HAEMOLYTIC DISEASE OF THE NEWBORN*

PART I: A CLINICAL-PATHOLOGICAL STUDY OF 157 CASES

BY

ALBERT CLAIREAUX, M.D., M.R.C.P.(Edin.)
From the Department of Child Life and Health, University of Edinburgh

(RECEIVED FOR PUBLICATION, JULY 28, 1949)

Since the discovery of the Rh blood groups (Landsteiner and Weiner, 1940) many articles on the subject of haemolytic disease of the newborn have been written. Gilmour (1944) in this country and Potter (1944; 1946) in America have presented authoritative accounts of the pathology of haemolytic disease, but, as the former points out, this aspect of the condition has received far less attention than it has merited.

Present Investigation

The clinical records of all cases of haemolytic disease in the Simpson Memorial Maternity Pavilion, Edinburgh, the Royal Hospital for Sick Children, Edinburgh, and the Elsie Inglis Memorial Maternity Hospital, Edinburgh, in the period 1937-47 have been studied. Also all necropsy material from fatal cases has been examined in detail. The serological investigation of the Rh blood groups in affected families was carried out at the clinical laboratories of the Royal Infirmary, Edinburgh. It must be emphasized, however, that most of the cases in this series occurred before serological Rh testing of mothers and their affected offspring had become a routine antenatal procedure.

Analysis of Cases

The total number of patients was 157, the offspring of 149 women.

Sex. Eighty were male and 67 were female. In ten cases no record was available. The slight preponderance of males over females has probably no statistical significance.

Maturity. One hundred and eleven infants were mature and 39 were premature. Prematurity does not appear to play a conspicuous part in the aetiology but it does add to the hazards of affected infants.

Twin Pregnancies. The disease occurred in six sets of twins and in four of these sets both twins were affected. One pair were examples of hydrops foetalis; two pairs were cases of icterus gravis neonatorum with kernicterus. In the fourth pair, one twin exhibited icterus gravis neonatorum while the other was an example of hydrops foetalis. In the remaining two sets, the first twin was spared while the other was affected by icterus gravis and kernicterus. This indicates that each twin may not necessarily suffer to the same extent, although exposed to the same concentration of maternal antibody. When one twin escapes altogether the father is usually heterozygous (Rh rh), one twin is Rh positive and affected, while the other is Rh negative and escapes.

Obstetrical Histories of the Mothers. A full history of previous pregnancies, miscarriages, and abortions was obtained from 128 of the 149 women. These 128 women gave birth to 478 infants and foetuses, and had borne a total of 278 infants and foetuses before a case of haemolytic disease was diagnosed among their offspring. Of 278 early pregnancies, 37 ended in miscarriages and 23 in stillbirths. Of the remainder, 14 infants died in the neonatal period. Thus only 204 pregnancies resulted in the birth of normal, healthy children. The number of unsuccessful pregnancies, 60 out of 278 (21.5%), is considerably above that in an unselected series (11% according to Potter, 1948). In this series 10 of the 149 women (7%) were pregnant for the first time. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Type of First Pregnancy in Ten Women</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrops foetalis</td>
<td>3</td>
</tr>
<tr>
<td>Icterus gravis and kernicterus (died)</td>
<td>2</td>
</tr>
<tr>
<td>Icterus gravis neonatorum (died)</td>
<td>3</td>
</tr>
<tr>
<td>'  (recovered)</td>
<td>1</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

A history of previous blood transfusion or of intramuscular injections of blood was not obtained from these ten women.

* Part of a thesis presented for the degree of Doctor of Medicine of the University of Edinburgh.
ARCHIVES OF DISEASE IN CHILDHOOD

A further five women (3.5%) had had previous pregnancies ending in miscarriages and had no viable children before the first child with haemolytic disease was born (Table 2). One of these women had after a miscarriage been transfused with blood not tested for Rh compatibility.

Table 2
RESULTS OF FIRST PREGNANCY IN FIVE SUBJECTS PREVIOUSLY TRANSFUSED

<table>
<thead>
<tr>
<th>Type of Haemolytic Disease</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrops foetalis</td>
<td>... 2</td>
</tr>
<tr>
<td>Icterus gravis and kernicterus (died)</td>
<td>... 2</td>
</tr>
<tr>
<td>Icterus gravis (recovered)</td>
<td>... 1</td>
</tr>
<tr>
<td>Total</td>
<td>... 5</td>
</tr>
</tbody>
</table>

It will be seen that the type of haemolytic disease in these early pregnancies is generally severe. This is not in accordance with the findings of Potter (1948) and other workers.

Twelve women had had one or more miscarriages between the birth of the last normal child and the first suffering from haemolytic disease. In three instances these miscarriages had led to severe haemorrhage which had been treated by blood transfusion, when the donor blood was not tested for Rh compatibility. These cases serve to emphasize that incompatible blood transfusion is more effective than pregnancy in sensitizing the mother and that the disease is liable to take a severe form in the results of a pregnancy following such a transfusion.

It will be seen from Table 3 that 66% of the women gave birth to their first affected child before their fourth pregnancy. Of the 129 affected children, 90 died or were stillborn, a mortality of 70%.

In this series 33 women had one or more pregnancies after the birth of a child with haemolytic disease. (Two of these later pregnancies ended in miscarriage.) These 33 women had a total of 70 children, of whom 68 were affected by haemolytic disease. Of the 68 children, 35 were stillborn, 13 died, and 20 were affected but survived. The survival rate of 30% is similar to that of first affected infants. Potter (1948) found a survival rate of only 10% among offspring of later pregnancies.

The 128 women in my series thus gave birth to 197 children affected by haemolytic disease. Of these, 59 survived, 79 died, and 9 were stillborn, a mortality of 70%.

Abnormalities in Pregnancy and Delivery. The majority of the women had uneventful pregnancies. Sixteen women (11%), however, had some disturbance of pregnancy. Their offspring showed 100% mortality. Six were stillborn and the remainder died after birth. The results of these pregnancies is shown in Table 4. In nine cases the delivery was not spontaneous. Three infants were delivered by forceps, two died, and one recovered. Two infants were delivered by caesarean section, one died and one recovered. Three cases were breech deliveries and all died. One was an assisted breech delivery and recovered.

Table 3
FIRST PREGNANCY IN WHICH HAEMOLYTIC DISEASE WAS DIAGNOSED

<table>
<thead>
<tr>
<th>Results</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant survived</td>
<td>4</td>
<td>13</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>Infant died</td>
<td>6</td>
<td>24</td>
<td>16</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>40</td>
<td>34</td>
<td>15</td>
<td>13</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>219*</td>
</tr>
</tbody>
</table>

* 1 pair of twins is included in this table. B.T. Case following transfusion in blood not tested for Rh compatibility.

Investigation of Rh Blood Groups. Serological investigations were carried out on 56 cases. In 50 cases (90%) the usual serological pattern of

Table 4
RESULTS OF ABNORMAL PREGNANCIES IN 16 WOMEN

<table>
<thead>
<tr>
<th>Complication</th>
<th>Stillborn</th>
<th>Died</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydramnios</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Pre-eclamptic toxoaemia</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>9</td>
<td>16</td>
</tr>
</tbody>
</table>
Rh negative mother and Rh positive father and child was present. In five out of the six remaining cases the mother and child were both Rh positive. In one case, the mother was Group 0 Rh negative and the child was Group B Rh negative. The mother's blood was tested for the presence of agglutinins in 13 instances.

Lack of sera prevented the full genotyping of the fathers of affected children, and so the number of results obtained in this series is too small to allow of comment.

**Previous Blood Transfusion in the Mother.** In three cases, Rh negative women had been treated by blood transfusion of unspecified blood after a miscarriage. The following pregnancy the women were delivered of infants suffering from icterus gravis neonatorum. Two of these infants recovered but one developed kernicterus and died.

**Clinical Features of Affected Cases.** Haemolytic disease in the foetus or infant usually takes the form of hydrops foetalis, icterus gravis neonatorum, or haemolytic anaemia. This classification is not rigid and some foetuses with hydrops may be slightly jaundiced, and some of the patients in this series with icterus were oedematous. For the purpose of this survey the 157 patients have been classified according to the type of disease they most closely resembled (Table 5).

<table>
<thead>
<tr>
<th>Analysis of Types of Haemolytic Disease in 157 Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stillborn Infants</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Hydrops foetalis</td>
</tr>
<tr>
<td>Icterus gravis</td>
</tr>
<tr>
<td>neonatorum</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

It will be seen that icterus gravis neonatorum is the most common and haemolytic anaemia the least common manifestation of the disease. This is in accordance with the findings of most workers. It is probable that a number of cases of both haemolytic anaemia and icterus gravis occurring during the earlier period of this survey were not diagnosed as they were clinically mild and serological tests were not available.

**Hydrops Foetalis.** From a study of Table 5 it will be seen that the foetus is usually stillborn (31 cases) and frequently macerated (10 cases). Less commonly a live birth occurs (5 cases), but death usually follows in a matter of hours. As the name implies, there is generalized subcutaneous oedema, the abdomen is distended with fluid, and the skin is pale or macerated. The degree of anaemia is severe. Jaundice is not the rule, but the umbilical cord may be yellow in colour (Fig. 1).

**Icterus Gravis Neonatorum.** Jaundice is the salient feature of this type, and until recently, 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 the vernix caseosa may be golden yellow in colour, or the jaundice may appear within the first 24 hours. It is infrequently delayed beyond 48 hours (Table 6).

<table>
<thead>
<tr>
<th>Time of Onset of Jaundice</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>At birth</td>
</tr>
<tr>
<td>Under 24 hours</td>
</tr>
<tr>
<td>24-48 hours</td>
</tr>
<tr>
<td>Over 48 hours</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

The relationship between the time of onset of jaundice and the outcome is shown in Table 7.

<table>
<thead>
<tr>
<th>Relationship between Time of Onset of Jaundice and Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Recovered</td>
</tr>
<tr>
<td>Died</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

In a proportion of patients with icterus gravis neurological signs were present, possibly the result of the onset of nuclear jaundice (kernicterus).

**Haemolytic Anaemia.** Haemolytic anaemia is the least severe and the least fatal form of the disease. The patient may be anaemic at birth or may suddenly develop a waxy pallor in the first few days of extra-uterine life. There may be a mild and transient jaundice for the first few days of the disease. The anaemia tends to persist for several weeks. In three instances, where the condition had been overlooked in its earlier stages, a fatal outcome resulted.

**Laboratory Investigations**

The cord blood was tested by the Wassermann and Kahn techniques to exclude syphilis. The maternal blood was tested for its reaction with anti-Rh (anti-D) serum and for the presence of agglutinins. In this series only the technique for the demonstration of saline antibodies was available,
More recently the test for the presence of albumin ('blocking') antibodies has come into general use. Wiener et al. (1948) have attempted to correlate the titre of albumin antibodies in the maternal serum with the severity of the disease process in the infant. Mollison and Cutbush (1949), however, do not believe that this correlation is reliable and many exceptions have been observed.

When the mother was Rh negative her serum was tested for the presence of antibodies at the third, seventh, and ninth months. A sudden rise in titre in the later months indicates a poor prognosis (Cummings, 1949).

In the later months of 1947 the direct Coombs test was carried out on the blood of all infants suspected of being affected by haemolytic disease. It was positive in all cases in which a diagnosis of haemolytic disease was finally made, and the strength of the reaction indicated the likely outcome. The reaction was strongest in the cases which terminated fatally. This is similar to the findings of Mollison and Cutbush (1949). It should be remembered, however, that the test is not infallible and a single negative result does not necessarily mean that the infant is not suffering from haemolytic disease. In cases of doubt the test should be repeated.

The blood was examined in all cases of haemolytic disease. In the case of live-born patients a full examination was carried out. Even in the case of the delivery of a macerated stillborn foetus a blood film, stained by the Leishman method, was examined, since the nucleated red cells retain their staining potential long after erythrocytes have become almost unrecognizable.

The findings vary from case to case. Different degrees of anaemia were present in affected patients and the degree of anaemia was not always related to the severity of the disease process. The haemoglobin values varied between 40% and 140% (Sahli). Some patients with icterus gravis and kernicterus had haemoglobin values of 120% and later died, whereas a few patients who were less severely affected and subsequently recovered had initial haemoglobin values of 70% or less. Nevertheless, as a general rule it was found that patients with a low haemoglobin level at birth were less likely to recover than those who had no initial severe anaemia. It is to be remembered that the haemoglobin level of an infant's blood is normally high at birth so that any value below 100% is indicative of anaemia.

The total red cell count also varied over a wide range from 1,000,000/c.mm. to 5,500,000/c.mm. The colour index was usually above unity. The reticulocyte count was high, and in two cases of haemolytic anaemia reached 50%.

When blood films were examined it was found that the red cells showed macrocytes, anisocytosis, and polychromasia. In two cases of haemolytic anaemia Howell-Jolly bodies were present.

Nucleated red cells were usually present in large numbers. There may be late or early normoblasts or erythroblasts. Occasionally haemocytoblasts were found.

Normally at birth nucleated red cells are less than 5% white cells. In haemolytic disease there were as many as 2,000% white cells. In general the most severely affected patients had large numbers of nucleated red cells in their circulating blood, but some exceptions were noted. These nucleated red cells usually disappear from the peripheral blood of normal infants by the third day of life. In haemolytic disease they are much more numerous and may persist for two weeks or more.

The patients affected by icterus gravis had low platelet counts (40-60,000/c.mm.) and the prothrombin index was frequently below 50% of the normal.

Biochemical investigations were carried out in the majority of cases of icterus gravis. It was found that the most useful tests were the icteric index and the estimation of serum (or plasma) bilirubin. The latter replaced the former as it is a more accurate procedure.

The icteric index was markedly increased in
icterus gravis and figures as high as 400 were achieved. When the icteric index rose above 200 the patient seldom survived. A very high icteric index was usually associated with a low haemoglobin level, but the correlation was not so close as in the series reported by Mollison and Cutbush (1949). The icteric index of cerebrospinal fluid ranged from 10 to 20.

The normal serum bilirubin level in newborn infants is 1-2 mg.%. In icterus gravis the range is 2-20 mg.% or even higher. Patients with a serum bilirubin level of over 7 mg.% seldom recover, whereas in those with a serum bilirubin level of 3 mg.% or less recovery is the rule. All patients with icterus gravis who subsequently developed kernicterus had a high serum bilirubin level.

These laboratory investigations can be most helpful in the diagnosis of haemolytic disease, but it is important to remember that no single test is diagnostic. A final diagnosis can sometimes only be reached after a careful study of all available information concerning the patient. In general the most useful information is that derived from the result of the direct Coombs test on the infant’s red cells, the presence of antibodies in the maternal serum, the haemoglobin value of the cord blood, the presence of numerous nucleated red cells in the peripheral blood of the infant, and a rise in the serum bilirubin level of the infant’s blood.

Treatment of Haemolytic Disease

Since the discovery of the Rh blood groups a considerable controversy has arisen regarding the respective merits of Rh positive and Rh negative blood in transfusion therapy and the correct technique by which such blood should be administered to the affected infant. Most clinicians in this country and in America prefer to use Rh negative blood, and in the present series all patients treated by blood transfusion received Rh negative blood when the latter became available. Among the earlier cases 13 patients received blood which was almost certainly Rh positive (e.g. father’s blood); seven recovered and six died.

Thirty-five patients were treated with Rh negative blood; 18 recovered and 17 died. The total amount of blood given to any one infant varied considerably. Some infants died after receiving only 90-100 ml. One infant received 1,100 ml. over a period of a month. The average amount transfused was 250 ml. and generally 15 ml. per lb. body weight was given at one time. The number of transfusions required varied according to the severity of the disease process and to the response of the infant to this form of therapy. A rapid fall in the haemoglobin level of the infant’s blood was taken as a need for further transfusion. If the haemoglobin continued to fall in spite of transfusions the outcome was not likely to be favourable.

In this series the transfusions were simple and direct, and the route used in the earlier cases was via the anterior fontanelle (a method now abandoned) or the saphenous vein at the ankle. In the later cases the umbilical route was used whenever possible. More recently Wallerstein (1947), Diamond (1947), and Mollison (1948) have described a new transfusion technique by which a 90% substitution of the infant’s blood may be effected. This technique had not been adopted during the period under review. Farquhar and Lewis (1949) have described the new technique, and it is now undergoing a period of trial but the results are less satisfactory than had been anticipated.

Mortality in Present Series

Of the 157 patients, 31 were stillborn (20%). Eighty-five (67%) of the remaining 126 live-born children died. The mortality figures analysed according to the type of haemolytic disease are shown in Table 8.

<table>
<thead>
<tr>
<th>Type of Disease</th>
<th>Died</th>
<th>Recovered</th>
<th>Total</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Icterus gravis</td>
<td>77</td>
<td>33</td>
<td>110</td>
<td>70</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>8</td>
<td>11</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>41</td>
<td>126</td>
<td>67</td>
</tr>
</tbody>
</table>

It must be emphasized that the majority of cases belonged to what may be termed the 'pre-Rh era,' and 78 of the 126 live-born cases were untreated. Of the 48 cases treated by blood transfusion, 23 died (48%). The newer methods of diagnosis and treatment were not in use during the period under review and time alone will tell whether they have reduced the mortality by any considerable degree.

Post-mortem Findings

Post-mortem examinations were carried out in 104 of the 116 cases of intra-uterine or extra-uterine death in this series.

Hydrops Foetalis. There were 36 cases of hydrops foetalis. Ten of these were macerated and five were live-born. Of these five only one lived for 24 hours. Eight were full-term foetuses and 28 were delivered prematurely. The average period of gestation of the latter was 33 weeks. Twelve were males and 19 were females. There was no record of the sex of the remaining five cases. Generalized subcutaneous oedema was one of the most constant features. It was less constant in the macerated foetus since there is a tendency for the foetus to dry in utero if birth is delayed. Hydrothorax, hydropericardium, and ascites were common. The fluid was generally clear and straw coloured in the stillborn and live-born, and blood-stained in the macerated
foetus. Petechial haemorrhages were found in the skin over the trunk, limbs, or face, and over the thymus, lungs, and heart. Occasionally subependymal petechiae led to intraventricular haemorrhage. Jaundice was not usually present, but in two cases there was a slight yellow tint of the skin and mucous membranes.

Icterus Gravis. There were 77 cases of icterus gravis and 35 of these showed kernicterus. Gilmour (1944) reported a predominance of four to three in favour of males. In this series there were 48 males and 29 females. Of these 59 were mature and 18 premaures. Naturally the main feature was jaundice. The skin and mucous membranes were a bright golden-yellow colour and many of the infants had been covered by a golden-yellow 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 lesion. All the cases with kernicterus were deeply jaundiced, but many equally jaundiced patients had not suffered from intracerebral lesions. Petechial haemorrhages into and under the pleura or pericardium were commonly found. Subcutaneous oedema was rare. The serous sacs sometimes contained bile-stained fluid, but the quantities were less than is usual in foetal hydrops.

Haemolytic Anaemia. There were three cases of haemolytic anaemia. Two were males, the third was a female, and all three were mature. One of the patients was slightly jaundiced, the others were not. There was slight subcutaneous oedema but no petechial haemorrhages were found. Nucleated red cells were numerous in the peripheral blood, and in one case numbered 200,000 c.mm. All types of red cell, from the pro-erythroblast to the mature erythrocyte, were found. Numerous reticulocytes were present and the erythrocytes showed anisocytosis and polychromasia.

The Organs in Haemolytic Disease. The liver was usually enlarged and reddish-brown in colour in all types of haemolytic disease. In a few cases it was too tough to cut, and in the severely macerated foetuses it was soft and diffusent. The Prussian blue reaction was usually positive. It was positive in all three cases of haemolytic anaemia. In icterus gravis the liver frequently showed some greenish discoloration as a result of bile-staining. No true examples of cirrhosis were found. On histological examination there was a marked amount of extramedullary haemopoiesis (Fig. 2). Foci of normoblasts and erythroblasts obscured the parenchyma in many cases. In hydrops foetalis the liver cells showed varying degrees of degeneration and had lost their structure in the macerated cases. In many cases there was a fine intercellular fibrosis and the cells in the central zones of the lobules showed early necrosis. In most instances the fibrosis was much less marked than may be found in congenital syphilis, but in two cases it was widespread and resembled cirrhosis of the liver in the macerated foetus described by Henderson (1942). In a few of the patients who had died from the effects of icterus gravis after the first week of life a fine diffuse fibrosis was found. In one case, the patient having died at the age of seven weeks, there was considerable perportal fibrosis with numerous new bile-ducts in the fibrous stroma. Plugs of bile in the intercellular canaliculi were not observed in hydrops foetalis and in haemolytic anaemia. In icterus gravis, however, the intercellular canaliculi were usually plugged with bile (Fig. 3). Haemosiderosis was present in nearly every case of hydrops foetalis (Fig. 4), in many cases of icterus gravis, and in all cases of haemolytic anaemia. The parenchymal cells and Kupffer cells in these cases contained the characteristic brown granules of haemosiderin, and the Prussian blue reaction was positive. The portal tracts showed numerous collections of haemopoietic cells. Normoblasts, erythroblasts, and eosinophil leucocytes were especially common, but myelocytes and lymphocytes were frequently observed.

The spleen was almost invariably enlarged and sometimes grossly so. The latter was especially true of the cases of haemolytic anaemia. The spleen was rather soft in hydrops foetalis, but firm in icterus gravis and haemolytic anaemia. The pulp was dark red in colour. The Prussian blue reaction was usually positive. In one case of icterus gravis the spleen had ruptured causing a massive fatal intraperitoneal haemorrhage. In one case of haemolytic anaemia there was a small superficial rupture of the spleen with extravasation of blood into the abdominal cavity. Microscopically, the capsule and trabeculae showed little change. The pulp was congested and numerous foci of haemopoiesis were present. The Malpighian bodies were usually absent in hydrops foetalis (Fig. 5), and poorly developed in the other types. Free haemosiderin was found in the subcapsular region or in histiocytes throughout the pulp.

The kidneys were often pale or macerated in hydrops foetalis, and bile-stained in icterus gravis. In one case of icterus gravis there was pyelonephritis. In a few cases histological examination showed that the lining epithelium of the convoluted tubes contained haemosiderin and the Prussian blue reaction was positive. Extramedullary haemopoiesis was less pronounced than in the liver or spleen. It was observed in the boundary zone of the cortex and in the perivascular connective tissue in the renal pelvis.

In approximately 60% of cases of hydrops foetalis the suprarenal glands showed a light yellow streaking of the inner cortex similar to that described by Gilmour (1944). Histologically, the cells in this region were vacuolated and distended with lipid (Fig. 6). This change is not found in icterus gravis or haemolytic anaemia. A few foci of haemopoiesis may be found in the suprarenal cortex in all three types.

The lungs were collapsed in hydrops foetalis as a result of the hydrothorax. Subpleural petechiae
FIG. 2.—Liver $3 \times 75$. Haematoxylin and eosin. Extramedullary haemopoiesis.

FIG. 3.—Liver $\times 375$. Haematoxylin and eosin. Plugs of bile in intercellular canaliculi. Case of icterus gravis.

FIG. 4.—Liver $\times 80$. Prussian blue. Deposits of haemosiderin in parenchymal and Kupffer cells.

FIG. 5.—Spleen $\times 85$. Haematoxylin and eosin. Marked haemopoiesis. Note absence of Malpighian bodies. Case of hydrops foetalis.

FIG. 6.—Suprarenal gland $\times 80$. Haematoxylin and eosin. Vacuolation of cells of the cortex. Case of hydrops foetalis.

FIG. 7.—Heart $\times 400$. Haematoxylin and eosin. Pronounced oedema of myocardial fibres. Case of hydrops foetalis.
were commonly found. In icterus gravis the lungs were occasionally the seat of massive haemorrhages. This occurrence was less frequent than might be expected. When present all the lobes may be firmly consolidated and dark red in colour. Large quantities of blood can be expressed from the cut surface. In a few instances it was complicated by a superadded bronchopneumonia. In haemolytic anaemia the lungs were pale and oedematous and areas of bronchopneumonic consolidation were present in two cases.

The heart was greatly enlarged in 12 cases of hydrops foetalis. This was not observed in icterus gravis or haemolytic anaemia. In the affected cases the myocardium was pale and the apparent hypertrophy was the result of oedema (Fig. 7).

The pancreas showed no change on macroscopic examination. Histological examination revealed the presence of a few foci of haemopoiesis in the perilobular connective tissue. In one case of hydrops foetalis the whole of the pancreatic stroma showed dense collections of nucleated red cells and eosinophil leucocytes. Large islets of Langerhans, as described by Gilmour (1944), were not a prominent feature in the hydrops foetalis cases in this series. They are more commonly found in infants whose mothers are diabetic or in the pre-diabetic phase.

Foci of haemopoiesis were frequently found in the thymus and thyroid glands.

Intracerebral lesions were uncommon in hydrops foetalis. In a few cases subependymal haemorrhages had ruptured into the lateral ventricles causing considerable damage. The basal ganglia showed no change. In icterus gravis diffuse bile-staining of the leptomeninges and cerebral cortex was frequently found. Kernicterus was present in 35 cases. Intracerebral damage was not present in haemolytic anaemia.

In two cases of hydrops foetalis the ossifying zone in the epiphyseal lines of the long bones was deeper than usual and rather yellow in colour. One case showed an abnormally broad layer of unossified cartilage in the epiphyseal region. The abnormalities of the bony trabeculae described by Gilmour (1944) were not observed.

The bone marrow was highly reactive and nucleated red cells were extremely abundant in all types of haemolytic disease. All stages of development from the haemocytoblast to the erythrocyte were seen. There was an increase in the number of eosinophil leucocytes.

The placenta in hydrops foetalis was enlarged, pale, and oedematous. The weight of this organ was greatly increased and frequently approximated to half the body weight of the affected infant. The villi were large, swollen, and oedematous. Foci of haemopoiesis were rarely found but the foetal capillaries contained large numbers of nucleated red cells. The stroma cells were enlarged, vacuolated, but not increased in number. Occasionally the syncytial cells were prominent.

Discussion

There have been great advances in knowledge concerning the development of haemolytic disease within the last 20 years. Before this the individual types of haemolytic disease were not recognized as belonging to a single disease complex. Diamond, Blackfan, and Baty (1932) assembled the three types, hydrops foetalis, icterus gravis, and haemolytic anaemia, under the heading 'erythroblastosis foetalis.' The work of Parsons et al. (1933) concerning what they termed the 'erythronoclastic anaemias,' served to demonstrate the importance of red cell destruction as a factor in the development of haemolytic disease. With the discovery of the Rh factor by Landsteiner and Wiener (1940) and of the aetiological importance of the Rh blood groups by Levine et al. (1941), the development of haemolytic disease in the foetus was more clearly understood.

The iso-immunization of the mother by a Rh positive child or by transfusion with Rh positive blood has now been clearly established. New serological methods of diagnosis have been introduced and transfusion of the infant with Rh negative blood has become the recognized method of treating affected subjects. In spite of these advances, however, there is still quite a high mortality. The figures obtained in this series are not truly representative. Nevertheless, the mortality is considerable and many cases come to post-mortem examination. It is for this reason that the pathological features of haemolytic disease have been described in some detail. Mistakes in serological diagnosis occasionally occur and the pathologist may be asked to confirm or refute a diagnosis of haemolytic disease.

Hydrops foetalis resulting from 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 erythroblastemia are found in both diseases. The Wassermann and Kahn tests to exclude syphilis, and the Coombs test to detect sensitization, have helped to distinguish the two diseases. A full post-mortem examination should be carried out in all doubtful cases. For example, I performed a post-mortem examination on an infant suspected of having haemolytic disease. Wassermann and Kahn tests had not been carried out, and the mother was Rh negative. The liver and spleen were enlarged and the former showed a marked degree of haemopoiesis of a primitive type. No spirochaetes were found in either organ. The pancreas,
however, showed well-marked fibrosis of a syphilitic type.

It is in the idiopathic form of hydrops foetalis that a full necropsy is most essential. Potter (1946) correctly pointed out that the diagnosis of hydrops resulting from haemolytic disease must be made only after very careful histological examination of the tissues. The condition of the placenta is also a valuable aid in correct diagnosis. Hydrops foetalis of the idiopathic type is of obscure origin. It occurs not infrequently in first pregnancies and may be associated with congenital malformations such as spina bifida or diaphragmatic hernia. These hydropic foetuses are nearly always followed by normal children. They show no evidence of erythroblastosis on post-mortem examination. Such a careful examination saves the parents much needless anxiety.

Wiener and Sonn (1946) have described an instance where a stillbirth was thought to have been caused by haemolytic disease but further examination revealed diabetes mellitus in the mother. Examination of the pancreas in the offspring of diabetic and pre-diabetic mothers will reveal the presence of giant islets of Langerhans.

Icterus gravis neonatorum must be distinguished from physiological jaundice of the newborn, congenital atresia of the bile ducts, congenital syphilis, and neonatal sepsis. The problem of differentiating between the various types of jaundice in the newborn is now a familiar one.

Neonatal sepsis was regarded as a cause of haemolytic disease before the Rh blood groups were recognized. In a case of neonatal infection jaundice may appear after a few days and anaemia may develop. Nucleated red cells are to be found in the peripheral blood. Neonatal sepsis may complicate an existing haemolytic disease in which case the serological investigations will be positive. This is the probable explanation of the cases described by de Bruyne and van Creveld (1948).

Haemolytic anaemia must be distinguished from haemorrhagic disease of the newborn and other blood diseases of the neonatal period. Haemorrhagic disease of the newborn is a result of hypoprotrombinaemia and reacts well to vitamin K therapy. There is neither enlargement of liver and spleen nor jaundice. The degree of anaemia is seldom severe. Primary blood diseases, such as aplastic anaemia and leukaemia, are rare in the neonatal period. Many cases at this stage of life formerly diagnosed as leukaemia were probably haemolytic disease.

Modern serological tests have enabled a correct diagnosis to be made to-day more often. This is especially true of the milder examples of haemolytic disease which may have been overlooked before. More rapid methods of diagnosis naturally lead to the institution of specific therapy under more favourable conditions. The mainstay of therapy in haemolytic disease is now Rh negative blood transfusion. This should be given as early as possible in severe cases. The amount of blood used should be adequate, but transfusions should not be repeated more frequently than is absolutely necessary. The technique is a matter of individual preference. The exchange-replacement transfusion technique is the more formidable procedure and is mainly suitable for use in well-equipped institutions. Unfortunately, transfusion therapy has little effect in hydrops foetalis. Whether the new techniques will redress the balance is a matter for further investigation. In icterus gravis the most favourable results follow the early institution of transfusion therapy. Haemolytic anaemia is the mildest form of the disease and may require no specific treatment, but these patients should be carefully watched lest a sudden fall in haemoglobin occur when prompt and adequate blood transfusion should suffice.

With regard to prognosis in haemolytic disease, it is important to consider the progress of both mother and infant. The mother is suffering from a state of sensitization to a blood antigen she herself does not possess, and the clinician must give careful advice about future pregnancies. The infant is affected by a disease process which may vary considerably in intensity. Potter (1948) and many others have stated that haemolytic disease is rare in the first pregnancy and when it occurs is likely to prove mild in character, provided the mother has not previously received Rh incompatible blood. In the present series this was not so clearly demonstrated. Ten of the 149 women were pregnant for the first time. Three of the offspring were stillborn exhibiting hydrops foetalis, five died from icterus gravis, and only two recovered. It is reasonable to suppose that women who bear offspring suffering from haemolytic disease in their first pregnancy are unduly susceptible to sensitization by a foreign blood antigen and thus the effect 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. It was found in the present series that the maximum incidence of haemolytic disease occurred in the offspring of the second and third pregnancies and so the maximum number of recoveries will also be found in this period. Stillbirths with hydrops foetalis are, however, proportionally more common
in later pregnancies. Whether the mother who has borne one child exhibiting haemolytic disease is likely to have a normal infant in a subsequent pregnancy is a complex question. If the father is homozygous Rh positive all the offspring will be Rh positive and liable to suffer from haemolytic disease. If, on the other hand, he is heterozygous Rh positive there is an even chance of future children escaping haemolytic disease. It must be remembered that genotyping is by no means absolutely accurate owing to the lack of rarer sera, and also errors in typing the father’s blood are not uncommon.

A study of the cases in this series shows that certain 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 stillborn hydropic foetuses will continue to produce such offspring in subsequent pregnancies. Such a mother should be advised against further pregnancies, and if one occurs a therapeutic abortion should be considered. 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 only mildly affected. One patient in this series had one infant suffering from severe icterus who subsequently died; a second infant also had icterus gravis with Kernicterus and subsequently died; the third was only very mildly affected and recovered. Thus, considerable care must be taken in venturing a prognosis.

One final danger to the mother must be mentioned. It is absolutely essential to ensure that any blood given in the treatment of severe post-partum or other haemorrhage is of the correct Rh type. If an Rh negative woman, who is sensitized to Rh antigen, is transfused with blood which is not Rh negative the result may well be disastrous.

The future outlook in affected offspring depends on the variety of haemolytic disease encountered. In hydrops foetalis the outlook is grave, whereas in haemolytic anaemia it is good. In icterus gravis the prognosis depends on the severity and on the success of transfusion therapy. In general, a very severe degree of jaundice at birth, a low haemoglobin level in the cord blood, and a high serum bilirubin level are signs of a poor chance of recovery. Patients with evidence of neurological involvement seldom survive.

Each patient, both mother and child, requires careful study and no set of rules can be devised to meet all contingencies.

Summary

This study has dealt with the features presented by 157 cases of haemolytic disease in the newborn, including 36 examples of hydrops foetalis, 110 of icterus gravis, and 11 of haemolytic anaemia. The pathological changes found in all forms of the disease have been noted. The obstetrical histories of the mothers of the affected infants have been investigated and the various factors of prognostic significance examined. The clinical features of the affected patients and the criteria of severity were similar to those reported by other workers. An unusual feature was the severity of the disease process in ten women who were pregnant for the first time.

References

Part I


PART II: NUCLEAR JAUNDICE (KERNIKTHERUS)

The first description of this unfortunate sequel to haemolytic disease was that by Orth (1875) who used the term nuclear jaundice. Schmorl (1903) introduced the word Kernicterus in his description which was the first detailed report of the pathological features of the cerebral lesions.

It soon became obvious that kernicterus was found in close association with icterus gravis neonatorum. The familial aspect of the condition was dealt with in the earlier literature (Beneke, 1907; Pfannenstiel, 1908; Esch, 1908; Pfältzer, 1914; Ylppo, 1918; Thorling, 1922; Heiligenberg, 1925). Spiller (1915) reported three cases of mental deficiency in which severe jaundice had occurred during the neonatal period. Occasional instances of kernicterus were reported by various authors (Hart, 1917; Palm, 1919; Paul, 1924; de Lange, 1925; Hoffmann and Hausmann, 1926; Greenwald and Messer, 1927; Diamond and van Creveld, 1937; Westrienen and de Lange, 1937). Detailed reports of the histological findings in kernicterus were given by Zimmerman and Yannet (1933; 1935) and by Fitzgerald, Greenfield, and Kounine (1939). Up to this date the association of kernicterus with icterus gravis had been generally recognized. With the discovery of the Rh blood groups a new approach to the problem was instituted. Recent reviews of the subject are those of Docter (1945), Vaughan (1946), Stiller (1947), and Lande (1948).

In spite of the great interest which has been taken in these cases many problems concerning the development of kernicterus have remained unsolved. One of these problems is the time of onset of nuclear jaundice, and this will be specially considered.

Clinical Features of Kernicterus

Of the 110 patients affected by icterus gravis in this series, 37 showed kernicterus (33.6%). The condition may be considered in two stages, the acute stage, which is the stage most frequently seen and occurs at the height of jaundice in the early neonatal period, and the chronic stage which follows the acute stage in the few patients who survive beyond the first week of extra-uterine life.

Acute Stage. This is the stage of acute irritation of the cerebral ganglia. The infants show evidence of severe cerebral irritation, with clonic and tonic movements going on to convulsions, hypertonia, and opisthotonos. 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 steadily weaker and the majority die before the fifth day of extra-uterine life.

In this series 28 of the patients (76%) died before the second week of life. They become jaundiced shortly after birth (or were jaundiced at birth) and the jaundice steadily deepened. The liver and spleen were palpable. Evidence of cerebral irritation soon became manifest and twitching of the limbs, convulsions, and opisthotonos were noted.

Examination of the peripheral blood revealed the presence of large numbers of nucleated red cells and the serum bilirubin level was 10-20 mg. %. The degree of anaemia was variable. One patient had a haemoglobin level of 70% (Sahli) whereas another had a haemoglobin level of 120%. All ranges between these extremes were noted. In spite of treatment the infants collapsed and died. A further four infants died in the second week of life, two at nine days, and two at twelve days. One other infant died on the fourteenth day. All showed clear signs of neurological involvement. Thus only four infants survived beyond the acute or early chronic stage.

Chronic Stage. After the first week of extra-uterine life, should the infant survive so long, irreversible change occurs in the basal ganglia and the other cerebral nuclei. Clinical evidence of derangement of the extrapyramidal system is now more 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, they take on an added significance. Lande (1948) divided her patients into four clinically distinct groups:

Group 1: Patients exhibiting choreo-atheosis as a result of involvement of the corpus striatum and globus pallidus.

Group 2: Patients showing persistent spasticity from the involvement of the pyramidal and extrapyramidal systems.

Group 3: Patients showing ataxia and disturbance of balance.

Group 4: Patients in whom atonic diplegia is the predominant sign.

As that author points out, many 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. These have also been noted by Zimmerman and Yannet (1935). Recently kernicterus has been cited as a cause of infantile cerebral palsy (Evans, 1948).

Mental retardation may be a prominent feature among patients in this stage, and it is difficult to assess how much this retardation is due to cerebral damage and how much to the physical incapacity which the disease promotes. The patients with severe choreo-athetosis, for example, 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 or even hold their head up at one year of age. Walking may be delayed up to the sixth year or longer (Lande, 1948). Feeding themselves is a difficulty and training in toilet habits is prolonged. In the present series only four patients survived to this stage. One patient improved considerably but had marked choreo-athetosis. Another child is also still alive at the time of writing. He has learned to walk at four years, though with some difficulty, and there is no evidence of mental impairment. The chief neurological feature is hypotonia rather than spasticity. The remaining two patients died at the age of 4 months and 17 months respectively. The former developed jaundice 24 hours after birth and exhibited head retraction and opisthotonos on the fourth 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. She died at the age of four months as a result of acute bronchitis. The last child 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 with icterus gravis before the onset of kernicterus.

**Time of Onset of Jaundice**

As will be seen from Table 1, jaundice was present in the great majority of patients by the end of the first day and was present at birth in 14 out of 37 patients.

<table>
<thead>
<tr>
<th>Time of Onset of Jaundice</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present at birth</td>
<td>14</td>
</tr>
<tr>
<td>Present within 6 hours after birth</td>
<td>2</td>
</tr>
<tr>
<td>Present within 6-12 hours after birth</td>
<td>5</td>
</tr>
<tr>
<td>Present within 12-24 hours after birth</td>
<td>13</td>
</tr>
<tr>
<td>Present within 24-48 hours after birth</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
</tr>
</tbody>
</table>

**Rhesus Blood Groups**

Serological investigations were carried out on 15 of the 37 patients. In 13 of these 15 patients the usual pattern of Rh negative mother and Rh positive father and child was obtained. Five of the 13 Rh negative mothers had antibodies in their serum.

**Age at Death in Fatal Cases**

The age of the infant when death supervened is shown in Table 2. 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 2**

<table>
<thead>
<tr>
<th>Time of Death</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 hours after birth</td>
<td>1</td>
</tr>
<tr>
<td>36 hours after birth</td>
<td>4</td>
</tr>
<tr>
<td>2 days after birth</td>
<td>6</td>
</tr>
<tr>
<td>3 days after birth</td>
<td>8</td>
</tr>
<tr>
<td>4 days after birth</td>
<td>1</td>
</tr>
<tr>
<td>5 days after birth</td>
<td>3</td>
</tr>
<tr>
<td>6 days after birth</td>
<td>1</td>
</tr>
<tr>
<td>7 days after birth</td>
<td>4</td>
</tr>
<tr>
<td>9 days after birth</td>
<td>2</td>
</tr>
<tr>
<td>12 days after birth</td>
<td>2</td>
</tr>
<tr>
<td>14 days after birth</td>
<td>1</td>
</tr>
<tr>
<td>4 months after birth</td>
<td>1</td>
</tr>
<tr>
<td>17 months after birth</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
</tr>
</tbody>
</table>

**Cause of Death in Fatal Cases**

Most of the patients died from the systemic upset associated with icterus gravis and aggravated by kernicterus. Complications such as intrapulmonary haemorrhage, pneumonia, and meningitis may also occur, and a summary of the cause of death in the 35 cases is shown in Table 3.

**Table 3**

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>General effects of icterus gravis</td>
<td>23</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2</td>
</tr>
<tr>
<td>Intrapulmonary haemorrhage</td>
<td>2</td>
</tr>
<tr>
<td>Subarachnoid haemorrhage</td>
<td>2</td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td>3</td>
</tr>
<tr>
<td>Acute leptomenigitis</td>
<td>1</td>
</tr>
<tr>
<td>Acute bronchitis</td>
<td>1</td>
</tr>
<tr>
<td>Late effects of kernicterus</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
</tr>
</tbody>
</table>

Vaughan (1946) stresses the importance of severe respiratory distress resulting from intrapulmonary haemorrhage as a factor in the sudden collapse. As may be seen from Table 3 only two patients suffered from this complication. Hawksley and Lightwood (1934) and Mollison and Cutbush (1949) are inclined to the view that medullary failure plays a part in the early fatal outcome.

**Treatment of Patients with Kernicterus**

Vaughan (1946) remarks that the only benefit from blood transfusion to patients with kernicterus is that resulting from the replacement of cells in those who are severely anaemic, and, as severe anaemia was not invariably present, such therapy had disappointing results.
In the present series the mortality was high. Thirty-three of the 37 patients died within a fortnight of birth (90%). Only two survive at the time of writing. One received a total of 265 ml. of Rh negative blood and the other was given blood of unknown Rh group. One other patient received a transfusion of blood whose Rh group was unspecified, but died. Five patients received transfusions of Rh negative blood and all died. The remaining 29 patients received no blood transfusions and all died. It is obvious that too small a number were treated by blood transfusion to permit of comment.

Vaughan (1946) found that no case of kernicterus occurred when infants with icterus gravis were transfused on the first day of life with Rh positive blood. The majority of workers, however, do not recommend the use of Rh positive blood in the treatment of haemolytic disease.

Blood transfusion therapy has obviously no place in the treatment of the chronic case as irreparable 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.

**Pathological Features of Kernicterus**

**Morbid Anatomy.** The morbid anatomy of what may be termed the chronic stage of kernicterus has been described in the papers of Zimmerman and Yannet (1935) and of Fitzgerald et al. (1939). The anatomical features of the acute stage are well known but have received less study.

In this series the anatomical findings in the 35 fatal cases will be described. Of these 35 patients, 23 were males and 12 females. (Litchfield, 1945, suggested that the lesion was less common in the female.) Twenty-five were full-term infants and only ten were premature. Kernicterus was found in two sets of twins, each twin being affected, and in two other pairs, one twin only was affected. These twins account for five of the eleven premature infants.

In the acute stage the infants are very jaundiced and a few subcutaneous petechiae may be present. One infant was also very oedematous.

The liver was usually moderately enlarged and brownish red in colour. The Prussian blue reaction was positive. The spleen was greatly enlarged in 26 cases and moderately enlarged in a further six cases. In three cases it was of normal size. The splenic pulp was dark red in colour, and the Prussian blue reaction was frequently positive. The kidneys were of normal size and were frequently bile-stained. The lungs showed intrapulmonary haemorrhage in two cases and in another two cases bronchopneumonia had developed. The leptomeninges were intensely bile-stained. The brain was usually found to be diffusely bile-stained and pale yellow in colour. On section certain nuclei were intensely bile-stained and were bright orange-yellow in colour. The nuclei liable to be affected were the corpus striatum, thalamus, corpus Luysii, globus pallidus, cornu ammonis, putamen, corpora mamillaria, subthalamic nuclei, hippocampus, third nerve nuclei, the nuclei in the floor of the fourth ventricle, the inferior olives, the grey matter of the medulla, the dentate nuclei, and the flocculus of the cerebellum. The anterior and posterior horns of the spinal cord were also frequently involved. These nuclear areas were not necessarily all affected in any one case. The cornu ammonis, the basal ganglia, inferior olives, and dentate nuclei were usually stained. In the great majority of cases the inferior olivary nucleus was bright orange-yellow in colour and had a more striking appearance than that shown by the basal ganglia. On rare occasions the white matter around the posterior horns of the lateral ventricles was also deeply stained by bile. This latter feature was observed in one case. The other organs of the body showed no pathological change.

Two patients in this series survived beyond the neonatal period. One died at the age of four months as a result of acute bronchitis. At post-mortem examination there was evidence of acute bronchitis and pulmonary congestion. There was no bile-staining of any part. The second patient died at the age of 17 months. No satisfactory cause of death was found at necropsy and none of the organs showed any pathological change. In neither patient did the brain show any abnormality on external examination. On section the only change found was in the cornu ammonis. This was rather smaller than usual.

**Morbid Histology.** There were many foci of extramedullary haemopoiesis in the liver in just over half the cases and fairly numerous foci were found in the remainder (Fig. 1). Many of the intercellular canaliculi were plugged with bile and the liver cells contained bile pigment. More occasionally the parenchymal cells contained haemosiderin and the Prussian blue reaction was positive. In the older infants there was slight fatty degeneration or early necrosis of cells in the central zones of the lobules and a slight increase in the perportal fibrous tissue. There was no cirrhosis in any case. Haemopoiesis was not a marked feature in patients who had died after the first week (Fig. 2). In the patients who had died in the chronic stage the liver was perfectly normal.

Numerous foci of extramedullary haemopoiesis were present in the spleen in the acute cases. Numerous histiocytes in the pulp, especially in the subcapsular region, contained haemosiderin, and the Prussian blue reaction was positive (Fig. 3). No fibrosis had occurred.

The cells lining the convoluted tubules contained bile-pigment in a few cases. In some others there were foci of haemopoiesis in the boundary zone of the cortex and in the peripelvic connective tissue.
Numerous foci of haemopoiesis. Case of icterus gravis showing kernicterus (acute stage).

Only a few foci of normoblasts present. Case of icterus gravis showing kernicterus (early chronic stage).

Extensive haemosiderosis. Kernicterus (acute stage).

Ganglion cells are vacuolated. Case of kernicterus (early chronic stage).

Neuronal loss in dentate nucleus and astrocytic replacement. Kernicterus (chronic stage).

Neuronal loss and astrocytic replacement. Kernicterus (chronic stage).
Intrapulmonary haemorrhage was severe in two cases and very slight in a third. The interstitial tissue and alveoli were flooded with fresh blood. Pneumonia was present in two cases.

The definition of the acute stage includes those patients who died before the end of the first week of extra-uterine life. Histological examination of the brain in these cases failed to reveal any features which differed from those of normal brains in infants of the same age. Most of the sections examined showed no structural change whatsoever. A few showed chromatolysis of the neurones and a few ganglia showed cell loss, but these changes are common necropsy findings in children of this age. Two cases showed yellow staining of the cells in the cornu ammonis, but the cells were not otherwise altered. In one case an acute leptomeningitis and ventriculitis masked the histological appearance.

The early chronic stage corresponds to the clinical stage when signs of extrapyramidal involvement are more striking features than generalized cerebral irritation. It occurs about the end of the first week of life. The areas which were seen to be pigmented on macroscopical examination now showed some loosening up of the cerebral tissue and the formation of globules of lipoid (Fig. 4). Slight astrocytic reaction may be present. It is interesting to note that Zimmerman and Yannet (1933) examined the brain of a child with kernicterus who had died aged 11 days. Even at this age, they stated 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.

There are two patients in this series who died in the chronic stage. In the first, who died at the age of four months, there was severe cell loss and astrocytic replacement in the dentate gyrus. There was loss of large cells and astrocyte replacement in the globus pallidus. Focal neuronal loss had occurred in the dentate nuclei (Fig. 5). In the putamen, inferior olives, and third nerve nucleus there were small foci of polymorphonuclear leucocytes, lymphocytes, and microglial cells.

In the second patient (died at the age of 17 months) the spinal cord, medulla, frontal, parietal and occipital cortex showed no abnormality. In the basal ganglia there were focal areas of nerve cell loss with a marked reparative gliosis (Fig. 6). In the cornu ammonis there was complete disappearance of the nerve cells of the fascia dentata and of the terminal nerve cells of the subiculum. There was an increase of astrocytes in this region.

It is interesting to compare these findings with those of Zimmerman and Yannet (1935). The changes in their case were even more widespread. The optic nerves, reticulo-spinal, rubrospinal, and spino-cerebellar tracts showed demyelination. On the other hand, 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 found in gross sections, no microscopic abnormality whatsoever was encountered.' This is not surprising because nearly all his fatal cases were patients dying in the acute stage when no irreversible histological change occurs.

Discussion

There were 37 patients showing evidence of kernicterus in a total of 110 patients suffering from icterus gravis, an incidence of 33%. Of these 110 jaundiced patients, 47 died within the first week of life and, of these 47 patients, 31 were found to have kernicterus at post-mortem examination.

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 might be expected in 40% of cases of icterus gravis in the neonatal period and among 12% of the survivors. His figures and those of 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%).

Kernicterus does not occur in hydrops foetalis, even when these foetuses are jaundiced. It is not seen in patients with icterus gravis who die within an hour or two of birth. The earliest death in a child showing kernicterus occurred 18 hours after birth and the jaundice was first noted six hours after birth. The kernicterus therefore probably developed within the first 12 hours after birth. Parsons (1947) also reported a patient with kernicterus who died 18 hours after birth. The nuclear staining, while causing intense cerebral irritation, does not appear to cause immediate irreversible damage to the affected cells. On the contrary, at necropsy, the affected cells may be bile-stained but not structurally altered. This lack of histological change may persist as late as the sixth to eighth day of extra-uterine life or slightly later. At the end of this period irreversible cellular damage occurs with neuronal destruction and gradual astrocytic replacement. The cornu ammonis is the first to be so affected, and the optic nerves and grey matter of the spinal cord are among the last to undergo such structural alteration. Thus in the acute stage of the disease the presence of bilirubin in the cells causes intense cerebral irritation which leads to tonic and clonic movements and opisthotonos. These severe spasms are broken by periods of lethargy and drowsiness. In the early chronic stage the corpus
striatum shows the most severe cytological change and this leads to the development of choreoathetosis which is such a well-marked feature of the condition. In the later chronic stage the changes are widespread and affect structures outside the basal ganglia or even the extrapyramidal system. Lande (1948) has described patients with deafness, blindness, strabismus, and cranial nerve palsies. Such symptoms are not surprising in view of the histological findings in the case of Zimmerman and Yannet (1935) and of the changes present in the patient in this series who died at the age of four 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, in addition to evidence of lesions in the basal ganglia and extrapyramidal system. The condition affects not only the latter structures but is widely present throughout the nuclear aggregations of cerebrum, brain stem, cerebellum, and grey matter of the spinal cord.

Even at the beginning of this century it became recognized that kernicterus was almost always associated with icterus gravis neonatorum wherever 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 atresia of the bile ducts. The author makes it quite clear, however, that the patient was also affected by haemolytic disease and he stresses the latter, rather than the former, as the primary aetiological factor in the development of the nuclear jaundice.

At one time neonatal sepsis was believed to be an aetiological factor in the development of icterus gravis and of kernicterus. Beneke (1907) believed that organisms entered the blood stream via erosions (stigmata) in the gastric mucous membrane. Knoepfelmacher (1910), Pfältzer (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. Pfältzer (1914) even went so far as to name Bact. coli as the causative organism. Dunham (1933) found jaundice in 14 instances out of 40 cases of neonatal sepsis. Zimmerman and Yannet (1933) obtained Bact. coli from the blood in Case 2 and Case 3 of this series. They were inclined to favour the sepsis hypothesis for the development of nuclear jaundice. Biemond and van Crevel (1937) reported two cases of kernicterus and icterus gravis where severe umbilical sepsis was also present. Since the discovery of the Rh blood groups the influence of sepsis in the aetiology of icterus gravis and kernicterus has received less attention. Recently, however, a further series of four cases of kernicterus complicating icterus gravis has been reported by de Bruyne and van Crevel (1948). In all these cases severe umbilical sepsis was present. Cases 1 and 2 of their new series were siblings of the earlier patients of van Crevel (1937) and evidence of iso-immunization to Rh antigens was now present in both mothers. The mother of Case 3 also had anti-Rh agglutinations in her serum. The mother of Case 4 was Rh positive but her serum had an anti-A agglutination titre of 1/512. These serological findings throw some doubt on the validity of the infective hypothesis. Sepsis played no part in the development of kernicterus in the patients in my series.

Orth (1875) believed that there was a primary necrosis of parts of the brain and that these areas were subsequently pigmented. Schmorl (1903) suggested that the primary change was a cell necrosis either resulting from vascular damage or from a toxic degeneration of the cell. He believed that the toxin responsible might be bile itself. Hart (1917) also thought that the ganglion cells were injured by bile or became pigmented with the latter following injury by some unknown toxin. 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 changes in the nervous system.' The link between the liver upset and the brain injury had already attracted the attention of workers in this field. Hoffmann 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 such an experiment and found an inflammatory reaction in the basal ganglia with neuronal loss. The damage was diffuse, however, and not wholly 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. While these experiments failed to reproduce kernicterus they did seem to indicate a link between hepatic dysfunction and cerebral damage.

As an alternative to the theory that kernicterus resulted from the action of a toxin on the basal ganglia, Schmorl (1903) and Beneke (1907) both suggested that vascular damage caused by thrombosis or other change led to ischaemia of the nuclear masses and subsequently to pigmentation. Thorling (1922) also thought that ischaemia played a part and suggested a combination of low blood pressure and respiratory failure as the underlying cause. 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 disease affecting the latter. Meyer (1936) showed that the globus pallidus, inferior olives, dentate nuclei of the cerebellum, and cornu ammonis are the most commonly injured in anaemia of the brain, whether the latter was caused by morphine, carbon dioxide, or carbon monoxide poisoning. That asphyxia and anoxia affected these regions severely was also demonstrated by Wolff (1937) and by Putnam (1937). These findings were interpreted by various authors as indicating that some type of vascular damage was responsible for the changes in kernicterus. In a study of the blood-brain barrier, Broman (1941) decided that vascular injury was an essential preliminary before cerebral tissue could be induced to take up trypan blue. This latter dye had been shown by Friedemann (1942) to resemble the action of bilirubin. With the discovery of the Rh blood groups a great impetus was given to the study of the disease process, and many workers attempted to establish a relationship between vascular changes and the development of kernicterus. Diamond and Denton (1945) and Liber (1945) suggested that the terminal capillaries to the nuclear masses might become blocked by cellular debris resulting from haemolysis. Wiener (1946), in his theory concerning the pathogenesis of haemolytic disease, postulated the blocking of these terminal capillaries by agglutination thrombi with subsequent cell damage. Unfortunately as Levine (1946), Vaughan (1946), and Cappell (1947b) pointed out there is no histological evidence to support these theories. Recently Wiener and Gordon (1948) retracted the thrombosis hypothesis.

The discovery of the Rh blood groups explained the familial incidence of the disease which had puzzled so many previous investigators. It was then shown that the antibody in the mother's serum acted on the antigen of the infant's red cells causing haemolysis of the latter. This has led some authors to suggest that a direct antigen-antibody reaction occurs in kernicterus with the cerebral cells acting as the antigen. Yannet and Lieberman (1946) believe that this is a possibility, but there is no clear proof that such antigen is present in the cerebral neurons or that such a reaction occurs. Darrow (1938) had suggested that anaphylaxis might play a part in the aetiology of kernicterus. Recently, Darrow and Chapin (1947) have repeated the suggestions that in addition to the antigen-antibody reaction there is an anaphylactic process which accounts for the onset of icterus gravis and the development of kernicterus in some cases. Their explanation of the development of haemolytic disease is not a very convincing one.

Orth (1875) had suggested that there might be some congenital inferiority of the brain predisposing to the localization of bile in the nuclear masses. This suggestion has met with considerable support in recent years. Fitzgerald et al. (1939) believed that a degree of 'maldevelopment' was a primary factor in the onset of kernicterus. Frolich and Mirsky (1942) produced convulsions in young rats but not in older ones by administration of bilirubin. Vaughan (1946) was inclined to believe that 'immaturity of cerebral and cerebrovascular tissue' played a part in the aetiology 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 quoted a few examples in support of her belief. It is not a simple matter to prove or disprove such a theory but no cases in the present series showed any evidence of cerebral immaturity.

The main difficulty in accepting one or other of the theories which have just been considered lies in the fact that the majority lack a sound histological basis for some of their premises. This may partly result from the lack of many detailed histological reports in the literature. Zimmerman and Yannet (1933; 1935) and Fitzgerald et al. (1939) have presented the most detailed reports of their cases, but histological data were only obtained in four instances. 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:

'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 localize in zones of injury, leaving unstained tissues which are not damaged.

This is by far the most reasonable view, as Vaughan (1946) has remarked. The ganglion cells are almost certainly damaged in the first instance and thereafter assimilate bilirubin to a greater extent than the surrounding areas which are undamaged. If this assumption is correct, it is then necessary to decide what agent or agents are responsible for the original damage to the cell. In so doing, we must take into account (1) that kernicterus does not occur at birth but may occur within a short period of time following the onset of jaundice; and (2) that irreversible cellular damage did not appear to occur before the end of the first week of life in the present series of cases.

Yannet and Lieberman (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 anaoxaemia causes permanent injury to the developing neurone, and the 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. As Cappell (1947b) has remarked, if the neurones are damaged during foetal life it is remarkable that no histological evidence of neuroglial reaction to neuronal death is present in the brains of patients who die within the first day or two of life.

It will be remembered that several experimental workers, notably Spielmeyer (1930) and Meyer (1936), have shown that the areas involved in kernicterus are those most commonly injured by anaemia or by anoxia of the cerebral tissue. Thus 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 recent work of Mollison and Cutbush (1949) shows that some degree of anaemia is present in nearly all cases of icterus gravis resulting from haemolytic disease, but this anaemia may be concealed in the early stages. It would seem probable, therefore, 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 sufficiently severe to render the cell membrane unduly permeable to bilirubin but is not so great as to cause cell death.

As Zimmerman and Yannet (1933) point out, groups of cells in the cerebral cortex, in the white matter around the lateral ventricles, and in the cerebellar cortex may show similar changes to those in areas more commonly affected. As the lesions are so very 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 cell. On histological grounds there is reason to believe that this process of gradual cell death occurs over the first week of extra-uterine life. It occurs first in the very vulnerable cornu ammonis and then in the other affected areas. After the death of the neurones the cerebral tissue undergoes necrosis with the formation of lipoid globules. These globules are then removed by the phagocytic action of the microglial cells and ultimately astrocytic replacement occurs with some shrinkage of the affected areas.

From a consideration of the manner in which kernicterus develops it is obvious that it is in the acute stage that therapy is likely to prove most useful. It is known that kernicterus can occur within 18 hours of birth, so if severe jaundice is present at birth treatment should be begun immediately. Since there are few reliable clinical criteria to indicate which patients are likely to develop kernicterus, all cases of severe icterus resulting from haemolytic disease should be treated within 12 hours of birth. Since the cerebral pigmentation would appear to result from anoxia, the obvious solution is to supply blood with full oxygen-carrying capacity, i.e. by the transfusion of Rh negative blood. Rh positive cells are quickly destroyed (Mollison, 1943) and so their beneficial oxygen-carrying power would soon be lost. Darrow and Chapin (1947), who have suggested an anaphylactic condition being responsible for the development of kernicterus, and Vaughan (1946) believe that better results are achieved by the use of Rh positive blood. This requires further confirmation and it would probably be wiser at present to use Rh negative blood. Whether the direct transfusion or exchange-replacement transfusion method is used will depend on the preferences of the clinician and the resources at his disposal. Wallerstein (1947) has suggested that if exchange-replacement therapy using Rh negative blood is begun immediately after birth kernicterus will not develop. Further work is necessary to substantiate his opinion. Whichever technique is used it must be emphasized that it must be instituted at the earliest possible moment in order to have any hope of success. Since the findings in the present series appear to indicate that irreversible
cytological change does not occur before the end of the first week, it may be worth while transfusing patients who were not examined until signs of cerebral irritation had become apparent. If the cerebral anoxia were overcome, further involvement of other nuclear masses might be prevented. By the time irreversible histological change has occurred and the process is fully established, there is no benefit to be derived from blood transfusion therapy. Treatment of this stage of the process should be confined to the prevention of secondary infection and to the care of the child's mental and physical development. Docter (1945) has observed that if great care is exercised with these unfortunate children a striking improvement may occur within the limits of their physical disability. Nevertheless, in spite of the early diagnosis of haemolytic disease and the institution of Rh negative blood transfusion therapy, the outlook in patients with kernicterus is not good. It is hoped that further improvements in methods of treatment in the early stages may achieve better results.

Recently, Yannet and Lieberman (1946) suggested that some cases of mental deficiency might be due to haemolytic disease. Cappell (1947b) discounts the evidence collected by Yannet and Lieberman, and does not believe that cases of idiopathic mental deficiency are the result of haemolytic disease. Cappell (1947b) has suggested, however, that the diffuse bile-staining of the cerebral cortex associated with an underlying kernicterus may cause mental deficiency in some instances. This diffuse bile-staining of the cortex is commonly found in patients with severe icterus who die in the neonatal period quite irrespective of the 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 deficiency in later life.

If mental deficiency should result from the effects of haemolytic disease in infancy it is most likely to occur in the instances where there is deep icterus of groups of cortical cells. This has been described by Zimmerman and Yannet (1933), and some deep staining of the subcortical white matter was present in one case in this series.

Conclusions

From a study of the cases in this series it can be seen that nuclear jaundice is always associated with icterus gravis neonatorum resulting from haemolytic disease. It was not observed in any of the patients with hydrops foetalis or haemolytic anaemia nor has it been found in connexion with severe icterus other than that resulting from haemolytic disease. It was not present at birth in any case, but was found at necropsy in a patient who died 18 hours after birth. It would seem most probable that the antigen-antibody reaction in the foetus causing haemolysis of red cells results in a sufficient degree of anaemia to cause cerebral anoxia. This anoxia severely affects the cells of the nuclear masses, which are extremely sensitive to anoxic state, and is sufficient to cause increased permeability of the cell to bilirubin but not to cause cell death. No evidence of cell death was found on histological examination of the brains of patients who died within a few days of birth. Cell death ultimately occurs, and is first observed in the cornu ammonis towards the end of the first week of life. Thereafter the affected nuclear masses undergo gradual astrocytic replacement and the typical neurological signs of the condition become apparent. Up to the present time the outlook in patients with kernicterus has been exceedingly grave. It is to be hoped that modern methods of transfusion therapy, if instituted soon after birth, will produce more satisfactory results. Once irreversible cytological change has occurred no specific therapy is of any avail. In such patients careful attention must be given to the prevention of secondary infection and to the promotion of maximum mental and physical activity within the limits of their disability.

Summary

The clinical and pathological features of 37 cases of nuclear jaundice occurring among 110 patients with severe icterus neonatorum are described.

Twenty-eight of these 37 patients died by the end of the first week of life. Only four survived the neonatal period, and only two are alive at the time of writing.

The histological changes present in the brain in nuclear jaundice are described in detail, and an attempt is made to explain the development of the condition on the basis of these histological findings.

The prognosis in nuclear jaundice is exceedingly grave both as to the immediate chances of survival and the future development of the affected child. It is too early to judge the effect of modern methods of transfusion therapy.

I wish to thank Dr. A. R. Macgregor and Prof. R. W. B. Ellis for much useful advice and encouragement. I am indebted to the clinical staffs of the Simpson Maternity Pavilion, Edinburgh, the Royal Hospital for Sick Children, Edinburgh, and the Elsie Inglis Memorial Maternity Hospital, Edinburgh, for access to their clinical records. The serological investigations were carried out by the staff of the Blood Transfusion Department, Royal Infirmary, Edinburgh, under the supervision of the Director, Dr. R. Cummings. The photomicrographs were produced by Mr. T. C. Dodds, of the Pathology Department, University of Edinburgh.
REFERENCES

Part II


