positive blood. The merits and demerits of these opposing views will be dealt with in a later chapter (Chapter 11).

2. Varieties of Rh.

When the importance of Rh was realised a great number of serological investigations were carried out in all countries. It soon became obvious that all anti-Rh sera were not identical in every respect. Levine (1942) showed that one serum which agglutinated 87% of bloods among the population in New York contained two agglutinins which he designated Rh_1 and Rh_2 . These were thought to be similar to A_1 and A_2 (Wiener 1942), but the subject was found to be more complex and the nomenclature was revised by Wiener, Sonn and Belkin (1944) and Wiener (1944a).

An elementary knowledge of the genetic relationships of the blood groups is essential for the understanding of some of the reactions which will be mentioned later. As Boyd (1945) points out this knowledge is most easily achieved if the simpler groups/

groups are considered first.

The inherited characteristics of animals and humans alike are determined by genes. These genes are carried on chromosomes. All the genes on any one chromosome are transmitted from parent to offspring. The cell nuclei of the offspring contain two sets of chromosomes - one set derived from the father and one from the mother - and therefore two sets of genes, one maternal and one paternal. Furthermore each chromosome has a particular locus on it for certain genes. If there is only one gene available for this locus the offspring will not differ from its parents in respect of that particular characteristic. If, however, more than one kind of gene may occupy the particular locus, there is a chance for offspring to differ from its parents with regard to this particular characteristic.

It is the latter possibility which is concerned in the inheritance of the blood groups.

The simplest genetic relationships occur in respect of the M-N blood groups. At a certain locus on a certain chromosome there may occur a gene " M " at the corresponding locus on the other member of the chromosome pair there may occur a gene " N ". Thus the result of mating may lead to the formation of one of three different types of chromosome pairs - a pair with M genes on both, a pair with N genes on both and a pair with one M gene and one N gene. i. e. MM, NN, or MN. These are the three types of blood found in the general population. This is summarised in/

in the following table.

Table 35. (after Boyd 1945)

Gene Series	Genotype	Phenotype
M	MM	M
	MN	MN
N	NN	N

Persons whose blood cells contain only M genes (genotype MM) are classified as " type " or "phenotype " M ; those whose blood cells contain only N genes are phenotype N and those whose cells contain both are phenotype MN. These may be further classified into homozygous and heterozgous types. Phenotypes M and N are homozygous MM and NN respectively. Phenotype MN is heterozygous. The homozygous and heterozygous Rh individuals are of some importance clinically and this matter will be recalled later.

A further complication may occur. This is the availability of more than two genes to fill the two loci on the chromosome pair. Since one person can only have two genes on these particular loci, a number of different genotypes and phenotypes will be found in the population. Genes which are members of such a series and all capable of occupying the same chromosome loci are known as allelomorphs. This type of allelomorphic series is found in the A-B-O blood groups. In the case of these genes, A and B/
B/

B are dominant over O. The genotypes and phenotypes are as follows :

Table 36

Gene Series	Genotype	Phenotype
O	OO	O
A	AO)	A
	AA)	
	AB	AB
B	BO)	B
	BB)	

Further serological investigation showed that this was an oversimplification and that A was a combination of A_1 and A_2 , the former being dominant over the latter. This may be summarised as follows

Table 37.

Gene Series	Genotype	Phenotype
O	OO	O
A_2	A_2O)	A_2
	A_2A_2)	
A_1	A_1A_2)	A_1
	A_1O)	
	A_1A_1)	
B	A_2B	A_2B
	A_1B	A_1B
	B O)	B
	B B)	

Further work has suggested the presence of an A_3 and A_4 gene and of division of the B gene into B_1 and B_2 , but this is not germane to the present discussion./

discussion.

With regard to the Rhesus blood groups it was originally thought that they closely resembled the M-N blood groups, as in the next table.

Table 38.

Gene Series	Genotype	Phenotype	Reaction
rh	rh rh	rh	Homozygous Rh neg.
	rh Rh	Rh rh	Heterozygous Rh. pos.
Rh	Rh Rh	Rh	Homozygous Rh. pos.

Unfortunately, when more testing sera became available it was found that the Rh blood groups resembled the A-B-O groups in their behaviour rather than the simpler M-N ones. Indeed, even at the time of writing, rare Rh groups are being discovered. The Rh groups are more numerous than their A-B-O counterparts, even after the new subdivisions of the latter are taken into account, and are located on a different pair of chromosomes (Landsteiner and Weiner, 1941).

Working with new testing sera, Wiener (1944a) found six principal genes which he called rh, Rh₀, Rh¹, Rh¹¹, Rh₁ and Rh₂. He found that there genes occurred in the population with the following frequency.

Table 39.

Gene	Incidence %
rh	35.9
Rh ₀	3.5
Rh ₁	43.4
Rh ¹	1.2
Rh ₂	15.7
Rh ¹¹	0.4

Of these genes Rh₀ symbolises the one responsible for the antigen detected by the original rabbit and guinea-pig anti-Rh sera and thus all bloods reacting with anti-Rh₀ serum are Rh-positive (85%) and those failing to react are Rh-negative (15%).

These six principal genes can give rise to $\frac{6}{2} (6 + 1) = 21$ genotypes as in the following table.

Table 40. /

Table 40.

Gene Series	Genotype	Phenotype	Phenotype reaction with anti-Rh ₀ serum.	Phenotype Incidence % (New York)
rh	rh	Rh neg.	Neg.	12.4
	Rh ₁ ^l Rh	Rh ^l	Neg	0.8
	Rh ₁ ^{ll} Rh ₁ ^{ll}	Rh ^{ll}	Neg.	0.3
Rh ₀	Rh ^l Rh ^{ll}	Rh ^l Rh ^{ll}	Neg.	Rare
	rh Rh ₀	Rh ₀	Pos.	2.5
Rh ₁	Rh ₁ ^l Rh ₁ ^l	Rh ₁ ^l	Pos.	53.6
Rh ^l	Rh ₁ ^l Rh ₁ ^l	Rh ₁ ^l	Pos.	
Rh ^{ll}	Rh ₂ ^{ll} Rh ₂ ^{ll}	Rh ₂	Pos.	13.4
	Rh ₂ ^{ll} Rh ₂ ^{ll}	Rh ₂	Pos.	
	Rh ₁ ^l Rh ₁ ^l	Rh ₁ ^l Rh ₂	Pos.	16.9

Since the genes Rh_1 and Rh_2 were also capable of reacting with anti- Rh_0 serum they are alternatively written Rh_0^1 and Rh_0^{11} , although this is rather confusing. They are therefore " double antigens " as they react with anti- Rh_0 and anti- Rh^1 or anti- Rh^{11} sera, as their full connotation implies. The six genes in the series act as six antigens producing six antisera anti- Rh_0 , anti- Rh^1 , anti- Rh^{11} , anti- Rh_1 , anti- Rh_2 and anti-Hr (Levine 1941). The latter (Rh reversed) was used by Wiener to signify sera which reacted with Rh negative bloods (i.e. rh rh etc.) It was originally thought that Rh negative cells merely failed to contain Rh_0 and were not antigenically active. This was later proved to be erroneous and the rh antigen is active producing anti-Hr sera. It enables one to distinguish between homozygous and heterozygous patients in a large percentage of cases.

From Table 40 it will be seen that phenotype Rh_1 is by far the commonest (53.6%).

This very brief summary of the genetic relationships of the Rh blood groups was felt to be necessary as many accounts in the literature tend to make the subject seem very complicated. Pride of place has been given to the American version of the Rh blood groups for two reasons.

1. They were first in the field with the brilliant work of Landsteiner and Wiener (1940).

2. Their terminology of Rh_1 and Rh_2 etc. closely resembles/

resembles the A_1 and A_2 subdivisions of the A-B-O groups and allows the development of the argument to proceed smoothly.

It was not in any way meant to cast a slur on the equally brilliant work of the British workers, an account of which now follows.

3. The British Classification of the Rhesus Blood Groups.

The discovery of the Rhesus blood groups was not only important from the clinical point of view but also helped to aid the study of genetics. About the same time as Wiener (1944a) in America was re-classifying the Rhesus blood groups, Race and Taylor (1943) and Race, Taylor, Boorman and Dodd (1943) in this country using a strong Anti-Hr serum (called St), had produced a similar classification of the Rh genes. Fisher (1944) then noticed that if a gene was positive with Anti-Hr serum it was negative with Anti-Rh¹ serum. He then supposed that the antigens responsible for these reactions were allelomorphic genes which he labelled c and C respectively. He further suggested that anti-Rh₀ serum was produced by a gene D which had an allelomorph d and that anti-Rh¹¹ serum was the result of a gene E with an allelomorph e. This theory presupposed the existence of three (not two as in the original hypotheses) loci on the chromosome which were responsible for the Rh characteristic. This new scheme/

scheme using the letters Cc, Dd and Ee instead of the Rh₀ etc., was published by Race (1944) and is known as the Fisher-Race classification. Fisher believed that loci were "closely linked" as the phenomenon of "crossing over" (with consequent change in characteristic) did not often occur. It was later shown that crossing over did in fact occur, but is rare, and is responsible for the rarer varieties of Rh.

Quite naturally, this new classification was not immediately accepted by Wiener and his co-workers, but the controversy has now been amicably settled by the adoption by the American authorities of both schemes (Wiener's and that of Fisher and Race) in the classification of Rh testing sera.

A short account follows of the more important developments (from the clinical point of view) arising out of the Fisher-Race classification. It has been taken largely from the Medical Research Council Memorandum No. 19 by Mollison, Mourant and Race (1948).

It will be remembered that the Rh-positive antigen was known as Rh₀. This is now known as antigen D and its antibody is anti-D. In England anti-D serum reacts with the blood cells of 83.2% of the population. These latter are the Rh positive bloods.

These Rh positive people may be homozygous DD or heterozygous Dd. If the former, all their chromosomes will carry the gene D. If such a person marries/

marries an Rh negative woman, all their offspring will be liable to suffer from haemolytic disease once the mother has become sensitised. If however the father is heterozygous Dd one half of his chromosomes will carry D and the other d. This allows the possibility of such a family having a normal Rh negative child if the mother is Rh negative.

In the unselected male population DD : Dd occurs in the ratio of 3 : 4 ; among fathers of children with haemolytic disease however, there are 3 DD : 1 Dd. This is owing to the fact that Rh negative mother's are more likely to become sensitised if all their offspring are Rh positive than if some are positive and some negative.

Since Fisher (1944) was able to show that the six main antigens - Cc, D d and E e, occurred as allelomorphic genes at three closely linked loci on the chromosome, there were thus 8 possible combinations on one chromosome. These combinations are, in order of frequency, CDE, cde, cDE, cDe, cde, Cde, CDE and CdE. This last is rare and has, until recently, been difficult to identify owing to the lack of anti-d serum. Since each cell in the adult organism has two such chromosomes, there are $\frac{8}{2} (8 + 1) = 36$ possible genotypes. The five commonest of these, accounting for 90% of the English population are listed below.

Table 41. /

Table 41.

Five Common Rh Genotypes.

Fisher-Race Classification.	Wiener Classification	Wiener short symbols	Reaction with anti-D serum	Frequency %
CDe/cde	Rh ₁ rh	R ₁ r	Pos.	34.9
CDe/CDE	Rh ₁ Rh ₁	R ₁ R ₁	Pos.	18.5
cde/cde	rh rh	r r	Neg.	15.1
CDe/cDE	Rh ₁ Rh ₂	R ₁ R ₂	Pos.	13.4
cDE/cde	Rh ₂ /rh	R ₂ r	Pos.	14.1

At this time there were four available sera for testing - anti-C, anti-c, anti-D and anti-E. There were no anti-d or anti-e sera. Therefore there was some error in guessing the genotype where the presence or absence of d or e was uncertain.

It will be remembered that antigen A of the A-B-O blood groups was subsequently shown to be composed of A₁ and A₂. A similar occurrence was found with regard to the Rh blood groups. C was shown to be composed of C and C^W, and most anti-C testing sera were anti C + C^W. (Callender and Race, 1946). More recently C^u and C_v have been found (Race et al. 1948) These have no clinical importance. A variation of D, D^u has been described by Stratton (1946).

Thus there are now 12 main combinations CDe, cde, cDE, cDe, C^WDe, cdE, Cde, CDE, CD^ue, cD^uE, cD^ue, and C^Wde. These can give rise to $\frac{12}{2} (12 + 1) = 78$ genotypes./

genotypes. A table could be composed of these according to principles already followed but would be too lengthy for this work.

4. The Rh antibodies.

It has been shown that the Rh blood groups were slightly more complex than was originally believed. Testing of pregnant Rh negative women soon showed that the Rh antibodies also consisted of more than one type. It was found that some women had sera containing antibodies which were not detected by ordinary techniques.

The commonest antibody, anti D (anti Rh₀) was shown to exist in two forms. The first form will agglutinate appropriate red cells when the latter are suspended in saline. The second form, known as the "incomplete" (Race 1944) or "blocking" antibody (Wiener 1944b) will agglutinate red cells when the latter are suspended in a protein medium (e.g. 2% ox albumin) and not if they are suspended in saline. This albumin agglutinin is thought by Diamond (1947) to be a later product of the immunisation process than its saline counterpart. Anti-c albumin agglutinins and anti-C^W albumin agglutinins have been reported but are rare.

The reason for the term "blocking" antibodies is the fact that when D-positive cells have been exposed to these antibodies in saline they fail to react at once and also fail to react if subsequently exposed to saline agglutinins.

The/

The discovery of these albumin antibodies is of clinical importance as they enable a serological diagnosis of maternal isoimmunisation to be made in a higher proportion of cases than was formerly possible. Other terms for the " blocking antibodies " are " glutinins " or " univalent antibodies " (Wiener 1946 b) and hyper-immune antibodies (Diamond 1947).

5. The effect of A- B - O antigens on the formation of Rh antibodies.

It was pointed out by Levine (1943 a) that mothers of children suffering from haemolytic disease were more frequently A-B-O compatible with their husbands than unselected women. In Britain Race and Mourant (1948) have found that A-B-O compatibility in present in 84 % of the former compared with 66% of the latter.

The explanation may be that Rh positive cells with A antigen invading the blood of a mother who has developed anti-A are quickly destroyed, before the maternal organism can manufacture anti-Rh (Race 1948). Alternatively A-B-O incompatible foetuses are destroyed before anti-Rh is developed (Fisher 1944).

Unfortunately in the present series insufficient serological data were available to allow of any comment on this interesting phenomenon.

6. Geographical Distribution of Rhesus Group.

As already stated, the percentage of Rh positive persons is greater in the white than in the coloured races. This has been shown by Wiener, Belkin and Sonn (1944)/

(1944); Wiener, Sonn and Yi (1944). Wiener, Zepeda, Sonn and Polivka (1945) and Wiener, Unger and Sonn (1945).

7. Development of Isoimmunisation to Rh.

This may occur in one of two ways

1. In Rh-negative women who have borne Rh positive children or foetuses.

2. In Rh negative persons of both sexes who have received transfusions or injections of Rh positive blood. Incompatible transfusion is the better sensitising stimulus. This is shown by the fact that some mothers in this series delivered children suffering from severe haemolytic disease following one or more incompatible blood transfusions. They had had numerous uneventful pregnancies prior to these transfusions. The latter are not commonly the cause of haemolytic disease since repeated transfusions themselves are not common, although their frequency has greatly increased during the last decade.

Some persons develop sensitisation after only one transfusion, others may show no sensitisation after many. Diamond (1947 a) has shown that only 0.05 cc. of Rh positive blood is sufficient to produce an antibody response. If the reaction to an incompatible transfusion is slight, only mild haemolysis will result. If it is severe there may be rigors, post-transfusion jaundice or even renal failure with death from uraemia. Mollison (1948) has shown that false serological reactions may be found if the patient is tested/

tested soon after a transfusion with Rhesus positive blood.

With regard to sensitisation as a result of pregnancy it has been found that women are more liable to become sensitised if they have already borne one or more Rh positive children. This explains the low frequency of haemolytic disease in first pregnancies. Miscarriages, as well as full-term foetuses and children, aid the development of sensitisation (Potter, 1948).

Since some women require many pregnancies before sensitisation develops and others never become sensitised, an unknown factor (eg. constitutional factor K of Wiener, 1946 a) would seem to be at work.

Furthermore, haemolytic disease occurs in about 1 in 200 pregnancies, whereas the calculated frequency based on genetic relationships, is 20 in 200. However, if first pregnancies are excluded the incidence is about 1 in 130 pregnancies.

Women who deliver a child with haemolytic disease as a result of their first pregnancy often give a history of previous blood transfusion. Thus women in the child bearing period should not be transfused with Rhesus positive blood without careful serological grouping first being performed to exclude the Rh negative members. Therapeutic injections of blood (eg. intramuscular injection of blood in the neonatal period in the treatment of haemorrhagic disease) may also give rise to subsequent disasters in the child-bearing/

bearing period. Rather ironically this mishap occurred in a case reported by Wiener (1946) who had administered the blood himself to the patient some twenty years previously.

Rh antibodies have also been produced by intravenous injections of blood (Rh positive) into Rh negative males (Diamond 1947 b, Wiener and Gordon 1947) and also in Rh negative women past the menopause who have born children with haemolytic disease (Hill et al, 1945).

8. Detection of Isoimmunisation.

a) Detection of antibodies in the Mother.

Saline or albumin antibodies may be detected in the maternal serum by appropriate techniques. If they are present before the 3rd. month, the mother has been sensitised before the pregnancy commenced and the outcome is likely (though not invariably) to be unfavourable. If sensitisation develops during a pregnancy the antibodies are not usually found before the 5th. month but are almost always present by the end of the 7th month.

b) Detection of Antibodies in the Child.

" Coating " of the child's erythrocytes with Rh antibody can be demonstrated by the anti-human globulin test (Coombs, Mourant and Race, 1946), also known as the " Coombs' test ". This test is positive for at least 7 days and often for as long as 3 months after birth in affected cases. The test depends on the fact that the antibodies are composed of the globulin/

globulin fraction of human serum. When Rh positive cells are exposed to albumin-type (incomplete) antibody, they specifically absorb antibody globulin on to their surface. This renders them agglutinable by a rabbit anti-human globulin serum.

If the exposure to the antibody has occurred in vivo a direct Coombs test in the child's cells will demonstrate this fact. A positive result in a newborn infant indicates that its cells have been sensitised to some kind of antibody globulin.

An indirect Coombs test can also be used to demonstrate antibody in a suspected serum. Washed Rh positive cells are exposed to the unknown serum for 30 minutes at 37°C and then added to anti-human globulin serum. The presence of agglutination indicates the presence of incomplete antibodies in the original serum under test.

In both direct and indirect tests cells giving known positive and negative reactions are set up as controls.

9. Rare Types of Isoimmunisation.

It will be recalled that bloods which fail to react with anti-D sera are classed as Rh-negative. If however, the testing serum is not pure and contains say C + D the blood of a person of genotype Cde/cde will react with such a serum and may be wrongly classified as Rh-positive. If such a person were transfused with Rh-positive blood, the results might well be disastrous. Thus women at ante-natal clinics should/

should only be typed with pure anti-D sera. If any unusual serological reactions are obtained the blood should be forwarded to large serological laboratories which have facilities for complete genotyping (Mollison 1948).

Cde/cde types are uncommon but should not be used as Rh negative donors as homozygous Rh negative women (cde/cde) could develop anti-C after a transfusion of such blood.

Occasionally Rh-positive persons may become immunised against Rh negative blood. For example a person whose blood is CDE/CDE may develop antibodies against c or e. A female of blood type CDe/CDe may mate with a cde/cde male and develop anti-c. In such a case the child would be CDe/cde and should be transfused with Rh-positive blood, should such therapy be required.

These unusual reactions are fortunately excessively rare.

10. The Rh Blood Groups in the Present Series.

When this investigation began it was hoped that complete serological data would be obtained in a large number of the more recent cases. This unfortunately did not materialise. This was in part the result of technical difficulties such as a lack of the rarer antisera of sufficient potency and partly an outcome of the tragic death of the Director of the Blood Transfusion Laboratory. This latter occurrence led to a temporary break in the follow-up of/

of cases.

The results of serological tests were available in 56 cases. In 50 of these the mother was Rh negative (D negative) and the husband and child were Rh positive (D positive). In 5 of the remainder both mother and child were Rh positive and in 1 case both were Rh negative (D negative).

It must be pointed out that these 5 D-positive cases and the 1 case where both mother and child were D negative may not have been tested with complete accuracy. In particular, one patient (Case 39, Series I) exhibited kernicterus at necropsy and the mother was said to be Rh positive. As Cappell (1947) points out kernicterus is seldom, if ever, found in cases of neonatal jaundice which are not attributable to Rh incompatibility. A few of these Rh positive cases may therefore have been actually D negative or belonged to one of the rarer Rh genotypes which could not be identified with the testing sera available at that time.

The remainder were possibly the result of A-B-O incompatibility but this also was not definitely established.

As was mentioned in an earlier section, it seems that a greater number of cases of haemolytic disease occur where mother and child are compatible in respect of their A-B-O groups than when the opposite is the case. In the present series this information was obtainable in 6 cases. In 3 cases mother and child were A-B-O compatible and in 3 cases incompatible. But this number of cases is too small to permit/

plasma-albumin conglutination test which they claim to be superior to the Coombs test. Unfortunately, it is sometimes negative owing to the absorption of "conglutinoid" by sensitised cells in an analagous manner to the masking of saline agglutinins by the "blocking" antibodies referred to above. In such cases the Coombs test is still positive and therefore would seem to be more generally useful.

In the present series where the maternal serum contained antibodies the foetus or infant was severely affected and in the majority of cases died. The lack of full serological investigations prevents any conclusions being drawn from these findings.

11. Iso immunisation as a result of A-B-O group incompatibility.

Haemolytic disease would seem to be the result of iso immunisation of the mother against a blood group antigen which she herself does not possess. In the great majority of cases (90% at least) the missing antigen belongs to the Rhesus blood groups. In a few instances, however, the antigen responsible may be one of the A-B-O groups. For example an O mother may have an A husband and A child. This could lead to the development of anti-A in the mother's blood.

Such isoimmunisation could account for the development of haemolytic disease in some of the 5 cases in the present series where both mother and child were Rh positive. In one case the mother was group/

group O and the child group B.

A case of maternal iso immunisation with B antigen and the subsequent delivery of a child affected with haemolytic disease has been reported by Abraham (1947) and by Gruber, Litvak and Jacobi (1946). Sensitisation due to antigen A has been reported by Halperin, Jacobi and Dubin (1945).

It is interesting to note in connection with A-B iso immunisation that Wiener (1948) believes that albumin agglutinins are formed in such cases and are similar in reaction to those occurring in Rh isoimmunisation. He further suggests that the reason why A-B sensitisation is so rarely a cause of haemolytic disease is that the placenta is not permeable to the ordinary saline anti A and anti B agglutinins. The validity of his hypotheses requires further investigation.

12. Serological Tests used in the Present Series.

a) Rhesus Typing.

The blood of infants and mothers, and fathers also when this was possible, was tested for its reaction to a potent anti-D serum. Occasionally this serum was of the anti-C + D type. The rare anti-d and anti-e sera, which have only recently been identified, and anti-E sera were not available for testing.

The reaction to anti-D serum was used to differentiate Rhesus positive from Rhesus negative bloods. Any cases where the reaction was not clear cut/

cut were referred to the Lister Institute for full genotyping.

b) Serological Tests on the Mothers. The sera of Rhesus negative mothers suspected of being sensitised to Rh were examined for the presence of antibodies. The ~~saline~~ agglutinins only were investigated, using a modification of the technique of Boorman et al (1942).

Albumin titrations are now being carried out at the Blood Transfusion Department but these techniques were not in use until 1948.

It should be emphasised, therefore, that references to agglutinins in the maternal serum in patients in this series refers only to saline agglutinins. There is little doubt, however, that albumin agglutinins would also have been present in the majority of cases if a technique capable of demonstrating them had been in use at that time.

Indirect Coombs testing was not carried out.

c) Direct Coombs Test :

Towards the middle of the year 1947 the direct Coombs test was performed on a number of bloods from children suffering from congenital haemolytic disease. It was positive in all cases.

It is important to remember that the Coombs test only demonstrates the presence of an antibody globulin and is not diagnostic of congenital haemolytic disease. The antibody could be formed by a process of isoimmunisation other than that caused by Rh antigen or A-B antigen. In practice of course a direct/

direct Coombs test is almost conclusive evidence of Rh iso immunisation. Nevertheless, Cummings (1949) has stated that among a group of recent cases the Coombs test gave erroneous information in a few instances.

Chapter 8.

The Etiology and Pathogenesis of Congenital Haemolytic Disease.

Congenital Haemolytic Disease, especially the condition known as Hydrops foetalis, attracted the attention of many physicians in days gone by. Lieutand (1761), Orth (1875), Jakesch (1878) and many others drew the attention of their colleagues to some of the aspects of the disease complex. Since their time much has been added to the literature on this subject and at no time has the latter evoked the greater interest of the profession than during the present decade. The comparatively recent discovery of the Rhesus blood groups and of their part in the etiology of haemolytic disease has undoubtedly promoted this enthusiasm. This discovery makes a suitable division of the theories concerning the etiology and pathogenesis of haemolytic disease. They may be considered under two groups - those which were postulated before the discovery of Rh and those which have been developed since.

A. Pre-Rh Theories concerning the Development of Congenital Haemolytic Disease.

As has been stated elsewhere (Chapter 1) it was not until 1932 that Diamond, Blackfan and Baty collected the three types of haemolytic disease under the title of "erythroblastosis foetalis." Before their important contribution to the study of the disease, hydrops foetalis, icterus gravis and haemolytic/

haemolytic anaemia were regarded either as separate entities or as members of other disease groups. For instance hydrops foetalis was linked with congenital syphilis, icterus gravis with other types of neonatal jaundice and haemolytic anaemia was loosely grouped in company with many types of anaemia in infancy.

Nevertheless it was recognised prior to 1932 that these diseases did have factors in common.

Extramedullary haemopoiesis was known to occur in all three types of the disease even although it might not invariably be present. Numerous instances of the occurrence of the disease in families had been reported, and this aspect had been stressed by Hampson (1929).

Some of the early workers confused hydrops foetalis with congenital syphilis and as jaundice also may result in the latter disease, icterus gravis was at one time thought to be a form of congenital syphilis. Such theories were later disproved, by serological and histological investigations.

More recently Rolleston (1910) and Hoffman and Hausman (1926) believed that eclampsia and pre-eclamptic toxæmia were responsible. As this series has shown (Chapter 5), toxæmia is no more frequent in mothers of children affected by haemolytic disease than would be expected in a normal unselected group of mothers.

The early difficulty of distinguishing hydrops foetalis from congenital syphilis, and the greater frequency/

frequency of icterus gravis neonatorum than either hydrops or haemolytic anaemia, tended to focus the attention of workers on this form of neonatal jaundice. Some of the theories proposed as to the etiology of the latter condition have only recently been discounted and they will be mentioned briefly.

1) Theory of Neonatal Sepsis :

As will be mentioned in the section dealing with kernicterus this theory still has some adherents (van Creveld, 1948). Pfaltzer (1915), following the work of Knoepfelmacher (1910) and Beneke (1912) believed that Bacillus coli or related bacteria were responsible for icterus gravis. Even Zimmerman and Yannet (1933) believed the work of Dunham (1930) on neonatal sepsis showed that infection played a major role. Hawksley and Lightwood (1934) did not think the evidence in favour of infection was sufficiently strong.

Ylppo (1910) and Palm (1919) were in favour of the theory that post-natal sepsis was important (vide van Creveld 1948). Klemperer (1924) thought that the focal necrosis sometimes found in the liver in icterus gravis was caused by an infective process. Darrow (1938) did not think that this was the primary cause.

2. Disturbances of the Liver.

Pfannenstiel (1908) believed that icterus gravis was merely an intensification of the normal physiological jaundice of the newborn, but this was disproved by the much higher values for the serum bilirubin in icterus gravis and the coexistence of severe/

severe anaemia as well as by the difference in the underlying pathology.

Some early workers believed that the jaundice was the result of biliary obstruction caused by the blocking of intercellular caniculi in the liver with inspissated bile. This undoubtedly occurs in some cases of icterus gravis (Fig. 32) but is a secondary phenomenon.

3. Unknown Toxin.

De Lange and Arntzenius and Parsons, Hawksley and Gittens (1933) believed that some toxic factor caused the disease. They considered that both the liver damage and the haemolysis were the result of action by a chemical substance. The fact that some children were apparently unaffected till birth was explained by assuming that toxins produced in utero were eliminated by the mother. After birth this convenient avenue for toxin removal no longer existed and a rapidly fatal course might ensue in the child. If, however, the toxic production in utero was so great that the removal mechanism was overcome, intra-uterine death could occur. This was a useful hypothesis but no attempt was made to explain the nature or source of the toxin.

Von Reuss (1914) had suggested that possibly toxins were derived from the intestinal tract in live-born cases, but could not explain the delivery of a still born oedematous or macerated foetus.

Hoffmann and Hausmann (1926) suggested food allergy to explain the nature of the toxic substance/

substance. They based this view on the severe reaction of an icteric infant to its mother's milk, the milk of another woman having no effect (This phenomenon is of course now explained by the presence of Rhesus antibodies in the milk of sensitised mothers).

Palm (1919) believed that toxic substances released by hepatic dysfunction in the newborn infant might cause some of the symptoms but was not able to elucidate the cause of the liver upset.

3. Disturbances of the Haemopoietic System :

The presence of primitive red cells in the liver, spleen and other organs in haemolytic disease is well known. Early workers, such as Ballantyne (1902) believed they were leucocytes and did not belong to the red cell series. Woolley (1916) compared the erythroblastosis with the growth of leucoblastic tissues in leukaemia and suggested the two conditions might be related. Salomonsen (1931) also considered this possibility.

Von Gierke (1930) believed that hydrops foetalis and icterus gravis were a " primary constitutional anlage defect of the haemopoietic system." This theory was supported by Salomonsen (1931) and Clifford and Hertig (1932). They believed that the red cell formation was reverted to its embryonic form and this form was more easily destroyed than the mature cell. This theory also served to explain the familial incidence.

Diamond, Blackfan and Baty (1932) believed that/

that the excessive extramedullary erythropoiesis was the result of some metabolic disturbance of the haemopoietic system. Increased haemolysis was, in their view, also part of this disturbance and oedema resulted from the consequent anaemia. Since there was no arrest of the developing erythrocytes at the megaloblast stage (Haden 1935) ; and administration of liver extract was shown to have little effect (Abbot and Abbott 1935, Henderson 1938); and since iron was not found to be lacking, the nature of the metabolic disturbance was obscure.

Abt (1933) was also inclined to the belief that the erythroblastosis was the primary lesion. He called it " embryonal haematopoietic persistence. " This he believed was either due to a defect in the anlage (Gierke 1930) or in the germ plasm in familial cases and to a toxin in the sporadic cases. These primitive cells had a reduced oxygen-carrying power and caused damage to the liver by anoxia. They were also fragile and being easily destroyed led to anaemia and hyperbilirubinaemia. This latter led to the formation of bile thrombi and a " regurgitation " jaundice with positive van den Bergh reaction.

Parsons, Hawksley and Gittens (1933) felt that extramedullary haemopoiesis was merely an outstanding feature of the disease and was a response to a call for new blood formation. It was a sign and not a cause of the disorder. They believed that the primary lesion was an " erythronoclastic " anaemia of toxic origin.

Hampson (1929) on the other hand believed two processes were at work. Firstly, bilirubin might be excreted via the placenta from the foetus in utero and after birth some time might be required for the child to adjust itself to eliminate such substances. Secondly, there might be some hormone in the maternal blood which restrained haemolysis of the foetal cells in utero but which might be absent after birth. He believed that the recovery of 17 out of 18 cases treated with injections of 5-15 cc. of maternal serum supported this view.

Hawksley and Lightwood (1934) did not agree with his findings and were certain that any transfused blood acted by virtue of its cell replacement and not by the supply of an antihaemolytic substance.

4. Familial Aspects of the Disease.

It had long been recognised that a number of cases of hydrops or icterus gravis may occur in one family. This was followed by the suggestions of von Gierke (1930) and Abt (1933) that an anlage defect or a defect in the germ plasm might be a factor in the causation.

Hawksley and Lightwood (1934) suggested the possibility of a Mendelian recessive characteristic being responsible, but as Macklin (1937) pointed out, the incidence is too large. This latter author attempted to show that the disease was inherited as a dominant mutation. She further suggested that the disease group was the result of an adjunction of multiple/

multiple allelomorphs to the normal gene or genes for the formation of blood in the foetus. The actual genetic relationships of the Rhesus blood groups are not, in fact, dissimilar to the general principles outlined by Macklin (vide Chapter 7). Her facts and figures, however, were strongly challenged by Darrow (1938) who evolved an interesting hypothesis which will now come under consideration.

5. Antigen-antibody Reaction in Haemolytic Disease.

Darrow (1938) did not believe that any of the hypotheses outlined above adequately explained the causation of the disease. For instance she regarded erythroblastosis as merely one of the signs of the disease not as a factor of primary importance. She further cited the work of Judd et al. (1935) to show that the liver damage in icterus gravis might be the cause and not the result of anoxaemia. She believed that the two main processes responsible for the disease in the child were (1) " abnormal destruction of erythrocytes " and (11) " injury of the liver. " The mother was regarded by her as " the one constant factor to be found. " when haemolytic disease occurs in a series of offspring. She believed the erythrocyte destruction was caused by an immune reaction and that the mother was immunised " against foetal red cells or some component of them. " The foetal cells or their haemoglobin entered the maternal sinuses of the placenta, and, gaining access to the mothers blood, set/

set up a state of sensitivity in the mother. Antibodies were then transferred to the foetus via the placenta and reacted on the foetal cells. She thought that there was some difference between foetal haemoglobin and adult haemoglobin and that the former might be the antigen responsible. The additional features of haemolytic disease, nervous excitability, gastro-intestinal irritability, haemorrhagic diathesis and respiratory distress she attributed to a state of anaphylaxis to the foreign protein of foetal haemoglobin.

This was a remarkable piece of objective reasoning and proved to be very close to the truth. It was subsequently shown by Levine and Katzin (1940) that a case of haemolytic disease reported by Levine and Stetson (1939) had been the result of sensitisation of Rh negative mother to the red cells of a Rhesus positive child. Thus a new stage in the study of haemolytic disease was reached.

B. Theories involving the Rhesus Blood Groups.

The discovery of Rh by Landsteiner and Wiener (1940) had led to a tremendous spate of papers concerning the Rhesus factor and haemolytic disease. Indeed Race (1945) has remarked that "too much has already been written about the Rhesus factor. "

It is not possible to discuss all the varying shades of opinion on the subject of Rh and haemolytic disease, therefore this section will be strictly limited to an analysis of some of the more important work which has been contributed to the knowledge of the/

the pathogenesis of this condition.

The theoretical aspects of the Rhesus blood groups and some of the earlier results have been discussed elsewhere (Chapter 7). Many authors disagree about some of the particular aspects of haemolytic disease, but the general etiological background of the condition has been clarified by the work of Davidsohn, Wiener and Darrow in America and by Mollison and his associates in this country. Some of the theories proposed by these authors will now be discussed.

1. Davidsohn (1945).

By the time this author's work was published, the major importance of the Rhesus blood groups as factors in the etiology of haemolytic disease was already established. Davidsohn (1945) attempted to fill in some of the gaps in the knowledge of the pathogenesis of this condition.

Commencing with the assumption that Rhesus incompatibility leads to the sensitisation of the mother and that the antibodies so produced cause a severe haemolytic anaemia in the foetus, he proceeded to link up the various manifestations of the disease. If the anaemia was very severe it caused severe anoxaemia of the tissues, damaged the capillary endothelium, and oedema of the foetus (hydrops foetalis) resulted. It also led to a stimulation of the haemopoietic centres in the body and the characteristic erythroblastaemia. Davidsohn thought that/

that the extramedullary erythropoiesis in the liver caused pressure atrophy of the liver cells and disturbed the normal circulation of that organ. The resultant liver damage had a three fold result. Protein formation was impeded and the subsequent hypoprotinaemia led to oedema ; intrahepatic biliary stasis caused an obstructive jaundice ; inhibition of normal liver function produced a hepato-cellular jaundice. He thus considered that icterus gravis neonatorum was in part the result of a process of haemolysis (haemolytic jaundice), in part of biliary stasis (obstructive jaundice) and in part of parenchymal damage (hepatocellular jaundice). He regarded the haemorrhagic manifestations of the disease as being caused by endothelial damage resulting from anoxia or by liver damage or by both.

One of the great drawbacks of his hypothesis is that it lays too much stress on the importance of erythroblastosis which is only a frequent, and not an invariable, feature of the disease. Furthermore, actual damage to the liver cells of such severity as to be demonstrable histologically is rather rare. In addition he fails to tackle the problem of the pathogenesis of kernicterus.

2. Wiener (1946a, 1946 b)

This author proffered a most ingenious theory concerning the pathogenesis of haemolytic disease and its sequelae. He pointed out that while it was an accepted fact that foetal blood, containing an antigen lacked by the mother, gained access to the maternal circulation/

circulation and set up an antigen-antibody reaction, it was not clear how the foetal cells reached the maternal circulation or why the incidence of haemolytic disease was only about 1 in 300 deliveries. He also drew attention to the fact that in 10 % of cases the mother was Rhesus positive. This group of Rhesus positive mothers were held to be sensitised to rare genotypes of Rh or to A or B blood groups. He stated that A and B are better antigens than Rh for man and the rarity of isoimmunisation to A or B as a cause of haemolytic disease could not be ascribed to the protective action of the tissue fluids as 15% of the populace do not contain A or B in their tissues (i.e. are non-secretors).

Wiener attempted to explain these anomalies by demonstrating the actions of the additional varieties of Rh agglutinins - the albumin agglutinings. He believed that the saline agglutinins were bivalent i.e. their molecule contains more than one combining group for the specific antigen. Albumin agglutinins (blocking antibodies) on the other hand were simpler structures with a smaller molecule containing only one combining group for the specific antigen. The albumin agglutinins were thus univalent and were stated to traverse the placental barrier more easily and earlier in pregnancy than the saline agglutinins.

Wiener was able to demonstrate the presence of the albumin antibodies in patient's serum by means of the " conglutination " test. He believes this test/

test to be superior to the Coomb's test. Briefly it consists in exposing a 2% suspension of Rhesus positive cells to the action of the suspected serum. Both serum and cells are diluted in inactivated AB serum and not in saline solutions. The albumin antibodies are adsorbed on to the surface of the red cells and a colloidal substance present in all normal sera causes the cells to stick together in clumps indistinguishable from normal agglutination. Wiener believes this colloidal substance to be X protein composed of aggregates of albumin, globulin and phospholipide. It dissociates readily in water and thus serum alone is a suitable diluent for the last reagents. He states that a negative conglutination test " practically rules out the presence of Rh sensitisation " even although the patient is Rh negative.

Wiener explained the rarity of isoimmunisation to A or B antigens on two grounds. Firstly, anti-A and anti-B agglutinins would frequently be of the saline type and would not traverse the placental barrier easily. Secondly, 85% of persons are secretors i.e. A and B substances are present in the body tissues so the foetal red cells would be better protected against albumin antibodies in cases of A-B-O incompatibility than in cases of Rh incompatibility where all persons are non-secretors. Furthermore even non-secretors have A and B substances in alcohol-soluble form in their tissues.

That/

That infants affected by congenital haemolytic disease may not show any symptoms for the first few days after birth was explained by assuming that X-protein may require a few days to develop, and thus there may be a delay in the haemolysis of the infant's cells. If, on the other hand, X protein is formed in sufficient amount in utero, a still-birth will result.

Since sensitisation of the mother is rare in the first pregnancy, unless there is a history of a previous incompatible blood transfusion, Wiener thought that there was little or no escape of foetal blood into the maternal circulation during pregnancy but that such a leak did occur during parturition as a result of changes at the placental site during labour and delivery. He supported this theory by the statement that Rh antibody titres tend to rise after parturition. This last statement is undoubtedly correct but as has been shown in this series quite severe cases of haemolytic disease can occur in the foetus without a previous pregnancy or blood transfusion in the mother.

The fact that haemolytic disease occurs less frequently than is theoretically possible, Wiener ascribes to the lack of a constitutional factor K in the mother. Persons with K respond easily to antigen stimulus by producing the appropriate antibodies. Those who lack K do not. He was also of the opinion that A-B-O incompatible pregnancies (eg. Mother O and Child A) "protected" the foetus against/

against Rh antibodies as the mother would form anti-A in preference to anti-Rh since the former is the more powerful antigen (see Chapter 7).

Finally Wiener explained the causation of the 3 main types of haemolytic disease on the basis of his theory concerning the nature of the albumin antibodies.

Hydrops foetalis he regarded as the outcome of severe antigen-antibody reaction in utero where a large amount of antibody is available. If the amount of antibodies in the maternal serum is small the child will be born alive. Congenital haemolytic anaemia will result if these antibodies are of the albumin (univalent) variety. If, however, the antibodies are saline agglutinins (bivalent) " agglutination thrombi " will form with subsequent liver damage and kernicterus. These latter he regards as almost certainly fatal types of the disease.

These latter statements have been refuted by Levine (1946) and by Cappell (1947) since no capillary thrombi have been found in the liver, brain or elsewhere in haemolytic disease. Consequently Wiener and Gordon (1948) have modified this conception somewhat and now disregard the action of saline agglutinins entirely. They believe that only univalent antibodies cross the placenta and that their titre in the maternal serum is closely related to the severity of the disease. Mollison and Cutbush (1949) on the other hand are not so certain that the antibody titre and the severity of the disease process/

process are so closely interrelated.

The theories of Wiener are undoubtedly most ingenious and there is reason to believe that some of them may be true. For instance, his interpretation of the rarity of A-B-O isoimmunisation seems to fit the facts. On the other hand, he introduces many imponderables such as X protein and the K factor into his arguments. Furthermore, although he has indicated a possible sequence of events leading up to the haemolysis of the foetal cells, he does not provide a satisfactory explanation of the subsequent reactions of the foetal organism and he leaves the problem of kernicterus unsolved.

3. Darrow (1947)

It will be remembered that Darrow (1938) was among the first to postulate an antigen-antibody reaction as an etiological factor in the development of haemolytic disease. More recently Darrow and Chapin (1947) have considered the pathogenesis of this disease subsequent to the discovery of the Rhesus blood groups. They disagree with the hypothesis of Davidsohn which stresses the importance of anaemia leading to anoxia and liver damage as a result of biliary obstruction and pressure atrophy caused by erythropoietic cell masses. Nor do they agree with Diamond and Denton (1945) or with Wiener (1946a) that the severity of the disease is related to the concentration in the maternal serum of albumin antibodies.

They describe cases where quite severe anaemia is/

is present without erythroblastaemia, hepatic damage or anoxia. There was no oedema or jaundice and the authors consider that anaemia is only a minor contributory cause to the production of haemolytic disease. They also describe cases with severe jaundice which did not appear to be the result of haemolysis as the erythrocyte count was over 4 million/cu. mm. They believe that another factor is at work. They point out that antibodies other than agglutinins may play a part. For instance Sennot (1946) has shown that erythrophagocytosis occurs in haemolytic disease and Darrow and Chapin believe that this is the result of opsonic activity. In fact this phagocytosis of red cells by Kupffer cells of the liver, histiocytes of the spleen and monocytes of the blood seems to them to be more frequent than true haemolysis. They believe that sensitisation effects of the anaphylactic type play an important part in the development of the disease.

They suggest that liver damage is caused by this means and not by anaemia. Even when the latter is present they did not always find erythroblastaemia which they believe to be the result of anaphylaxis. They point out that nucleated red cells appear in the peripheral circulation in dogs after anaphylactic shock. Darrow and Chapin further suggest that the difference between a case of haemolytic anaemia and one of hydrops or icterus gravis is the difference between a non-sensitised patient and a sensitised one. The severe intrapulmonary and intracerebral haemorrhages/

hages found in some cases of icterus gravis they also attribute to anaphylaxis.

The mechanism of this anaphylactic reaction is said to be a reaction within the tissue cells, especially the liver cells, between " specific sensitising antibody and the Rh antigen freed by the destruction of Rh positive erythrocytes." The destruction of erythrocytes is the result of previous antigen- antibody reaction according to the hypothesis of Levine et al (1940).

The intracellular reaction (in the liver and elsewhere) produces cell injury with the release of histamine and allied substances which then enter the circulation and have a deleterious effect on tissues elsewhere in the body (eg. lungs and brain).

They believe, therefore, that the haemorrhagic diathesis found in icterus gravis is the result of prolonged liver damage caused by anaphylaxis. Liver damage leads to hypoprotinaemia and oedema. The action of histamine on the lungs is said to cause an anoxic anoxaemia and on the placenta to cause intra-uterine death from asphyxia. These assumptions indicate the development of hydrops foetalis. Kernicterus is said to be the result of anoxia, intoxication or of the direct sensitisation of the neurones which then develop a " greater avidity for the circulating bile pigment " than the surrounding cerebral tissue.

Since they believe in the anaphylactic concept of the disease process they use Rhesus positive blood in the treatment of the condition in order to achieve/

achieve " desensitisation " of the affected patient.

Briefly, Darrow and Chapin, believe that haemolytic disease is the result of a " dual antibody reaction." Erythrocyte - destroying antibodies (agglutinins) destroy Rhesus positive cells by phagocytosis. Sensitising antibodies react on the cells of the liver, brain, lungs etc. and cause severe injury to these organs.

It is, of course, not impossible that an anaphylactic reaction does occur in haemolytic disease, but Darrow and Chapin have not demonstrated any conclusive proof of this. Liver damage sufficient to cause recognisable cellular change on histological examination is rare in icterus gravis and it will be shown that erythroblastosis can occur in a variety of conditions where there is no reason to suspect anaphylaxis. It would seem therefore that these authors have gone out of their way to assume that anaphylaxis is the cause of certain events which can be explained without invoking this type of reaction. It would seem there is a strong trend in modern medicine to explain the occurrence of any unusual feature on the basis of allergy or anaphylaxis and it is felt that these agencies should not be held responsible unless there are clear indications that no other explanation is possible.

4. Mollison (1948-1949)

The British workers concerned with the investigations into the Rhesus blood groups have also discussed/

discussed the effect on the foetus of isoimmunisation in the mother to a Rhesus antigen she herself does not possess.

Mollison (1948) has pointed out that when an Rh-negative woman becomes pregnant with an Rh-positive foetus she may become sensitised to Rh antigen and produce antibodies or she may not. This, the author believes, depends on two factors 1) The number of previous stimuli such as previous pregnancies with Rh-positive foetuses or previous transfusions or injections of Rhesus positive blood. 2) An unknown factor.

This latter factor is said to explain the reason why some women become sensitised in an early pregnancy, others in a late pregnancy and others not at all. (It is rather reminiscent of Wiener's constitutional factor K).

Mollison stresses the fact that the incidence of haemolytic disease is only 1 in 200 pregnancies (Boorman, Daley and Dodd 1947) or 1 in 180 (Potter 1948) whereas the expected frequency is 1 in 10 pregnancies. He states that only 1% cases occur in first pregnancies although Potter (1948) found an incidence of 7% and in this series it is even higher (Chapter 5).

Mollison believes that the presence of Rh antibody (Saline or albumin) in a pregnant woman's serum "almost always causes damage to the Rh-positive foetus." The damage commences with the destruction of erythrocytes as the result of the action of antibody/

body on red cells. This destruction seems to be greatest at birth and thereafter gradually lessens. The author had shown in an earlier article (Mollison 1943) that in some affected infants the rapid rate of destruction ceases towards the end of the first week but in others it persists for 6 weeks. In mild cases this accounts for the anaemia, jaundice and erythroblastaemia ; in the more severe cases there is damage to the liver, brain and lungs. The author regards the latter features as secondary manifestations and not to direct antibody action on the tissues concerned.

More recently Mollison and Cutbush (1949) have considered the criteria of severity in cases of haemolytic disease. They confirm the findings of Davidsohn and Stern (1948) that no severe cases occur when the titres of albumin antibodies in the maternal serum is low and many deaths occur when this titre is high. They do not believe, however, that the correlation between antibody titre in maternal serum and the severity of the disease in the foetus is as close as Wiener and Gordon (1948) have suggested, although they are in favour of the theory that saline agglutinins play little part in the etiology of haemolytic disease.

Mollison and Cutbush have shown that severe anaemia is present at birth although the regurgitation of blood into the cord from the placenta may mask this feature in the early hours after birth. They believe/

believe that the severity of the haemolytic process determines the likelihood of the infant dying from anaemia in utero or shortly after birth (i.e. cases of hydrops foetalis) and also " largely determines the possibility of the infant developing kernicterus 2-5 days later." Deaths during that latter period they ascribe to medullary failure.

Lastly, they believe the fall in the haemoglobin value and red cell count in infants after birth is not so much the result of increased blood destruction as the result of decreased blood production. Faxen (1937) and Shapiro and Bassen (1941) have shown that there is a normal diminution in the production of erythrocytes after birth. Mollison and Cutbush believe that it is this normal process which accounts for the apparent fall in haemoglobin values during the first week of extra-uterine life.

These findings are important and serve to explain the development of anoxic effects in cases of haemolytic disease in which anaemia did not appear to be present on routine examination.

5. The Pathogenesis of Haemolytic Disease.

From this study of some of the more important theories concerning the etiology and pathogenesis of haemolytic disease certain facts emerge.

Firstly, all modern authors are agreed that the most important etiological factor in the causation of the disease is sensitisation of the mother to an antigen present in the blood of the foetus but not in her own. In at least 90% of cases, this antigen is/
is/

is one of the Rhesus blood groups, the mother being Rh negative and the father and child Rh positive ; in the remaining cases the antigen may be one of the A-B-O groups.

Secondly, there would seem to be an additional factor at work which causes some women to become sensitised easily, while others in whom it is lacking are sensitised with difficulty or not at all. Whether this factor is of the nature of the constitutional factor K of Wiener (1946a) remains to be seen. It is unfortunate that such extra factors have to be introduced into an already rather complicated system but no other explanation is available in the present state of our knowledge.

Thirdly, the albumin antibodies seem to be more potent in the development of the disease in the foetus than do the saline agglutinins, although there is no direct correlation between the antibody titre in the maternal serum and the severity of the disease in affected offspring.

Fourthly, most authors, while accepting the antigen-antibody concept of the disease process, are at variance with regard to the subsequent events in affected subjects.

It is felt that a study of the cases in this series is ^{of} some value in determining the development of haemolytic disease, from the commencement of the haemolytic process in utero, to the clinical and pathological features exhibited by the foetus at birth, and in the child during the neonatal period.

Before/

Before offering such a hypothesis for the pathogenesis of haemolytic disease a word must be said about three features of the disease process which have been greatly stressed by all workers in this field, namely anaemia, erythroblastosis, and liver damage.

a) Anaemia :

This is the basic pathological change in the disease. The reaction between the antibodies in the maternal blood and the foetal red cells causes haemolysis of the latter with resultant anaemia.

If this haemolytic process is marked in early pregnancy and the anaemia severe, the foetus will die from cardiac failure and a macerated still-birth will result

If the anaemia is slightly less severe, but still a marked feature, a pale hydropic foetus will be delivered, either still-born or dying within a few hours of birth. It is well known that infants with hydrops are severely anaemic at birth. In cases where the anaemia is not so severe as to immediately endanger the life of the foetus but is sufficiently severe to cause considerable upset of the foetal organism, the condition known as icterus gravis will result. The changes in the various organs which occur in this form of the disease are the result of anoxia caused by the anaemia. This view was challenged by previous workers such as Vaughan (1946) and others who pointed out that severe cases of icterus gravis showing kernicterus may not have a demonstrable anaemia/

anaemia. This difficulty has been removed by the recent work of Mollison and Cutbush (1949) who have shown that even a haemoglobin value of 16 Gm.% in a capillary sample of an infant's blood taken on the 3rd. day of life is indicative of anaemia if that infant has received placental blood. Infants with a haemoglobin value of under 8 gm.% at birth are likely to die within 24 hours and those with over 14.5 gm.% at birth are likely to recover. Similar results were obtained in this series (Chapter 9, Graph 2.).

It is obvious therefore that the previous criteria for the presence of anaemia in haemolytic disease will have to be reviewed in the light of this recent work.

The development of the least fatal type of the disease, congenital haemolytic anaemia, is also the result of anoxia. In this type, however, the anoxia is less severe and chiefly affects the bone-marrow and haemopoietic system. In a few cases the liver is mildly affected and a slight jaundice is present. The action on the marrow and haemopoietic system is such as to cause initial stimulation of the system with resultant production of extra medullary foci of haemopoiesis in various organs and the outpouring of nucleated red cells into the peripheral circulation. When this stimulus is removed after birth, the diminution in production, which occurs in normal infants during the neonatal period, comes into full play. Such a development explains the gradual onset of the anaemia in some cases and the reason for the/

for the slow recovery, since the haemopoietic system has to make good the amount lost during the initial period of haemolysis in addition to the normal loss during the neonatal period. It also explains the lack of other symptoms and signs.

b) Erythroblastosis :

There is no doubt that erythroblastaemia and the presence of extramedullary foci of erythroblasts in various organs in cases of haemolytic disease has attracted more attention than any other feature of the disease with the exception of the Rhesus blood groups. It is a great pity that the term ever came in to general use as it has been vested with a significance it does not merit. It is true that extra medullary haemopoiesis and nucleated red cells in the peripheral blood are a prominent, indeed a striking feature of the disease, but they are not in themselves pathological phenomena. They are the natural response of the haemopoietic system at this stage of development to the stimulus of oxygen lack.

Many workers seem to imagine that the presence of erythroblasts is a significant pathological feature of the disease in the same manner as megaloblasts are features of pernicious anaemia. The two are not comparable. In the latter case there is an intrinsic deficiency in the maturation of the erythrocyte in its early stages as a result of lack of the anti-anaemic principle. In the former instance there is no such deficiency.

If/

If an analogy is required it should clearly be that of the appearance in the peripheral blood of an occasional metamyelocyte or myelocyte in cases of acute infections. Nobody, however, would dream of labelling such a case as leukaemia on such slender grounds. It is true that in haemolytic disease there are often very many nucleated red cells in the peripheral blood but after all there are many more red cells than white cells in the blood in any case and stimulation of the erythropoietic portion of the haemopoietic system will be much more noticeable than a stimulation of the leucopoietic system of similar intensity.

Part of the confusion is undoubtedly due to the nomenclature of nucleated red cells. A normoblast is regarded as a "normal" cell whereas an erythroblast is regarded as an abnormal one. This is not the case. An erythroblast is merely an earlier stage in the development of the erythrocyte.

Darrow and Chapin (1947) have suggested that erythroblastosis is the consequence of a state of anaphylaxis in the affected foetus. Extramedullary haemopoiesis, however, occurs in a variety of conditions and it is stretching the bounds of credulity too far to suggest that anaphylaxis is responsible for them all.

In congenital syphilis, foci of normoblasts and erythroblasts are found in the liver (Fig. 51) and elsewhere. There is probably a degree of anaemia in such/

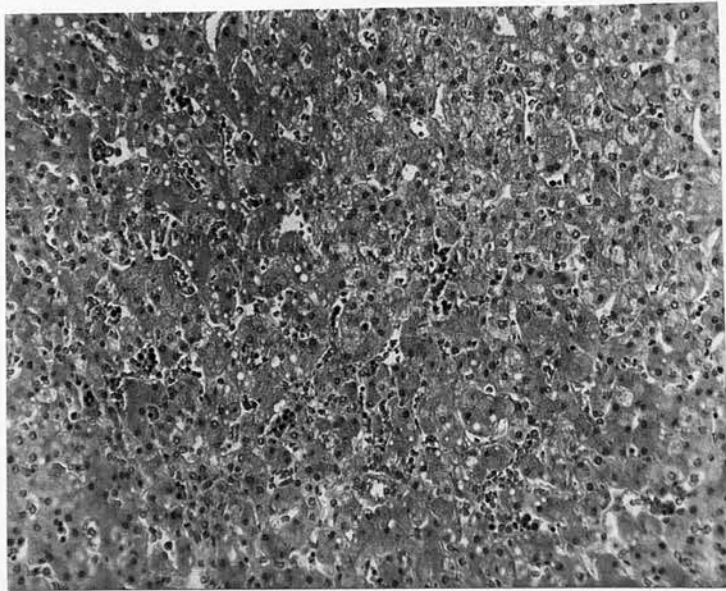


Fig. 49. LIVER x 120. Haematoxylin and Eosin.
Numerous foci of haemopoiesis. Case of
congenital heart disease.

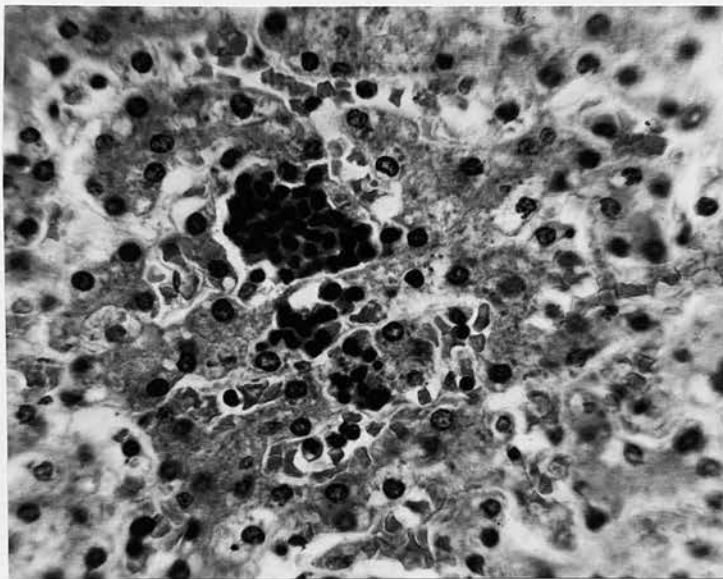


Fig. 50. LIVER x 375. Haematoxylin and Eosin.
Foci of haemopoiesis, mainly normoblastic in
character. Case of neonatal asphyxia.

such cases as a result of the toxic effects of the spirochaetes and anoxia causes stimulation of the blood forming tissues.

More important still, in neonatal asphyxia and in congenital heart disease (during the neonatal period) there is extensive extramedullary haemopoiesis in the liver and elsewhere and nucleated red cells are found in the peripheral blood.

In the case of neonatal asphyxia the foci of haemopoiesis are normoblastic in character (Fig. 50) and the cells in the peripheral blood are normoblasts. This is owing to the fact that the anoxic stimulation is very short lived and only the first line of replacements (the normoblasts) are called into action. In congenital heart disease, on the other hand, the anoxia is prolonged and the second line of replacements (the erythroblasts) are called into action and foci of both normoblasts and erythroblasts are found in the liver (Fig. 49).

These foci of haemopoiesis in the liver and elsewhere are always stimulated by anoxia in the neonatal period before they have atrophied. They are merely the remnants of the more immature haemopoietic system of the foetus which was in full play before the bone marrow was able to take over its function. That these foci had pathological significance is an idea which has been carried over from the days when haemopoiesis was less well understood and some workers (Wooley 1915) regarded them as collections similar to those found in leukaemias. Nothing is farther/

farther from the truth and it must be stressed that these collections of haemopoietic cells are merely the physiological response of the foetal organism to lack of oxygen.

It has been stated that these nucleated red cells are poor carriers of oxygen and thus a vicious circle is set up in which the formation of nucleated red cells increases the degree of anoxaemia and the subsequent production of more of their number.

Davidsohn (1945) has suggested that these collections of haemopoietic cells cause pressure atrophy of the liver cells and circulatory disturbances in the liver itself. This is an extraordinary suggestion. If the liver of a very premature infant (weight 1-2 lbs.) is examined, it will be found that haemopoiesis is so extensive that it is often difficult to see parenchymal cells at all. But no cellular damage is visible. It is true that the majority of the nucleated red cells are normoblasts but many erythroblasts are also seen and more would be found if anoxia was present.

It is to be hoped, therefore, that the presence of erythroblastosis, will in future be accorded a less prominent place in the pathogenesis of this disease. This should not be difficult now that serological tests furnish a more accurate method of arriving at a correct diagnosis.

c) Liver Damage :

The term liver damage has been loosely applied in haemolytic disease. Some authors regard it as synonymous/

synonymous with liver cell necrosis or atrophy of the liver cells (Davidsohn 1945). Injury to the liver cells sufficient to cause necrosis of the latter is a rather rare finding. Nearly a hundred blocks of liver sections from 30 fatal cases of icterus gravis neonatorum showing kernicterus were examined. The blocks from one case only showed histological change in the liver parenchyma which could be classified as severe. In 17 cases the blocks showed moderate cellular damage and in 12 cases the change was either very slight or not demonstrable.

It is true that Hawksely and Lightwood (1934) found evidence of " cirrhosis " in a large proportion of their cases of icterus gravis who had survived the acute stage of the disease and had died some weeks after birth. It is doubtful if the term " cirrhosis " is correct usage in this condition. Two patients in the present series died at the age of $3\frac{1}{2}$ weeks and 7 weeks respectively, having exhibited severe jaundice from birth (Case 3 and Case 4, Series 2). Each of these showed evidence of increase in the fibrous tissue in the liver on histological examination (Fig. 36). In the case of the patient dying at 7 weeks there was considerable periportal fibrosis or a fine pericellular fibrosis but even so this hardly warrants the term cirrhosis.

In two patients out of the 30 showing icterus gravis and kernicterus death ensued some time after the jaundice had disappeared (Case 17 and Case 48, Series 2). In one, dying at the age of 4 months, the liver/

liver is absolutely normal and the parenchymal cells show no change whatsoever. The portal tracts were normal and there was no increase in the fibrous tissue (Fig. 57a). Likewise in the patient dying at 17 months there was no histological change in any organ except the brain.

Henderson (1942) has described cases of hepatic cirrhosis and maceration in still-born infants which he regards as a separate type of haemolytic disease and the most severe form of the latter. As Mollison (1948) points out, it is doubtful if any clear cut distinction can be made between such cases and other forms of hydrops foetalis. It is possible that such cases result from fluctuations in the antibody content of the maternal serum during early pregnancy. An initial high output of antibodies causes severe anaemia and anoxic damage to the liver cells. During a period of low antibody output the liver undergoes repair with fibrosis. A further rise in the production of antibodies causes another period of severe anaemia and intrauterine death from cardiac failure.

There is no doubt that in the majority of cases of icterus gravis liver damage is functional and not organic in nature.

The excretory capacity of the liver is poor in newborn infants. In infants suffering from haemolytic disease there are additional factors which interfere with the function of an organ which is already working at a low level of output. The haemolysis of erythrocytes is increased and leads to a greater production/

production of bilirubin than the liver can excrete. Furthermore, the effect of anoxia on the liver cells causes a great decrease in the capacity of the organ to exercise its functions. Lastly, the onset of biliary stasis with formation of bile plugs in the intercellular canaliculi introduces an element of obstruction. When all these processes are considered, it is small wonder that the infant develops jaundice and there is little necessity to postulate "pressure atrophy of liver cells" or "anaphylactic shock" to explain the mechanism of disturbed function.

In a number of cases the anoxia is severe and actual histological change occurs in the liver cells, notably those in portal zones and, provided the infant survives, repair will occur by fibrosis and by occasional parenchymal cell regeneration.

With regard to the occurrence of intra-pulmonary haemorrhage, similar processes are involved. Intra-pulmonary haemorrhage is a frequently fatal complication of icterus gravis but is also found in very many other patients dying in the neonatal period. This is particularly true of infants showing asphyxia, whether the result of inhalation of liquor amnii or of other damage. In icterus gravis there is damage to the capillary endothelium in the lungs and elsewhere (eg. skin petechiae and intracerebral haemorrhage). This "haemorrhagic diathesis" is caused by anoxia and by lack of prothrombin which the liver is unable to produce in sufficient quantity.

The onset of kernicterus may also be explained on/

on the basis of cellular anoxia (Chapter 14).

6. Summary :

The principle etiological factor in the development of haemolytic disease is the sensitisation of the mother to an antigen in the blood of the foetus which she herself does not possess. The consequent antigen-antibody reaction on the foetal erythrocytes causes severe haemolysis of the latter with the production of anaemia. If the latter is severe and develops early in pregnancy, the foetus will die from cardiac failure (Hydrops foetalis with maceration). If the anaemia is severe but not immediately lethal the foetus will be still-born or will be moribund at birth (Hydrops foetalis). If sufficiently severe to cause anoxia but not so marked as to cause death, the infant will exhibit icterus gravis with or without kernicterus. If the anaemia is only sufficient to cause mild anoxia, there will be an initial stimulation of the haemopoietic system alone, without liver involvement. When this stimulus is removed the subsequent delay in regeneration of erythrocytes (an exaggerated normal neonatal occurrence) reveals the condition known as congenital haemolytic anaemia. It is probable that haemolysis is minimal by the time the condition is diagnosed and that diminished production is the main operative factor (Mollison and Cutbush 1949).

There would seem to be little necessity for the assumption that anaphylaxis or other unusual antibody reactions play a significant part in the pathogenesis of haemolytic disease.

Chapter 9.

Clinical Findings in Haemolytic Disease.

It has been shown that the pathological findings in fatal cases of haemolytic disease are very variable. Thus it is not surprising that great variations in the signs and symptoms may be found in the living patient. In this chapter the clinical features will be briefly reviewed and the more useful laboratory investigations will be discussed.

A. Hydrops Foetalis : (Fig. 1).

The foetus is usually still-born and frequently macerated. Less commonly a live birth occurs but death usually follows in a matter of hours. As the name implies there is generalised subcutaneous oedema, the abdomen is distended and the skin is pale. The degree of anaemia is usually severe. Jaundice is not the rule but a yellow umbilical cord may draw the attention of the obstetrician to the nature of ^{the} condition. Litchfield (1945) cites a case of hydrops foetalis which lived for 6 days.

B. Icterus gravis neonatorum :

It goes without saying that the most salient feature of this type is jaundice. Indeed, until recently it might be said to be the only constant feature. Modern serological tests, however, have altered the picture.

The jaundice may be present at birth, in which case/

case the vernix caseosa may be golden yellow in colour, or the jaundice may come on within the first twenty-four hours. It is seldom delayed beyond 48 hours.

Table 42.

Time of onset of Jaundice.

Time	No. of cases.
at Birth	32
under 24 hours	55
24 - 48 hours	14
over 48 hours	9
Total	110

Table 43.

Relationship between severity of Disease
and Time of Onset of Jaundice.

	At birth	In less than 24 hrs.	In more than 24 hrs.
Total	32	55	23
Recovered	5	14	13
Dead	27	41	10
Kernicterus	12	21	3

The relationship between the time of onset of jaundice and the outcome is shown in Table 43.

The skin, tongue, conjunctivae and buccal mucous membrane show an icteric tint. The colour is usually golden-yellow in the typical case. In a mild case the jaundice is much less intense and, if the case is severe, there may be a greenish tint visible./

visible. Ellis (1938) and Parsons (1947) have reported a greenish discoloration of the teeth after icterus gravis. This bizarre sign was not present in any case in this series. The stools are usually, but not invariably pigmented and the urine contains urobilin and uroblinogen. The jaundice comes on earlier than physiological jaundice. It generally commences to fade by the 10th day after birth but may persist for 3-6 weeks.

The liver and spleen are usually enlarged and may be easily palpable. The spleen is rather more frequently palpable than the liver.

Anaemia is usually present but in not a few cases the haemoglobin values and red cell counts are within normal limits. Mollison and Cutbush (1949) have shown that an influx of placental blood into the infant's blood stream at parturition may mask the existence of anaemia. The findings on examination of the blood will be discussed later. Oedema is occasionally present.

Haemorrhagic manifestations are not uncommon and petechial haemorrhages into the skin may be seen. In a number of severely affected patients haemorrhage from the cord and into the lungs or brain may lead to a fatal termination.

In a proportion of patients neurological signs are present. According to Cappell (1947) this may be expected in 40% of patients in the acute stage and in 12% of survivors. The subject is fully discussed in Chapter 14.

With/

Rh negative or Rh positive. If both are Rh positive haemolytic disease in the child is not excluded but is rather less likely to be present.

c) Maternal antibodies :

If the mother is Rh negative tests may be carried out on her serum at the 3rd., 7th and 9th months for the presence of antibodies. Albumin antibodies in high titre are usually a bad prognostic sign. Again this is not invariably the case.

Antibody titres usually rise following delivery.

d) Direct Coombs Test:

The direct Coombs test should be carried out on the red cells of all infants suspected of haemolytic disease. In the great majority of cases it is positive when haemolytic disease is present. Furthermore, the strength of the reaction is some indication of the likelihood of a fatal termination. It may be positive up to 90 days after birth.

It must be kept in mind, however, that in a few cases the Coombs test is negative (Cummings 1949) or gives an equivocal result when compared with a control. It is thus not infallible. In addition, it must be remembered that the test merely demonstrates the presence of globulin in the foetal blood. Any globulin could theoretically give the reaction although fortunately Rh antibody globulin is the commonest found in this connection.

e) Indirect Coombs Test.

The presence of free Rh antibody in the infant's serum may be detected by this means.

2. Haematology :

a) Haemoglobin : Varying degrees of anaemia may be present at birth. Haemoglobin values of 40-140% may be expected. Broadly speaking, a low haemoglobin at birth is a poor prognostic sign but there are exceptions to this rule. It is to be remembered that the haemoglobin is normally high at birth and any subnormal value is indicative of quite a degree of anaemia.

b) Red Cell Count : The total red cell count varies also over a wide range (see Tables 1-28). The colour index is generally above unity and an anaemia of macrocytic type is the rule. It has been suggested that macrocytosis is only " apparent " and results from large numbers of reticulocytes in the peripheral blood.

c) Reticulocyte Count : The reticulocyte count may be raised in normal infants at birth, but falls to under 1% by the end of the first week. In haemolytic disease it is greatly raised and may be as high as 50%. As a general rule a good reticulocyte response is an index of recovery.

d) Blood Film : The red cells show macrocytosis, anisocytosis, poikilocytosis and polychromasia. Howell-Jolly bodies may be found in cases of haemolytic anaemia.

Nucleated red cells are usually present. The cells may be late normoblasts, early normoblasts or erythroblasts (Fig. 42). The normoblasts have an acidophile/

acidophile cytoplasm with a small central darkly staining nucleus. The erythroblasts have a basophile cytoplasm with slightly eccentric, darkly staining nucleus which shows reticulation. Occasionally haemocyto blasts may be found.

The number of nucleated red cells compared with the number of white cells varies considerably. (Tables 1-28). In the present series and in the series of Mollison and Cutbush (1949) the majority of the most severe cases of haemolytic disease showed a pronounced erythroblastaemia. Normally at birth nucleated red cells are less than 5 per 100 W.B. C., in haemolytic disease there may be as many as 2000 per 100 W.B.C.

A high eosinophil count is a not infrequent finding.

Nucleated red cells usually disappear from the peripheral blood of normal infants by the third day. In haemolytic disease they are more numerous and persist for a much longer period, sometimes 2 weeks or more.

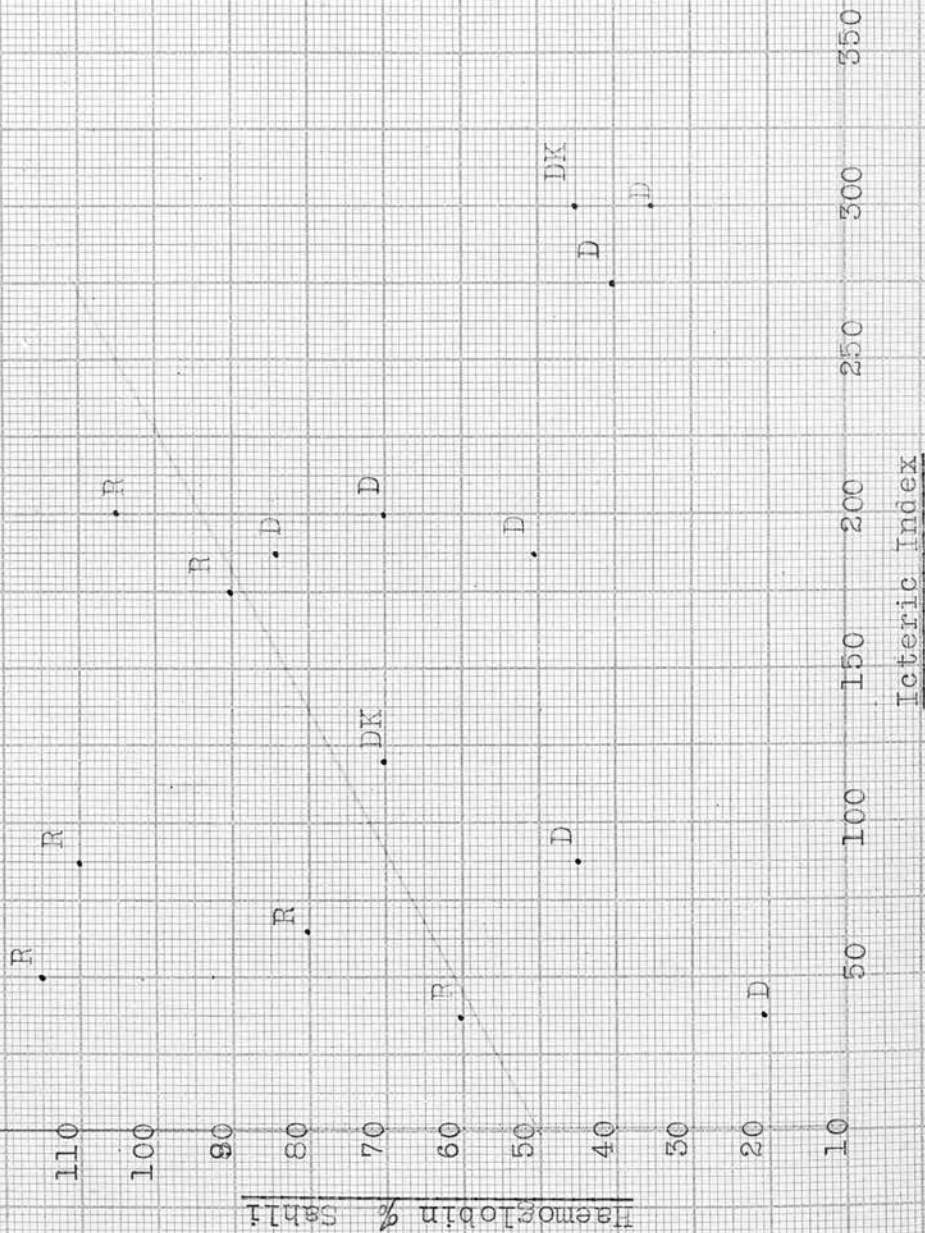
e) Fragility Test : There is no increase in the fragility of the erythrocytes in haemolytic disease.

f) Prothrombin Index : The prothrombin index is usually below 50 % of normal in icterus gravis. This denotes poor liver function and the lower prothrombin level is in part responsible for the haemorrhagic tendencies. The response to Vitamin K injections is poor and the prothrombin levels rise less/

GRAPH 2

HAEMOGLOBIN VALUES AND ICTERIC INDEX FROM 15 PATIENTS

R - Recovered
 K - Kernicterus
 D - Died



less rapidly after such injections than in normal subjects (Leonard, 1945).

g) Platelet Count : Patients with icterus gravis and showing purpuric lesions have a low platelet count (40, - 60,000/cmm.).

h) Bleeding Time : Diamond et al (1932) assert that in icterus gravis the bleeding time is prolonged. The observation was carried out too infrequently in this series to permit of comment.

3. Biochemistry :

a) Icteric Index : The icteric index is moderately raised in haemolytic anaemia and very markedly increased in icterus gravis. In the latter condition figures as high as 400 may be achieved. A correlation of haemoglobin levels and icteric index in 15 patients is shown in Graph 2. A very high icteric index is a poor prognostic sign.

b) Serum (or Plasma) Bilirubin : This is a more accurate means of following the rise of bilirubin in the blood than the icteric index. The normal serum bilirubin in infants is 1-2 mgm%. In icterus gravis the range is from 2-20 mgm.% or higher.

Patients having a serum bilirubin of 7 mgm.% or over were unlikely to survive. Those with a serum bilirubin of 3 mgm.% or less usually recovered. These findings are similar to those of Mollison and Cutbush (1949). As will be shown later (Chapter 14) a high serum bilirubin is found in patients with kernicterus.

Mollison and Cutbush (1949) plotted the values for/

for cord blood haemoglobin against those for cord blood bilirubin. They found a direct correlation and the majority of their patients who died from kernicterus had a high blood bilirubin and a low haemoglobin. Leonard (1945) found no such correlation (she used icterus index values instead of bilirubin levels, as has been carried out in Graph 2 in 15 patients in the present series.) Sufficient serum bilirubin figures were not available in the present series.

c) Cerebro-spinal fluid Examinations : It is difficult to distinguish between kernicterus and cerebral irritation resulting from haemorrhage during the early days of life. Cerebro-spinal fluid examinations were carried out on a few patients showing kernicterus. Sugar and chlorides are little affected. The colloidal gold reaction is normal. The fluid is light yellow in colour and the icteric index ranged between 10 and 20. The van den Bergh reaction gave a weak biphasic response.

d) Van den Bergh reaction : This test is used less frequently nowadays owing to the indefinite nature of the results. In the present series of cases three responses were elicited. An indirect reaction is congenital haemolytic anaemia and mild cases of icterus gravis and a direct biphasic response in the majority of icterus gravis patients. A few very severely affected icterus gravis patients gave an immediate direct response. These results are to be expected/

expected from the nature of the upset in liver function (Chapter 8).

e) Plasma Proteins : The plasma proteins gave moderately low values in a few patients with severe icterus. A range of 4 gm.% to 6 gm.% was found. The albumin/globulin ratio was not reversed. Wallerstein (1947) found a reversal of the albumin/globulin ratio in 5 patients with icterus gravis.

f) Liver Tolerance Tests : The laevulose tolerance test was carried out in 4 patients with icterus gravis. The results were poor, indicating derangement of liver function. Wallerstein (1947) has found a high alkaline phosphatase and a positive cephalin-cholesterol reaction in patients with icterus gravis.

From this description it will be found that laboratory investigations fall under two heads - those which are of diagnostic importance and those which have a prognostic significance.

A. Tests of Diagnostic Importance :

1. Rh blood typing of maternal and child's cells.
2. Direct Coombs test on infant's red cells.
3. Indirect Coombs test on infant's blood to detect presence of antibodies.
4. Antibody titre in maternal serum.
5. Wassermann and Kahn to exclude syphilis.
6. Haemoglobin and red cell count of cord blood.
7. Film of infant's blood to detect the presence of nucleated erythrocytes.

B./

B. Tests of Prognostic Significance :

1. Strength of reaction of infant's red cells to the Direct Coombs test.
2. Titre of albumin antibodies in maternal serum.
3. Cord haemoglobin. Very low values are prejudicial to recovery.
4. Reticulocyte count. Good reticulocyte response in patients who recover.
5. Plasma bilirubin. Very high values are found in patients developing kernicterus or who subsequently die. There is some correlation between the haemoglobin values and the level of plasma bilirubin (Graph 2).
6. Blood Film. A very high proportion of nucleated red cells weighs against the child's chance of recovery.

The laboratory investigations in congenital haemolytic disease can be most helpful but it is important to remember that no single test is diagnostic of the disease. A diagnosis can only be made with certainty by a careful study of all available information concerning the patient. It is important that all the tests listed above should be carried out if facilities are available since they will reduce the chances of error in diagnosis. Furthermore, a full trial of these tests, none of which present any great technical difficulty, will enable an accurate assessment of their value to be made.

Case	Family history	Rh groups			Age (days)	Weight (lbs)	Enlargement of liver	Enlargement of spleen.	Initial Hb (Sahli)%	Nucleated R.B.C. /100 W.B.C.	Total transfusion cc.	Recovered	Died	Autopsy	Abnormalities of pregnancy	Delivery
		father	mother	child												
59	+	0	0	0	7	7 $\frac{3}{4}$	+	+	0	0	90	+	0	0	0	Spont.
60	+	0	0	0	S.B.	5 $\frac{3}{4}$	+	+	0	0	0	0	S.B.	+	Toxaemia	"
61	+	+	-	+	S.B.	2	+	++	0	0	0	0	S.B.	+	0	"
62	0	+	-	+	2	9	0	0	0	0	265	+	0	0	0	Caesari. Section.
63	+	0	0	0	2	6 $\frac{3}{4}$	+	+	0	0	335	+	0	0	0	Spont.
64	0	+	-	+	2	2 $\frac{3}{4}$	0	+	120	0	0	0	+	+	A.P.H.	"
65	0	0	0	0	$\frac{1}{2}$	8 $\frac{1}{2}$	+	++	70	800	200	0	+	+	0	"
66	0	+	-	+	2	7 $\frac{1}{4}$	0	0	0	0	0	+	0	0	0	"
67	+	+	-	+	1	8 $\frac{3}{4}$	0	0	0	0	120	+	0	0	0	"
68	0	+	-	+	1	4 $\frac{1}{2}$	+	+	0	0	0	+	0	0	0	"
69	0	+	-	+	1	6 $\frac{3}{4}$	0	+	111	0	0	+	0	0	0	"
70	0	+	-	+	14	3 $\frac{3}{4}$	+	++	140	++	0	0	+	+	0	"
71	0	0	0	0	2	6 $\frac{1}{2}$	+	++	0	0	15	0	+	+	0	"
72	0	0	0	0	16	7	0	+	45	0	0	+	0	0	0	"
73	0	0	0	0	38 hr	8 $\frac{1}{4}$	+	++	84	1620	75	0	+	+	0	"
74	0	0	0	0	3	8	0	+	105	0	0	+	0	0	0	"
75	+	0	0	0	2	5 $\frac{1}{2}$	+	++	105	150	150	+	0	0	0	"
76	0	0	0	0	2 $\frac{1}{2}$	7	+	++	0	0	0	0	+	+	0	"
77	0	0	0	0	8	9	+	++	15	0	0	0	+	+	0	"
78	0	0	0	0	6	6 $\frac{1}{2}$	0	+	20	0	0	+	0	0	0	"
79	+	0	0	0	$\frac{1}{4}$ hr	6 $\frac{1}{2}$	+	++	0	0	0	0	+	+	0	"
80	0	0	0	0	1	9	++	++	55	120	100	+	0	0	0	"

Case	Family history				Age (days)	Weight (lbs)	Enlargement of liver	Enlargement of spleen	Initial Hb (Sahli) %	Nucleated RBC /100 W.B.C.	Total transfusion. c.c.	Recovered	Died	Autopsy	Abnormalities of pregnancy	Delivery
	Father	Rh groups mother	Rh pos neg	child												
1	0	0	0	0	10	-	+	++	0	0	0	0	+	+	0	Spont.
2	0	0	0	0	160	7½	0	+	70	0	0	0	+	0	0	"
3	0	0	0	0	24	-	++	++	0	0	0	0	+	+	0	"
4	0	0	0	0	50	-	+	+	0	0	0	0	+	+	0	"
5	0	0	0	0	21	-	+	+	0	0	0	0	+	+	0	"
6	0	0	0	0	1	-	+	++	0	0	0	0	+	+	0	"
7	0	0	0	0	8	7	+	+	0	0	245	+	0	0	0	"
8	0	0	0	0	21	-	+	++	0	0	0	0	+	+	0	"
9	0	0	0	0	28	-	+	+	0	0	0	0	+	+	0	"
10	0	0	0	0	35	-	++	++	0	0	0	0	+	+	0	"
11	+	0	0	0	1	4½	+	+	0	0	60	+	0	0	0	"
12	0	0	0	0	17	-	+	++	0	0	0	0	+	+	0	"
13	0	+	-	+	90	6¼	0	+	0	0	0	0	+	+	0	"
14	0	0	0	0	2	7½	0	+	20	0	140	+	0	0	0	"
15	0	0	0	0	3	-	0	+	0	0	0	0	+	+	0	"
16	0	0	0	0	1	7	+	+	25	++	0	0	+	+	0	"
17	0	+	-	+	120	-	0	0	0	0	0	0	+	+	0	"
18	+	0	0	0	30	9	0	0	0	0	90	0	+	0	0	"
19	0	0	0	0	42	7	0	+	0	0	100	0	+	0	0	"
20	0	0	0	0	7	7½	+	++	0	0	0	0	+	+	0	"
21	+	0	0	0	24	7	+	+	0	0	100	0	+	+	0	"
22	0	0	0	0	60	10	0	0	65	0	40	0	+	+	0	"
23	0	0	0	0	11	7	+	+	0	0	90	0	+	+	0	"
24	0	0	0	0	6	7	+	+	15	0	5	0	+	+	0	"
25	0	0	0	0	4	6½	+	++	0	0	0	0	+	+	0	"
26	0	0	0	0	14	7½	+	+	0	0	100	0	+	+	0	"
27	0	0	0	0	40	6¾	+	+	105	0	0	0	+	+	0	"
28	+	0	0	0	1	6¾	+	+	0	0	++	+	0	0	0	"
29	0	0	0	0	3	7¼	++	++	45	++	0	0	+	+	0	"
30	+	+	-	+	5	7	0	0	0	0	180	+	0	0	0	"
31	0	+	-	+	7	-	+	++	76	62	0	0	+	+	0	"
32	+	+	-	+	13	6½	+	+	90	0	420	0	+	+	0	"

Case	Family history	Rh Groups			Age (days)	Weight (lbs)	Enlargement of liver	Enlargement of spleen.	Initial Hb (Sahli) %	Nucleated RBC. /100 W.B.C.	c.c. Total transfusion	Recovered	Died	Autopsy	Abnormalities of pregnancy	Delivery
		father	mother	child												
33	0	+	-	+	2	7½	+	+	0	0	200	+	0	0	0	Spon
34	0	+	-	+	23	-	+	+	0	0	650	0	+	+	0	"
35	0	+	-	+	2	9	0	0	0	0	150	+	0	0	0	"
36	0	+	-	+	28	7½	0	+	35	0	120	+	0	0	0	"
37	+	+	-	+	2	-	+	++	100	12	0	0	+	+	0	"
38	0	+	+	+	26	-	+	++	50	0	0	0	+	+	0	"
39	0	+	-	+	12	7¾	+	+	80	0	320	0	+	+	0	"
40	+	+	-	+	6	-	+	+	0	0	210	+	0	0	0	"
41	0	+	-	+	5	9¼	+	+	85	++	0	0	+	+	0	"
42	0	0	0	0	7	6¼	+	++	20	60	100	0	+	+	0	"
43	0	+	-	+	2	-	0	+	0	0	380	+	0	0	0	"
44	+	+	-	+	2	6½	+	++	70	50	100	0	+	+	0	"
45	0	+	-	+	21	-	0	+	-	-	180	+	0	0	0	"
46	0	0	0	0	2	-	+	++	0	0	0	0	+	+	0	"
47	+	+	-	+	16	8	+	++	0	0	100	0	+	+	0	"
48	0	0	0	0	17 mths	-	0	0	0	0	0	0	+	+	0	"
49	+	0	0	0	12	9	+	++	44	0	200	+	0	0	0	"
50	+	0	0	0	24	7	+	++	29	0	0	+	0	0	0	"
51	0	0	0	0	3	7	0	+	53	0	0	+	0	0	0	"

SERIES 3. (Elsie Inglis Hospital)

Case	Family history			Age (days)	Weight (lbs)	Enlargement of liver	Enlargement of spleen	Initial Hb (Sahli) %	Nucleated R. B. C./W. B. C.	Total transfusion	Recovered	Died	Autopsy	Abnormalities of pregnancy	Delivery	
	father	mother	child													
1	+	0	0	0	S. B.	-	+	+	0	0	0	0	S. B.	0	0	Spontaneous
2	0	0	0	0	2	6 $\frac{3}{4}$	0	0	0	0	0	0	+	0	0	"
3	+	0	0	0	S. B.	-	+	+	0	0	0	0	S. B.	0	0	"
4	0	0	0	0	14	7	0	+	0	0	10cc.	+	0	0	0	"
5	0	0	0	0	S. B.	-	0	0	0	++	0	0	S. B.	0	Hydran nios.	Forceps
6	0	0	0	0	1	7 $\frac{1}{2}$	+	+	0	0	60cc.	0	+	0	0	Spontaneous
7	0	0	0	0	1	7 $\frac{3}{4}$	0	0	0	0	0	+	0	0	0	"
8a	0	0	0	0	5	5 $\frac{3}{4}$	+	++	0	0	0	0	+	+	0	"
8b	0	0	0	0	S. B.	-	0	0	0	0	0	0	S. B.	0	0	"
9	0	0	0	0	S. B.	-	+	0	0	++	0	0	S. B.	0	0	Breech
10	0	+	-	+	7	8 $\frac{3}{4}$	0	+	0	++	4 trans.	+	0	0	0	"
11	0	0	0	0	5	8 $\frac{1}{2}$	+	+	0	++	160 cc	+	0	0	0	Forceps
12	+	+	-	+	S. B.	4	0	0	0	0	0	0	S. B.	0	0	Spontaneous
13	0	+	-	+	2	5 $\frac{3}{4}$	+	+	0	++	0	0	+	+	0	"
14	+	+	-	+	$\frac{3}{4}$	6 $\frac{1}{2}$	+	+	0	0	0	0	+	+	0	"
15	0	+	-	+	$\frac{1}{4}$	5 $\frac{3}{4}$	+	+	0	0	0	0	+	+	0	"
16	0	0	-	-	8	7	0	+	120	++	0	0	+	0	0	"
17	0	+	-	+	5	8 $\frac{3}{4}$	+	+	0	0	1080cc	+	0	0	0	"
18	+	+	-	+	2	8 $\frac{1}{2}$	0	+	0	0	0	+	0	0	0	"
19	+	+	-	+	7	7 $\frac{3}{4}$	+	+	0	0	250cc.	+	0	0	0	"
20	+	+	-	+	1	8	0	0	0	0	0	+	0	0	0	"
21	+	+	-	+	S. B.	6 $\frac{1}{2}$	+	++	0	0	0	0	S. B.	+	0	Breech
22	0	0	0	0	2	7	+	++	40	0	0	+	0	0	0	Spontaneous

Chapter 10.

Differential Diagnosis of Congenital Haemolytic Disease.

At the present time, as a result of the use of serological methods, haemolytic disease in the newborn may be diagnosed with greater accuracy than was formerly possible. Clinical and laboratory methods of diagnosis were outlined in the previous chapter. Post-mortem diagnosis was discussed in a previous chapter (Chapter 6).

a) Hydrops Foetalis :

Hydrops foetalis as a result of haemolytic disease must be distinguished from congenital syphilis, congenital heart disease and idiopathic hydrops. Occasionally the offspring of a diabetic mother may be hydropic.

Congenital syphilis was confused with haemolytic disease for many years. Hepato-splenomegaly and erythroblastaemia are found in both diseases. Serological tests, namely the Wassermann and Kahn to exclude syphilis and the Coombs test to detect sensitisation, have helped to distinguish the two diseases. A full post-mortem examination should be carried out in all cases where there is doubt. One still-born infant showed changes at post-mortem which were consistent with a diagnosis of haemolytic disease. No spirochates were found in the tissues but the pancreas showed severe syphilitic fibrosis (Figs./

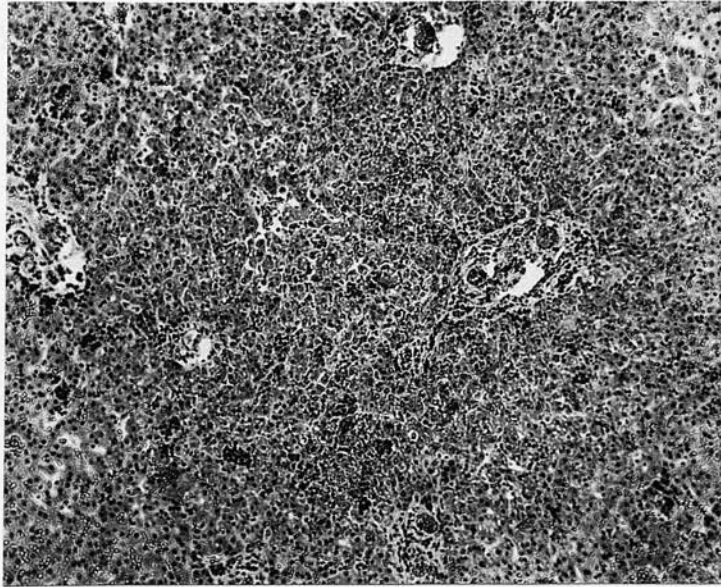


Fig. 51. LIVER x 90. Haematoxylin and Eosin.
Numerous foci of normoblasts and erythroblasts
in the parenchyma and portal tract.
Case of congenital syphilis.

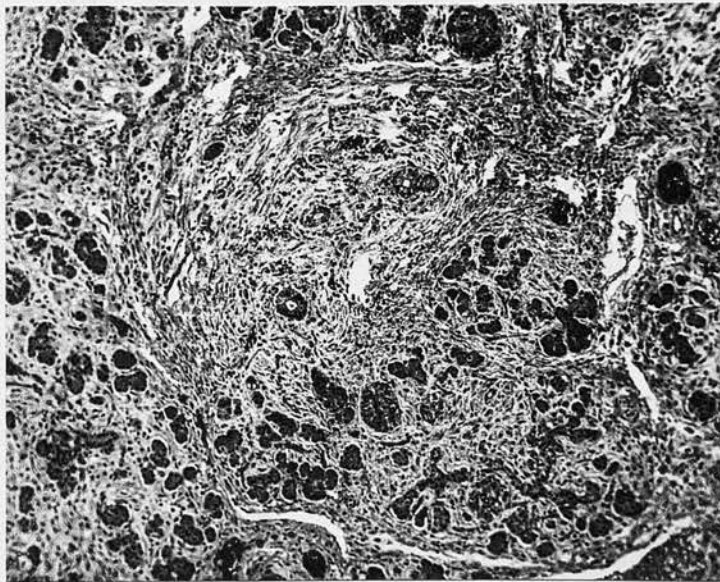


Fig. 52 a. PANCREAS x 90. Haematoxylin and Eosin.
Note extreme degree of periacinar fibrosis.
Case of congenital syphilis.

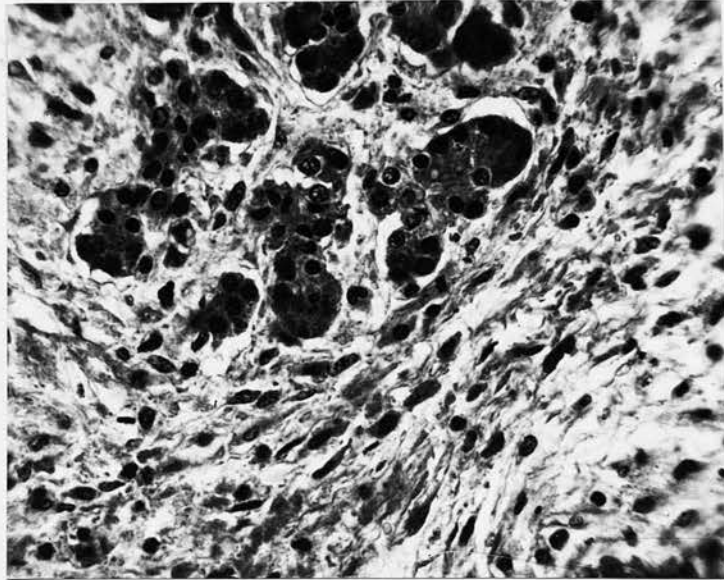


Fig. 52 b. PANCREAS x 350. Haematoxylin
and Eosin. Marked interstitial fibrosis.
Case of congenital syphilis.

(Figs. 52a and 52 b). It is important to remember that a mother with frank syphilis may be also Rh negative and bear an Rh positive child (Case 24, Series I). In such a case a full post-mortem examination is essential. Again, the proof that the child did in fact die as a result of haemolytic disease and not from congenital syphilis will spare the mother much unnecessary anguish.

Congenital heart disease may occasion the delivery of a still-born, oedematous infant. The serological tests will be negative, however, and the placenta will not be greatly enlarged. Hepato splenomegaly and extra medullary haemopoiesis (Fig.49) may be present, and early nucleated red cells are found in the peripheral blood. Anaemia may be severe. A full post-mortem examination will reveal the true lesion.

It is in the idiopathic form of hydrops foetalis that a full necropsy is most essential. Potter (1946) has shown that most cases of this type are the offspring of primiparae (Chapter 6).

Hellman and Irving (1938) have shown that an intrauterine diagnosis of hydrops foetalis can be performed by radiography. In the X-ray film the subcutaneous tissues are increased in density and a corona surrounds the skull as a result of oedema of the scalp. It is doubtful if idiopathic hydrops can be excluded by this means.

Wiener and Sonn (1946) have described an instance where a still-birth was thought to have been/

been caused by haemolytic disease but further examination revealed diabetes mellitus in the mother. The examination of the pancreas in offspring of diabetic mothers will reveal the presence of giant islets of Langerhans.

b) Icterus gravis neonatorum :

This condition must be distinguished from physiological jaundice of the newborn, congenital atresia of the bile-ducts, familial acholuric jaundice, congenital syphilis and neonatal sepsis.

Physiological jaundice is seldom present at birth and if it occurs within the first 24 hours it is generally mild in character. The serum bilirubin levels are lower than in icterus gravis and the van den Bergh reaction is indirect. In icterus gravis the latter is frequently biphasic or even direct. Severe anaemia, erythroblastaemia and neurological sequelae never accompany physiological jaundice. The latter is usually transient but Beskow (1941) reported an instance in which it persisted for four weeks. Isoimmunisation plays no part in physiological jaundice which is the result of functional immaturity of the liver (Rich 1930) ; the serological tests are negative.

Congenital atresia of the bile ducts may cause some difficulty (Case 47, Series II). In this condition the jaundice rarely appears till the second week of life and thereafter steadily deepens. The stools are acholic from birth. There is no severe anaemia/

anaemia and no erythroblastaemia. Serological tests are negative. Acholia may develop in icterus gravis but is exceptional.

Congenital syphilis may produce jaundice and erythroblastaemia; hepatosplenomegaly and anaemia are found. The family history, serological tests and post-mortem examination in fatal cases will establish the diagnosis.

Familial acholuric jaundice may be present in the first two weeks of life. There is mild jaundice, anaemia and enlargement of the spleen. In this disease, however, the erythrocytes show increased fragility in saline solutions. There is no evidence of maternal isoimmunisation and the serological tests are negative.

Neonatal sepsis has been mentioned as a cause of haemolytic disease in the days before the Rh blood groups were discovered and even more recently has been described as a cause of icterus gravis and kernicterus (de Bryne and van Creveld, 1948). Jaundice may appear towards the end of the first week and anaemia is common. Nucleated red cells may be found in the peripheral blood. A positive blood culture may help to establish the diagnosis. Since neonatal sepsis and haemolytic disease may coexist the serological tests are frequently positive. It is this fact which has led the Dutch workers to ascribe kernicterus to neonatal sepsis.

c) Congenital Haemolytic Anaemia.

This form of haemolytic disease must be distinguished/

distinguished from haemorrhagic disease of the newborn and from other blood diseases in the neonatal period.

Haemorrhagic disease of the newborn is a result of hypoprothrombinaemia. The latter causes purpuric manifestations and melaena. It is most marked between the 3rd and 6th days of life and there is neither enlargement of liver and spleen nor jaundice. The anaemia is seldom severe. As Mollison (1948) points out the treatment of haemorrhagic disease by injection of whole blood without first determining the Rh type of the recipient may cause great harm to the latter in adult life. Vitamin K injections are a much safer therapeutic procedure.

Primary blood diseases such as aplastic anaemia, leukaemia etc. are rare in the neonatal period. Cases of leukaemia diagnosed at this stage of life were probably examples of haemolytic disease. Full serological examinations should establish the diagnosis.

It will be seen from this brief review that serological tests play a great part in the diagnosis of haemolytic disease in the living patient. The Wassermann and Kahn tests exclude syphilis and the Coombs test indicates sensitisation of the infant's erythrocytes.

Let it again be emphasised that a post-mortem examination in fatal cases must be extremely thorough.

Chapter 11.

The Treatment of Haemolytic Disease.

Since the discovery of the Rhesus blood groups there has been considerable controversy regarding the respective merits of Rhesus positive and Rhesus negative blood in transfusion therapy. It is not possible to examine all the claims in detail and this section will be confined to a summary of the chief methods of therapy in haemolytic disease.

1. Prevention of Haemolytic Disease.

It would be a considerable achievement if some means were found of preventing the onset of haemolytic disease in the foetus. Unfortunately, this has not been possible by any methods used up to the present time.

The disease commences in the foetus as a result of an antigen-antibody reaction caused by the development of a state of isoimmunisation in the mother. It has been suggested, therefore, that it might be possible to "desensitise" the mother and thus prevent the development of the disease in the foetus. This line of attack has, so far, met with little success.

Darrow (1938) remarked that Hampson's (1929) therapeutic success with small intramuscular injections (5-15 cc.) of mother's serum daily into the affected infant might be explained by his achieving a state of desensitisation in the infant.

Parsons/

Parsons (1947) pointed out that Hampson's recovery figures (17 out of 18) were far in excess of anything achieved since, and that Hampson must have included cases of physiological jaundice in his series.

Wiener (1946) thought that the Rhesus antigens were less potent in inducing a state of iso-immunisation in the mother than were some other antigens. He postulated the " overthrow " of Rh isoimmunisation by the simultaneous development of isoimmunisation to a more powerful antigen, if the latter could be introduced. He thus considered that typhoid or pertussis vaccine might be used therapeutically to prevent the development of maternal isoimmunisation to Rh antigen by utilising all the mother's capacity to produce antibodies with typhoid antigen (or pertussis antigen). Parsons (1947) did not believe that Wiener's premises were correct and certainly little has been achieved by this form of therapy. Wiener (1946) himself admitted that once isoimmunisation to Rh antigen had developed, counter immunisation had little effect. It is just possible, however, that further work along these lines will achieve results of greater therapeutic value.

One of the most important aspects of prevention of haemolytic disease, is the avoidance of any unnecessary sensitisation of the mother to Rh antigens. It is a well-known fact that transfusion of an Rh negative person with Rh positive blood is the easiest method of inducing isoimmunisation to Rh antigen in the/

the former. If the recipient be a woman in the childbearing period or earlier she may be delivered of an affected infant in a subsequent pregnancy.

This was clearly illustrated in a case in this series where a woman had normal children until she was transfused with blood (not tested for Rh incompatibility) after a miscarriage. In the following pregnancy she produced an infant exhibiting icterus gravis and kernicterus which subsequently died. Such tragedies can be prevented by testing all women who have not reached the menopause for the reaction of their blood to standard Rh antisera. Any blood must be adequately tested for compatibility (A-B and Rh) before it is given to such a patient. The tests are not difficult to perform and may be used routinely. Whole blood injections in the treatment of haemorrhagic disease of the new born are also dangerous and this form of therapy should be replaced by treatment with Vitamin K. The dangers of incompatible blood transfusions in women who are already immunised has been stressed elsewhere (Chapter 7).

Haemolytic disease could of course be prevented if all Rh-negative women chose Rh-negative mates. It is hardly likely, however, that this method of selection will meet with any approval.

Once isoimmunisation has occurred, and in particular, once a series of still-born hydropic foetuses have resulted from pregnancies, the only chance the mother has of producing a normal infant is by/

by remarriage to an Rh-negative male or by artificial insemination from an Rh-negative donor. This last method introduces questions of a social nature and will not be pursued further. Such a method has been used successfully in America (Potter and Wilson 1945).

Many authors have recommended the induction of abortion or of premature labour or the Caesarian section method of delivery in cases where the infant is thought likely to be affected by haemolytic disease.

A therapeutic abortion may be advocated in the case of women who have had a series of still-born hydropic foetuses and whose husband is homozygous Rh positive. The outlook regarding the outcome of future pregnancies in such subjects is very poor but not invariably hopeless.

Induction of premature labour at the 37th-38th week, or the delivery by Caesarian section at the same period is held by some workers (Diamond 1947), Harville (1945), to improve the chances of the infant's recovery and allows early transfusion therapy to be instituted. Parsons (1947), on the other hand, points out the hazards to the infant in such premature deliveries. It has been shown in this series that any extra hazard to the infant at birth militates against the subsequent recovery (Chapter 5). Where the offspring is likely to exhibit hydrops such therapy is justified as it is the only slender hope of achieving a live child. So far such therapy has met with little success in cases of hydrops foetalis but/

but some further period of trial is required.

2. Transfusion Therapy in Affected Subjects :

Blood transfusion is the mainstay in the treatment of infants suffering from haemolytic disease.

a) Hydrops Foetalis.

As has been stated in the preceding section, little success has been achieved in the live-born infants affected by this form of the disease. It is to be hoped that by induction of premature labour at the 37th - 38th week combined with immediate exchange-replacement transfusion of the Rh-negative blood, the prognosis may be improved. It is to be remembered, however, that severely oedematous infants will have a massive bilateral hydrothorax. This is a great mechanical impediment to respiration and these infants commence with a great disadvantage.

b) Icterus gravis neonatorum :

It is in this type of haemolytic disease that blood transfusion is most useful and in which the latter has had the most striking success.

Parsons (1947) has suggested that the chief use of blood transfusion in haemolytic disease is the replacement of red cells to compensate for those lost by haemolysis. He points out that many cases fail to show any marked anaemia and believes that transfusions are not indicated in such cases and may even do harm by overloading the circulation. Mollison and Cutbush (1949) have shown that the previous criteria for the presence or absence of anaemia may have been inadequate and suggest that anaemia is a more frequent occurrence/

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occurrence in icterus gravis than was previously realised. Furthermore, it has been demonstrated in this series that a degree of anoxia is probably present in most severe cases of icterus. Thus blood transfusion will be of value in such cases by virtue of the oxygen carrying capacity of the transfused blood.

It is obvious that severely affected patients should be transfused immediately. Unfortunately the criteria of severity are not absolutely definite and exceptions occur. These have been discussed elsewhere (Chapter 9). Briefly all cases severely jaundiced at birth or within 24 hours of birth and all cases with a subnormal haemoglobin and high plasma bilirubin in their cord blood should be transfused immediately.

It will be shown later (Chapter 14) that kernicterus may occur within 12 hours of birth and thus prompt treatment is essential as there is no means of separating patients liable to develop nuclear jaundice from those which are not. Parsons (1947) believes kernicterus, or at least the cerebral damage, to be established before birth and thus treatment is likely to be of little value. There is reason to believe this is not entirely true (Chapter 14). In a later section it will be shown that in the present series 66% of patients dying of icterus gravis in the neonatal period showed kernicterus post-mortem. If it is possible to avert the development of the latter, the reason for prompt treatment of/

of severely jaundiced cases is clear.

Mild cases of icterus gravis may require no treatment. The clinician will use his own discretion in cases of this type which are often first affected infants, are in good condition at birth, and show mild jaundice and no anaemia. Such infants will require careful observation but in the majority no active measures will be required.

Although transfusion therapy has been accepted in principle, two great controversies have raged over its application. The first is concerned with whether Rh positive or Rh negative blood should be used. The second is a question of technique ; some workers prefer ordinary direct transfusion while others advocate the use of the slightly more complicated exchange- transfusion technique.

(1) Rhesus positive blood :

This has few advocates in this country at the present time. In America, however, Darrow and Chapin (1947), Sandford (1946) and a few others are staunch supporters of this type of therapy. Vaughan (1946) believed it to be of some value in the prevention of kernicterus provided it was given within 24 hours of birth. Darrow and Chapin (1947) defend their use of Rh-positive blood on the grounds that it " desensitises " the infant. This " desensitisation " is a necessary part of the therapy since they believe severe icterus gravis is a anaphylactic phenomenon. The grounds for their belief are not at all certain.

In all fairness it must be pointed out that
Rhesus/

Rhesus positive blood can be a therapeutic success in some cases of haemolytic disease. Some cases in this series were successfully treated by means of blood transfusion before the Rhesus blood groups were discovered (Table 44). It is highly probable, in view of the distribution of these groups among the population, that the blood used was in fact Rhesus positive. In one case, however, which was being treated by multiple transfusions of Rhesus negative blood, the infant was given a transfusion of Rhesus positive blood in error. A severe transfusion reaction ensued. The jaundice which had been clearing, returned with added intensity, the spleen and liver became more enlarged, the haemoglobin fell sharply and the infant was very ill. Fortunately an immediate transfusion of Rh negative blood restored the balance. It is obvious, however, that the use of Rh positive blood in such cases may be highly dangerous.

Finally, it will be remembered that iso-immunisation to rare genotypes may occur. If a mother becomes immunised to c-antigen or e-antigen and develops anti-c or anti-e agglutinins the child, if affected with haemolytic disease, should be transfused with Rh positive blood (Chapter 7).

(11) Rh negative blood :

This is the generally accepted type of blood used in the treatment of haemolytic disease. Wiener, Wexler and Gamrin (1944) have stressed the dangers of using Rh positive blood and have shown that Rh negative blood is a safer and a more effective method/

method of treatment. Mollison (1948) has summarised the beneficial effects of Rh negative blood as follows " The transfusion of Rh-negative blood provides a stable population of erythrocytes not susceptible to rapid destruction. The raising of the haemoglobin value above the previous level diminishes the stimulus to the bone marrow, and the infant only needs to maintain a small concentration of Rh-positive erythrocytes, but, more important, the total amount of destruction of Rh-positive erythrocytes is diminished, and this is sometimes reflected in a rapid diminution of jaundice. "

(111) Direct Transfusion :

This form of therapy was used long before the Rhesus blood groups were identified. Sometimes the father's blood was used and sometimes that of a donor. Many cases recovered but equally as many developed a reaction to the transfused blood and died. With the discovery of the Rhesus blood groups, direct transfusions of Rh-negative blood were given with some success. (Gimson 1943, Wiener and Wexler, 1943).

Various routes and techniques have been used. The anterior fontanelle was greatly favoured at one time and the earlier cases in this series were transfused via this route. A possible danger is the injection of blood into the subdural space.

Intravenous drip transfusions (via saphenous vein) largely replaced the fontanelle route as the method of choice. This was used extensively in cases in/

Table 44.

Results of Blood Transfusion Therapy with Different types of Blood.

Type of Blood Transfused.	No. of Patients Recovered.	No. of patients Died.	Total No. Patients	Mortality %
Father's blood (Probably Rh positive).	3	3	6	50 x
Mother's Blood (Probably Rh negative).	1	0	1	Nil x
Rh Unknown (Probably Rh positive).	4	3	7	43 x
Rh negative	17	17 +	34	50
Total	25	23	48	48

+ Two patients died from gastro-enteritis; one patient was adversely affected by an abdominal operation.

The technique used in all cases was that of simple direct transfusion via anterior fontanelle, arm vein or umbilical vein.

x Series too small to have statistical significance.

in this series with fairly good results (Table 44). The amount given varied with the preference of the individual physician and no fixed amount was given at any one time. In general 15 cc. per lb. body weight was given at one time. In a few cases this was reduced to 10 cc/lb. body weight (Table 45). Transfusions were repeated as often as were required. A rapid fall in the haemoglobin of the infant was taken as a need for further transfusion. In all cases Group O Rh negative blood was used. Mollison (1948) points out that accurate A-B matching is very important as anti-A or anti-B agglutinins in the donor's plasma may cause further haemolysis of the infant's erythrocytes. It is also important to remember that Rh negative donor's blood may contain anti-Rh agglutinins.

The total amount of blood given to any one infant varied considerably. Some infants died after receiving only 90-100 cc. One infant received more than 1,000 cc. over a period of a month. The average amount transfused was 250 cc. (Table 45).

Naturally the number of transfusions required will vary according to the severity of the disease process and the response of the infant to transfusion therapy. The amount to be given and the frequency of transfusions are matters within the province of the clinician concerned.

Mayes (1946) has recommended the use of direct blood transfusion via the umbilical vein in the treatment of haemolytic disease. A cannula or plastic catheter/

Table 45.

Relationship between Amount of Blood Transfused and the Outcome.

Amount	Recovered	Died	Total	Mortality %.
0 - 50 cc.	2	4	6	66 x
60 cc.- 100 cc.	2	15	17	88
110 cc.- 200 cc.	11	1	12	8
210 cc - 300 cc.	6	1	7	14 x
310 cc.- 400 cc.	2	2	4	50 x
Over 400 cc.	1	1	2	50 x

x Series too small to have statistical significance.

catheter (vide infra) is inserted into the vein and 100 cc. or so of blood may be given by this route.

Mollison (1948) points out that the scalp vein route can be used with advantage and 80 cc. may be transfused in 10 minutes. This route was not used in the present series.

One of the criticisms of the direct method of blood transfusion is the danger of overloading the infant's circulation and causing right sided heart failure. The haemoglobin should be raised to 100 % during the first week and thereafter it is unnecessary to achieve a level above 70% (Mollison, 1948) It must be noted, however, that acute right sided heart failure may occur after exchange-replacement transfusion, although the avoidance of this catastrophe was considered to be one of the advantages of such a technique. I have recently (1949) performed a post-mortem examination on a child affected with haemolytic disease who had been treated in this manner. The cause of death was undoubtedly right sided cardiac failure.

If due care is exercised, the direct method is probably not any more likely to overload the circulation than the newer method. The danger is, however, a very real one.

The effect of simple direct blood transfusion on the haemoglobin levels in affected infants is shown in Graphs 3- 14. It will be seen from these graphs that a more sustained response to blood transfusion is obtained when the initial transfusion is/

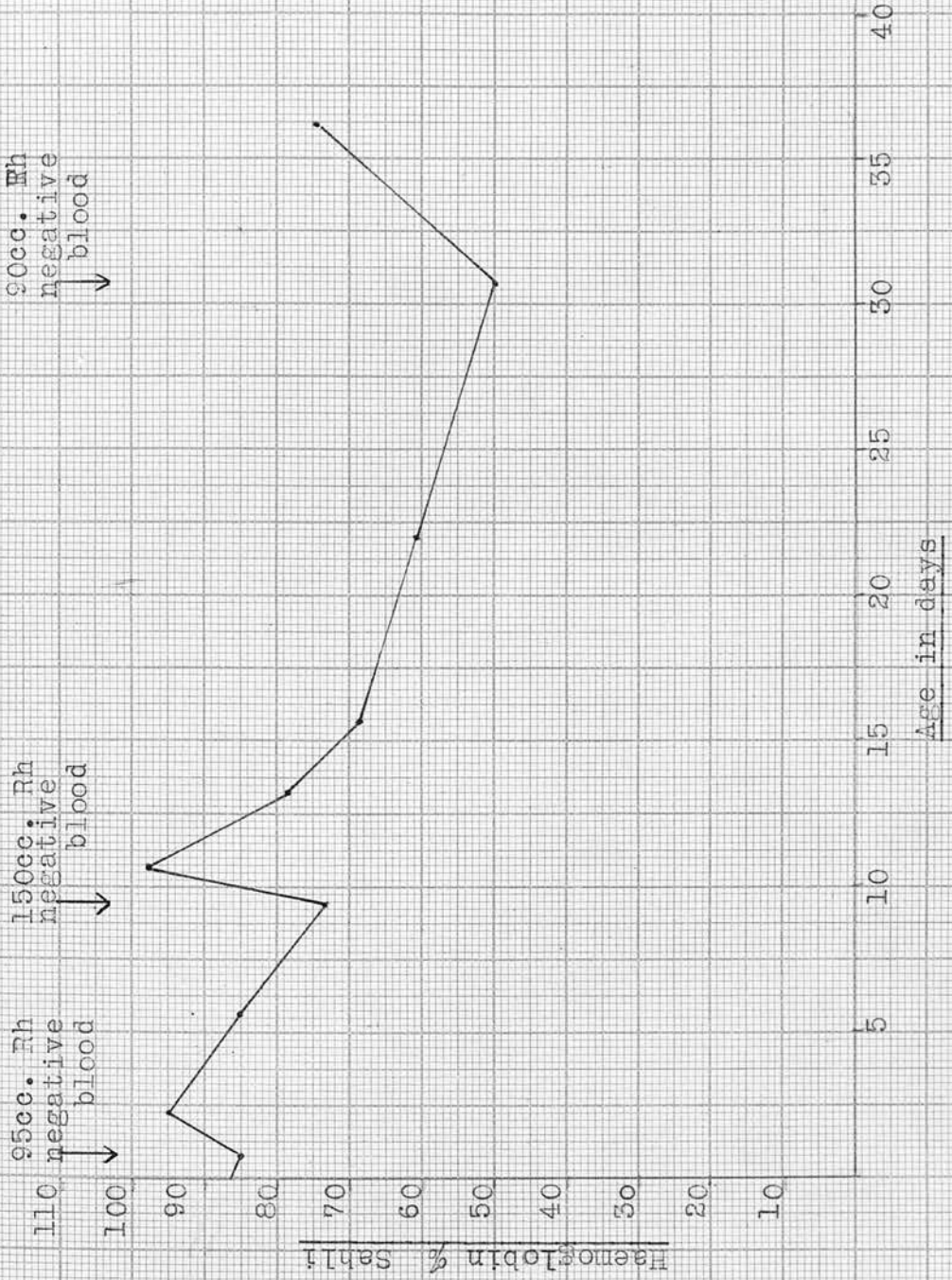
GRAPH 3

Effect of Rh negative blood transfusion on Haemoglobin Levels.
Case of Haemolytic Anaemia (Recovered)



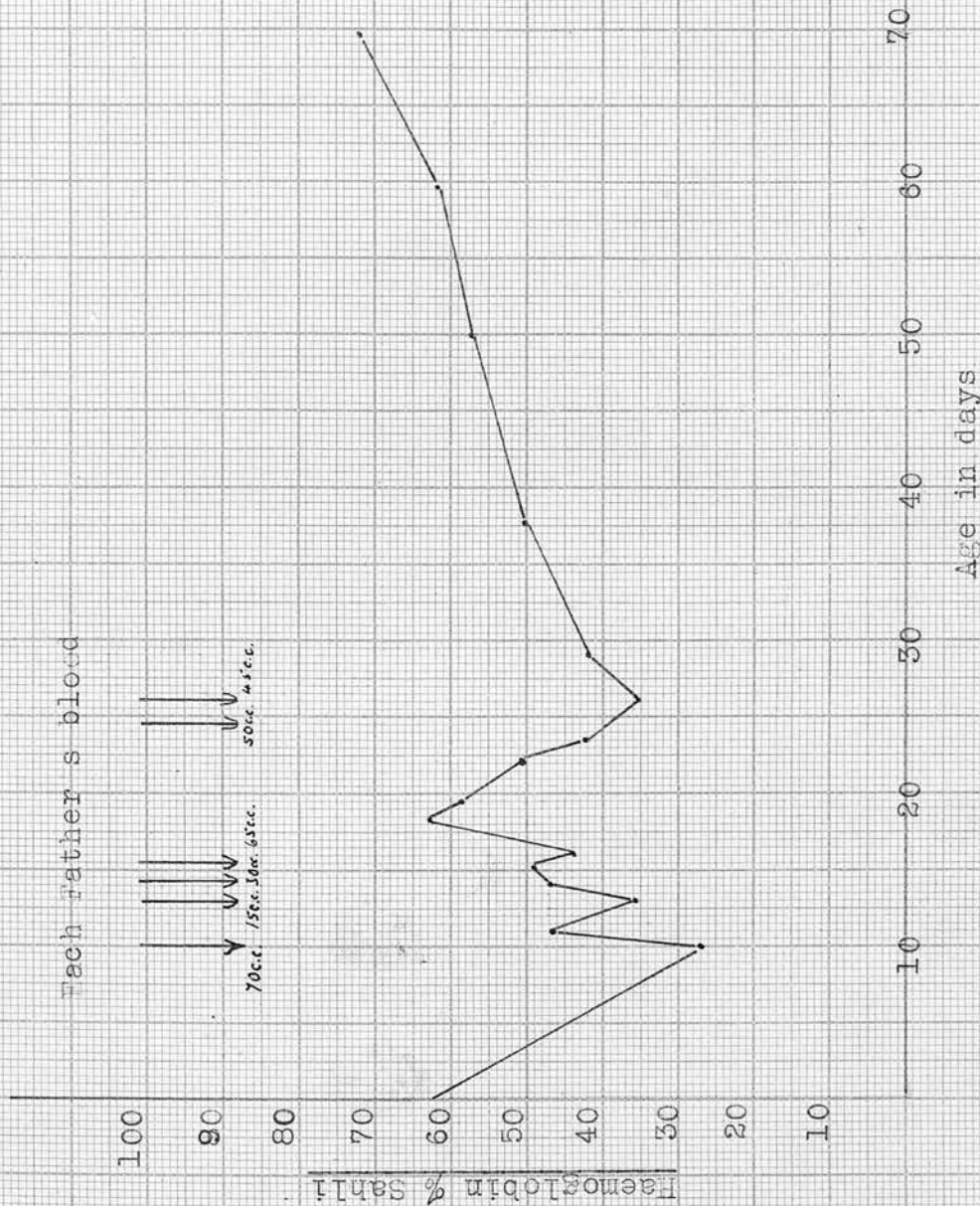
GRAPH 4

Effect of Rh negative Blood Transfusion on Haemoglobin
Levels: Case of Icterus Gravis (Recovered)



GRAPH 5

Effect of transfusion of father's blood on Haemoglobin levels
Case of Icterus Gravis (Recovered)

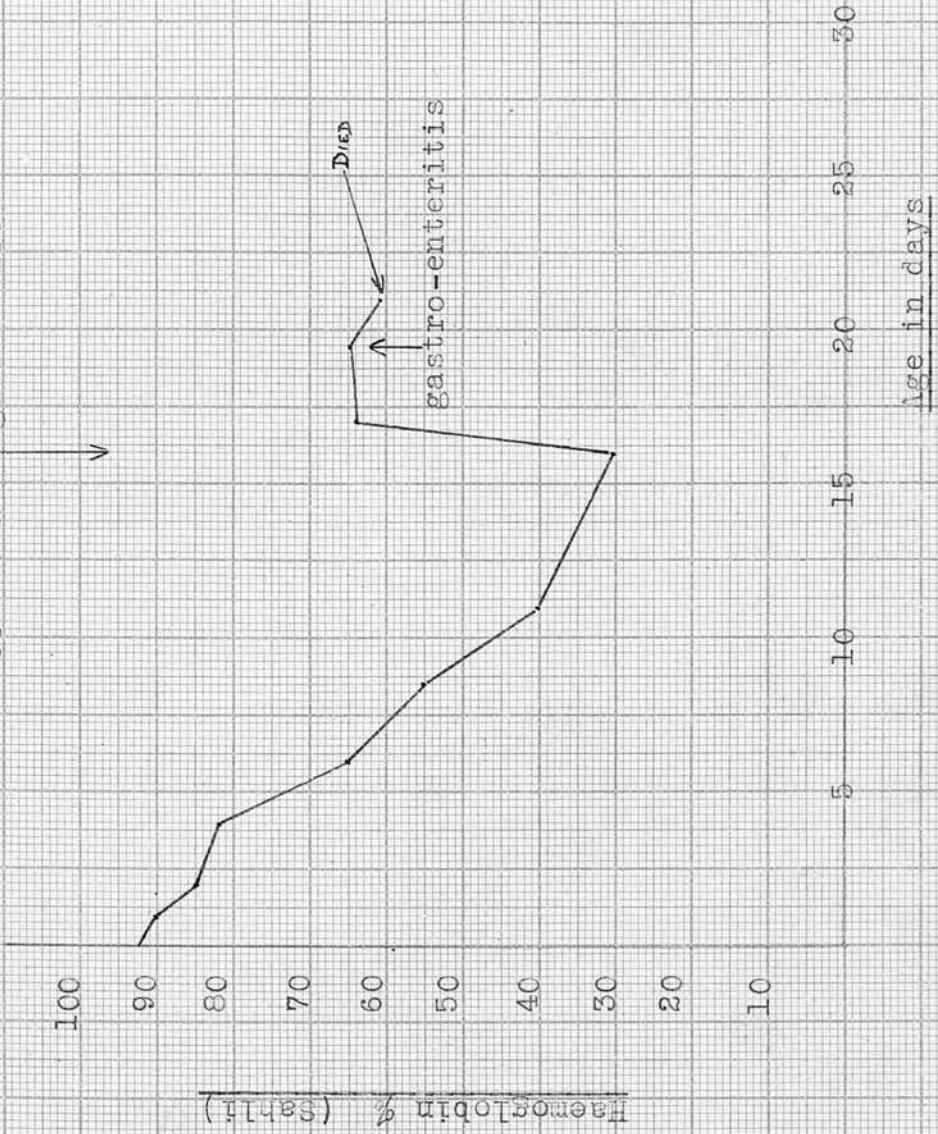


GRAPH 6

Effect of Rh negative Blood Transfusion on Haemoglobin Levels

Case of Icterus Gravis (died)

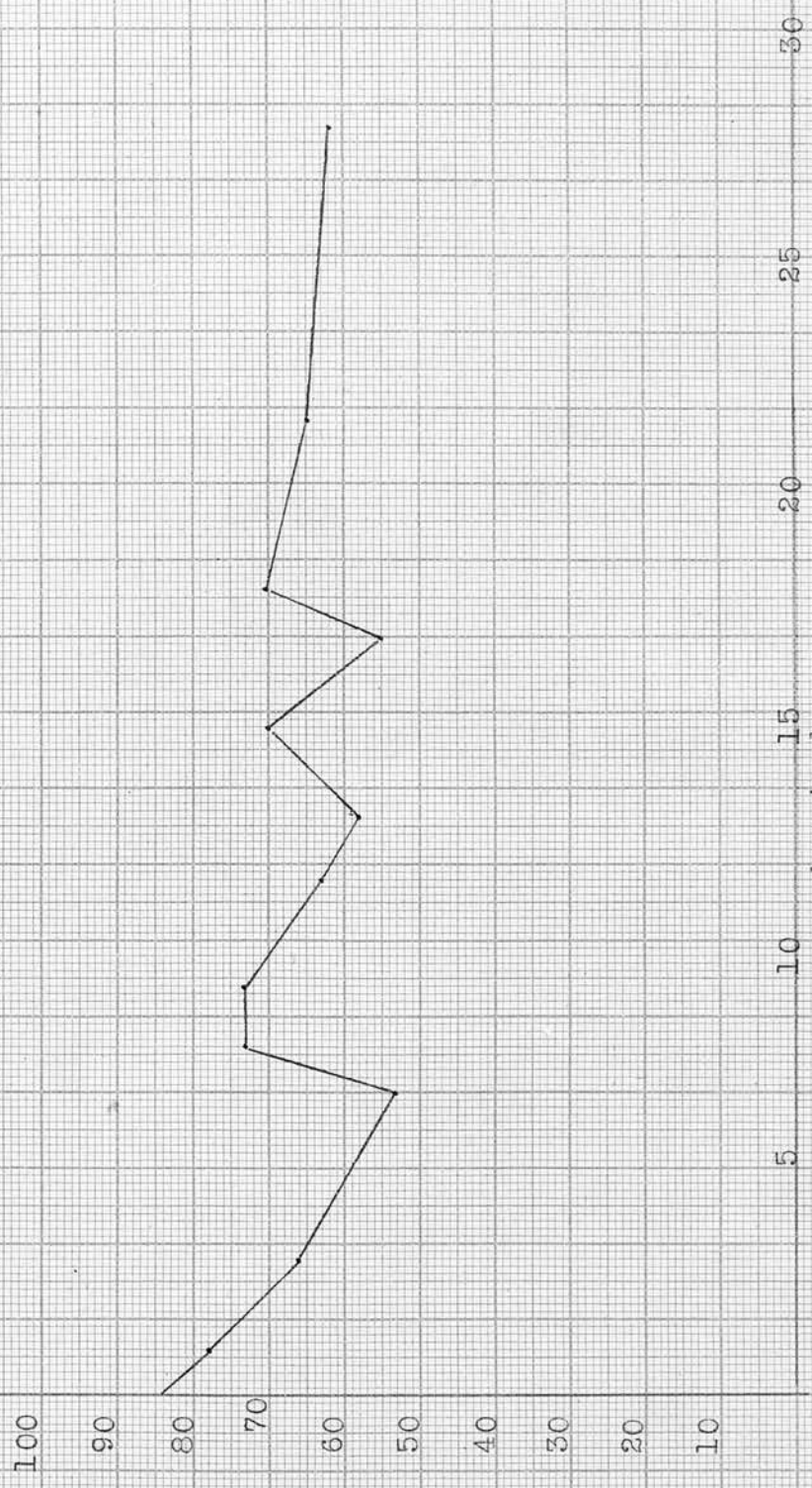
100 cc. Rh negative blood



GRAPH 7

Effect of Rh negative Blood Transfusion on Haemoglobin Levels
Case of Icterus Gravis (Recovered)

Each 60 cc. Rh negative blood

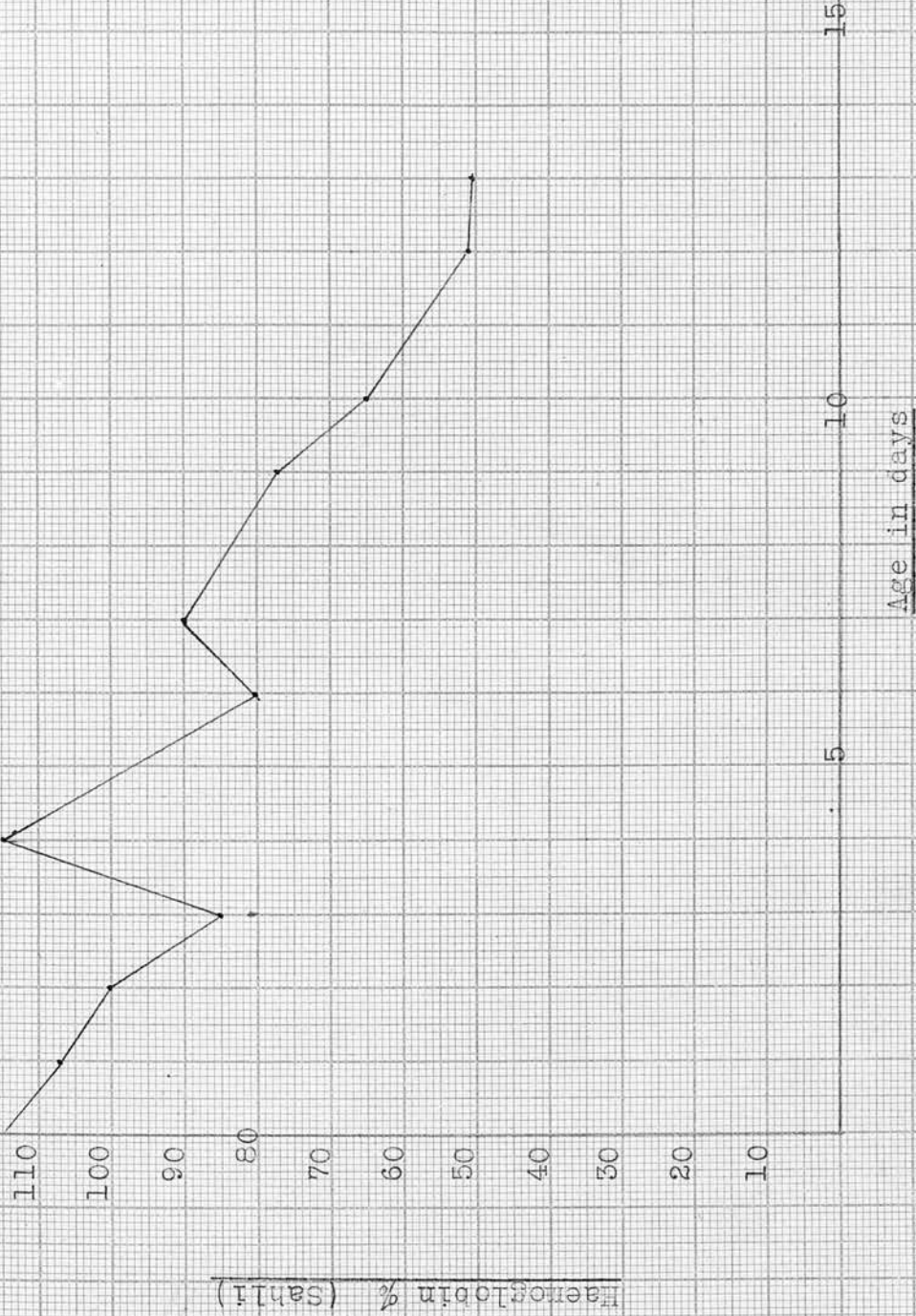
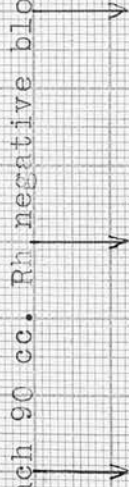


GRAPH 8.

Effect of Rh negative Blood Transfusions on Haemoglobin Levels

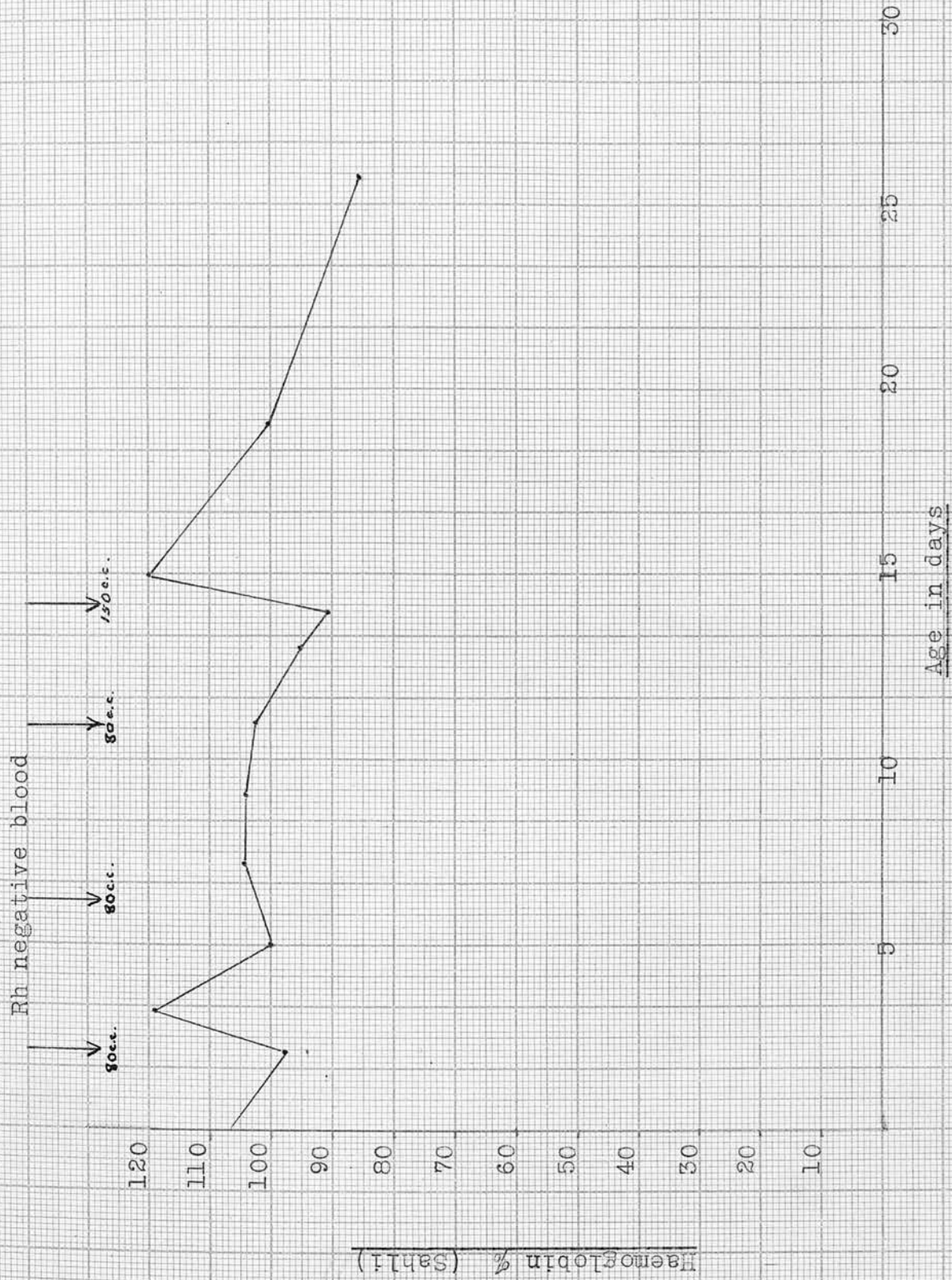
Case of Icterus Gravis (died)

Each 90 cc. Rh negative blood



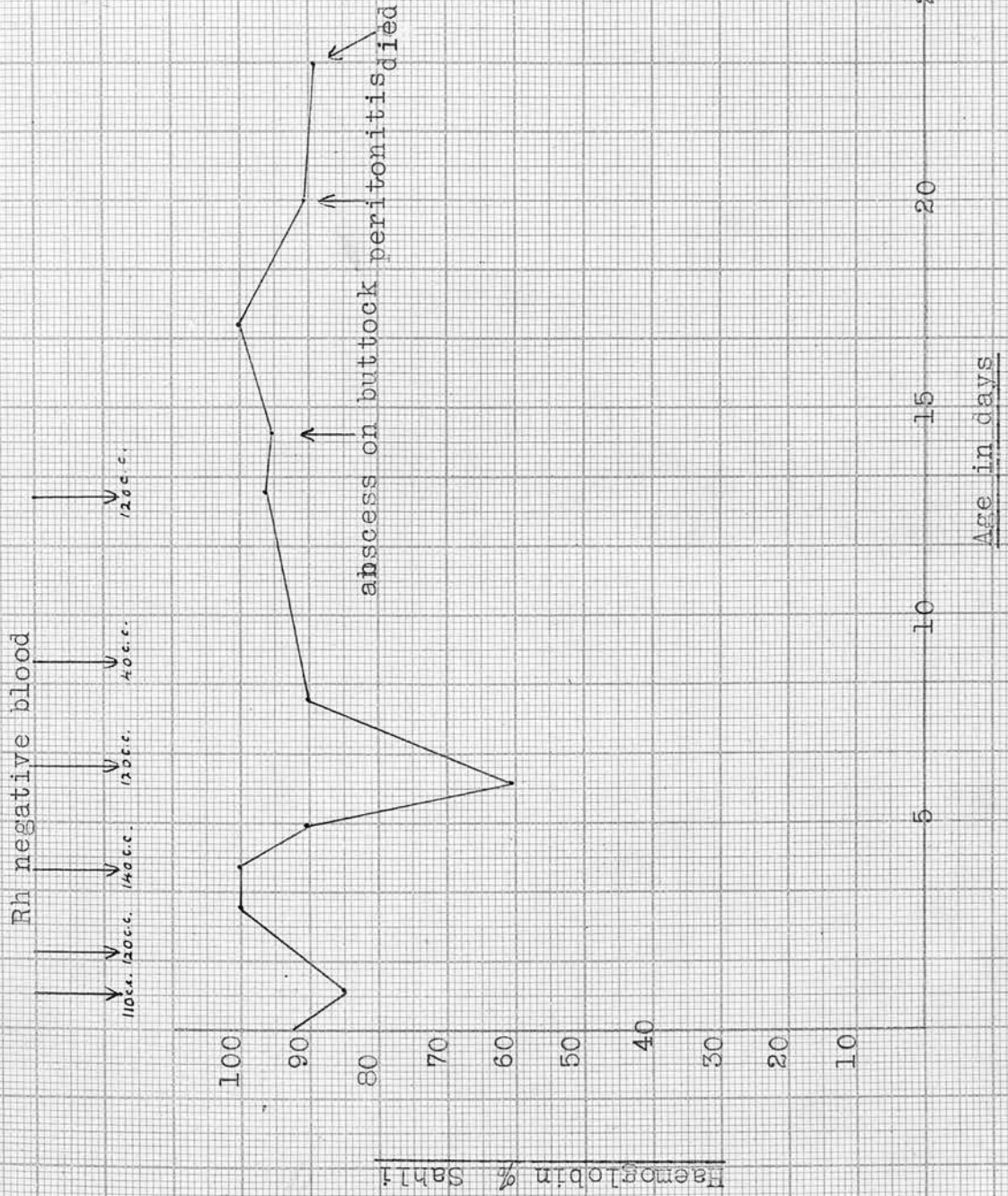
GRAPH 9.

Effect of Rh negative blood transfusion on haemoglobin Levels
Case of Icterus Gravis (recovered)



GRAPH 10

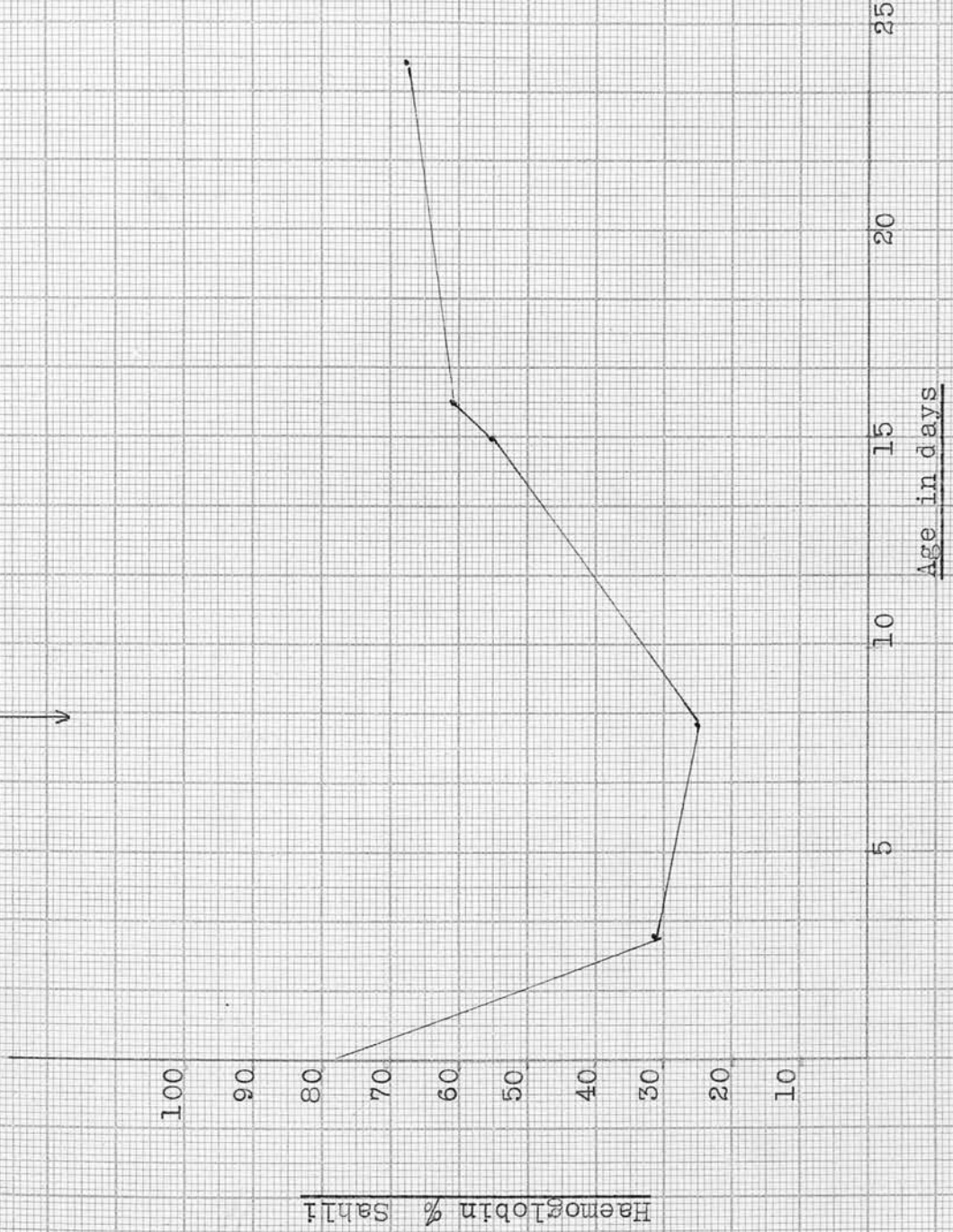
Effect of Rh negative blood transfusion on Haemoglobin Levels
Case of Icterus Gravis (died)



GRAPH 11

Effect of blood Transfusion on Haemoglobin Levels
Case of Icterus Gravis (recovered)

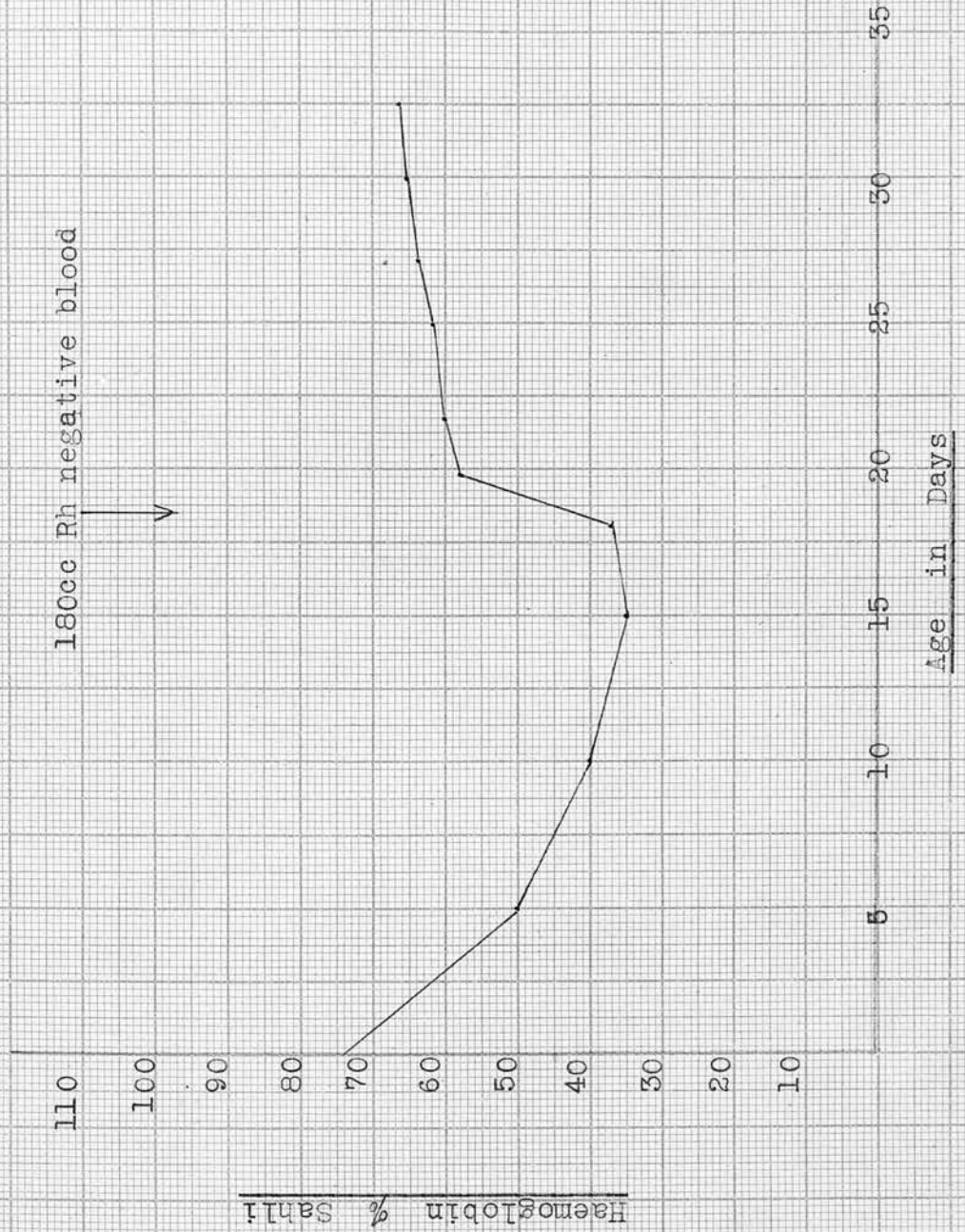
150 cc. Rh negative blood



GRAPH 12

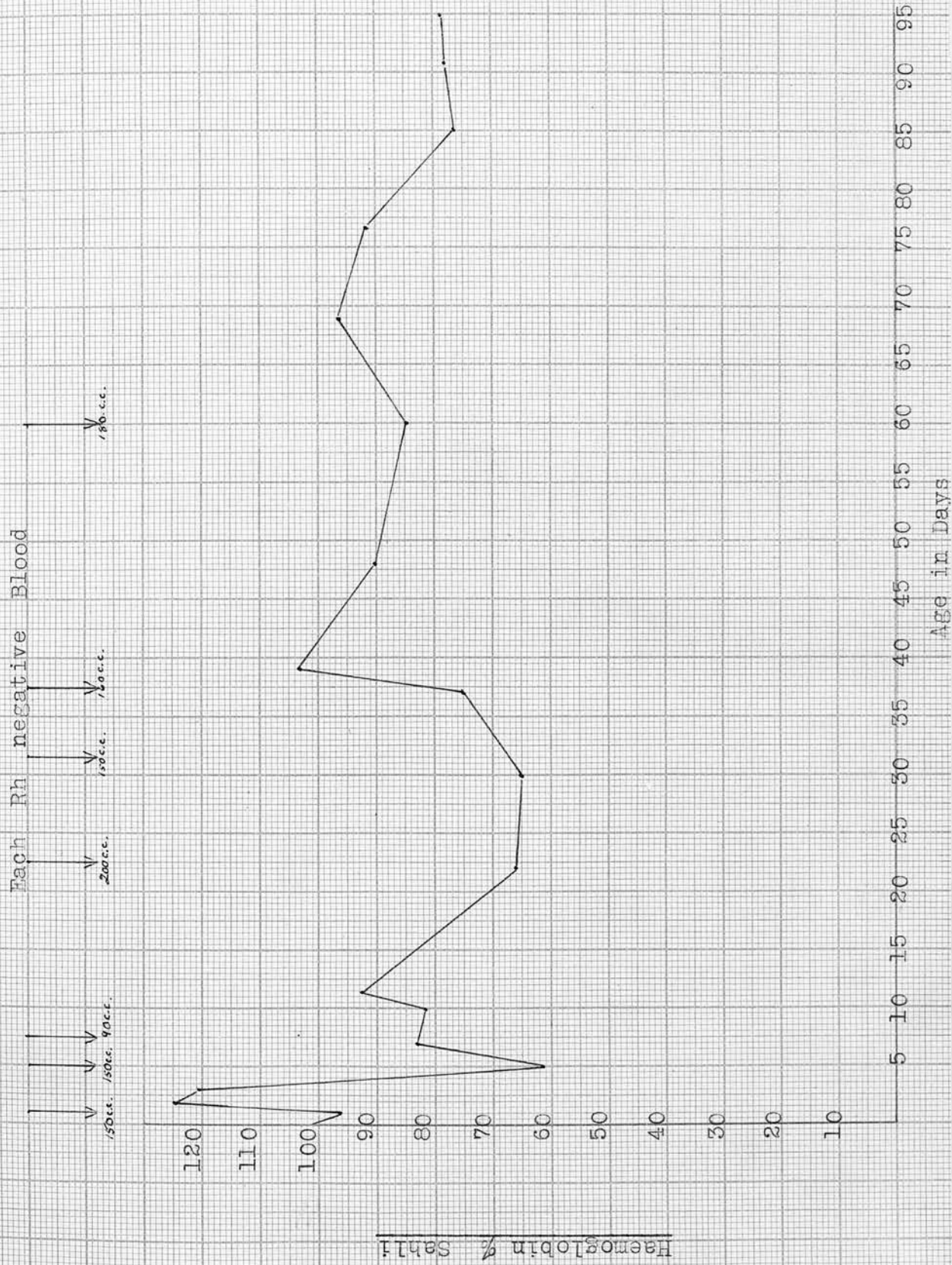
Effect of Blood Transfusion on Haemoglobin Levels

Case of Haemolytic Anaemia (Recovered)



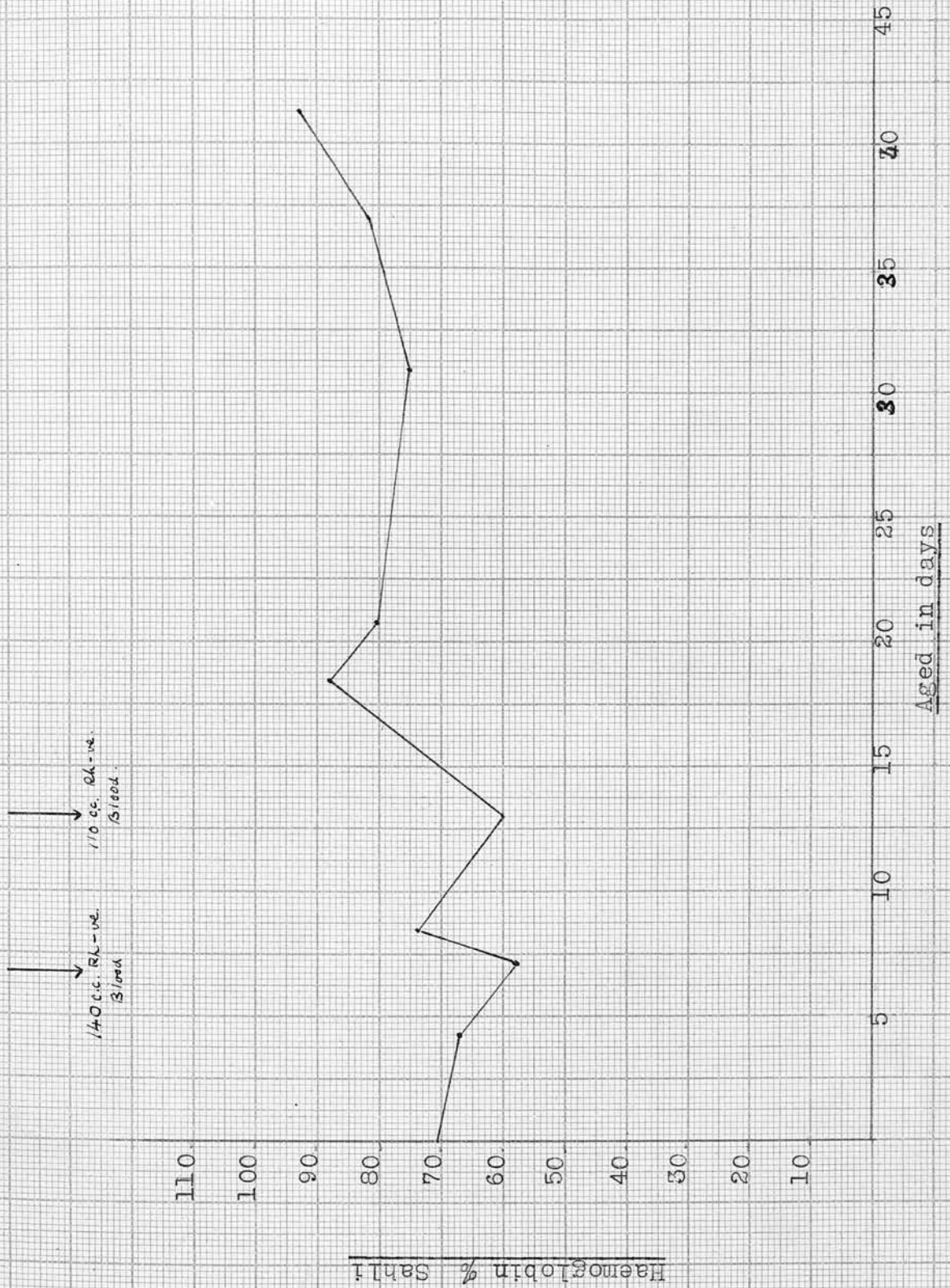
GRAPH 13.

Effect of Rh negative Blood Transfusion on Haemoglobin Levels
Case of Icterus Gravis (recovered)



GRAPH 14

Effect of Blood Transfusion on Haemoglobin Levels. Case of Icterus Gravis (Recovered)



is large (120 - 150 cc.). This is especially true in the infants affected by haemolytic anaemia (Graph 12) where one large transfusion may be sufficient to stabilise the blood levels.

If blood transfusion merely results in a temporary rise in the haemoglobin levels and this rise is followed by a rapid, profound fall (Graph 13), multiple transfusions may be required in order to stabilise the blood levels. There is a danger in giving multiple transfusions where a low blood level does not exist. An instance of this is seen in Graph 9, where the patient received nearly 400 cc. of blood over a period of 15 days although the fall in haemoglobin between transfusions was never severe. In such cases there is a danger of overloading the child's circulation and some of the transfusions may not be required.

If the fall in the haemoglobin level persists in spite of transfusion therapy, the outcome is not likely to be favourable (Graph 8).

The effect on the haemoglobin levels of transfusing the infant with father's blood is shown in Graph 5.

In all cases the transfusions were of the ordinary simple direct variety and the route was via the anterior fontanelle or via the saphenous vein at the ankle.

(1V) Exchange-Replacement Transfusion :

The technique of exchange-replacement transfusion, or exsanguination transfusion as it is sometimes/

sometimes called, was first used in the treatment of haemolytic disease by Hart (1925). This worker treated a child who was the 8th offspring of a mother who had had seven previous children affected with icterus gravis. The 8th child developed jaundice on the second day after birth. Hart removed 300 cc. of blood from the anterior fontanelle and at the same time introduced 335 cc. of blood from a healthy male donor into the saphenous vein at the left ankle. The jaundice faded by the 4th day and the child made an almost uninterrupted recovery. The patient is now alive and well.

The use of this technique was not widely known and the method fell into abeyance until it was reintroduced by Wallerstein (1946) who transfused patients with Rh negative blood by this means. In a more detailed report, Wallerstein (1947) described the technique in a number of successful cases. He believed that such a mode of therapy would remove the end products of haemolysis or prevent the formation of the latter in excessive amounts. Thus it was hoped to avoid the continual assault on the liver of such noxious substances. Wallerstein based his premises on the theory of the pathogenesis of haemolytic disease evolved by Davidsohn (1945) (vide Chapter 8). He was able to show that "if a continuous transfusion and withdrawal are carried out for sixty minutes and if the baby's blood volume is approximately 250 cc. only 36.7% of the original blood will remain. " This was shown experimentally by/

by the dilution of 250 cc. N. H Cl to 0.0367 N. H Cl by this method in vitro.

In actual practice this author removed 250 cc. of blood from the anterior fontanelle and substituted simultaneously 350 cc. Rh negative blood via a cannula in a superficial arm vein. By this technique only 20 - 25 % of the original Rh positive blood remained in the infant. The blood was removed and Rh negative blood replaced in five exchanges of 50 cc. each. Finally a further 100 cc. of Rh negative blood was administered. The procedure occupied 1- 1½ hours. Polayes (1947) used the radial artery for exsanguination instead of the fontanelle and this technique was followed by Wallerstein (1947) in his later cases. This form of therapy was used by the latter in 7 infants who were severely affected by haemolytic disease. Recovery took place in all 7 cases. Two further cases were treated but both died. Kernicterus was found at autopsy. The author believed that these later cases might have been saved had treatment been instituted within the first twenty four hours of life (If the theory of the pathogenesis of kernicterus outlined in Chapter 14 proves to be correct, this opinion of Wallerstein will be confirmed). He stressed the need for the early institution of therapy, preferably immediately after birth.

Diamond (1947) has introduced a technique whereby removal and replacement is carried out via a plastic catheter in the umbilical vein. By this method/

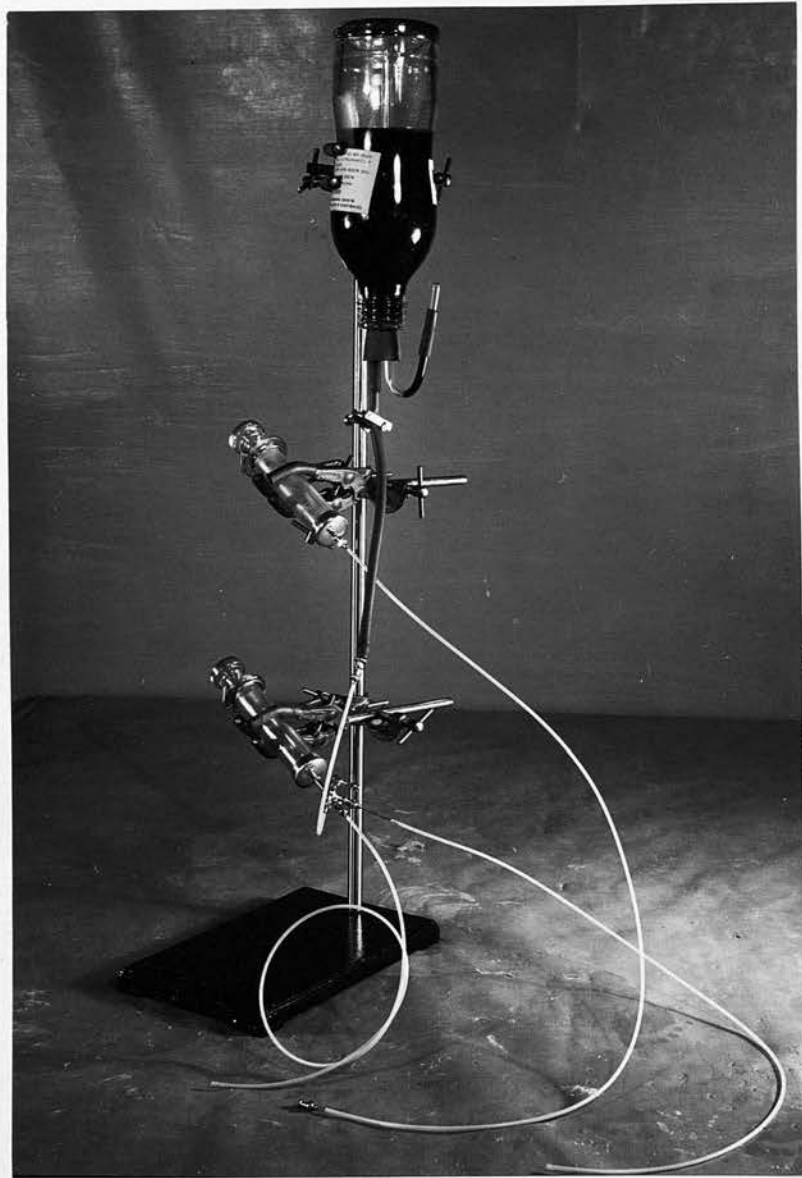


Fig. 53. Apparatus for Exchange-Replacement Transfusion.

(by kind permission of Dr. I.C. Lewis).

method some 450 cc. of the infant's blood is withdrawn and 500 cc. of Rh-negative blood is replaced over a period of $1\frac{1}{2}$ hours. This turnover of blood effects a 90% substitution. A full description of the technique involved, with some modifications, is given by Mollison (1948).

This technique was not performed upon patients in this series but is now undergoing a period of trial in the Simpson Maternity Pavilion under the care of Dr. I. C. Lewis. An illustration of the apparatus used is seen opposite (Fig. 53). The lower 3-way syringe in the photograph is used for replacement transfusion. The upper syringe contains heparin (1/100 saline solution) which is used to flush the transfusion apparatus, and prevent coagulation (Wiener and Wexler 1946). The catheter (made of a plastic known as polyethylene) is inserted into the umbilical vein and the technique is similar to that of Mollison (1948).

Wiener et al (1948) have stressed the danger of air embolism but the use of transparent plastic catheters ensures that such a complication will be easily detected and the air can be sucked back into the syringe.

More recently Arnold and Alford (1948) have modified the technique of this form of therapy by introducing the plastic catheter into the inferior vena cava via the great saphenous vein instead of via the umbilical vein. They point out the latter undergoes thrombosis early and the route is no longer/

longer practicable after the first twelve hours of life.

Lewis (1949) informs me that although this latter method would seem to be more formidable, it is actually easier to use than the umbilical route as the introduction of a catheter into the inferior vena cava via the umbilical vein is not always such a simple procedure as ~~as~~ Diamond (1947) would suggest.

Furthermore, I have examined the position of a plastic catheter at post-mortem in a number of still-births. The catheter had been introduced into the vena cava via the umbilical vein. In one instance the tip of the catheter had passed through the foramen ovale into the left auricle. It is thus obvious that the catheter should not be introduced too far and this danger is less likely to occur if the saphenous route is used. Direct overloading of the heart could occur if the umbilical route were used and the catheter introduced into the heart itself.

Mollison (1948) summarises the advantages of exchange-transfusion as follows :-

" in (1) Removing most of the Rh-positive erythrocytes from the circulation and thus saving the infant's organs from being overloaded with breakdown products;

(2) enabling a far more extensive replacement of Rh-negative erythrocytes to be affected in a short time ; and

(3) removing a certain amount of free Rh antibody. "

The/

The final assessment of the merits of exchange-transfusion therapy will have to await further study. It is a possibility that its prompt use may prevent kernicterus and promote recovery in live-born patients suffering from the hydropic type of haemolytic disease. A period of further trial is necessary before its value in these cases can be assessed.

c) Haemolytic Anaemia.

This is the mildest form of haemolytic disease and many cases will not be sufficiently severe as to require transfusion therapy.

If blood transfusion is required, Rh negative blood should be used and simple direct transfusion technique may be employed. The amount of blood required and whether this should be repeated will depend on the clinical findings in the individual case. Even the most severe cases generally respond well to transfusion therapy but blood regeneration is slow (Parsons, 1947) and more than one transfusion may be required.

3. Other Forms of Therapy :

(1) Breast Feeding :

Hoffman and Hausmann (1926) noted that infants with icterus gravis showed an exacerbation of symptoms when suckled by their mother's but not if suckled by other women who have no infants so affected. They attributed this phenomenon to a toxin in the mother's milk. It is, of course, now established that the mother's /

mother's milk contains antibodies. Wallerstein (1947) and other writers have suggested that infants suffering from haemolytic disease should not be breast fed to avoid transference of maternal agglutinins in the breast milk. Parsons (1947) has discounted the possible danger from this source and believes that the disadvantages of bottle-feeding far outweigh the doubtful advantages derived from the removal of a possible source of fresh antibodies. More recently Cathie (1947) has shown that Rh positive infants who received high-titre anti-Rh serum by mouth failed to show any antibody-absorption when an anti-globulin test was performed. On the other hand Bessis (1947) has shown that rats can develop a fatal haemolytic anaemia when given antibody by mouth. Further experimental work is required before all the facts can become known. In the present state of knowledge regarding this subject it seems desirable to allow breast feeding. If any anxiety is felt the breast milk may be boiled before feeding (Parsons, 1947).

(11) Supplementary Nutritional Factors :

Many authors have stressed the part played by liver dysfunction in severe haemolytic disease.

Jacobi et al (1946) have noted pronounced oedema in some cases of icterus gravis and have correlated this feature with low plasma albumin values in the blood. For this reason they recommend the use of human serum albumin by the intravenous/

intravenous route to counteract the plasma protein loss.

Danis and Anderson (1942) recommend the oral administration of choline chloride (0.3 Gm. daily) as an adjuvant to transfusion therapy.

The haemorrhagic manifestations of icterus gravis have also been noted. Mayman (1940) and Leonard (1945) strongly advise that intramuscular injections of Vitamin K be given until the prothrombin index returns to normal. This is probably the most valuable accessory to transfusion therapy.

Burnham (1944) believes that Vitamin C administration should be carried out during the neonatal period.

If artificial feeding has replaced breast feeding Leonard (1945) recommends a high carbohydrate, high protein, low fat diet based on powdered half-skimmed milk with glucose added.

Liver extract has little effect on blood regeneration. This is not surprising as there is no maturation defect in the development of the erythrocyte.

With regard to the therapeutic use of iron, Henderson (1938) correctly points out that there is an abundance of iron in the initial stages of the disease in the widespread tissue deposits of haemosiderin. It is in the later stages when recovery is taking place, especially if the latter is protracted, as it may be in haemolytic anaemia, that iron will be most useful. This is well illustrated/

Chapter 12.Prognosis in Congenital Haemolytic Disease.

There are, in cases of haemolytic disease, two subjects whose further progress is worthy of close consideration. The mother is suffering from a state of sensitisation to a blood antigen she herself does not possess and any advice regarding future pregnancies requires careful thought on the part of the clinician.

The child is suffering from the effects of an antibody reaction on its erythrocytes and a careful study of data obtained from clinical examination and laboratory investigations will be required before weighing the chances of its recovery.

A. Further outlook in the case of the Mother :

Potter (1948) and Mollison (1948) and many others have stated that haemolytic disease is rare in the first pregnancy and when it occurs is likely to be mild in character, provided the mother has not been transfused previously with Rh incompatible blood. In the present series this was not so clearly indicated. Thirteen women were for the first time pregnant. Three of the offspring were still born and a further 6 died of severe icterus gravis. Only 4 recovered. It is reasonable to suppose that women who deliver offspring suffering from haemolytic disease in their first pregnancy are unduly susceptible to sensitisation by a foreign blood antigen and/

and thus the affect on the foetus is likely to be severe.

Potter (1948) has also remarked that the offspring of early pregnancies exhibit a milder form of the disease than those born in later pregnancies. This she attributes to the fact that the antibodies take some time to develop in the maternal blood and are likely to be more potent following the repeated antigen stimulus from several pregnancies. It should be pointed out, however, that the maximum incidence of haemolytic disease occurs in the offspring of the 2nd and 3rd. pregnancies (i.e. " early pregnancies") and so the maximum number of recoveries will also be found in this period. One fact does appear from a consideration of the cases in this series. Still births showing hydrops foetalis are definitely commoner in later pregnancies. This is also borne out by the findings of Potter (1948).

It is only natural that a mother, who has borne one child exhibiting haemolytic disease, will wish to know what the chances are of her having a normal infant in a subsequent pregnancy. This is not a simple question to answer, since a number of factors are involved.

Firstly, if her husband is homozygous Rh positive (DD) all the offspring will be Rh positive and liable to suffer from haemolytic disease. On the other hand if the father is heterozygous Rh positive (Dd) the children of subsequent matings will/

will be either Rh positive or Rh negative and these variables will occur with almost equal frequency. Thus there is an even chance of future children escaping haemolytic disease.

Secondly, cases are known in which a mother of an infant showing haemolytic disease has given birth subsequently to a normal child, although her husband was homozygous Rh positive. Furthermore, mothers who have been delivered of still-born hydropic infants may occasionally give birth to a child with only mild icterus in a later pregnancy.

Thirdly, genotyping is by no means 100% accurate owing to a lack of the rarer sera, and mistakes in typing the father's blood may not be uncommon.

Thus it is obvious that no hard and fast rule can be laid down with regard to the outcome of future pregnancies. It can be said, however, that a few probabilities are of some help in judging the prognosis.

For instance, it is highly probable that a mother who has been delivered of a number of consecutive still-born hydropic foetuses will continue to produce such infants in subsequent pregnancies. One mother in this series (Case 41, Series I) was delivered of a still-born hydropic foetus in her 3rd. pregnancy. A similar issue resulted from the 4th, 5th, 6th, 7th, 8th, 9th and 10th pregnancies. The outlook in such patients is very grave and a therapeutic abortion should be considered/

considered in future pregnancies. Such a mother should be advised against further pregnancies.

In contrast to these patients, it is very probable that women who have had one child mildly affected with haemolytic disease will produce future infants who are also only mildly affected. Thus a mother who has had an infant suffering from mild haemolytic anaemia may have others in future pregnancies who are also mildly affected. The same process occurs in cases of mild icterus.

It is true that there is a general trend for subsequent children to be more severely affected than their earlier siblings, but exceptions to this general rule may be found.

With regard to toxæmia of pregnancy in mothers carrying children likely to be affected by haemolytic disease Javert (1942) has stated that the incidence of such toxæmia is higher in mothers of hydropic infants than in unselected cases. This was not noticed in the present series, where the great majority of pregnancies were free from complications.

One important danger to a mother of an infant suffering from haemolytic disease must be mentioned. That is the treatment of any severe post-partum or other haemorrhage by blood transfusion. It is now a common procedure to treat a severe haemorrhage following the birth of a child or a miscarriage by blood transfusion. If an Rh negative woman, who is sensitised to Rh antigen, is transfused with blood which/

which is not Rh negative, the results may well be disastrous. At the best a severe transfusion reaction will result and at worst death from renal failure.

B. Future Outlook in the Affected Offspring :

Even with all the benefits accruing from modern methods of diagnosis and treatment, the mortality of haemolytic disease of the newborn is considerable. It has been pointed out that the mortality rate in the present series is rather high and a true picture cannot be obtained from these figures (Chapter 5). Nevertheless, Mollison and Cutbush (1949) had 5 still-births and 19 deaths in their series of 74 cases which were most carefully studied. Thus a mortality of about 30% is to be expected in this disease even under the most favourable conditions.

The prognosis naturally varies according to the type of disease exhibited.

1. Hydrops Foetalis :

The outlook in this type is grave. The majority are still born and the life span of those who survive can be measured in hours. It is possible that the termination of pregnancy a few weeks before term and the prompt treatment of the affected infant by exchange-replacement transfusion will reduce the mortality, but such methods of therapy require a further period of trial.

2. Icterus Gravis Neonatorum :

With the advent of new methods of transfusion therapy and the prompt application of these, the outlook/

outlook in this, the commonest form of the disease, has been greatly improved.

As was noted in an earlier chapter (Chapter 9) the degree of severity in individual cases and the nature of the outcome may be judged from a number of observations.

In general, a very severe degree of jaundice at birth, a low haemoglobin value in the cord blood (under 50%) and a high serum bilirubin are signs indicating a poor chance of recovery. In the present series of cases a high degree of stimulation of the erythropoietic foci throughout the body and large numbers of nucleated cells in the peripheral blood were found in most patients who failed to survive.

Conversely, a delay in the onset of jaundice and a mild form of the latter with a high haemoglobin value in the cord blood (100% or over) are favourable signs.

Exceptions do occur, but the observations cited above are of considerable value.

It has also been stated by Wiener (1946) and others that the presence of albumin antibodies in a high titre in the maternal blood indicate that the foetus will be affected severely. This observation is of some value but no close correlation between the antibody titre and the severity of the disease has been shown to exist in all cases.

With regard to kernicterus, Capell (1947) states that it can be expected in 40% of patients with icterus gravis in the neonatal period and in 12%/

12% of those who recover from the initial acute stage. Thus the mortality is very high among the patients who develop this complication. The subject is discussed in some detail in a later chapter (Chapter 14).

3. Congenital Haemolytic Anaemia :

This, the mildest type of the disease, has a favourable prognosis. The patients reported in this series were very severely affected and would probably not have been admitted to hospital had this been otherwise. Thus they do not give a fair indication of what may be expected in this form of the disease.

The course in haemolytic anaemia may be very protracted and some of the more severely affected patients show mild jaundice in the early stages. A good reticulocyte response is one of the more reliable guides to the efficacy of treatment.

In conclusion, it must be emphasised that each case requires careful study and no set of rules can be devised to meet all contingencies.

Chapter 13.Sequelae.

In any disease affecting infants in the neonatal period, intercurrent infection is liable to occur as a complication, and in haemolytic disease the outcome is likely to be unfavourable if the former develops.

Ten cases of icterus gravis in the present series were complicated by an acute infective process and in all 10 instances the patients died. Two suffered from acute bronchopneumonia, two from acute leptomeningitis, one from acute peritonitis, one from acute pyelonephritis and one from pyaemia following the development of an abscess in the buttock at the site of therapeutic injections.

In addition there were 3 patients suffering from gastro-enteritis. This latter complication has been stressed by Darrow (1938) and Leonard (1945) but it is liable to occur in any child at this age period.

Apart from intercurrent infection, there are certain events which occur with special frequency in patients suffering from icterus gravis. These are kernicterus, haemorrhage into the lungs, alimentary tract and brain or from the umbilicus, and the development of hepatic cirrhosis in later infancy.

Kernicterus is probably the only feature which is/

is pathognomonic of congenital haemolytic disease. It may occur in a proportion of the more severely affected infants (Chapter 14).

The so-called " haemorrhagic diathesis " has received considerable attention by many writers. Leonard (1945) and Darrow and Chapin (1947) have stressed this feature which is exhibited by some patients suffering from icterus gravis.

It would seem that far too much emphasis has been placed on the occurrence of haemorrhage as a complication of icterus gravis. It was anything but an outstanding feature among the patients in this series and some very doubtful conclusions have been drawn from the occurrence of this particular complication (viz. Darrow and Chapin 1947).

Intrapulmonary haemorrhage is found in a variety of conditions in the newborn. It may be very severe in neonatal asphyxia, bronchopneumonia, congenital heart disease and inhalation pneumonia to mention only a few examples. In these conditions it is equally extensive as in icterus gravis and presents the same characteristics.

Intracranial haemorrhage was no more frequent than is generally found in this age period. In only two patients was subdural haemorrhage sufficiently severe to affect the outcome. In one patient with hydrocephalus foetalis the brain had been severely damaged as a result of venous thrombosis.

No instance of severe bleeding from the umbilicus or into the alimentary tract was reported.

When/

When cerebral haemorrhage occurs in patients with icterus gravis it may be very difficult to determine whether the neurological signs are the sequel of vascular damage or whether kernicterus has developed. In both there may be evidence of mental defect, muscular hypotonia and delay in walking. Choreo-athetosis and other evidence of striatal involvement is less common after cerebral haemorrhage than after the onset of kernicterus.

The enlarged spleen which is such a characteristic feature of haemolytic disease is liable to rupture. This is not a common complication but occurred in two cases in this series. In one instance the rupture was very large and a considerable intraperitoneal haemorrhage resulted. This was not an effect of the "haemorrhagic diathesis." In the other instance only a superficial rupture had occurred and was found post-mortem. Little haemorrhage had ensued.

It has been shown in a previous chapter that cirrhosis of the liver is not such a constant finding in recovered cases of icterus gravis as Hawksley and Lightwood (1934) would suggest. It is doubtful if a true "cirrhosis" occurs as a sequel to icterus gravis. A fine pericellular fibrosis or an increase in the periportal connective tissue does occur in a proportion of recovered cases. To what extent this increase in fibrous tissue interferes with liver function has not been established but if the examples presented in this series are any criteria, the/

The disturbance is not likely to be very severe.

Parsons, Hawksely and Gittens (1933) have discussed the anaemias of infancy and childhood in some detail. They suggest that congenital haemolytic anaemia may merge into a subchronic haemolytic anaemia in which recovery is considerably delayed. This does not seem to be a very frequent complication as the majority of patients with haemolytic anaemia recover within 1- 2 months.

Chapter 14.Kernicterus. (Nuclear Jaundice).

The first description of this unfortunate sequel to congenital hæmolytic disease was that of Orth (1875) who used the term nuclear jaundice. Later Schmorl (1903) introduced the word "kernikterus" in his description of the condition. He gave the first detailed report of the morbid anatomical and histological features of the cerebral lesions in 6 cases.

It soon became obvious that kernicterus was found in close association with icterus gravis neonatorum. Beneke (1904) reported jaundice in premature twins, both of whom later died. He performed a post-mortem on one of them and found nuclear jaundice. The mother had had a severe fright before term and the twins were born prematurely. The author ascribed the cerebral lesions to ischaemia following shock with resultant neuronal necrosis.

Pfannenstiel (1908) described cases of neonatal icterus occurring in one family. The first child showed severe jaundice after birth but survived. The second child developed severe jaundice and later showed clinical evidence of kernicterus and died on the fourth day. The third died on the 6th day after 4 days of jaundice and the brain showed kernicterus at post-mortem examination. The 4th. child/

child died of severe jaundice but there was no kernicterus.

Esch (1908) reported one case of kernicterus in a jaundiced child who died on the 5th day after birth. Pfaltzer (1914) described a case of a child who developed jaundice on the second day of life and at the same time became feverish (Temp. 103°F). It died the following day. The cerebral ganglia were bile-stained when examined post-mortem, but the liver was said to have been normal.

Spiller (1915) reported three cases of mental deficiency in which severe jaundice had occurred in the neonatal period and he considered the jaundice to be of prime importance.

Hart (1917) reported one case where kernicterus was found in a jaundiced infant at post-mortem.

Ylppo (1918) described a family whose mother had lost five out of eight children from icterus gravis. One of the 5 children at post-mortem examination showed kernicterus and atresia of the cystic duct and of one of the hepatic ducts. Palm (1919) had a patient who developed severe jaundice shortly after birth and died on the third day. Severe kernicterus was found at necropsy. Thorling (1922) also reported on a family showing repeated incidence of icterus gravis. The first two children were normal. The third had severe jaundice and showed clinical evidence of kernicterus. It did not survive but no post-mortem examination was carried out. The fourth child was jaundiced at birth and died on the 4th day.

Kernicterus/

Kernicterus was found post-mortem. The 5th pregnancy ended in the delivery of a still-born macerated foetus. The 6th. child died on the 11th day after severe jaundice. Kernicterus was also found post-mortem.

Paul (1924) had a patient showing clinical evidence of kernicterus following on severe neonatal jaundice. De Lange (1925) reported a case which she ascribed to kernicterus. The child had suffered from severe jaundice commencing 48 hours after birth and there were clinical signs of damage to the extrapyramidal centres. Helgenberg (1925) reported fourteen fatal cases of icterus gravis. Three showed kernicterus at necropsy.

Hoffmann and Hausmann (1926) reported two cases showing clinical evidence of kernicterus following severe neonatal jaundice. Greenwald and Messer (1927) reported one case showing clinical evidence of kernicterus. Huwer (1928) reported the necropsy findings of one set of post-mature twins who had died after severe jaundice on the second day of extra-uterine life. Both showed kernicterus. Zimmerman and Yannet (1933) reported four cases of kernicterus. Two of these patients had died and the necropsy findings were described in detail. Two at this time were alive but had definite clinical signs of kernicterus. In 1935 the same authors described the necropsy findings in Case 4 of their original series, the patient having died in the interval at the age of three years.

Biemond and van Grevelde (1937) reported two cases/

cases of kernicterus. These authors found a lack of normal cerebral development in their cases and suggested this was of considerable etiological importance. Westrienen and de Lange (1937) had one case which showed neurological evidence of kernicterus.

Fitzgerald, Greenfield and Kounine (1939) described the pathological findings in two cases of kernicterus. Their patients had died at the age of $4\frac{1}{2}$ months and 1 year respectively.

Up to this date the association of kernicterus with icterus gravis neonatorum and erythroblastosis had been generally recognised although some authors such as de Lange (1935) and van Creveld (1937) stressed the importance of infection in the etiology. With the discovery of the Rhesus blood groups a new approach to the problem was instituted. Leonard (1945), Docter (1945), Wiener and Brody (1946), Vaughan (1946) have all stressed this newer aspect of the problem. Docter (1945) reported on five patients with neurological signs of kernicterus. Vaughan (1946) reviewed the incidence of erythroblastosis in his hospital over a period of seventeen years. He reported twenty cases of kernicterus, sixteen of his patients had died and four were still living with clinical evidence of neurological involvement. More recently, Stiller (1947) described four cases of patients exhibiting kernicterus with marked neurological involvement. Lande (1948), after a brief review of the literature, described eight cases/

cases of clinical kernicterus and stresses the widespread nature of the condition in the nervous system. The oldest of her series was $11\frac{1}{2}$ years of age.

Kernicterus has thus not been neglected in the literature during the last fifty years but many problems concerning it have remained unanswered. Various theories concerning the etiology and pathogenesis of kernicterus have been formulated and the more important of these will be discussed later.

1. Clinical Features of Kernicterus.

The clinical features of a disease are hardly within the province of a pathologist but a brief summary is necessary in view of what follows. The condition may be divided into two stages :- a) the acute stage which is most frequently seen and occurs during the height of the jaundice in the early neonatal period and b) the chronic stage which follows on from the acute stage in the few patients who survive beyond the first week of extra-uterine life. The clinical features are described in some detail by Zimmermann and Yannet (1933), by Fitzgerald et al (1939) and by Lande (1948).

a) Acute Stage :

Only six of the thirty-seven patients in the present series survived beyond this stage. The remainder died within a week of birth. This stage is that of acute irritation of the cerebral ganglia. It appears during the height of the jaundice. The infants/



Fig. 54. Child during spasm caused by severe cerebral irritation. Case of kernicterus in the acute stage.

infants show evidence of severe cerebral irritation with clonic and tonic movements going on to convulsion, hypertonia and opisthotonos (Fig. 54). In between these spasms of hyperactivity the patients are drowsy and apathetic. They cry a good deal and have difficulty in taking their feeds. The infants become weaker steadily and the majority die before the fifth day of extra-uterine life.

The general clinical signs are those of icterus gravis neonatorum. The jaundice is almost invariably severe, and the majority of patients in this series showed a marked or moderate degree of erythroblastosis. The serum bilirubin is raised and may reach a level of 10 - 20 mgm% or more. The degree of anaemia is variable and as Lande (1948) remarks, there seems at first glance to be no correlation between the degree of anaemia and the incidence of kernicterus. One patient in the present series had a haemoglobin of 25% (Sahli) and a red cell count of 2.0 million/cu.mm whereas another had a haemoglobin of 110%.

Recently Mollison and Cutbush (1949) have shown that the haemoglobin values in the cord blood at birth in patients with severe haemolytic disease is usually below normal. Furthermore, they point out that if the child has received a backflow of blood from the placenta at birth, a haemoglobin of 100% on the third day will indicate anaemia. This interesting work throws considerable light on the development of kernicterus since it indicates that anaemia/

anaemia may well have been present in cases where haemoglobin values were within normal limits.

The maternal blood shows little of diagnostic significance and the Rhesus antibody titre bears no close relationship to the severity of the disease. Wiener and Gordon (1948) stress the importance of a high albumin antibody titre in the maternal serum. Indeed it may be said that there are few clinical features in cases of icterus gravis which would enable us to anticipate the onset of kernicterus.

Treatment seems to have been of little avail up to the moment and there is little to choose between patients who have been transfused and those who have not. Nevertheless some reasons will be put forward later to suggest that in future some amelioration may be expected.

Up to the present time little benefit has accrued from the use of blood transfusions in kernicterus. The mortality rate in this series has remained unchanged. Darrow and Chapin (1947) and Vaughan (1946) prefer to use Rhesus positive blood. This method of treatment is hotly contested by Wiener (1946) and other workers. The method of exsanguination-replacement transfusion of Diamond (1947) and Wallerstein (1947) using Rhesus negative blood has not been studied for a sufficiently long period.

b) Chronic Stage :

After the first week of extra-uterine life, should/

should the infant survive so long, irreversible change occurs in the basal ganglia and other cerebral nuclei. Clinical signs of lesions in the extra-pyramidal system are now obvious. Before this, in the acute stage, the signs were those of cerebral irritation and were not in themselves pathognomonic of kernicterus, although in association with icterus gravis neonatorum they take on an added significance.

Lande (1948) has divided the chronic stage into four clinically distinct groups :-

Group I : Cases exhibiting choreo-athetosis as a result of involvement of the corpus striatum and globus pallidus.

Group II : Cases showing persistent spasticity from the involvement of the pyramidal and extra-pyramidal systems.

Group III : Cases where the predominant features are ataxia and disturbance of balance owing to cerebellar change.

Group IV : Cases in which atonic diplegia due to extrapyramidal lesions predominates.

As Lande (1948) states these groups must not be regarded as watertight compartments and most patients show a combination of physical signs from all groups. In addition, cranial nerve lesions may be present and blindness, strabismus and deafness are not uncommon. This has been noted also by Zimmerman and Yannet (1935).

Mental retardation is a prominent feature among patients in this stage and it is difficult to assess/

assess how much is due to cerebral damage and how much to the physical incapacity which the disease promotes. The patients with severe choreo-athetosis for instance have difficulty in remaining still for an instant.

The further development of patients in the chronic stage is very slow. They may not be able to sit up and hold the head up at one year of age. Walking may be delayed up to six years or more (Lande 1948, Case 1). Feeding themselves is difficult if not impossible and training in toilet habits is prolonged.

Some have a happy disposition, many are nervous and irritable, and the majority show emotional instability. Treatment of patients in this stage of the disease by blood transfusion has no effect, as irremediable damage to the cerebral cortex has already occurred.

Careful training of these patients to use their mind and body as much as their physical disabilities will permit has done much to mitigate the degree of functional incapacity. Docter (1945) states that patients showing severe spasticity in the early weeks tend to improve as they grow older if adequate care is exercised. This view has been supported by Henderson (1948). Some advances from these methods of treatment may be expected.

2. Cases of Kernicterus in the Present Series.

There were 37 patients showing evidence of kernicterus from a total of 110 patients suffering from/

from icterus gravis, an incidence of 33%. Of these 110 jaundiced patients, 47 died within the first week of life. Of these 47 patients 31 were shown to have kernicterus at postmortem examination. Thus 66% of patients with severe icterus who died soon after birth also had kernicterus.

Docter (1945) and Vaughan (1946) both found a high incidence of kernicterus in their patients suffering from icterus gravis.

Thirty-three patients who had suffered from icterus gravis survived beyond the neonatal period. Four of these (12%) exhibited neurological signs of kernicterus. Cappell (1947a) stated that kernicterus may be expected in 40% of cases of icterus gravis in the neonatal period and among 12% of the survivors. His figures and those obtained from this series are very similar.

The total number of cases of haemolytic disease of all types in the present series was 157, and kernicterus was present in 37 instances (23%).

a) Clinical Features of the Thirty-Seven Patients.

Twenty eight of the patients exhibited the form of the disease associated with the acute stage outlined in a previous section. The infants become jaundiced shortly after birth (or were jaundiced at birth) and the jaundice steadily deepened. The liver and spleen were usually palpable. Evidence of cerebral irritation soon became manifest and twitchings of the limbs, convulsions and opisthotonos were/

were noted. Intervals of lethargy and drowsiness were seen and there was difficulty in getting the infants to feed. Kernig's sign was frequently positive. In spite of treatment the children collapsed and died by the end of the first week of extra-uterine life.

Vaughan (1946) stresses the importance of severe respiratory distress resulting from intrapulmonary haemorrhage, as a factor in the sudden collapse. This has not been reported in the case records in this series and only two patients showed the lesion post-mortem.

Four infants died in the second week, two at nine days and two at twelve days after birth. One became lethargic on the 4th day and then showed evidence of spasticity before dying on the 9th day. One of the second group, dying at twelve days, showed clear signs of cerebral involvement. One other infant died on the 14th day.

Only 4 infants survived beyond the acute or chronic early/stage. One (Case 62, Series I) apparently improved considerably but was left with a marked athetosis of the left arm. There is some doubt as to whether this was owing to kernicterus or was the result of a transfusion leak from the anterior fontanelle. There was reason, however, to believe that it was a true example of kernicterus. Another child (Case 75, Series I) is still alive at the time of writing. He has learned to walk, although with some difficulty, and there is as yet no evidence of/

of mental impairment. The chief neurological feature is hypotonia rather than spasticity.

The other 2 survivors (Case 17, Series II and Case 48, Series II) died at 4 months and 17 months respectively. The first child (Case 17) died as a result of acute bronchitis at the age of 4 months. This child developed jaundice 24 hours after birth and exhibited head retraction and opisthotonos on the 4th day. The jaundice disappeared after six weeks but evidence of cerebral involvement persisted. She had severe spasms of the limbs, and the arms and legs were spastic. The reflexes were absent and Kernig's sign was present. This is a typical example of ^{the} chronic stage of the condition. The other child (Case 48) died at the age of 17 months and no satisfactory cause of death was found at post-mortem examination.

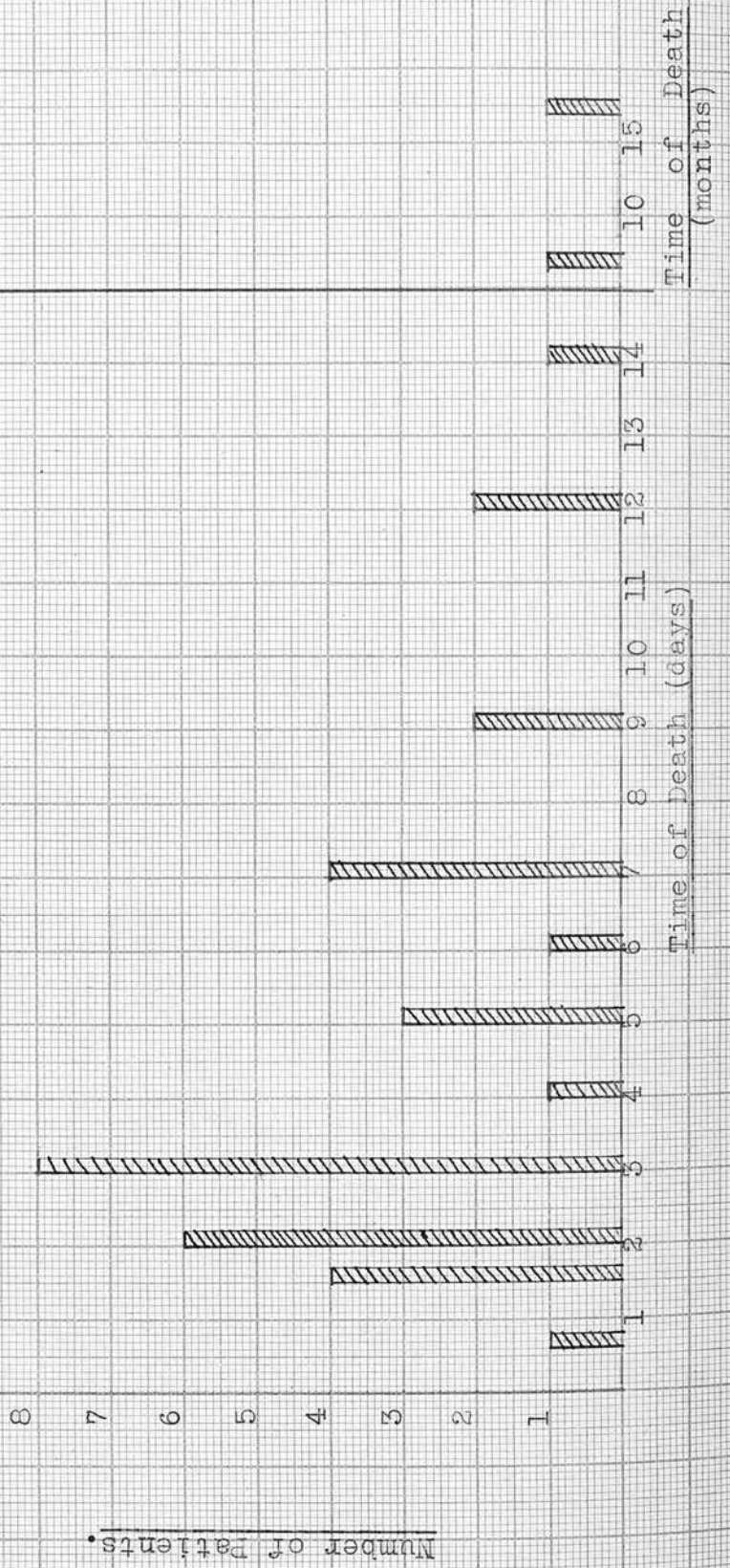
In no instance did any general sign or symptom differentiate these patients from others of severe icterus gravis before the onset of kernicterus. The jaundice was severe in 33 of the patients. Vaughan (1946) found no correlation between the severity of jaundice and the onset of kernicterus. The converse was true in this series. This is not surprising as a high blood bilirubin is necessary to stain the cerebral nuclei as severely as is generally found.

b) Time of Onset of Jaundice.

As will be seen from the table, jaundice is present in the great majority of cases by the end of/

GRAPH 15 -

Time of Death in 35 Fatal Cases of Kernicterus



of the first day, and was present at birth in 14 out of 37 patients.

Table 46.

Time of Onset of Jaundice.	No. of patients.
Present at birth	14
" within 6 hrs. after birth.	2
" " 12 " "	5
" " 24 " "	13
" " 48 " "	3
Longer	0
Total	37

c) Rhesus Blood Groups :

Serological investigations were carried out in 15 out of the 37 patients. The usual pattern of Rhesus negative mother and Rhesus positive father and child occurred in 13 of the 15 cases. Five of the 13 Rh negative mothers had antibodies in their serum. It is almost certain that the other 2 mothers tested were in reality Rh negative and an error was made in the serological reports

d) Age at Death in Fatal Cases : (Graph 15)

The age of the infant when death supervened in the thirty-five fatal cases is shown in the following table. It will be noted that the great majority succumb by the end of the first week of extra-uterine life. Of these the greater number die within the first 72 hours.

Table 47/

Table 47.

Time of Death.	No. of patients.
18 hours after birth	1
36 " " "	4
2 days " "	6
3 " " "	8
4 " " "	1
5 " " "	3
6 " " "	1
7 " " "	4
9 " " "	2
12 " " "	2
14 " " "	1
4 months " "	1
17 " " "	1
Total	35

e) Cause of Death in the Fatal Cases :

Most of the patients died from the systemic upset associated with icterus gravis and aggravated by the kernicterus. Complications such as intrapulmonary haemorrhage, pneumonia and meningitis may also occur and a summary of the causes of death is contained in the following table (Table 48). Hawksley and Lightwood (1934) and Mollison and Cutbush (1949) are inclined to believe that medullary failure plays a part in the early fatal outcome.

Table 48.

Cause of Death.	No. of Cases.
General effects of Icterus gravis	23
Pneumonia	2
Acute suppurative bronchitis	1
Acute Depto-meningitis	1
Intrapulmonary Haemorrhage	2
Subarachnoid Haemorrhage	2
Intraventricular Haemorrhage	3
Late effects of Kernicterus	1
Total	35

f)/

f) Treatment of Patients with Kernicterus :

Vaughan (1946) remarks that the only benefit to be derived from blood transfusion in these patients is that resulting from the replacement of cells in those who are severely anaemia. He stated that severe anaemia was not invariably present and that such therapy had disappointing results.

In the present series two patients survive at the time of writing. Both were treated with blood transfusions. One (Case 65) received 265 cc. Rh negative blood and the other (Case 75, Series I) was treated with blood of unknown Rh group. One other patient received a transfusion of blood whose Rh group was unspecified ; the patient died. Five patients received transfusions of Rh-negative blood but all died. The remaining twenty-nine patients received no blood transfusion and all died.

It is known that some degree of anaemia is present in the majority of patients with severe icterus (Mollison and Cutbush, 1949). The rationale of blood transfusion in kernicterus is thus more clearly understood. Vaughan (1946) found that no case of kernicterus occurred when children were transfused on the first day of life with Rhesus positive blood. The majority of workers do not advocate the use of Rhesus-positive blood except in special cases. This question is dealt with elsewhere (Chapter 11). The importance of transfusing patients early is dealt with later in this chapter.

3. Morbid Anatomy of Kernicterus.

The morbid anatomy of what may be termed the chronic stage of kernicterus has been fully described in the papers of Zimmerman and Yannet (1933) and Fitzgerald et al (1939). The anatomical features of the acute stage has been less fully investigated, although the general features are well known to most writers.

In this series the anatomical findings in the 35 fatal cases will be described.

a) Sex :

Of the 35 patients 23 were males and 12 females. This preponderance of males is not reflected in the cases of congenital haemolytic disease taken as a whole. Litchfield (1945) however, has suggested that the disease is commoner in the male. Potter (1948) is doubtful if any valid statistical difference is present.

b) Maturity :

Twenty-five were full-term infants and only ten were premature. This finding is against the view of Vaughan (1946) who thought premature infants to be more liable to develop the disease. He did not stress the point, however.

c) Occurrence in Twins :

Kernicterus was present in two sets of twins (Cases 26a and b and 29 a and b, Series I). It was also found in one of two other sets of twins (Cases 4 and 39, Series I) the second twin being normal in both instances. These twins account for 5 out of/

of the 11 premature infants.

d) General Appearances.

The bodies are those of very jaundiced infants and a few subcutaneous petechiae may be present. One infant (Case 14, Series III) was also very oedematous. There is no difference on external examination between patients with kernicterus and those of icterus gravis neonatorum without kernicterus.

e) Organs.

(i) Liver : The liver is usually moderately enlarged and brownish red in colour. The Prussian blue reaction may be positive. There is seldom any sign of liver damage on macroscopic examination.

(ii) Spleen : The spleen was very enlarged in 26 cases and moderately enlarged in a further 6. This does not correspond to the findings of Vaughan (1946). The spleen is dark red in colour and the Prussian blue reaction is often positive.

(iii) Kidney : The organ is usually bile-stained and the Prussian blue reaction is occasionally positive.

(iv) Lungs : Intrapulmonary haemorrhages may be very severe. This only occurred in two cases, but Vaughan (1946) stresses the importance of this finding.

(v) Other organs : These show little change on macroscopic examination.

It will be seen from this description that no changes occur which would not fit a diagnosis of icterus gravis.

f)/

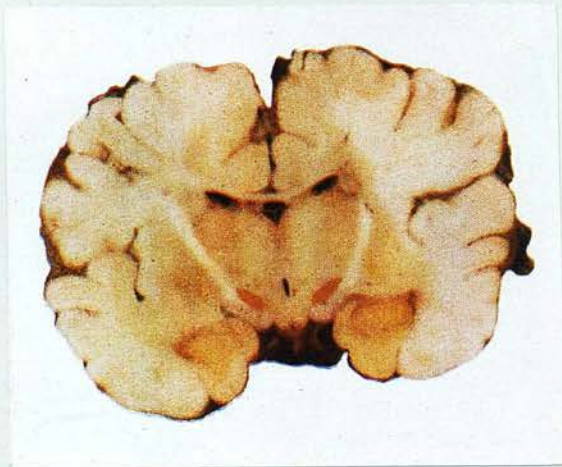


Fig. 62 b. BRAIN. Nuclear staining of
basal ganglia and cornu ammonis.
(By kind permission of Dr. W. Blackwood).

bile-stained. (Case 31, Series I).

(ii) Chronic Stage :

This has been fully described by Fitzgerald et al. (1939) and Zimmermann and Yannet (1935). The latter found there was no deformity of the brain on external examination (Their patient had died at the age of 3 years). The cornu ammonis was small, the substantia nigra was absent and there was some shrinkage of the white matter around the posterior horns of the lateral ventricles. In addition the optic nerves and chiasma were rather soft. There was no bile-staining of any part.

The only patients in the present series coming into this group were the one dying at the age of 4 months (Case 17, Series II), and the one at the age of 17 months (Case 48, Series II). The brains showed no abnormality on external examination. On section the only change was in the cornu ammonis which was rather small. There was no bile-staining of any part.

4. Morbid Histology of Kernicterus.

Organs :

(i) Liver : Damage to the liver parenchyma was only moderate or slight in the majority of cases. It is interesting to note that in the cases showing chronic cerebral damage (Case 17, Series II and Case 48 Series II) no hepatic lesion was present (Fig 57a and 57b). Very numerous foci of extramedullary haemopoiesis of erythroblastic type were found in just over half the cases and fairly numerous foci were/

were found in the remainder (Fig. 55). This is contrary to the findings of Vaughan (1946) who failed to find severe extramedullary haemopoiesis in the majority of his cases.

Many of the inter cellular canaliculi may be plugged with bile and bile pigment is contained in the hepatic cells. The Prussian blue reaction may be positive.

In the older infants there may be slight fatty degeneration and early necrosis of cells in the central zones of the lobules and a slight increase in the periportal fibrous tissue. There was no cirrhosis in any case. Erythropoiesis is not a feature of patients dying after the first week (Fig. 56).

(ii) Spleen : Numerous foci of extramedullary haemopoiesis are usually present, except in the chronic cases (Fig. 58). Numerous histiocytes in the pulp and the subcapsular region contain haemosiderin and the Prussian blue reaction was frequently positive (Fig. 59). No fibrosis had occurred.

(iii) Kidney : The cells lining the convoluted tubules may contain bile-pigment or haemosiderin. In the latter instance the Prussian blue reaction will be positive. A few cases show extramedullary erythropoiesis in the boundary zone of the cortex (Fig. 60).

(iv) Lungs : Intrapulmonary haemorrhage was severe in two cases and very slight in a third. The interstitial tissue and alveoli are flooded with blood/

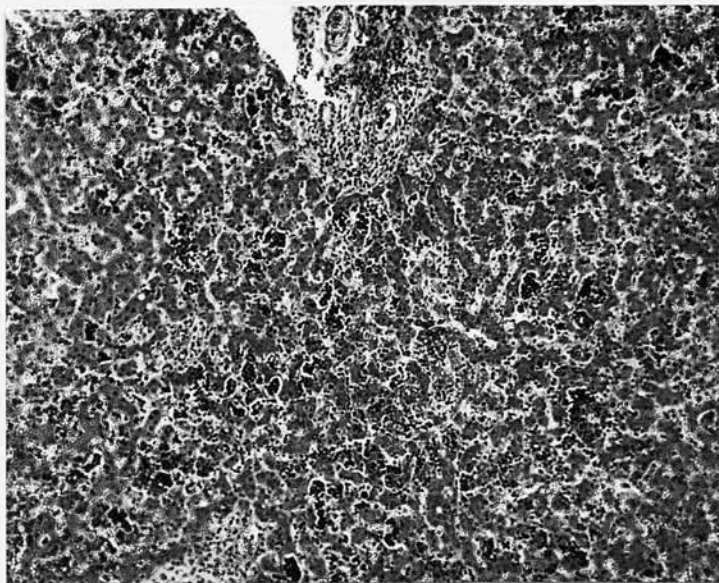


Fig. 55. LIVER x 80. Haematoxylin and Eosin.
Numerous foci of extramedullary erythropoiesis.
Case of icterus gravis showing kernicterus.
Died at the acute stage.

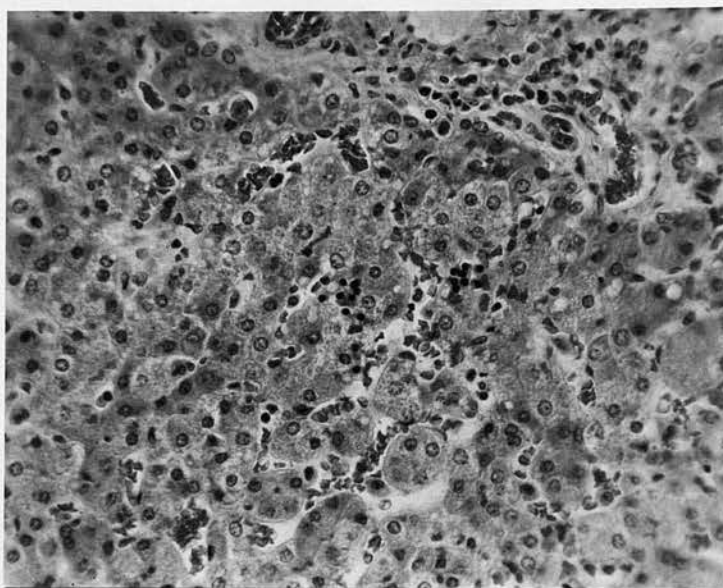


Fig. 56. LIVER x 240. Haematoxylin and Eosin.
Only a few foci of normoblasts are present.
Case of icterus gravis showing kernicterus.
Died in the early chronic stage.

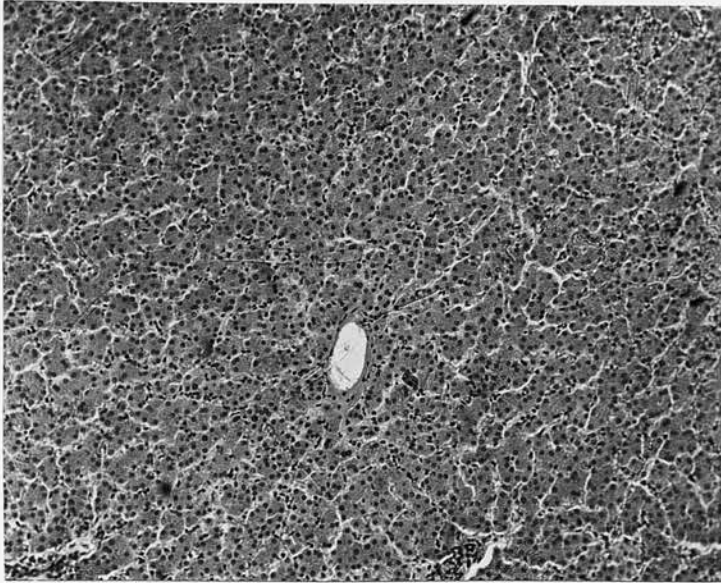


Fig. 57 a. LIVER x 80. Haematoxylin and Eosin.
No pathological changes seen. Case of
icterus gravis with kernicterus. Died in
chronic stage.

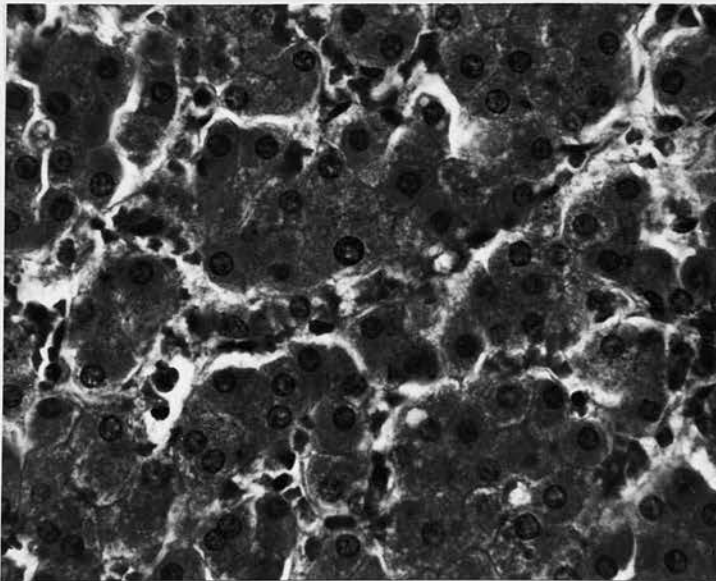


Fig. 57 b. LIVER x 400. Haematoxylin and Eosin.
No pathological change. Case of icterus
gravis with kernicterus. Died in chronic stage.

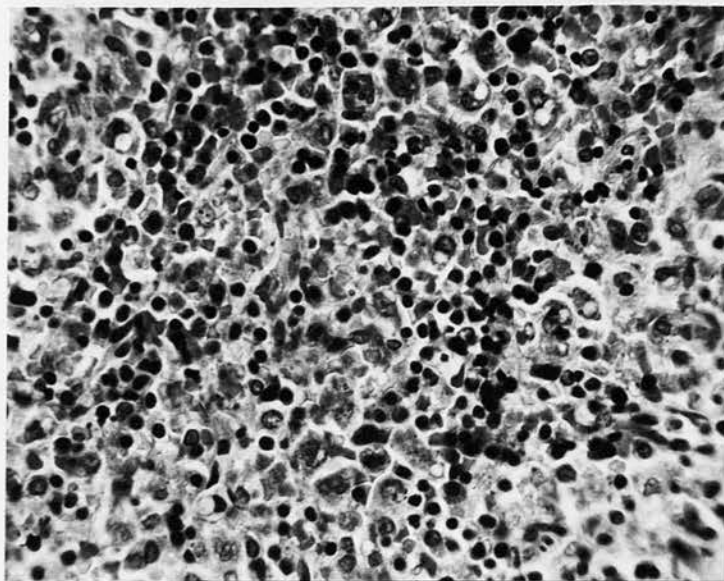


Fig. 58. SPLEEN x 400. Haematoxylin and Eosin.
Numerous foci of erythropoiesis in pulp.
Case of icterus gravis with kernicterus.
Died in acute stage.

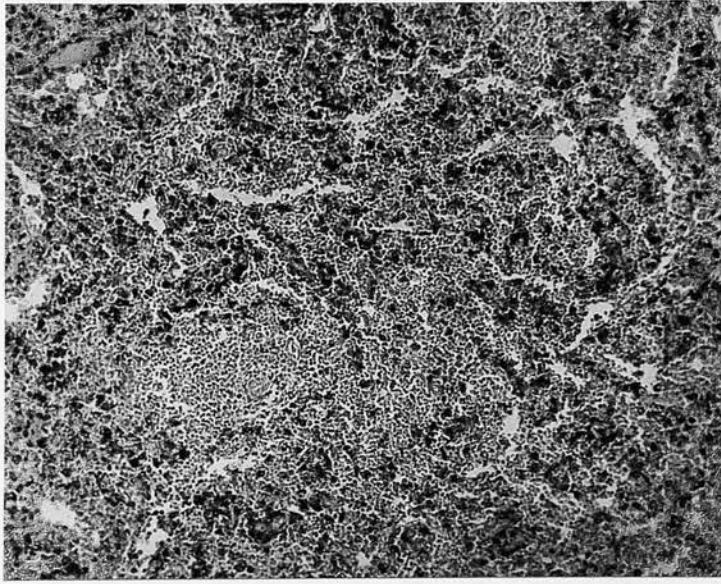


Fig. 59. SPLEEN x 90. Prussian Blue.
Extensive haemosiderosis. Case of icterus
gravis with kernicterus. Acute stage.

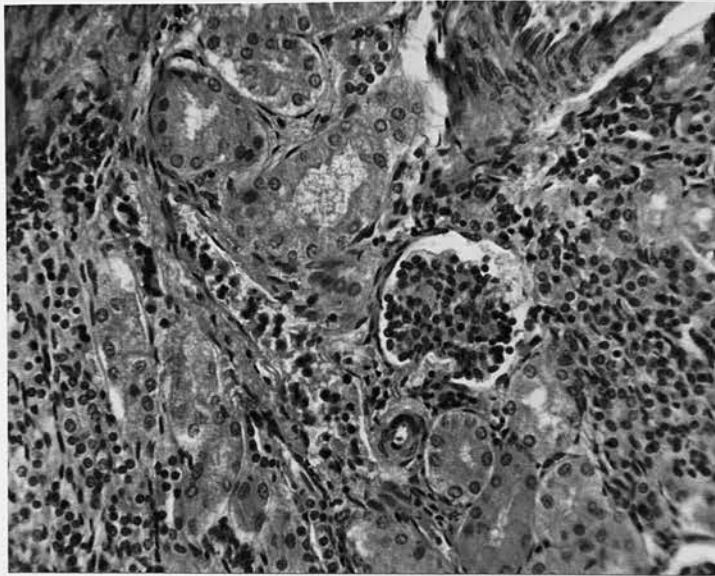


Fig. 60. KIDNEY x 250. Haematoxylin and Eosin.
Foci of haemopoiesis in boundary zone of the
cortex. Case of icterus gravis with
kernicterus. Acute stage.

blood. Pneumonia is liable to occur but, is of course, not a specific sequel to this disease.

(v) Suprarenal Glands (Fig. 61), Thymus Glands and Pancreas may show foci of haemopoietic cells.

In one case a severe acute leptomeningitis and ventriculitis masked the histological findings in the brain.

The histological appearances of the cerebral lesions in kernicterus were described by Fitzgerald et al (1939) and Zimmermann and Yannet (1933 and 1935). These authors, however, were describing the changes in the chronic stage of the lesions. For the convenience of description the histological appearances may be classified as belonging to one of three stages (i) acute stage (ii) early chronic stage, and (iii) late chronic stage.

Brain :

(i) Acute Stage : This comprises cases where the patients died before the end of the first week of extra-uterine life. Histological examination of the affected areas of the brain in these cases failed to reveal any features which were different from those of normal brains in infants of the same age. Most of the sections examined revealed no structural change whatsoever. A few showed chromatolysis of the ganglion cells and a few ganglia showed cell loss but these changes are common post-mortem findings in children of this age period. Two cases showed yellow staining of cells in/

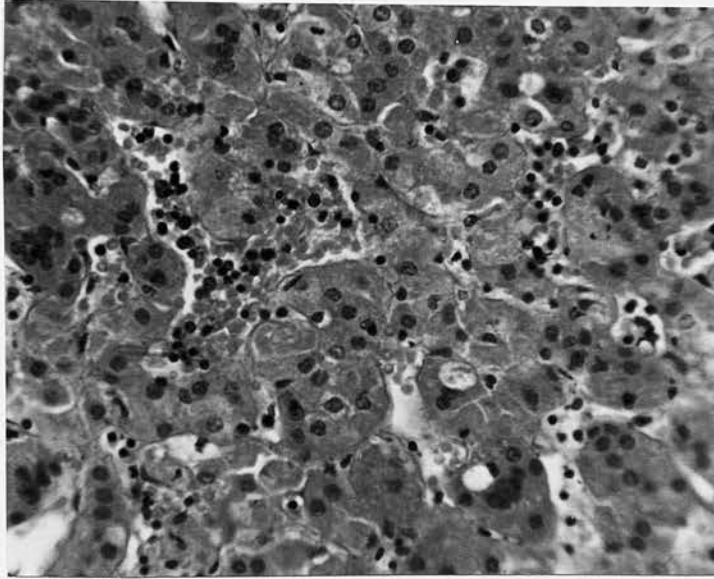


Fig. 61. SUPRARENAL GLAND x 280. Haematoxylin and Eosin. Nucleated red cells in the vessels of the cortex.

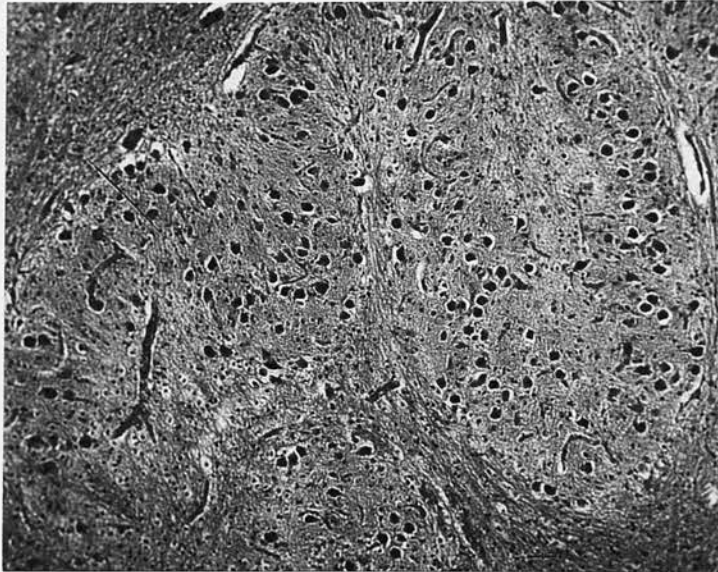


Fig. 62 a. BRAIN STEM x 80. Haematoxylin and Eosin. Normal brain in neonatal period.

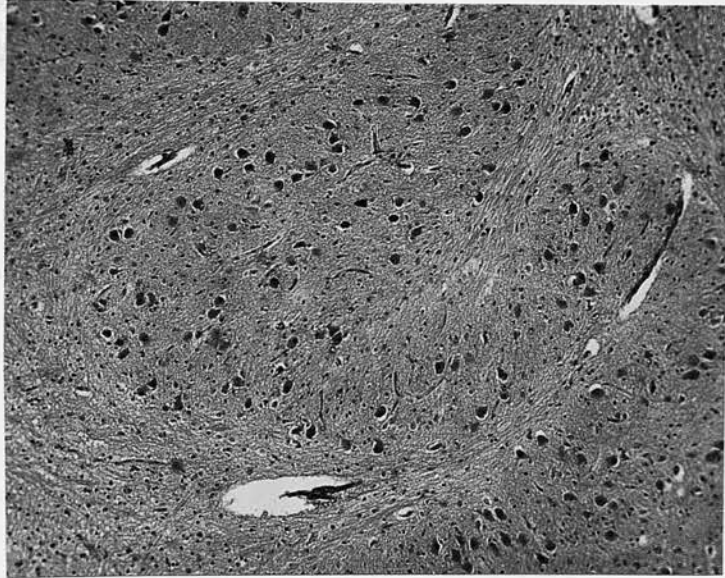


Fig. 63. BRAIN STEM x 80. Haematoxylin and Eosin. Case of kernicterus in acute stage. Note absence of histological change.

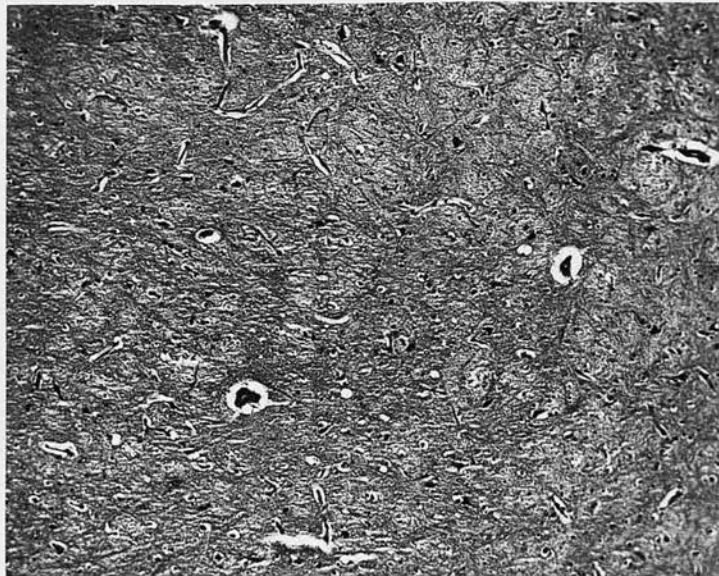


Fig. 64. BRAIN (basal ganglia) x 80. Haematoxylin and Eosin. Case of kernicterus in acute stage. No histological change.

in the cornu ammonis but the cells were not otherwise altered (Figs. 63 and 64).

These important facts have been confirmed by Dr. A. R. Macgregor (Pathologist to the Royal Edinburgh Hospital for Sick Children) and Dr. W. Blackwood (formerly Neuropathologist to the Royal Infirmary Edinburgh) who examined many of the brains in this series.

(ii) Early Chronic Stage. (Fig. 65).

This corresponds to the clinical stage where signs of extrapyramidal involvement are more striking features than the general cerebral irritation of the earlier period. It occurs about the end of the first week and before the end of the first month of life.

The icteric areas now show some loosening up of the cerebral tissue and formation of globules of lipoid material (Fig. 67) Slight astrocytic reaction may be present (Fig. 66).

It is interesting to note that Zimmermann and Yannet (1933) examined a kernicteric brain of a child dying at the age of eleven days. They stated, even at this age, that " it was impossible to determine whether the icteric ganglion cells were injured. " They did, however, find numerous fat granule cells in the centrum ovale.

Thus the earliest " irreversible " change in the affected areas would seem to be a necrosis of cerebral tissue with slight astrocytic replacement.

(iii) Chronic Stage. (Figs. 68 - 77)

The/



Fig. 65. BRAIN (Basal ganglia) x 80.
Haematoxylin and Eosin. Ganglion cells are
vacuolated and an early astrocytic reaction
is present. Case of kernicterus. Early
chronic stage.

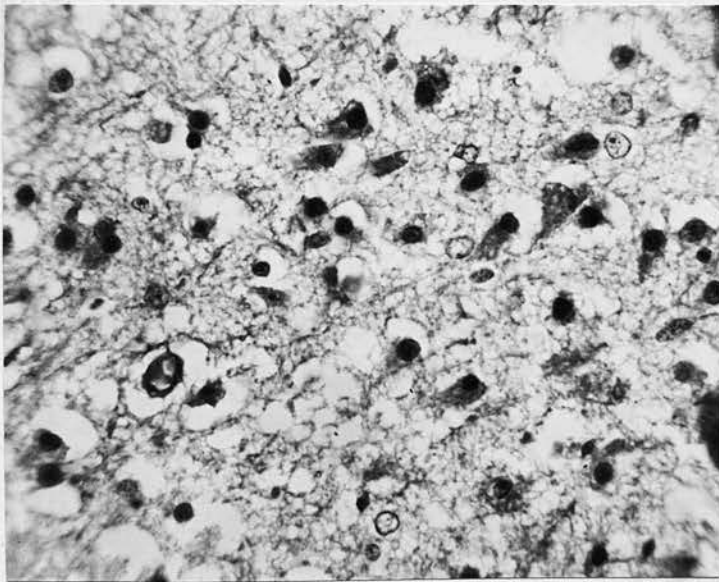


Fig. 66. BRAIN (Basal ganglia) x 375.
Haematoxylin and Eosin. Vacuolated neurones
with early astrocytic replacement. Case of
kernicterus. Early chronic stage.

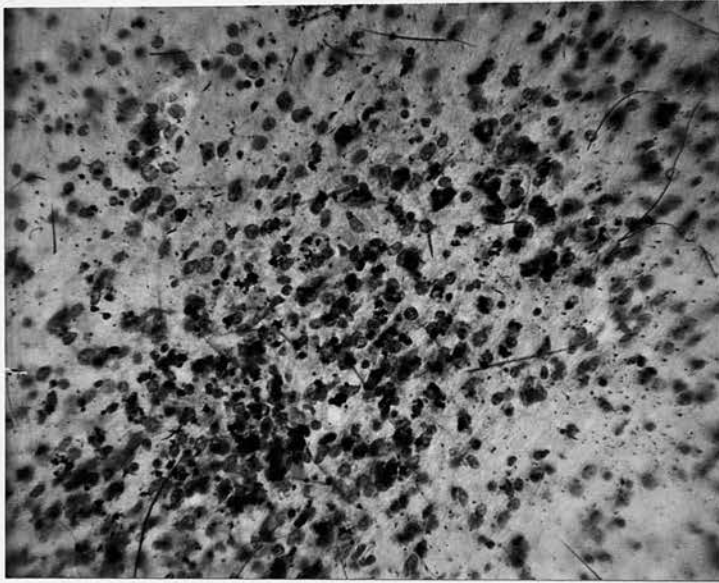


Fig. 67. BRAIN (Basal Ganglia) x 220.
Scharlach R. Early fatty change in cerebral
tissue. Case of kernicterus. Early
chronic stage.

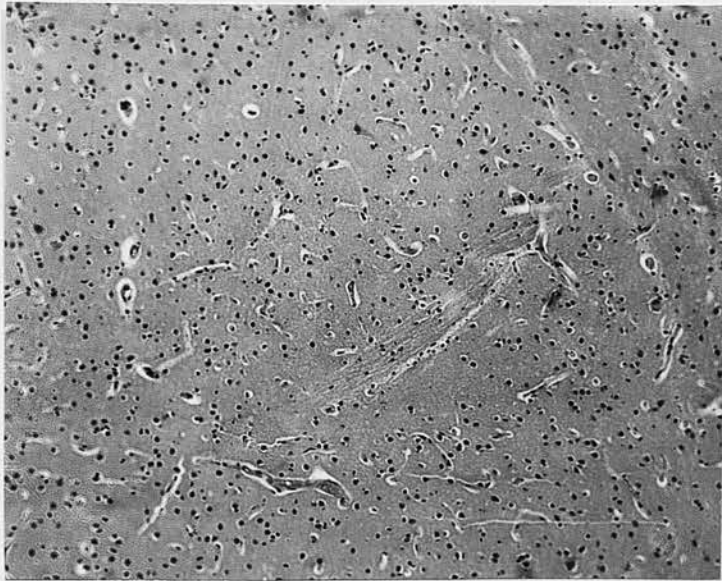


Fig. 68. BRAIN (Basal Ganglia) x 80.
Haematoxylin and Eosin. Note loss of ganglion
cells and astrocytic replacement. Case of
kernicterus. Chronic stage.

The lesions of the affected areas in this stage have been fully described by Fitzgerald et al (1939) whose two patients died at the age of 4½ months and 1 year respectively - and by Zimmerman and Yannet (1935) whose patient died at the age of three years.

There are only two cases in the present series which fall into this group (Case 17, Series II and Case 48, Series II). These children died at the age of 4 months and 17 months respectively. The histological findings were as follows :-

a) Case 17, Series II. (Figs 72-75).

There was severe cell loss and well marked astrocytic replacement in the dentate gyrus. There was loss of large cells and astrocytic reparative overgrowth in the globus pallidus. Focal neuronal loss had occurred in the dentate nuclei (Figs. 73, 74 and 75). In the putamen, inferior olives, thalamus and third nerve nucleus there were small foci of polymorphonuclear leucocytes, lymphocytes and microglial cells (Fig. 68).

b) Case 48, Series II.

The spinal cord, medulla, frontal, parietal and occipital cortex showed no abnormality. In the basal ganglia there were focal areas of myelin degeneration in the globus pallidus associated with an apparent loss of large nerve cells ^{and} ~~unassociated~~ with a marked reparative gliosis. In the cornu ammonis there was a complete disappearance of the nerve cells of the fascia dentata and of the terminal nerve cells of the subiculin. There was an increase of/

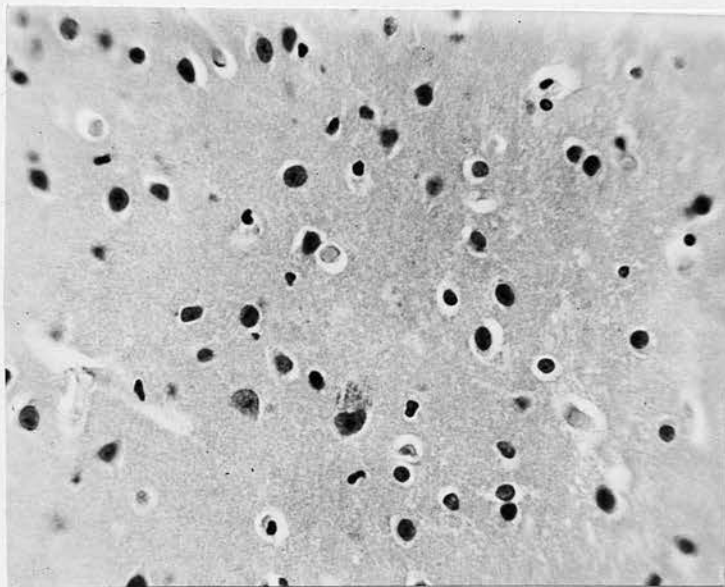


Fig. 69. BRAIN (Basal ganglia) x 350.
Haematoxylin and Eosin. Marked neuronal loss.
Case of kernicterus. Chronic stage.

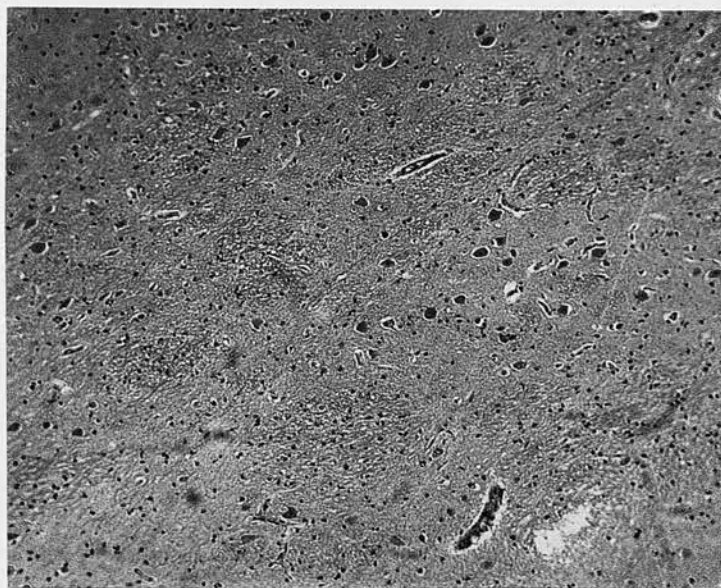


Fig. 70. BRAIN (Basal ganglia) x 80.
Mallory. Severe neuronal loss and
astrocytic replacement. Case of kernicterus.
Chronic stage.

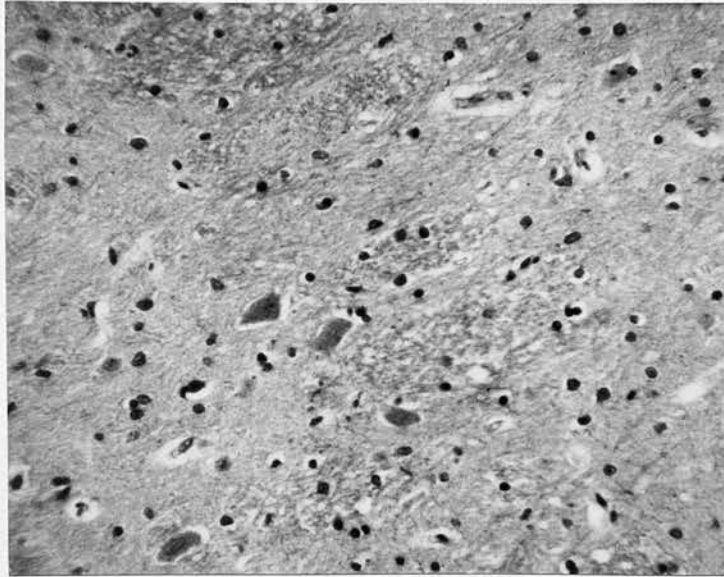


Fig. 71. BRAIN (Basal ganglia) x 250.
Mallory. Neuronal loss and astrocytic
replacement. Case of kernicterus.
Chronic stage.

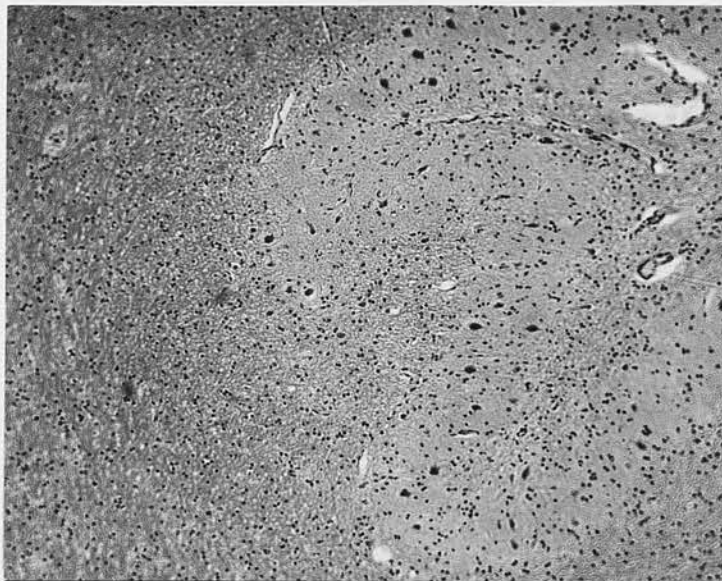


Fig. 72. BRAIN (Cerebellum) x 80.
Haematoxylin and Eosin. Severe neuronal
loss. Case of kernicterus. Chronic stage.

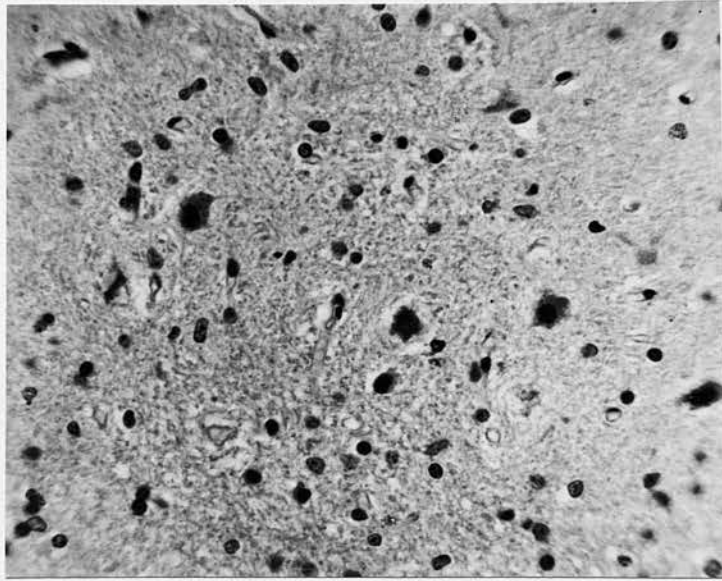


Fig. 73. BRAIN (Cerebellum) x 350.
Haematoxylin and Eosin. Neuronal loss.
Case of kernicterus. Chronic stage.

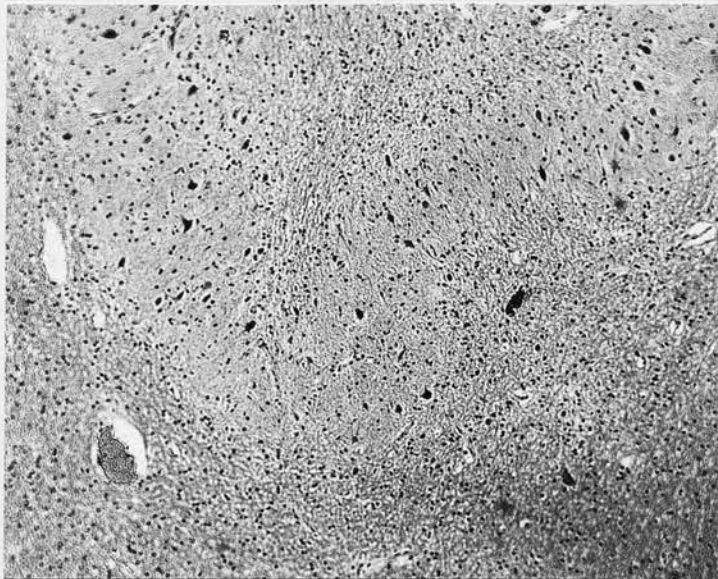


Fig. 74. BRAIN (Cerebellum) x 80. Mallory.
Neuronal loss and astrocytic replacement.
Case of kernicterus. Chronic stage.

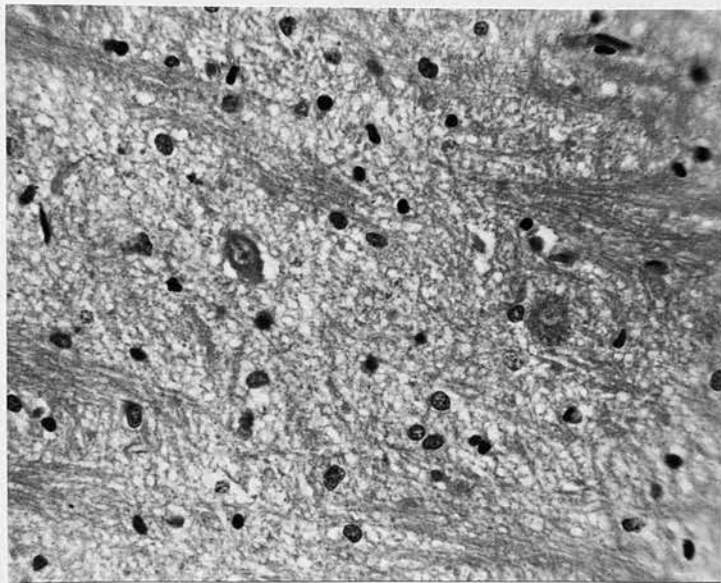


Fig. 75. CEREBELLUM x 350. Mallory.
Neuronal loss and astrocytic replacement.
Case of kernicterus. Chronic stage.

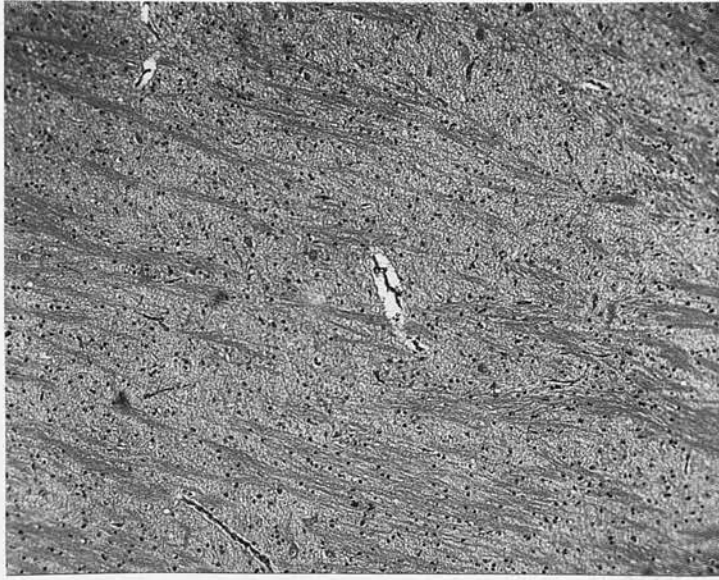


Fig. 76. BRAIN (Basal ganglia) x 80.
Haematoxylin and Eosin. Note loss of
neurones. Case of kernicterus. Chronic
stage.

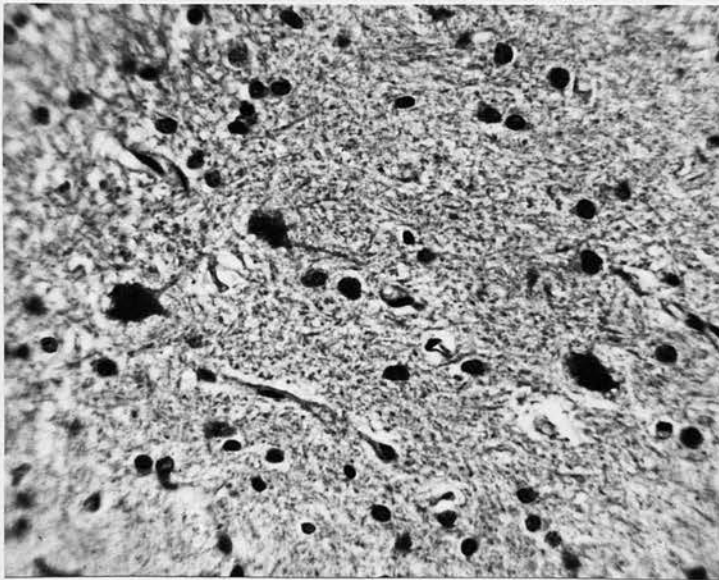


Fig. 77. BRAIN (Basal ganglia) x 350.
Haematoxylin and Eosin. Severe neuronal loss.
Case of kernicterus. Chronic stage.

of astrocytic nuclei in this region (Figs. 69, 70, 71).

It is interesting to compare these findings with those of Zimmermann and Yannet (1935) whose case was referred to in the previous section. The changes in their case were even more widespread than those described above. The optic nerves, reticulo-spinal, rubrospinal and spino cerebellar tracts showed demyelination. The fascia dentata of the cornu ammonis was destroyed and there was neuronal loss in the corpus striatum, globus pallidus, subthalamic nuclei and the red nucleus. They found no astrocytic replacement in any region and describe the destructive process as one of "necrobiosis."

Vaughan (1946) failed to find "necrobiosis" in his fatal cases of kernicterus and states that "in certain of the brains where kernicterus was intense in gross sections, no microscopic abnormality whatsoever was encountered." This is not at all surprising in view of the fact that nearly all his fatal cases were patients dying in the acute stage when, as has been shown, no irreversible histological change occurs.

5. Time of Onset of Kernicterus.

Now that the pathological features and clinical appearances have been described it is necessary to try to ascertain at what period the cerebral damage occurs.

Kernicterus does not occur in hydrops foetalis even when examples of the latter are jaundiced. It is not seen in patients with icterus gravis who die within/

within an hour or two of birth. As the present series of 157 cases covers a period of 10 years, and no case of kernicterus occurring at or before birth has been found, it is reasonable to suppose that nuclear staining occurs some time after birth. The earliest death in a child with kernicterus occurred 18 hours after birth and the jaundice was first noted six hours after birth (Case 14, Series III). The kernicterus therefore probably developed sometime between the time of birth and twelve hours after birth. Thus nuclear staining in patients who are jaundiced at birth or within a few hours of birth probably develops within the first twelve hours. In patients in whom jaundice is later in appearing, nuclear jaundice may occur within twelve hours of the first clinical sign of jaundice.

This nuclear staining, while causing intense cerebral irritation, does not appear to cause immediate irreversible damage to the affected cells. On the contrary, at post-mortem examination, the affected cells may be bile-stained but are not structurally altered. This lack of histological change may persist as late as the 6th to 8th day of extra-uterine life or perhaps even slightly longer.

Finally, towards the end of the first week of life, or the beginning of the second, irreversible cellular damage occurs with nuclear destruction and gradual astrocytic replacement. This process first involves the cornu ammonis and then the other icteric areas. The change apparently becomes more widespread over/

over the first two months of life, until the majority of the icteric areas show cell loss with a variable degree of astrocytic replacement. Thereafter if the child survives, the jaundice disappears but the cellular destruction remains. The optic nerves and the spinal cord tracts seem to be among the last of the affected areas to undergo gross structural alteration.

These gradual changes are of some importance in the treatment of the disease and the probable facts may be summarised as follows :-

(1) Kernicterus does not occur at birth

(2) Kernicterus can occur within twelve hours of birth in cases which are jaundiced at birth or soon after and probably occurs within twelve hours of the first appearance of jaundice in cases where the latter is delayed.

(3) Irreversible cytological change is delayed until the end of the first week of life.

(4) Once this cytological change has commenced it spreads throughout the affected icteric areas causing widespread destruction, provided, of course, the child survives for a sufficiently long period.

6. Correlation of the Clinical and Pathological Findings.

In the acute stage of the disease the presence of bilirubin in the cells caused intense cerebral irritation which leads to tonic and clonic movements or even opisthotonos. These severe spasms are broken by periods of lethargy and drowsiness. In the/

the early chronic stage the corpus striatum shows the most severe cytological change although this area is frequently not the most severely bile-stained, when early cases are examined post-mortem. This damage leads to the choreo-athetoid movements which are such well-known features of the disease.

In the later chronic stage the changes are very widespread and affect structures outside the basal ganglia or even the extrapyramidal system as a whole. Lande (1948) has described deafness, blindness, strabismus and cranial nerve palsies. She believes she is the first to describe these clinical signs in older patients with kernicterus. These signs are not surprising, however, in view of the histological findings in the late case of Zimmermann and Yannet (1935) and of the changes present in the patient in this series who died at the age of 4 months. In the former there was demyelination of the optic nerves, optic chiasma and spinal tracts as well as damage to the basal ganglia and extrapyramidal system; in the latter the third cranial nerve nucleus and the dentate nuclei of the cerebellum were severely involved. Thus in the later cases of kernicterus neurological signs referable to damage in the cranial nerve nuclei, optic nerves and spinal cord are to be anticipated as well as evidence of lesions in the basal ganglia and extrapyramidal system. The lesion is not merely one affecting the latter structures but is widely present throughout the nuclear aggregations of cerebrum, brain stem, cerebellum/

cerebellum and grey matter of the spinal cord.

7. Pathogenesis of Kernicterus.

Even at the beginning of this century it became recognised that kernicterus was almost always associated with icterus gravis neonatorum where erythroblastosis was a prominent feature. The other major cause of severe jaundice of the newborn, congenital obliteration of the bile ducts, had failed to yield a single instance of kernicterus. It is interesting to note that Pasachoff (1935) has described a case of kernicterus in which the patient also had congenital obliteration of the bile-ducts. The author makes it quite clear, however, that icterus gravis with erythroblastosis was also present, and he stresses the latter, rather than the former, as the primary agent in the etiology of the nuclear jaundice.

At the beginning of the century, neonatal sepsis was believed to be the etiological agent in the development of icterus gravis and naturally this theory was invoked to explain the kernicterus.

a) The " Sepsis " Theory :

Beneke (1904) believed organisms entered the blood stream via erosions ("stigmata") in the gastric mucous membrane. Esch (1908), however, thought these lesions were secondary to the cerebral jaundice. Knoepfelmacher (1910), Pfaltzer (1914) and Thorling (1922) all obtained positive cultures from post-mortem material in kernicterus cases and were strongly in favour of an infective basis for the disease. Pfaltzer (1914) even went so far as to name *Bacillus coli*/

coli as the causative organism. Dunham (1933) found jaundice in 14 cases out of 40 instances of neonatal sepsis. Zimmerman and Yannet (1933) obtained B. coli from the blood in Case 2 and Case 3 of their series. They did not stress this feature but were inclined towards a theory of infection for the etiology of icterus gravis. Biemond and Van Creveld (1937) reported two cases of kernicterus in icterus gravis neonatorum where severe umbilical sepsis was also present.

Since the discovery of the Rhesus blood groups the influence of sepsis in the etiology of icterus gravis and kernicterus has received less attention. Recently, however, De Bryne and Van Creveld (1948) have reported a further series of four cases of kernicterus complicating icterus gravis. In all of these severe umbilical sepsis was present. It is interesting to note that Cases 1 and 2 of the new series were siblings of the earlier cases of Van Creveld (1937) and that evidence of iso-immunisation to Rhesus antigens was now present in both mothers. The mother of case 3 also had anti-Rh antibodies in her serum. The mother of case 4 was Rhesus positive but her serum had an anti-A agglutination titre of 1/512. These cases throw considerable doubt on the validity of the infective theory.

Sepsis played no part in the development of kernicterus among the patients in this series.

b) Toxic Theory :

Orth/

Orth (1875) believed that there was a primary necrosis of parts of the brain and these areas were subsequently pigmented. Schmorl (1903) suggested that the primary change was cell necrosis either due to vascular damage or to a toxic degeneration of the cell. He believed that the toxin could be bile itself. Hart (1917) also thought that the ganglion cells were injured by bile or some unknown toxin and then stained by bile pigment. Beneke (1907) believed that either bile pigment, bile salts or ischaemia were responsible.

As Zimmerman and Yannet (1933) correctly pointed out " any consideration of the pathogenesis of kernicterus must take into account the factors underlying the development of icterus as well as those responsible for the changes in the nervous system." The link between the liver disease and the brain injury soon attracted the attention of workers in this field.

Hoffman and Hausmann (1926) suggested that the liver damage took the form of a hepatitis which resulted in the liberation of lipolytic substances which were responsible for cerebral necrosis. Several experimental workers investigated the possibility of liver disease leading to cerebral damage. Fuchs (1917) fed experimental animals on guanidine which caused liver damage and subsequent changes in the brain. Pollak (1921) examined the brains of the animals who had undergone the above experiment/

experiment and found an inflammatory reaction with loss of ganglion cells. The damage was diffuse, however, and not confined to the nuclear masses. Mella (1924) injected manganese intraperitoneally into *Macacus rhesus* monkeys and produced nuclear loss in the putamen and caudate nuclei. In two of his animals hepatic-fibrosis occurred. Crandall and Weil (1933) ligated the common bile-duct in rats and caused degeneration of the corpus striatum.

These experiments failed to reproduce kernicterus but did seem to suggest that there was a link between hepatic dysfunction and cerebral damage. The toxic theorists have so far failed to name the toxin which causes the lesions.

c) Vascular Theory :

Schmorl (1903) and Beneke (1907) both thought that vascular damage resulting from thrombosis or other change led to ischaemia of the nuclear masses and subsequently pigmentation of their cells took place. Thorling (1922) also thought that ischaemia played a part and suggested a combination of low blood pressure and respiratory failure as causes.

In the experimental field Spielmeyer (1930) found that the corpus striatum and cornu ammonis have a relatively poor blood supply and are liable to damage in diseases affecting the latter. Meyer (1936) showed that the globus pallidus, inferior olives, dentate nuclei and cornu ammonis are the most commonly injured in anaemia of the brain/

brain whether it be caused by Morphine, carbon monoxide or carbon dioxide poisoning. That asphyxia and anoxia affected these regions severely was also demonstrated by Wolff (1937) and by Putnam (1937).

These facts were siezed upon by various investigators who interpreted them as showing that some vascular damage was responsible. In a study of the blood-brain barrier Broman (1941) decided that vascular injury was necessary before cerebral tissue would stain with trypan blue. This dye was shown to resemble bilirubin in its effects by Friedman (1942).

With the discovery of the Rhesus blood groups a great impetus was given to the study of the disease and many workers tried to fit the new data to a vascular theory of the origin of kernicterus. Diamond and Denton (1945) and Liber (1945) suggested the blocking by cellular debris of the terminal capillaries to the nuclear masses as a possible cause. Wiener (1946), in his theory of the pathogenesis of congenital haemolytic disease, postulated the blocking of these terminal capillaries by agglutinated red cells as the causative factor.

Unfortunately, as Levine (1946) and Vaughan (1946) pointed out, there is no histological evidence to support these theories. In no case in the present series was there any evidence of capillary thrombosis.

d) Antigen-antibody Reaction :

The discovery of the Rhesus blood groups explained/

explained the familial incidence of the disease, a problem which had exercised the minds of earlier authors. It was then shown that the antibody in the mother's serum acted on the antigen of the infants red cells causing destruction of the latter. At first it was believed that the Rhesus antigen was present only in the red blood corpuscles. More recently Boorman and Dodd (1943) showed that it was not very soluble in water but considered it was probably distributed in the tissues in the same manner as the A and B factors. This had led some authors to believe that a direct antigen-antibody reaction occurs in kernicterus with the cerebral cells acting as the antigen. Yannet and Liebermann (1946) believe this is a possibility. Their hypothesis is not yet based on very firm ground.

e) Anaphylactic Theory :

Darrow (1938) believed that anaphylaxis alone could account for some of the findings in congenital haemolytic disease. Even after the discovery of the Rhesus blood groups Darrow and Chapin (1947) still believe that in addition to the antibody-antigen reaction in the disease there is an anaphylactic process which accounts for severe icterus gravis and the development of kernicterus in some cases of the latter. It is very difficult to either prove or disprove such a theory but the grounds for its belief do not seem to be as definitely established as the authors would suggest (see Chapter 8).

f)/

f) Theory of Inherent Constitutional Defect.

Orth (1875) was the original exponent of this theory which has cropped up in the literature from time to time ever since. He believed that there might be some congenital inferiority of the brain predisposing to the localisation of bile. Fitzgerald et al (1939) believe that a degree of "mal-development" is the primary factor responsible for the onset of kernicterus. Frohlich and Mirsky (1942) produced convulsions in young rats but not in older ones by administering bilirubin. Vaughan (1946) was inclined to believe that "immaturity of cerebral and cerebrovascular tissues" played a part in the etiology of kernicterus. More recently Lande (1948) thought that in some families the nervous system was more liable to toxic or emotional upset than in others and lists a few cases in support of her belief in a constitutional hypothesis.

The two chief disadvantages of this theory are 1) that it is very difficult to produce concrete evidence for or against; 2) that it is a counsel of despair. If you believe in this type of theory then you tend to avoid pursuing further investigations which may lead to results of therapeutic importance.

g) Theory based on the Clinical and Pathological findings of cases in the present series.

The main difficulty in accepting one or other of the theories listed above lies in the fact that the majority of them lack a sound histological basis for their premises. This may be partly due to/

to the fact that in the recent literature there are only four cases in which a thorough histological examination has been carried out. These are the cases of Zimmerman and Yannet (1933 and 1935) dying at the ages of 11 days and 3 years respectively and of Fitzgerald et al (1939) whose cases died at the age of $4\frac{1}{2}$ months and 1 year respectively. As was shown in the section on the morbid histology of kernicterus these age periods do not cover all the stages of the development of kernicterus.

One of the first problems to be solved is whether the pigmentation of the cerebral masses is a primary occurrence or whether it is secondary to nerve cell injury. Zimmerman and Yannet (1933) state that "opinion is almost unanimous that following some injury, the nerve cells are subsequently stained with the bile pigments carried to them by the blood stream. This differs in no way from the well known fact that any intravital dye will localise in zones of injury, leaving unstained tissues which are not damaged." This is by far the more reasonable view, since to assume that bile pigment tended to pick out certain cerebral structures and leave the remainder unaffected would be contrary to all the known facts concerning intravital staining. It is highly probable, therefore, that the ganglion cells are damaged first. Thereafter they pick up bilirubin in greater measure than the surrounding cells which are undamaged.

Granted that this assumption is correct, it is then/

then necessary to discuss what agent or agents are responsible for the original damage to the cell. Any theory, which is evolved regarding this injury, must take into account the following :

(i) That kernicterus does not occur at birth but may occur within a short period of time following the development of jaundice.

(ii) That irreversible cellular damage does not appear to occur before the end of the first week of life.

These facts are borne out by the cases in this series and by the findings of Zimmerman and Yannet (1933) and Vaughan (1946).

Yannet and Libermann (1946) hold diametrically opposed views. They believe that cerebral injury is entirely secondary to the destruction of red blood cells during certain periods of foetal life. The resultant anoxaemia causes permanent injury to the developing neurone. They further believe that cerebral damage is well established before birth. This belief is also supported by Parsons (1947). There seems to be no histological evidence to support this view. Further, it would be expected that nuclear jaundice would be found in patients dying at birth from icterus gravis if such a hypotheses were correct. This is not the case.

It will be remembered that several experimental workers, notably Spielmeier (1930) and Meyer (1936), have shown that the areas affected in kernicterus are those most commonly injured by anaemia or anoxia of/

of the cerebral tissue. There is no histological evidence to support the theories of Wiener (1946) and others that vascular damage, caused by local capillary thrombosis or by lesions of an allied nature, occurs in kernicterus.

It would seem most probable that the antigen-antibody reaction in the foetus results in the production of a sufficient degree of anaemia to cause cerebral anoxia. Vaughan (1946) pointed out that he was unable to correlate the degree of anaemia with the onset of kernicterus. The more recent work of Mollison and Cutbush (1949) shows that the findings of the former are not the whole story. Some degree of anaemia is present in all cases of severe icterus (resulting from haemolytic disease) but the anaemia may be concealed in the early stages.

Thus it would seem that anoxia is responsible for the original cell damage which leads to subsequent pigmentation. This damage is of such a nature that the cell as a whole is not destroyed but is merely sufficiently changed to permit the bile-staining to occur. The oxygen lack is sufficient to render the cell membrane unduly permeable to bilirubin but is not so great as to cause cell death.

Cobb (1946) has stated that jaundice and kernicterus have resulted from phosgene poisoning of soldiers during the 1914-1918 War. This is the only known instance of kernicterus occurring in adults./

adults. It is interesting to note that such a development occurred in patients likely to be suffering from anoxaemia.

Lande (1948) has invoked a constitutional factor to explain the incidence of kernicterus in some cases of icterus gravis and not in others. This seems to be unnecessarily complicated. It will be remembered that 66% of patients dying in the neonatal period from icterus gravis showed kernicterus at post-mortem examination. It is very probable that careful studies of the serum bilirubin and haemoglobin values in such patients will show that a close correlation exists between these values and the development of kernicterus. Thus patients with a high serum bilirubin and a subnormal haemoglobin content of their blood in the first few days of life are very likely to develop kernicterus. If both these findings are absent the patient will escape this form of the disease.

Finally as Zimmerman and Yannet (1939) point out, cells in the cerebral cortex, in the white matter around the lateral ventricles, and in the cerebellar cortex show similar change to those in the areas more commonly affected. As the lesions are so extremely widespread, they are most probably the result of anoxia.

Once pigmentation has occurred, the combination of anoxia and the presence of pigment in the cell eventually leads to the death of the latter. On histological grounds there is reason to believe that/

that this process of gradual cell death occurs over the first week of extra-uterine life. It occurs firstly in the very vulnerable cornu ammonis and then gradually throughout the other affected areas.

The anoxic theory of the pathogenesis of kernicterus would seem to fit the cases in this series and may be summarised briefly as follows :

1) Anoxia causes increased permeability of the cell membrane to bilirubin but is not sufficient to cause cell death. This explains the absence of cellular necrosis in cases dying in the acute stage.

2) A combination of anoxia and the presence of bilirubin leads to death of the affected cell after a period of about a week.

3) That cell death first occurs in the vulnerable cornu ammonis and eventually occurs in the other affected areas.

8. Treatment of Kernicterus.

The following points with regard to the treatment of kernicterus are put forward only as suggestions. It is fully realised that it is the responsibility of the clinician in charge of the case and not of a pathologist to institute the treatment. In view of the pathogenesis of the condition, however, a few points are worth noting.

a) Acute Stage.

This is the stage when treatment is liable to be most useful. It is known that kernicterus can occur/

occur within 12 hours of the first clinical appearance of jaundice. Thus if severe jaundice is present at birth treatment should be instituted immediately. Since there are few reliable clinical criteria to indicate which patients will have kernicterus and which not, it is suggested that all cases of severe icterus gravis are treated within 12 hours of the development of jaundice.

Since cerebral cell pigmentation is apparently the result of anoxia the obvious solution is to supply blood with a full oxygen carrying capacity. This would mean a transfusion of Rhesus negative blood. Rhesus positive cells are quickly destroyed (Mollison 1943) and so their beneficial oxygen-carrying power would soon be lost. Darrow and Chapin (1947), who suggest an anaphylactic basis for kernicterus, and Vaughan (1946) believe that better results are achieved by the use of Rhesus-positive blood. This requires further confirmation and it would probably be wiser to use Rhesus negative blood.

Whether the direct transfusion or exchange-replacement transfusion method is used will depend on the preferences of the clinician (see Chapter 11).

As erythroblasts and normoblasts have a low oxygen-carrying capacity and since erythroblastosis is a marked feature in the patients with kernicterus in this series, prompt blood transfusion should be of considerable value.

Since irreversible cell damage does not occur before/

before a period of a week has elapsed it might be worth while treating cases which were not examined until signs of cerebral irritation had commenced. If the anoxia was overcome further involvement of other cell groups might be prevented.

Other methods, in addition to blood transfusion, which may be helpful in the treatment of kernicterus are mentioned elsewhere (Chapter 11).

b) Chronic Stage :

By the time this stage is established irreversible cellular change has occurred. The only treatment to be considered in this stage is

(i) The prevention of secondary infection

(ii) The care of the child's mental and physical development. Docter (1945) believes that if great care is exercised with these unfortunate children striking improvement occurs within the limits of their physical disability.

9. Prognosis.

There were 37 cases of kernicterus in this series. Twenty-eight patients died in the acute stage within one week of birth. Five more had died by the end of the second week. One lived for four months but then succumbed to an acute suppurative bronchitis. Another patient died at the age of 17 months. Only two survive at the time of writing.

This is a mortality of 95% so the prognosis in cases of kernicterus is grim indeed. It is to be hoped that early blood transfusion will not only save/

save life but prevent the unfortunate sequelae as well.

10. Diseases associated with kernicterus :

No account of kernicterus would be complete without a reference to other diseases which clinically resemble the findings in kernicterus.

The most important one is Progressive Hepato-Lenticular degeneration or Wilson's disease. The association in this disease of liver damage and degeneration of the corpus striatum has along aroused the interest of neurologists. Wilson(1912) in his paper on Progressive Lenticular degeneration suggested a relationship between it and kernicterus. This view was supported by Guthrie (1914), Hoffmann and Hausmann (1926) and de Lange (1925).

Zimmerman and Yannet (1933) and Brouwer (1936) did not agree with this view and discounted any such association on the grounds that cirrhosis of the liver is absent in kernicterus and that the lesions in the latter are not confined to the basal ganglia.

Some degree of cirrhosis of the liver in cases of kernicterus has been noted by Hawksley and Lightwood (1934) and by Diamond and Van Creveld (1935). Furthermore Greenfield et al (1926) were able to show that the lesions in Wilson's disease were not confined to the corpus striatum.

It would seem unlikely, however, that there is any actual relationship but an investigation of the Rhesus blood groups in cases of Wilson's disease might/

might yield further information.

C. and O. Vogt (1920) and Meyer and Earl (1936) in a study of the basal ganglia in mental defectives suggested a correlation between status dysmyeliniatus (Hallervorden-Spatz disease) and kernicterus. The distribution of the lesions is not dissimilar but no jaundice or liver lesions occur in the former condition.

Schuster (1925) found lesions in the caudate and lenticular nuclei in cases of familial cerebral degeneration. Davison and Goodhart (1938) suggested that dystonia musculorum deformans gave clinical signs similar to those found in the later stages of kernicterus.

More recently Yannet and Liebermann (1946) found that in a group of undifferentiated mental defectives 22% of their mothers were Rhesus negative, while they themselves were Rh positive. The authors therefore suggested that some cases of mental deficiency may be due to Rhesus incompatibility, as this figure of 22% is twice the expected incidence. Cappell (1947b) discounts the evidence of Yannet and Lieberman entirely and does not believe that cases of idiopathic mental deficiency are in reality cases of Rhesus incompatibility.

Cappell (1947 b) has suggested the diffuse bile-staining of the cerebral cortex may cause mental deficiency. This diffuse bile-staining is very commonly found in patients with severe icterus who die in the neonatal period, irrespective of the presence/

presence or absence of kernicterus. The incidence of such diffuse bile-staining is too frequent for it to be closely associated with the development of mental defect in later life.

Zimmermann and Yannet (1933) pointed out that a few cortical cells may be deeply bile-stained and in the rare instances where this occurs there may be subsequent mental defect in surviving patients.

The question of the relationship between congenital haemolytic disease and mental deficiency is one which requires more accurate study before definite conclusions are drawn.

Chapter 15.

Summary and Conclusions.

This study has dealt with features presented by 157 cases of haemolytic disease in the newborn. These 157 cases included the following

Hydrops Foetalis	36
Icterus gravis	110
Haemolytic Anaemia	11

Of these 157 patients 116 died or were still-born (74%). This is a very high mortality and the reasons for this figure are fully discussed.

The clinical features of the affected infants, and the laboratory investigations carried out, are described in detail. A post-mortem examination was carried out on the bodies of infants who had been still-born or had died from the effects of the disease process and the pathological lesions found in all forms of this disease have been noted.

It has been shown that isoimmunisation of the mother to blood antigens which she herself does not possess is the main etiological factor responsible for the development of haemolytic disease in her offspring. Serological investigations on the blood of a number of the mothers and affected infants and foetuses were carried out from 1943 onwards. In patients so tested the usual pattern of Rhesus negative mother and Rhesus positive child and father was found in most/

most instances (90%). These findings are compared with those of other authors and the subject of maternal isoimmunisation and the role of the Rhesus blood groups has been studied.

The obstetrical histories of the mothers of affected children in this series have been investigated and the various factors of etiological and prognostic significance have been elucidated. It has been shown that the number of unsuccessful pregnancies in mothers who subsequently bear children affected by haemolytic disease is greater than that in an unselected group. The maximum incidence of haemolytic disease occurs in the offspring of the 2nd, 3rd and 4th pregnancies. Haemolytic disease is less common in offspring of the 1st. pregnancy but should it occur in a 1st. pregnancy, it may quite well take a severe form. The dangers of incompatible blood transfusion in women in the childbearing period have been stressed.

It has been shown that the antibodies produced as the result of isoimmunisation in the mother react upon the red cells of the foetus causing haemolysis of the latter. If this haemolysis is very severe the foetus will die in utero. If only slightly less severe a still-born foetus will be delivered. In cases where haemolysis is marked, but not so severe, as to cause foetal death, a condition of anoxia will develop in the foetus. It is felt that the clinical and pathological features/

features of icterus gravis and haemolytic anaemia can be explained on an anoxic basis. The different degrees of involvement, seen clinically and at necropsy, correspond to the different degrees of anoxia which have been present.

The clinical features of the main varieties of haemolytic disease have been described and the criteria of severity are discussed. The most useful serological investigations have been noted. It has been pointed out that there is no single diagnostic feature by which it is possible to recognise, or to exclude haemolytic disease.

Of the serological tests, the Coombs anti-human globulin reaction is the most generally useful but it is not absolutely diagnostic of haemolytic disease when positive, nor does it necessarily exclude the latter when negative.

The most useful investigations upon which an opinion of the severity of the disease may be formed are the haemoglobin level of the blood and the level of the serum bilirubin. In general a low blood haemoglobin and a high serum bilirubin are signs of bad prognostic significance. The majority of severely affected patients have a large number of nucleated red cells of primitive type in their peripheral blood, but this feature is rather variable.

The treatment of patients suffering from haemolytic disease has been described. Blood transfusion, using rhesus negative blood, is the chief/

chief form of therapy. The relative merits of simple direct and exchange-replacement transfusions have been discussed. It is thought that the latter may be of special value in the treatment of hydrops foetalis and in the prevention of kernicterus. It is emphasised that whichever form of transfusion therapy is used it must be instituted at the earliest possible moment.

The prognosis of both mother and child depends on a number of interrelated factors. The importance of genotyping the husband in order to discover whether he is homozygous Rh positive or heterozygous Rh positive has been shown. The result of such an investigation will allow a more accurate forecast of the outcome of future pregnancies in sensitised mothers to be made.

The most important complications in patients affected with haemolytic disease are intercurrent infections and the development of kernicterus.

Thirty-seven of the 110 patients suffering from icterus gravis developed kernicterus, and 35 of these 37 infants died. Post-mortem examinations were carried out on the majority and a full description of the histological features at varying stages in the development of the cerebral lesion has been given. It would seem that the damage occurs after birth or not long before birth and the affected cells subsequently become pigmented. Histological evidence of irreversible cellular damage is not found before the end of the first week/

week of extra-uterine life.

On the basis of these findings a theory has been evolved regarding the pathogenesis of the cerebral lesions.

Granted that this hypothesis is correct, it would seem that energetic treatment by replacement transfusion of all cases jaundiced at or shortly after birth may prevent the incidence of this unfortunate sequel.

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