ERYTHROBLASTOSIS FOETALIS

and

ADDITIONAL PAPERS

by

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Volume I.

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VOLUME I.

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1. An Analysis of Nine Cases of Acholuric Jaundice in Childhood, with special reference to the Red Cell Morphology, and the effect of Splenectomy on it.

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Introduction and Acknowledgments.

The great interest which has been taken in haematology in the past ten or fifteen years and which has led to a rational classification of abnormal blood conditions, and to not a few therapeutic discoveries such as the liver treatment of pernicious anaemia, has not been confined to adult medicine. In paediatrics great strides have been made not only with regard to the diseases of the blood, but also in connection with the physiological changes in the haematology of the normal child from birth until the adult type of haematopoiesis has been acquired.

In the adult the dyshaematopoietic anaemias like pernicious anaemia and microcytic hypochromic anaemia, which is so common in women, are of greater importance than the haemolytic anaemias. In childhood, however, the haemolytic anaemias are relatively more important, although nutritional or dyshaemopoietic anaemias are, as in adults, of greater frequency.

Haemolytic anaemia in childhood is chiefly found in the neo-natal period, when it is known as erythroblastosis foetalis. This is a not uncommon condition, and, as I shall show, not less than 29 cases were observed in the Royal Hospital for Sick Children, Glasgow, in three years.

It is principally with erythroblastosis foetalis that I deal in this thesis, but additional papers - one on acholuric jaundice in childhood, with special reference to red cell morphology, and another on the red cell shape in conditions of acidosis and alkalosis - have been added.

The data which is used in the following pages were acquired at the Royal Hospital for Sick Children, Glasgow, by my personal observation and investigation between 1933 and 1936, with the exception of certain clinical details of cases treated in hospital in previous years.

I wish to express my thanks to Professor G.B. Fleming and Dr. Stanley Graham for permission to investigate cases in their wards. To Professor J.W.S. Blacklock and Dr. Noah Morris I am grateful for their courtesy in allowing me to work in their laboratories, and the opportunities afforded me to learn technical methods. To all of these gentlemen I am indebted for frequent critical discussions on the subject of anaemia in childhood.

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ERYTHROBLASTOSIS FOETALIS.

INTRODUCTION.

Erythroblastosis foetalis is a pathological conception, and signifies the hyperplasia or prolonged persistence of the extra-medullary sites of foetal blood formation. The term has been used to classify a group of neonatal anaemias, all of which have the same pathological basis, but which clinically are of somewhat divergent characteristics.

The foetal erythroblastoses may be subdivided into three groups:-

(i) Icterus gravis neonatorum.

(ii) Haemolytic anaemia of the new-born without oedema or jaundice.

(iii) Hydrops foetalis.

Icterus gravis was shown to be associated with pathological abnormalities of the haematopoietic system by Buchan and Comrie (1909). The second type was recognized by Ecklin (1919), and the pathological basis in hydrops foetalis was first appreciated by Schridde (1910).

The term erythroblastosis was invented by Rautmann (1912).

While working in the Royal Hospital for Sick Children, Glasgow, from 1933 to 1936, 23 cases of icterus gravis, and 6 of anaemia without oedema or jaundice came under my personal observation. I did not have the opportunity of seeing a case of hydrops foetalis, because, as the condition is very rapidly fatal, such cases are only very rarely encountered in a children's hospital. In order to complete the description of the erythroblastoses, however, I have included a brief summary of the history, clinical features, and pathology of the condition.

From the records at the Hospital from 1921 onwards I have been able to secure data from a further thirty-six cases of icterus gravis and two of anaemia without jaundice. Where applicable, such information has been used.

There are therefore two distinct series of cases, which shall hereafter be referred to as the 1921-33 series, and the 1933-36 series.

SECTION A.

I. THE FAMILY HISTORY IN ERYTHROBLASTOSIS FOETALIS.

(i) Miscarriages.

The family history in cases of erythroblastosis foetalis was particularly interesting. Often previous pregnancies had ended in miscarriage or stillbirth, and in the 1933-36 series of 28 families, 12 (42.86%) had been thus affected, while over the fifteen year period from 1921 to 1936 a similar history was found in 27 (40.90%) out of 66 families.

The remarkable incidence of miscarriages, stillbirths and deaths in early infancy in families in which one or other of the neonatal haemolytic anaemias had appeared, was commented on by Susstrunk (1924) and de Lange (1932).

(ii) Multiple cases of erythroblastosis foetalis in one family.

The occurrence of multiple cases of erythroblastosis foetalis in the families was striking, 7 (25%) out of 28 families of the 1933-36 series giving a history of two or more affected infants. Between 1921 and 1933 an examination of the case sheets revealed only two such families. It may be however that this point was not specially investigated in taking histories during this period.

A family history of hydrops foetalis was not obtained in either series.

In only one family of the 1933-36 group did both icterus gravis and neonatal anaemia without oedema or jaundice occur. The details were very striking. The first, fifth, sixth and seventh children were healthy, the second, third and fourth died of icterus gravis, and the eighth of anaemia without oedema or jaundice (Case 26).

In the 1921-33 series, there was one family which showed a history of eight healthy children, one miscarriage, one case of icterus gravis, and one of anaemia without oedema or jaundice.

More than two cases of either type of the condition were not infrequent. In one family (Case 25) the first four children were healthy, the sixth and seventh were stillborn, and the tenth died of an unknown cause six hours after birth, while the fifth, eighth and eleventh died of anaemia without oedema or jaundice.

There were four cases of icterus gravis in each of two families, all fatal in one (Case 7) and three fatal in the other (Case 23).

Occasionally all three types of anaemia, hydrops foetalis, icterus gravis, and anaemia without oedema or jaundice have been recorded in one family (Diamond, Blackfan and Baty, 1932). In the same family, hydrops foetalis and icterus gravis have been noted by de Lange (1932), hydrops foetalis and anaemia without jaundice or oedema by Pasachoff and Wilson (1935), and icterus gravis and anaemia without jaundice by Diamond et al. (1932).

Multiple cases of icterus gravis in one family have been recorded by Buchan and Comrie (1909), Rolleston (1910), Nason (1910), Pitfield (1912), Rosenbaum (1928), and Fordyce and McAfee (1924), while anaemia without oedema or jaundice in several infants of a family has been reported by Bonar and Smith (1933), and Segar and Stoeffler (1932).

(iii) The incidence of erythroblastosis foetalis in single families.

Erythroblastosis foetalis was not found in first children except in one instance, where the first-born developed anaemia without oedema or jaundice (Case 27). The fact that the first child is not usually affected has long been recognized (Rolleston, 1920). Occasional exceptions, like mine, have occurred, such as a case of anaemia without jaundice reported by Greenthal (1930), another by Åckerrén (1933), and two cases of icterus gravis published by Andrews and Miller (1935).

(iv) Erythroblastosis foetalis in collateral branches of the family.

A history of foetal jaundice, anaemia, or oedema was not obtained among the families of the parents' brothers and sisters or in more distant branches of the family. Icterus gravis and oedema of the new-born were however found by de Lange (1932) among the families of several brothers and sisters.

(v) The health of the mother during pregnancy.

Usually the mothers were healthy during pregnancy although occasionally a history of transient "kidney trouble" or "vomiting" was obtained. None of the mothers suffered from jaundice during pregnancy. Rolleston (1910) found that not infrequently the mothers of cases of icterus gravis had recurrent jaundice during pregnancy. Nason (1910) reported similar findings in a woman who had jaundice at parturition on three occasions. In each instance the child died of icterus gravis. The mother had jaundice during two other pregnancies but not at the time of parturition. One of the children resulting from these pregnancies developed icterus gravis and recovered, while the other was healthy.

Lately, however, maternal jaundice has not been reported.

(vi) Sex incidence of erythroblastosis foetalis.

The sex incidence has always been roughly equal.

(vii) Racial incidence of erythroblastosis foetalis.

All the reported cases have been in white children, except two cases of icterus gravis which occurred in negroes (Andrews and Miller, 1935).

(viii) Discussion on the family history in erythroblastosis foetalis.

The frequency of stillbirths, the occurrence of multiple cases of erythroblastosis foetalis in one family, and the occurrence of all permutations and combinations of its three types of manifestation in single families have impressed so many authors that mere accident or coincidence cannot be the explanation as has been suggested (Zimmerman and Yannet, 1933). Ample evidence has been accumulated from many sources to allow erythroblastosis foetalis to be classified as a familial disease. There has not been as yet a sufficiently long investigation of afflicted families to indicate whether transmission may be by a Mendelian factor.

Although many cases have been described as being isolated cases it is probable that if the mother's subsequent history were followed up, the incidence of multiple cases would be considerably increased. Already I have noted this in my series. Case 4 (icterus gravis) was the first affected child in the family, but just over a year later another infant was born and developed similar signs and symptoms (Case 5).

The tendency of erythroblastosis foetalis to occur among the later children of the family, and the frequent association with miscarriages and stillbirths, suggests that there may be a maternal toxin or a degree of exhaustion of the maternal reproductive organs. The possibility of a maternal toxin is favoured by the fact that sometimes the mother has been jaundiced during pregnancy. Against this, has been the occurrence of twin births where one child was healthy, and also that the mothers have very frequently been of good health during pregnancy.

Exhaustion of the reproductive organs is favoured by the fact that many of the families have been large and pregnancies too frequent. Not infrequently however, as I have shown, healthy children have been born between stillbirths and cases of erythroblastosis.

Diseases such as nephritis, syphilis, and tuberculosis of the mother may be excluded as causal factors because, although such may have occasionally been present in the mother of an affected child, the vast majority of the women have been healthy.

There is a further possibility that a toxin may be elaborated in the placenta (Goormaghtigh, 1925). If this were the case and the toxin originated from the foetal side, it would explain why the mother's health has been only occasionally affected. As to the existence or nature of such a toxin we do not have any direct evidence.

II. ICTERUS GRAVIS NEONATORUM.

A. CLINICAL FEATURES.

(i) Condition at birth.

Of the 23 cases of icterus gravis in the later (1933-36) series, 18 were full-time children, and 5 premature, and of the 36 cases in the older series (1921-33), 31 were born at full-time and 5 prematurely, the total being 49 full-time infants and 10 premature. Where there was doubt of the maturity of the child, and where the weight at birth or shortly afterwards was known, all children of 5½ pounds or under have been regarded as premature. From the statistics just given it would appear that the period of gestation had not any effect on the incidence of icterus gravis.

There was not any difficulty during parturition, and all babies born at term were well developed and lusty. Although it has been reported that the placenta may be enlarged, the amniotic fluid yellow, and the vernix caseosa of a golden yellow colour (de Lange and Arntzenius, 1929), none of these phenomena were noted by the accoucheur. Actually such observations have been comparatively rarely given in the literature.

(ii) Jaundice.

The first evident abnormality has been, in all cases, the onset of jaundice. Many of my cases were said to have been jaundiced at birth, and while this was certainly true in some, it is likely that in others the jaundice was not noticed until some hours afterwards. For instance, in one case the child was said to have been normal at birth, but after six hours a definite icteric tinge became noticeable in the face. At any rate jaundice was evident within the first twenty four hours of life in 31 out of 60 cases, on the second day in 4, on the third day in 5, and within the first 8 days in the remainder, with the exception of eight cases, of which those which occurred during my own period of observation (1933-36) will be discussed more fully later.

Jaundice appeared first in the face, and spread within twenty-four hours to the trunk and limbs. In one case the icterus was first noticed in the arms and legs (Case 4).

At the beginning the jaundice was of a pale yellow colour, but within a few hours it changed to a golden yellow, and then, if the child survived long enough, a greenish tinge sometimes followed. In two of my cases (Nos. 1 and 8) this change of hue was complete and the children became quite green in colour. The degree of jaundice varied, gradually clearing for some days and then quickly increasing.

The mucous membranes too, became definitely stained, and even in the early stages this could be detected in the downturned lower lip.

(iii) Pallor.

Anaemia was partially masked by the jaundice, but, quite early, a careful examination of the mucous membranes, especially of the mouth, but also of the palpebral conjunctivae, revealed a diminution of the natural red colour found in the normal baby. When the loss of blood became severe, say the red cell count was below 3.5 millions per c.mm., the pallor in spite of deep jaundice was quite evident, and in gross anaemia, e.g. one million red corpuscles per c.mm., the combination of pallor and jaundice was striking.

When anaemia became severe, or there had been a rapid destruction of blood, causing a considerable fall in the red cell count within a few hours, the child showed typical general signs of anoxaemia, such as cyanosis of the lips, skin, and finger-nails, increased respiratory excursion with occasional sighing, and indrawing of the lower costal margins. It was surprising, however, how often a child with a gross anaemia did not show any signs of distress, although slight cyanosis and increased depth of the respirations were usually present.

Occasionally a functional cardiac murmur was present, commonly a basal systolic bruit which was not conducted. This murmur sometimes ceased after transfusion.

In general the child was more drowsy than a normal infant, cried very little and was sometimes inclined to fall asleep during feeds, but if roused sucked well. There was occasionally an abnormally increased tone of the muscles, noticeable in the limbs with consequent exaggeration of the tendon reflexes. Further reference to the clinical findings in the nervous system will be made when discussing kernicterus.

(iv) Hepatomegaly and splenomegaly.

The commonest clinical findings excluding jaundice and anaemia were hepatomegaly and splenomegaly. Of the 23 cases of the 1933-36 series all except one had enlargement of the liver. Hepatomegaly in the newly-born, or in the child of a few weeks old, is less easily determined than enlargement of the spleen, because in all such children the liver occupies a very large part of the abdomen and the lower margin is normally palpable one finger breadth below the costal margin. Nevertheless when jaundice became marked the lower edge of the liver could be felt two or more fingers' breadth in 22 out of 23 cases. The organ was always quite smooth but abnormally firm. When the child survived for some weeks and became undernourished or emaciated the hepatic enlargement and hardness became relatively more striking.

Clinical splenomegaly, i.e. the spleen was palpable, was found in 15 of the 23 cases. It was noticeable that in only one of the 5 examples in premature children (Case 2) was splenomegaly found. In another 2 cases enlargement of the spleen occurred late in the disease - about fourteen days after birth. Both of these children had been transfused. The spleen never felt as hard as the liver, and the lower pole was usually found not more than two fingers' breadth below the costal margin.

The kidneys were not infrequently palpable, but never clinically enlarged. It is not uncommon to be able to palpate the kidneys in young babies.

Enlargement of the superficial lymphatic glands was not noticed in my cases.

(v) <u>The stools, the urine, and serum bilirubin</u>.

The stools, after the meconium was evacuated, were of a normal yellow colour in the breast-fed infant, and pale in the artificially fed child. As in the normal infant, urobilin was absent from the stools in the first week or two (Merritt, 1925), but thereafter was often found. Sometimes, however, urobilin disappeared from the faeces when the jaundice became very marked. In these cases small amounts of urobilin were again discernible after a short period.

It was necessary to test chemically for urobilin, either by the amyl alcohol extract and zinc acetate method, or by corrosive sublimate, because the pallor of the motions was often misleading, especially in bottle-fed children.

Bile pigments were nearly always present in the urine. They were found in 16 out of 20 cases where urine was available before death or transfusion. The amount of pigment varied from time to time, decreasing as the jaundice cleared, and quickly increasing when an exacerbation of blood destruction occurred.

Excessive urobilinuria was found less frequently: in

9 out of 18 cases examined before transfusion. Where bile pigments were present there was not any, or only a slight reaction for urobilin, but in 4 cases where bile pigments were absent a strong reaction was recorded. From Table I, which gives a rough impression of the amounts of bile pigments and urobilin found in the urine, it will be seen that usually the degree of urobilinuria varied inversely as the bile pigment content.

Table I. The relation between urinary and serum pigments.

Case No.	Bile pigments in urine.	Urobilinuria	Van den Bergh reaction.
1	++	-	
2	++	-	Biphasic: 88 units
8	++	-	Biphasic: 14 units
16	++	-	Biphasic: 34 units
21	++	-	
6	++	+	Biphasic: 18 units
12	++	+	Biphasic: 28 units
4	+	-	
5	+	-	Biphasic: 30 units
14	+	-	Biphasic: 46 units
23	+	-	
10	+	trace	
9	+	+	
15	+	+	?delayed +. Indirect +. 6 units.
11	-	++	
22	-	++	Delayed +. Indirect +. 32 units.
13	-	+	Biphasic: 20 units
17	-	+	

This suggested that there was a considerable degree of diminution of the outflow of bile from the liver into the intestinal tract, and consequently little and sometimes absence of formation of urobilin in the gut.

According to Astrachan (1937) the gut must still be considered as the sole site of urobilin formation. Some is reabsorbed into the portal circulation and returned to the liver, from which however a certain amount escapes into the general circulation, and is excreted by the kidneys.

The absence or diminished quantities of urobilin from the faeces presupposed the presence of destruction of the liver cells and rupture of the bile canaliculi, with consequent escape of bile into the systemic circulation. As we shall see later, considerable damage to the liver cells was constantly found post mortem. The presence of bile, i.e. bilirubin with bile salts and acids, in the serum in any quantity led to the expectation of a direct positive van den Bergh reaction, or at least a biphasic reaction. The latter term is used when immediately on addition of Ehrlich's diazo reagent to the serum, a slight red colour develops and slowly increases to blue. This type of reaction was usually found, and occurred in 8 out of 10 estimations (Table I). An immediate direct positive reaction was not encountered, although such have been reported in the literature (Ross and Waugh, 1936).

Two cases did not show a biphasic reaction, but merely an indirect and a delayed direct reaction. The latter is commonly regarded as not having any special significance, and like the indirect reaction does not indicate that any of the bilirubin in the serum has passed through the parenchyma cells of the liver, as does the bilirubin in the immediate and biphasic reactions (Astrachan, 1937).

One of the two cases (No. 22) did not show any reaction for bile pigments in the urine, although this was found in the other (No. 15) in which, however, urobilinuria was also present, indicating that some bile was being excreted into the intestines.

It would appear that in icterus gravis, owing to changes in the liver, there is usually a decreased flow of bile into the intestine, and some reabsorption into the bloodstream. In a few cases the damage to the liver is slight or absent and consequently bile is absent from the serum and urine, in which case increased urobilinuria is quite marked, and only bilirubin which has not passed through the liver parenchyma is found in the serum.

Further, as will be seen from Table I, the total amount of bilirubin in the serum was constantly and often greatly increased above the normal limit of C.5 units, even when damage to the liver, as indicated by an immediate direct or biphasic van den Bergh reaction, was lacking. This excess of bilirubin must therefore have come from increased destruction of haemoglobin by the reticulo-endothelial system.

B. THE HAEMATOLOGICAL FINDINGS.

(i) The red corpuscles.

(a) Numbers.

In this series of cases (1933-36) the children had usually been jaundiced for several days or as long as three weeks before they were brought to hospital. Only two cases were seen within forty-eight hours of birth. Most cases were transfused shortly after admission, and the blood picture consequently altered, or death occurred within a short period. For these reasons therefore, there is not any complete record of an untreated case from beginning to end available, and it is therefore only possible to infer the course of the anaemia from a consideration of data from many cases at different ages.

Table II shows the blood count on admission to hospital in nineteen cases, and the day of illness on which the figures were obtained. Unless where marked by an asterisk the onset of jaundice was within eight days of birth.

Anaemia was variable in degree, and in rate of development in individual cases. The only child admitted during the first 24 hours after birth (Case 23) had a red corpuscle count of 3.65 millions per c.mm., and one on the second day (Case 1) had 2.50 millions per c.mm. Even if the lower figures of 5.5 to 6 millions per c.mm. for red cells at birth (Lipmann, 1924;

Table II. Red cell counts in relation to the day of illness and the time of onset of jaundice.

Case No.	Day of illness.	Time of onset of jaundice.	R.B.C. per c.mm.	Nucleated red cells per c.mm.
23	1	Birth	3.65	4,680
1	2	Birth	2.50	20,500
14	4	1 day	3.5	1,176
10	5	2 days	3.27	Occasional
5	6	Birth	2.8	2,812
13	7	Birth	3.44	nil
4	7	4 days	1.63	2,900
2	8	Birth	1.5	nil
12	9	1 day	1.07	2,070
20	10	Birth	0.81	Occasional
17	11	Birth	1.48	300
19	13	Birth	1.48	Occasional
16	15	5 days	0.87	5,082
15	21	Birth	2.44	315
8	24	1 day	1.7	nil
9	25	2 days	1.44	560
*6	2	15 days	2.02	nil
*11	4	15 days	4.61	nil
*22	4	23 days	4.02	nil

* Jaundice of late onset.

Merritt and Davidson, 1933; Guest and Brown, 1936) are accepted instead of 6½ to 7¼ millions per c.mm. (Whitby and Hynes, 1935). The two cases quoted showed that 45 to 65% of the circulating red cells were destroyed in the first day or two of life. The figures for the remainder of the first week of illness varied between 1.63 and 3.5 millions per c.mm.. In the second week, the red cell counts fluctuated between 0.81 and 1.5 millions per c.mm.. Only two figures were available in the third week, one at the beginning (0.87 millions per c.mm.) and one at the end (1.44 millions per c.mm.), while in the fourth week the two counts observed were 1.44 and 1.70 millions per c.mm.. These examples show the variability in the degree of anaemia, and the rate of its development in different cases. They seem to suggest that the haemolytic process was most active in the first and second weeks, while in the third and fourth weeks, if the child had survived so long, the haemolysis was not nearly so active.

(b) Reticulocytes.

In all except two cases there was a reticulocytosis during the period of blood destruction. The extent of the reticulocyte increase varied at different stages, being as a rule high early in the illness, or during an exacerbation of blood destruction. The former observation has also been made by Ross and Waugh (1936), and the latter by Hawksley and Lightwood (1934). In my series the reticulocyte counts during brisk haemolysis varied between 12 and 36% of the red cells. Counts as high as 60% (Ross and Waugh, 1936) have been recorded. These figures are well in excess of the normal reticulocyte numbers during the first two months of life. At birth 5 to 6% are found (Friedlander, 1925; Krumbhaar, 1929; Merritt and Davidson, 1933; Heinio Paavo, 1933), but there is a rapid reduction in numbers during the first few days, and a significant increase does not normally occur until the end of the second, or in the third month.

When haemolysis was less acute or in abeyance, as shown by slowly falling or constant red cell numbers, the reticulocyte count dropped, perhaps remaining at about 3 to 7% or even falling to less than 1%.

The anaemia in icterus gravis was therefore of a true clinical haemolytic type, that is to say there was evidence of active blood regeneration, with a coincident diminution of the number of circulating red corpuscles.

(c) Erythroblastaemia.

Erythroblastaemia, or the presence of abnormal numbers of nucleated precursors of the erythrocyte in the peripheral blood, is one of the striking characteristics of the condition. It is not an essential feature, however, and many cases in which it was absent have been published (McLure, 1931; Greenwald and Messer, 1927; Zimmerman and Yannet, 1933).

Actually at birth, and during the first few days of life, nucleated red cells are present in the peripheral circulation. From five hundred to two thousand per c.mm. are regarded as the usual numbers at birth (Diamond, Blackfan and Baty, 1932; Forkner, 1929), but in normal infants up to five thousand per c.mm. have been observed (Lipmann, 1924; Hawksley and Lightwood, 1934). In premature infants the nucleated red cells tend to be more numerous and to persist longer, although they are not usually found after the first week (Herz, 1928, quoted by Josephs, 1936).

Of my two cases, seen within 48 hours of birth, one had a very marked erythroblastaemia, 20,500 per c.mm. (Case 1, Table II). In the other (Case 23), 4,680 per c.mm. were present. Probably some of the other cases had high counts at birth. At any rate three cases (Nos. 4, 5 and 14) first seen in the latter half of the first week had an erythroblast count between 1,100 and 2,900 per c.mm. One case (No. 12) showed 2,070 per c.mm. on the ninth day of life, and another (No. 16) 5,082 on the fifteenth day. Two other cases gave counts of a few hundreds late in the third week (Nos. 15 and 17) and in one (Case 17) the peripheral blood had been free of erythroblasts for some time. Their appearance at this time was coincident with a recrudescence of blood destruction. A count of 560 nucleated red cells per c.mm. was found in the fourth week of jaundice in one case (No. 9), while in several cases occasional erythroblasts insufficient to be accurately enumerated were seen in the later stages of the anaemia.

Various types of nucleated erythrocytes were represented.

In classifying them I have used the terminology of Doan, Cunningham and Sabin (1925). Only very rare megaloblasts were seen. Early erythroblasts were more common but the most frequent cells were the late erythroblasts and normoblasts. Normally, the last contain haemoglobin, but in many of the erythroblasts of both types, early and late, haemoglobin was present in the cytoplasm. The late erythroblasts and normoblasts showed frequently karyorrhexis of the nucleus and extrusion of the fragments (Plate I). Occasional mitotic figures were found in the early erythroblasts.

In stained films, anisocytosis and poikilocytosis were not marked, but when blood regeneration was in an active phase, polychromasia was frequent, and occasional punctate basophilia was seen. It has been proved that such cells are reticulocytes (Brookfield, 1928). The red corpuscles were evenly stained throughout, but nearly all cases during the periods of rapid blood production were characterised by the presence of fairly numerous cells containing very little haemoglobin - "ghost" cells - of varying size and shape.

Phagocytosis of red corpuscles by large mononuclear cells was not seen in the peripheral blood, although this phenomenon has been described in icterus gravis by Buchan and Comrie (1909), Abt (1933) and Abbot and Abbot (1935).

(d) <u>Fragility of the red corpuscles</u>.

The fragility of the red corpuscles in hypotonic saline was measured in four cases. In three, active blood destruction was progressing, while in the fourth haemolysis was temporarily in abeyance. In two instances (Table III) the corpuscles were slightly less resistant than the cells of an older and normal child, used as a control, while in the remaining two cases the degree of lysis was practically identical in patient and control. Similar results, with the addition that in a few cases increased resistance to hypotonic saline, have been recorded (Diamond et al, 1932; Hawksley and Lightwood, 1934; Ross and Waugh, 1936).

(e) The morphology of the red corpuscles.

Van Creveld (1932), studying the diameter of the red corpuscles after birth, found that the mean corpuscular diameter in the first week was 7.988μ, whereas the average in later childhood and adult life was 7.20μ (Price-Jones, 1929). During the first four weeks of life there was little difference between the curves for full time and premature children, but thereafter the mean diameter in the former increased, while in the latter there was diminution. During the fifth week of life the mean diameter of the corpuscles of full time children began to decrease and the degree of anisocytosis to diminish. These changes continued until the curve gradually assumed the adult form. Anisocytosis in premature children's blood remained very marked, and the mean corpuscular diameter showed further reduction until the eighth week of life, after which anisocytosis became less, and later the mean diameter

Table III. Fragility tests in icterus gravis.

Case No. 1. Aet 2 days: Haemolysis active: Washed r.b.c.

NaCl %	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58
Case 1	++	++	+	+	±	$\overset{+}{=}$	-	-	-	-	-
Control	++	++	+	$\overset{+}{=}$	-	-	-	-	-	-	-

Case No. 2. Aet 8 days: Haemolysis active: Washed r.b.c.

NaCl %	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58
Case 2	++	++	+	+	+	±	$\overset{+}{=}$	-	-	-	-
Control	++	++	+	±	-	-	-	-	-	-	-

Case No. 9. Aet 25 days: Haemolysis active: Unwashed r.b.c.

NaCl %	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58
Case 9	++	++	++	+	±	-	-	-	-	-	-
Control	++	++	+	+	±	-	-	-	-	-	-

Case No. 5. Aet 6 days. Haemolysis not brisk. Washed r.b.c.

NaCl %	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58
Case 5	++	+	+	+	±	-	-	-	-	-	-
Control	++	+	+	±	-	-	-	-	-	-	-

began to increase. About the twentieth week of life the curves from premature and full time infants were indistinguishable.

In icterus gravis, the same worker found an excessively high mean diameter and degree of anisocytosis at birth, and later an even greater reduction of the mean diameter than occurs in premature children. He was impressed by the lack of cells under 7μ in diameter in the earlier stages of the anaemia.

Hawksley and Lightwood (1934) confirmed the occurrence of an apparent microcytosis, but had no observations to make on the shape of the cells at an early stage.

I have constructed eleven Price-Jones curves from five cases of icterus gravis. Two of these (Figs. I and II) made from specimens obtained on the second day of life, confirmed van Creveld's observation that the mean corpuscular diameter was higher than normal, and that cells with a diameter of less than 7μ were absent. The base of the curves was wide, and cells as large as 13.5μ were found.

Later in the course of the anaemia great reduction in the mean corpuscular diameter was noted. In one case (Fig. I) a diminution of about 1.5μ was found in three weeks, normal figures for a full time child being almost reached. By the second or third week subnormal figures were found in the remaining cases (Figs. III, IV and V), and this persisted right into the eighth week (Figs. II and V) after which the normal mean diameters are not accurately known.

From the second week onwards cells varying from 5μ

upwards were present (Figs. I to V) but the degree of megalocytosis diminished and by the age of eight weeks very few cells above 9.25μ in diameter were found.

Further, as the later weeks were reached (Figs. II and IV) the peak of the curve was distinctly higher, i.e., anisocytosis was diminishing.

It will be seen from Table IV, where the normal mean corpuscular diameters of full-time children up to eight weeks of age, and the mean diameters in cases published by van Creveld and Hawksley and Lightwood, as well as my own are given, that the findings from all three sources are in agreement.

The initial post-natal haemolysis occurring in all babies has been attributed to the sudden increase of oxygen supply to which the child is subjected at birth (Goldbloom and Gottlieb, 1929 b). Van Creveld thought that the diminution of the cell diameter could be explained on similar grounds, and that the greater reduction of cell diameter in premature infants could be explained by the more prolonged and stronger stimulus occurring in these children as a result of the excessive haemolysis which they undergo. The fact that decrease of cell diameter did not occur for some time, he explained by the counter-action of dehydration which he said was common after birth.

In icterus gravis, van Creveld was of the opinion that the excessive fall in mean corpuscular diameter, and the lack

Table IV. M.C.D. in normal infants and cases of icterus gravis.

Age	Van Creveld			Hawksley and Lightwood				Paxton				
	M.C.D. in full-time infants (μ)	M.C.D. in icterus gravis (μ)		M.C.D. in icterus gravis (μ)				M.C.D. in icterus gravis (μ)				
		Case I	Case II	Case I	Case II	Case III	Case IV	Case I	Case II	Case III	Case IV	Case V
2 days			10.024					10.031				9.569
3 days		9.215										
1 week	7.998											
2 weeks	8.159		9.8365 9.19		7.36	8.09			8.263		7.5665	
3 "	8.243	7.431				7.79		8.4735	7.645	7.2565		
4 "	8.266			8.148			7.321					
5 "	7.945										7.316	
6 "	7.721											
7 "	7.858			7.30								
8 "	7.728	7.212					6.936			7.2435		7.37

M.C.D. = mean corpuscular diameter.

of anisocytosis, was possibly due to aplasia of the blood-forming tissues, because in at least one of his cases there was little evidence of blood regeneration. This conclusion however was wrong, because in my cases and those of Hawksley and Lightwood there was a reticulocytosis.

Again, if increased oxygen supply were the cause of microcytosis we would expect that in very anaemic children, where there would be a lack of oxygen, there would be an increase of cell diameter. This, as has been shown, was not the case.

It is not certain moreover if oxygen is the factor responsible for change in diameter of the red corpuscles. Price-Jones (1920) was of the opinion that the diurnal variation of cell diameter in human beings, and the increase or decrease after exertion or forced breathing respectively, was due to the variations of the carbon dioxide content of the blood. I showed however (Paxton, 1935) that in conditions of non-gaseous acidosis and alkalosis, there was an increase or decrease of cell diameter and cell volume, and that these phenomena seemed to be associated with the ratio of free or total carbon dioxide to combined carbon dioxide, or, in other words, cell diameter varied with the pH of the blood.

In one of my cases of icterus gravis (Fig. III) there was a partial shift to the left of the Price-Jones curve after transfusion. This seemed to be due merely to the mixture of normal adult cells with the baby's cells. If any factor such as increased oxygen or alkalosis were present the transfused corpuscles would also have been decreased in size and some cells smaller than 5.25 μ would have appeared.

Hawksley and Lightwood suggested that the diminution of mean corpuscular diameter was connected with the influence of the spleen, because as both they and I have shown, an increase of cell diameter occurs, and may persist, for some time after splenectomy in acholuric jaundice. It may be that in icterus gravis the red corpuscles have assumed a globular shape like those in acholuric jaundice. This contention is supported by the findings of Ross and Waugh (1936). It has been shown recently (Guest and Brown, 1936), that the mean volume of the red corpuscle at birth was from 91 to 123 cubic microns. Ross and Waugh found in five cases of icterus gravis that the mean corpuscular volume lay between 80 and 101 cubic microns. These figures were found at varying ages after, but not at birth, and therefore are not quite comparable with Guest and Brown's. It would seem however that in icterus gravis the cell volume was often within normal limits. Combining the proved decrease in cell diameter with the frequently normal cell volume, it would appear that in icterus gravis the corpuscle was more globular in shape than in normal infants.

On the other hand Haden (1934) observed that increased fragility of the red cells to hypotonic saline was dependent on the globularity of the cells. I have shown previously that there was not any constant change in fragility of the red corpuscles in icterus gravis.

Nevertheless it seems to me that change of shape of the corpuscle is a much more likely explanation of the apparent microcytosis than increase of oxygen supply, or presence of alkalosis, because the latter factors would be expected to be much more apparent at an earlier stage, as often, even in icterus gravis, there is not any dehydration to act in opposition after the first few days of life. Further transfused corpuscles would be expected to share in the process.

(ii) Haemoglobin.

The estimation of haemoglobin in young infants is unsatisfactory. The chemical constitution of foetal haemoglobin is apparently peculiar. Trought (1932) has shown that 53 minutes are required to convert the haemoglobin of newly-born children into alkaline haematin by the addition of sodium hydroxide, and Hawksley and Lightwood (1934) have shown that a similar long exposure to the action of N/10 hydrochloric acid, as in Sahli's method, is necessary to form acid haematin.

In Haldane's method of exposing laked blood to the action of coal-gas, only a 70% conversion of oxyhaemoglobin into carboxyhaemoglobin is obtained after an exposure of half-an-hour (Hawksley and Lightwood, 1934). Furthermore a true colour match with Haldane's standard tube is not obtained because the infant's blood, after carboxylation, has a brick red colour.

Of the two methods, Sahli's is preferable. The hydrochloric acid should be allowed to act for one hour. Even then there will be a further inaccuracy, in cases of icterus gravis, owing to the presence of large quantities of bilirubin in the serum.

The foetal type of haemoglobin gradually disappears from the blood, although Trought found that some is still present in normal infant's blood at the end of the first month. By the end of three and a half months all the haemoglobin was of the adult type.

In Table V I have compared the colour indices obtained by Haldane's and Sahli's methods. In eleven estimations by the former method an average colour index of 1.10 was obtained, with a range of from 1.03 to 1.20, while in nine estimations with the latter method an average of 1.38 was found, the range being from 1.24 to 1.57.

Because of the superiority of Sahli's method it was concluded that the corpuscles in cases of icterus gravis were hyperchromic, although it must be remembered that hyperchromia is also present in the normal infant's cells.

On the whole, the estimation of colour index was of little value in the neonatal anaemias.

<u>Table V.</u> A comparison of the colour indices obtained by the use of Sahli's and Haldane's methods of haemoglobin estimation, in cases of icterus gravis.

Case No.	R.B.C. (millions per c.mm.)	Hb. (Sahli)	Hb. (Haldane)	Colour Index. (Sahli)	Colour Index (Haldane)
1	2.5		60		1.2
2	1.5	45		1.5	
3	4.0		88		1.10
4	1.63	45		1.4	
5	2.38	65		1.38	
6	3.3	85		1.29	
8	3.7	92		1.24	
"	1.7		38		1.12
9	1.25		29		1.16
10	3.27		70		1.07
12	1.67		22		1.04
13	3.44		70		1.03
14	3.5		75		1.07
15	2.44		55		1.15
16	0.87		20		1.17
17	1.48		32		1.10
19	1.48	41		1.42	
20	0.81	25		1.57	
22	4.0	100		1.27	
23	3.65	100		1.37	

Colour Index (Sahli) : Average 1.38. Range 1.24 to 1.57

do. (Haldane): do. 1.10. do. 1.03 to 1.20.

(iii) <u>The leucocytes</u>.

The total leucocyte count at birth is about 18,000 per c.mm. and diminishes to around 14,000 per c.mm. in two days (Whitby and Britton, 1935 a). Over 60% (10,800 per c.mm.) of the leucocytes at birth are polymorphonuclear neutrophiles, but during the first week of life these undergo a great diminution in numbers, and the normal adult figure of about 4,500 per c.mm. is reached, and thereafter maintained. Increase of lymphocyte numbers however causes a rise in the total leucocyte count from the second day until the third week when the total count reaches about 17,000 per c.mm. Thereafter there is a slow decline of lymphocyte numbers until the normal adult figures are reached towards the twelfth year.

In the early days of life, too, a few primitive leucocytes --- myeloblasts and myelocytes --- are found, while the less mature polymorphs --- metamyelocytes --- are present during the first few weeks.

In Table VI I have arranged the leucocyte counts from cases of icterus gravis, according to the age of the child. The figures were from cases before any treatment was given.

Few of the total leucocyte counts were significantly raised, and although eleven out of twenty were above 20,000 per c.mm. only one exceeded 30,000 per c.mm.. Further in some of these cases the leucocytosis was probably due to associated infection. In eight instances (Cases 14, 10, 2, 17, 13, 6, 11 and 22) a leucopenia was observed.

Table VI. Leucocyte counts at various ages in icterus gravis.

Case No.	Age	Leucocytes per c.mm.	Myelo-blasts %	Neutrophiles			Eosino-philes %	Baso-philes %	Mono-cytes %	Lympho-cytes %	Plasma and Türck cells %	Neutrophile leucocytes		Lymphocytes	
				Myelo-cytes %	Meta-myelo-cytes %	Segmented poly-morphs %						per c.mm.	Normal per c.mm.	per c.mm.	Normal per c.mm.
23	1 day	23,400	0.75	1.25	3.5	69.5	0.75	0.5	0.25	23.5		17,374	10,800	5,499	2,700
1	2 days	20,500	1.0	2.5	3.0	64.0	2.0	0.5	0.5	26.0	0.5	14,247		3,330	
14	4 "	5,600	-	0.5	1.0	54.5	0.75	nil	1.75	41.5	-	3,126		2,324	
10	5 "	11,900	-	1.5	3.0	46.5	0.5	1.0	1.5	45.5	0.5	6,069		5,214	
5	6 "	22,600	-	0.5	3.25	70.0	-	-	2.25	24.0	-	15,637		3,424	
4	7 "	29,000	1.25	1.25	4.5	44.75	7.0	1.0	4.25	35.25	0.75	14,645	4,500	10,272	
2	8 "	9,300	3.0	9.5	13.0	50.5	2.5	-	4.0	17.5	-	6,789	"	1,627	
12	9 "	20,700	0.5	1.5	5.0	48.0	3.5	-	-	41.5	-	11,281	"	8,590	9,350
20	10 "	21,300	-	2.0	3.5	42.5	0.5	0.5	0.5	50.5	-	10,224	"	10,756	"
17	11 "	12,000	-	2.25	4.0	52.0	2.25	-	-	39.25	0.25	6,990	"	4,710	"
13	12 "	11,000	-	-	0.75	34.5	6.25	0.75	4.0	53.75	-	3,877	"	5,912	"
19	13 "	32,000	0.5	1.5	6.0	49.5	1.5	-	-	41.0	-	18,240	"	13,120	"
16	15 "	23,100	0.5	1.5	4.0	62.0	0.5	0.5	1.25	29.75	-	15,592	"	6,872	"
6	16 "	12,900	-	-	0.5	59.5	1.0	1.0	1.5	36.0	0.5	7,740	"	4,644	"
11	17 "	10,000													
15	21 "	14,200	1.0	0.75	2.0	60.25	1.5	0.5	4.0	29.75	0.25	8,946	"	4,224	"
8	24 "	17,000	-	1.0	1.0	40.5	5.5	1.0	3.5	47.25	0.25	7,225	"	8,032	"
19	25 "	28,000	0.25	0.5	4.0	51.25	3.75	0.5	2.5	37.25	-	15,610	"	10,430	"
22	4 wks.	13,600	-	-	1.25	31.25	1.25	1.0	0.5	64.25	0.5	4,420	"	8,538	"
3	6 "	21,000	-	0.5	1.0	58.5	-	0.5	3.5	36.0	-	12,600	"	7,560	"

The total numbers of neutrophile leucocytes and lymphocytes were of more significance, and it will be seen from Table VI, that there was an increase of the absolute numbers of polymorphs in fifteen out of nineteen cases, the figures being normal in two cases (Nos. 10 and 22) and subnormal in two cases (Nos. 14 and 13). Lymphocyte numbers were more variable and subnormal figures were frequent.

In one case with neutropenia (No.14) it is interesting to note that the child was dying of broncho-pneumonia and that erythropoiesis was active, as was shown by a reticulocyte count of 8.6%.

The differential counts showed little of importance, there being usually a few primitive cells --- myeloblasts and myelocytes --- and a few metamyelocytes. On only one occasion were large numbers of these cells present (Case 2). In this instance they formed 25.5% of the total leucocyte count. The child was premature, and there was not any infection.

Eosinophilia was noted in three cases (Nos. 4, 13 and 8). This has been reported in the literature, and usually occurred from the second or third week onwards (Diamond et al, 1932).

Of the other forms of leucocytes --- basophiles, monocytes, plasma and Türck cells --- little of importance was recorded. A high basophile count was seen in several cases (Nos. 23, 1, 10, 4, 20). Monocytes were scanty or normal in numbers. Twice, high figures for plasma and Türck cells were noted --- 102 and 227 per c.mm. in Cases 1 and 4 respectively.

In the literature a polymorphonuclear leucocytosis has usually been reported. The figures however have varied within very wide limits, and one count as high as 250,000 per c.mm. was reported by Buhrman and Sanford (1931). This however was exceptional, and figures above 60,000 per c.mm. have rarely been found. Leucopenia has not been a common feature, although cases have been published by Buchan and Comrie (1909), and Parsons et al. (1933).

Hawksley and Lightwood (1934) described a rise in the leucocyte count with each exacerbation of haemolysis. This was not my experience, although I found that commonly an intercurrent infection produced a ready polymorphonuclear leucocyte response.

The leucocytes, then, did not show much significant change as a result of the haemolytic process, there being only a slight, and sometimes transient, increase in the neutrophile forms. It is as well to remember in this respect, the observation of Carnegie Dickson (1908) that no matter what type of marrow reaction eventually occurs, there is first of all a hyperplasia of all the elements. This probably explains the leucocytosis in many cases of icterus gravis. In others, leucocytosis was due to pyogenic infection. Rarely, where there were both infection and excessive blood destruction, evidence of only one form of blood regeneration was present. The poor lymphocytic response was probably due to the changes in the lymph follicles, which I will describe later.

(iv) <u>The platelets</u>.

The normal platelet count at birth is between 200,000 and 300,000 per c.mm. (Lipmann, 1924, 213,000; McLean and Caffey, 1925, 278,000; Merritt and Davidson, 1933, 227,000 per c.mm.).

Platelet counts done on four of my cases showed normal counts in two cases (289,000 and 260,000 per c.mm.) and slightly subnormal numbers in the remainder (180,000 and 120,000 per c.mm.). In neither case however was the diminution of any great significance. Only occasionally have counts of less than 100,000 per c.mm. been recorded (Montford and Brancato, 1935) while sometimes comparatively high counts, over 400,000 per c.mm., have been observed (Ross and Waugh, 1936).

It would appear therefore that there is not a characteristic change in the numbers of platelets in icterus gravis.

(v) <u>Bleeding time and coagulation time</u>.

Various authors (Carr, 1929, Weiner and Bailey, 1934, and Merritt and Davidson, 1933) are in agreement that the average bleeding time in the new-born is between half-a-minute and two-and-a-half minutes, although in occasional cases an apparently normal infant will bleed for six minutes. I have noticed in three instances that the bleeding time in the later stages of fatal cases was considerably prolonged, ten minutes or more (Cases 1, 5 and 8).

Coagulation time at birth, according to the authors previously quoted, is on the average not more than four minutes if capillary blood is used. If blood is taken from the longitudinal sinus, the coagulation time is fifteen minutes (Lucas et al., 1921). In taking off blood by this latter method I have found that coagulation occurred within fifteen minutes.

Ross and Waugh (1936) have paid some attention to bleeding and coagulation times in icterus gravis. They found that a prolongation of the bleeding time, without alteration in the coagulation period, sometimes occurs in the later stages and is associated with a tendency to haemorrhage.

Of the three cases in my series where an excessive bleeding time was found, two died of intra-cranial haemorrhage, but bleeding was not evident either clinically or pathologically in the third (Case 8).

Haemorrhages occurring at a late period, often terminal, were frequent and varied in site. Thus, purpura of the skin, or even of the buccal mucous membranes, umbilical or nasal haemorrhages, melaena and haematemesis from bleeding into the intestines and stomach, have been noted clinically, while, as we shall see in the section on morbid anatomy, bleeding into the brain, meninges, lungs and serous membranes, has been observed. These haemorrhages are probably associated with prolongation of the bleeding time. They may occur when there is little anaemia, and also at times when blood destruction is slight or in abeyance (Cases 1, 5 and 8).

Icterus gravis was however sometimes associated with haemorrhagic disease of the new-born. The latter disease occurs usually in the first week of life, the greater number starting on the fourth or fifth day (Beveridge, 1928). The bleeding may occur from the umbilicus, the gastro-intestinal tract, the penis or vagina, or the urinary tract, and is usually quickly arrested by the subcutaneous injection of small quantities (10 c.c.) of parental blood. Rarely, especially in the gastro-intestinal form, transfusion may be necessary.

Between 1921 and 1936 there were three cases showing the association of icterus gravis and haemorrhage of the new-born. In the first, haemorrhage was from the prepuce on the fourth day, and in the second there was slight haematemesis at the same age. Recovery was spontaneous in both cases. The third case had profuse umbilical bleeding beginning on the seventh day, and lasting 48 hours, when it was quickly arrested by the subcutaneous injection of whole blood, although the haemolytic process continued unaffected.

A purpuric eruption was noticed at an early date on two occasions, in the face in the first, and over the genitals and lower abdominal wall in the second.

C. SUMMARY OF THE CLINICAL AND HAEMATOLOGICAL FEATURES OF ICTERUS GRAVIS.

The onset of anaemia and jaundice was usually at birth or within the first 48 hours, and later in a smaller proportion of cases, but rarely after the first week of life.

Most of the cases were of full-time children, born of healthy mothers, after a normal pregnancy. A few premature children were affected.

Splenomegaly and hepatomegaly were frequent features. The former was conspicuous by its absence in premature babies.

The anaemia was of the haemolytic type, showing progressive fall in the red cell count, a tendency to high colour index, and associated usually with signs of blood regeneration, reticulocytosis and erythroblastaemia. The haemolytic process was most active in the first two weeks.

The fragility of the red cells did not show any constant change. There was sometimes an excessive megalocytosis in the initial stages of the anaemia but later a more globular form was assumed by the red cells. This, I believe, may be due to an influence of the spleen, akin to what occurs in acholuric jaundice.

The leucocytes did not show any constant change, being either normal, increased or decreased in total numbers. There was nearly always an increase in the total number of polymorphonuclear neutrophile forms. Lymphocytes were frequently decreased in numbers. Eosinophilia was sometimes present.

Platelet numbers were not significantly changed.

Coagulation time was normal.

Not infrequently a tendency to haemorrhages occurred. This was associated with prolongation of the bleeding time which had been normal in the early stages of the anaemia.

Bleeding early in the course of the anaemia was due to haemorrhagic disease of the new-born, and was controllable by intramuscular injections of blood.

The deep jaundice, the presence of bile pigments in the urine, the intermittent absence of urobilin from the stools, and, not infrequently, the absence of urobilinuria, associated with excessive bilirubinaemia, commonly giving a delayed direct reaction with van den Bergh's test indicated that frequently there was grave damage to the liver.

III. HAEMOLYTIC ANAEMIA OF THE NEW-BORN WITHOUT OEDEMA OR JAUNDICE.

A. CLINICAL FEATURES.

(i) Number of cases and time of gestation.

Six cases of this type of erythroblastosis foetalis were admitted to the Royal Hospital for Sick Children, Glasgow, between 1933 and 1936. From an examination of the records I can find only two cases between 1921 and 1933. Some useful pathological material, proving the diagnosis, was available from them, and consequently such clinical facts as were recorded about them, have been used in the following analysis.

Seven of the cases occurred in full-time children and one in a premature infant (Case 27). Labour had been normal in all instances.

(ii) Jaundice.

In seven of the eight cases a history of jaundice was obtained. This was said to have been present at birth in three cases (Nos. 25, 31 and 37), on the second day in two (Nos. 24 and 29) and on the third and eighth days in the remaining two cases (Nos. 26 and 28). The jaundice was slight, and in five cases of transient duration. In three cases, very slight icterus --- a lemon yellow tinge --- persisted until death or the arrest of the haemolytic process. Recurrence of slight jaundice occurred suddenly in one case on the thirteenth day (Case 24).

(iii) Pallor.

As the jaundice receded, the child became increasingly pale, until in the second week of life the skin was of a waxy pallor, and the mucous membranes and finger-nails blanched. Pallor was never noticed by the parents until the child was very anaemic.

Meanwhile the child's nutrition was usually fairly well maintained although sometimes vomiting was troublesome. Later the patient became listless and drowsy, and sometimes slightly cyanosed. In spite of extreme anaemia, however, signs of distress were not apparent.

(iv) The liver and spleen.

Hepatomegaly and splenomegaly were constant findings, and similar in degree to what has already been described in cases of icterus gravis. Glandular enlargement was absent.

(v) The stools, urine and serum bilirubin.

The stools were of a normal colour, and a strong reaction for urobilin was obtained. The urine did not contain bile pigments, but excess of urobilin was found in all except one case (No. 27). These tests were made before giving any treatment, because after transfusion urobilinuria was always found, being due to the destruction of part of the injected blood.

The serum van den Bergh reaction was of the purely indirect positive type.

From the findings in the stools, urine and serum, it can be concluded that the grave degree of damage to the liver found in icterus gravis, was not present in anaemia without oedema or jaundice.

(vi) Haemorrhages.

Clinically, haemorrhages were not present, except in one case (No. 27) in which retinal haemorrhages were found shortly after birth.

B. THE HAEMATOLOGICAL FINDINGS.

(i) The red corpuscles.

Blood counts were available in five cases of the 1933-36 series, the other case (No. 26) having died on admission, and in one case (No. 36) from the 1931-33 series.

A very severe degree of anaemia was present by the second week of life (Cases 24, 25, 28 and 29 -- Table VII), the red cells numbering between 1.1 and 1.5 millions per c.mm., while a similar gross anaemia was found in the third week in one case (No. 36).

Table VII. Blood counts from cases of anaemia without oedema or jaundice.

Case No.	Age (in days)	Hb.%	R.B.C. (millions per c.mm.)	Colour Index	Leucocytes per c.mm.	Reticulocytes
29	9	28 (Sahli)	1.12	1.27	19,000	1.5%
24	13	42 (Sahli)	1.55	1.38	12,000	21.0%
25	13	32 (Sahli)	1.20	1.33	10,000	5.0%
28	13	24 (Haldane)	1.20	1.00	22,300	<1%
36	18	30 (Haldane)	1.33	1.1	28,000	<1%
27	63	19 (Haldane)	1.07	0.90	6,600	

Reticulocytes were numerous, 21% of the red corpuscles in one case (No. 24), and slightly excessive (5%) in another (Case 25). An appreciable increase in the remaining cases was not found.

Nucleated red cells were usually occasional, although in one case they varied between 660 and 3,500 per c.mm., while in a second in the older series (Case 36) it was noted that primitive red cells were numerous.

The colour index lay between 1.0 and 1.38, lower values being obtained with Haldane's than with Sahli's method. Films showed that the corpuscles were evenly stained, anisocytosis and poikilocytosis being slight, although polychromasia was sometimes frequent.

A Price-Jones curve from one case (No. 29) revealed a diminution of the mean corpuscular diameter or shift to the left (Fig. VI).

The fragility of the red cells in the same case was normal (Table VIII).

Table VIII. Fragility in a case of anaemia without oedema or jaundice.

NaCl %	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.60
Case No.29	+++	++	++	+	+	-	-	-	-	-	-	-
Control (Adult blood)	+++	++	+	+	$\pm$	-	-	-	-	-	-	-

(ii) The leucocytes.

During the first three weeks slight leucocytosis, between 19,000 and 28,000 per c.mm., was seen in three cases (Nos. 28, 29 and 36). Two cases (Nos. 24 and 25) had a slight leucopenia, although the majority of the leucocytes were polymorphonuclear neutrophiles.

(iii) Comment on the haematological findings.

It will be seen then that the haematological findings in my cases during the second and third weeks were very similar to those found in icterus gravis at the same age. The haemolysis seemed to have reached its maximum about this time. In this respect however it is pertinent to quote in some detail the findings of a case in the tenth week.

The child (Case 27) was first seen at the age of nine weeks. She had been prematurely born but had never been jaundiced. The date of establishment of the anaemia was doubtful. The mother, a woman of poor mentality, thought that pallor had been noticeable in the fifth week, but the grandmother insisted that the child had been pale at a much earlier date.

On admission the baby was found to be small but moderately well nourished. There was a slight café au lait tint of the skin, but the sclerotics were clear. The liver and spleen, especially the latter, were enlarged. Urobilinuria was absent.

The blood count showed a gross anaemia (Table VII) with a colour index of 0.90. This colour index is normal for the age of the child (van Creveld and Heybroek, 1932). Reticulocytosis and erythroblastaemia were not found. Leucopenia was present although the blood was not agranulocytic (42% neutrophile leucocytes).

This case seems to lie between the acute forms of neonatal haemolytic anaemia described previously, and the excessive and more chronic haemolysis of prematurity. In pure cases of the latter such a gross anaemia is not found, although the maximum anaemia occurs at a similar time (p. 166). Splenomegaly, too, is rare.

Before drawing any further conclusions, it seems desirable, owing to the comparatively small number of cases which I had the opportunity to study, to examine the literature at this point.

C. A COMPARISON OF THE PUBLISHED CASES WITH THE FOREGOING DATA ON HAEMOLYTIC ANAEMIA OF THE NEW-BORN WITHOUT OEDEMA OR JAUNDICE.

From the literature which is chiefly American, I have collected details of 40 cases of anaemia haemolytica sine icterus. These show a wider variety of types than was observed in my series.

(i) History and clinical findings.

The histories were much the same, gradually increasing pallor, with or without transient jaundice. Vomiting was not infrequent (Diamond et al., 1932; Segar and Stoeffler, 1932). Absence of splenomegaly was a not uncommon feature (Bonar, 1927; Greenthal, 1930; Ehrmann, 1929; Bonar and Smith, 1933; and Åckerrén, 1933). In some cases in spite of a falling blood count, urobilinuria was not present (Montfort and Brancato, 1935; Mannheimer, 1935; Åckerrén, 1933).

Haemorrhages were not seen.

(ii) Anaemia.

The blood counts of the cases from the literature have been tabulated (Table IX) according to the age at which the maximum anaemia was attained. It will be seen that anaemia was sometimes severe on the first day of life, e.g. 1.5 million red cells per c.mm.. Some very low red cell counts, under 0.5 million per c.mm., occurred in the first or early in the second week. None of the cases which died had been transfused.

The maximum degree of anaemia was found during the first week in twelve cases, in the second week in thirteen, and in the third week in seven cases. Thereafter the incidence of maximum anaemia became much reduced, only six cases being recorded between the fourth week and six weeks.

None of the recorded cases showed such a chronic course as my example (No. 27) although it is notable that at four weeks, Ducas and Jacquet's case had only 0.36 million red cells per c.mm.. An even more marked leucopenia than in my case was present, although 33% of the leucocytes were neutrophile polymorphs. In spite of the gross anaemia recovery followed.

(iii) Colour index.

The colour index was variable. Twenty-one of the tabulated cases had values between 1.0 and 1.5. Excessively high figures (1.9) were found in three cases (Gelston and Sappington, 1930; Segar and Stoeffler, 1932; and Happ, 1930), while very low indices, between 0.6 and 0.7, suggesting iron deficiency were not infrequent (Ecklin, 1919; Segar and Stoeffler, 1932; Pritchard and Smith, 1931; and Sidbury, 1922).

Some of the minor discrepancies of colour index might be explained by the difference between the standards in the various methods of estimating haemoglobin. The grosser irregularities however cannot be eliminated in this way.

<u>Table IX.</u> Data of published cases of haemolytic anaemia of the new-born without jaundice or oedema.

Author(s).	Date	Age	Hb.%	R.B.C. millions per c.mm.	Colour index.	Erythro-blastaemia.	Leucocytes per c.mm.	Treatment.	Result.
Sanford	1925	1 day	48	2.5	0.96	+	68,400	nil	Recovered.
Montford and Brancato	1935	1 day	43	1.75	1.11	++	17,000	trans.	Recovered.
do.	do.	1 day	38	1.5	1.26	+	27,000	trans.	Recovered.
Greenthal	1930	2 days	48	1.78	1.3		17,000	trans.	Recovered.
Segar and Stoeffler	1932	4 days	45	3.34	0.68		12,000	trans.	Recovered.
Ehrmann	1929	5 days	28	1.22	1.2		20,500	Intraper. blood	Recovered.
Pasachoff and Wilson	1931	5 days	8	0.399	1.0	Occas.	16,500	nil	Died 5/365.
Parsons et al.	1933	6 days	<20	0.328	1 +		15,200	nil	Died 6/365.
do.	do.	7 days	42	2.6	0.8			nil	Died 12/365
Susstrunk	1924	7 days	26	1.15	1.1	+	10,600	nil	Died 10/365
Gelston and Sappington	1930	7 days	42	2.3	1.9		11,200	Intraper. blood	Recovered.
Åckerrén	1933	7 days	35	1.9	0.92	nil	12,600	I.m. blood	Recovered.
Brown, Morrison and Meyer	1934	8 days	20	1.38	0.79	+	60,000	I.m. blood	Died.
Bonar and Smith	1933	8 days	79	2.87	1.39		14,300	nil	Recovered.

Table IX (contd.).

Author(s).	Date	Age	Hb.%	R.B.C. millions per c.mm.	Colour index.	Erythro-blastaemia.	Leucocytes per c.mm.	Treatment.	Result.
Abbot and Abbot	1935	8 days	17	0.74	1.21		26,000		Died 9/365.
Segar and Stoeffler	1932	10 days	45	1.15	1.95			Trans.	Recovered.
Montford and Brancato	1935	10 days	33	1.21	1.12	+++	39,000	Trans.	Recovered.
Pritchard and Smith	1931	10 days	10	0.75	0.67		74,000	Trans.	Recovered.
Diamond et al.	1932	10 days	<20	0.90	1+		26,000	Trans.	Recovered.
Ecklin	1919	12 days	32	2.5	0.64	+	40,000	Iron	Recovered.
Canino	1927	12 days	45	2.5	0.9	+	14,500	nil	Recovered.
Bonar	1927	13 days	31	1.2	1.3	±	11,000	nil	Recovered.
Bonar and Smith	1933	13 days	35	2.07	0.85		18,200	nil	Recovered.
Sidbury	1927	14 days	10	0.8	0.62		28,000	Trans.	Recovered.
Happ	1930	14 days	40	1.08	1.9	±	11,200	Intraper. blood	Recovered.
Stransky	1931	15 days	47	2.4		+	6,200	Trans.	Recovered.
Diamond et al.	1932	15 days	39	2.4	0.89		28,500	Trans.	Recovered.
do.	do.	15 days	35	1.3	1.3		16,000	Trans.	Recovered.
do.	do.	17 days	28	1.23	1.1	Occas.	18,700	Trans.	Recovered.
Stransky	1931	17 days	23	1.080		+	8,900	nil	Recovered.

Table IX (contd.).

Author(s)	Date	Age	Hb.%	R.B.C. millions per c.mm.	Colour index.	Erythro-blastaemia.	Leucocytes per c.mm.	Treatment.	Result.
Diamond et al.	1932	18 days	15	0.65	1.15	+	16,500	Trans.	Recovered.
do.	do.	18 days	30	1.5	1.0		16,800	Trans.	Recovered.
do.	do.	19 days	1.8	0.90	1.0	rare	11,100	Trans.	Recovered.
Donnally	1924	20 days	20	0.918	1.0		29,000	Trans.	Recovered.
Abbot and Abbot	1935	23 days	36	1.66	1.1		10,200	I.m.blood.	Recovered.
Noll	1934	26 days	34	1.92	0.90	+	7,500	nil	Recovered.
Huenekens	1936	27 days	42	1.38	1.5		30,500	Trans.	Recovered.
Ducas and Jacquet	1931	28 days		0.36		±	2,200	I.m.blood.	Recovered.
Åkerrén	1933	35 days	30	1.16	1.3	++	29,700	I.m.blood.	Recovered.
Segar and Stoeffler	1932	6 weeks		0.83				Trans.	Recovered.

Trans. = Intravenous blood transfusion.

Intraper. blood = Intraperitoneal blood transfusion.

I.m. blood = Intramuscular injections of blood.

Occas. = Occasional.

It may be that where there was a very high figure, an excessive degree of megalocytosis was present although there was not any data of cell measurement to prove this. Low colour indices suggest the coincidence of iron deficiency and haemolysis. Unfortunately an investigation of this hypothesis was not carried out.

(iv) Reticulocytes.

Regrettably, comparatively few reticulocyte counts, especially in the more acute phases of the anaemia, have been published. Reticulocytosis during increasing anaemia was sometimes seen (Donnally, 1924; Stransky, 1931; Diamond et al, 1932; Mannheimer, 1935; Abbot and Abbot, 1935), and later during the recovery phase (Huenekens, 1936; Montfort and Brancato, 1935).

(v) Erythroblastaemia.

Erythroblasts were frequently present in the peripheral blood, but rarely in such large numbers as in icterus gravis, even on the first day. Scanty nucleated red cells sometimes remained in the films throughout the period of increasing anaemia. On the other hand some cases showed an absence of reticulocytosis and erythroblastaemia at all stages (Brown et al, 1934; Gelston and Sappington, 1930; Stransky, 1931; Abbot and Abbot, 1935). Clinically, these cases, like some of my own, seem to have had a hypoplastic erythropoietic mechanism, although sometimes it was obvious that leucocyte formation was not affected (Brown et al., 1934, and Case No. 27 of my own series).

(vi) Fragility of the red cells.

Fragility of the red cells in hypotonic saline was reported as normal by Ehrmann and Diamond et al., the latter also finding that platelet numbers were unaltered.

(vii) Morphology of the red cells.

Only one reference to the morphology of the red cells in anaemia without jaundice was found. This was by van Creveld who found a practically normal cell diameter and Price-Jones curve in one case. In my case (No. 29), as I have already indicated, the cell size was very similar to what was obtained in icterus gravis.

(viii) The leucocytes.

The leucocyte counts showed widely divergent figures, the highest being 74,000 (Pritchard and Smith, 1931) and the lowest, 2,200 per c.mm. (Ducas and Jacquet, 1931). As a rule however the variation from the normal for the age has not been very great.

(ix) Coagulation and bleeding times.

Coagulation time, as in icterus gravis, was recorded as normal, and although the bleeding time rarely showed any alteration, two cases with prolongation have been published (Diamond et al., 1932; Montfort and Brancato, 1935).

D. SUMMARY OF THE CLINICAL AND HAEMATOLOGICAL FEATURES OF HAEMOLYTIC ANAEMIA OF THE NEW-BORN WITHOUT OEDEMA OR JAUNDICE.

The main features of haemolytic anaemia of the new-born, without oedema or jaundice, are clinically and haematologically very similar to those of icterus gravis.

The pallor did not become noticeable to the parents until the end of the first or in the second week, although blood counts obtained in the first few days of life in some of the published cases show that anaemia was present from an early date.

Splenomegaly and hepatomegaly were frequent features.

The anaemia was of the haemolytic type, showing a rapid fall in blood count, most marked in the early weeks. The colour index was usually high. Excess of urobilin in the urine and an indirect positive van den Bergh reaction were common. A high reticulocytosis was sometimes found but erythroblastaemia was not so obtrusive as in cases of icterus gravis.

Some cases showed little or no increase of reticulocyte numbers, and this suggested hypoplasia of the erythropoietic mechanism. This feature will be more fully discussed when the pathology of the erythroblastoses has been considered.

The red cell morphology in the only case investigated was similar to that in icterus gravis.

The fragility of the red cells was normal. Platelet counts, bleeding and coagulation times, showed little abnormality. Clinically haemorrhages were not noted in the literature and occurred in only one of my cases.

Certain cases published in the literature suggested from the very low colour indices that there may occasionally be an added element of nutritional anaemia. I did not find any suggestion of this in my series.

Although the ultimate anaemia may be equally as severe as in icterus gravis, it would appear from the published cases and one of my own that in anaemia without jaundice the course is often more chronic and that the general condition of the child is usually less seriously impaired.

The clinical findings indicated that there is very little damage to the liver such as was found in icterus gravis.

IV. HYDROPS FOETALIS.

A brief account of the clinical, haematological and pathological findings.

Oedema of the new-born has been recognised for centuries, but it was not until 1910 that Schridde associated one form of it with abnormalities of the haematopoietic system. Rautmann (1912) used the term erythroblastosis to cover the abnormal hyperplasia and persistence of the foetal depôts of blood formation, and since then many cases have been described by Woolley (1914); Goormaghtigh (1925); Salomonsen (1931); Ferguson (1931); Parsons et al (1933); Péhu et al (1934); Montfort and Brancato (1935); Pasachoff and Wilson (1935), and others.

I have already referred to the occurrence of the three forms of erythroblastosis foetalis in single families. Some interesting factors arising out of twin births have been recorded. One of Woolley's cases of hydrops foetalis was a twin, the other child being healthy, and de Lange (1932) noted a twin birth where one of the infants suffered from icterus gravis and the other from hydrops foetalis.

Woolley's instance seems to be excellent evidence against the theory that maternal toxaemia is a factor in the production of the neonatal haemolytic anaemias, although it does not exclude the possibility that the origin of the abnormality may be in the placenta. It is not known if in his case there were two placentas.

Frequently the mother's health has been good prior to and during the major part of the pregnancy. Nephritis, tuberculosis and syphilis of the mother have also been excluded. Therefore maternal toxaemia may be excluded as a cause of fatal oedema of the new-born.

There are several outstanding features of hydrops foetalis. Usually, the child was prematurely born and often varying degrees of hydramnios have been present in the mother. The foetus if not stillborn, lived at the most for only a few hours.

The placenta and cord were greatly enlarged and oedematous.

Generalised oedema of the child was present. The skin was oedematous and often slightly jaundiced. The subcutaneous tissues and muscles were water-logged with yellow fluid. Frequently great abdominal distension with yellow or bloody fluid, hydrothorax and pericardial transudation have been noted.

Anaemia was marked and counts as low as 0.5 million red cells per c.mm. have been found in the heart blood immediately after death (Parsons et al.). Numerous erythroblasts have been found in the circulating blood (Parsons et al, Goormaghtigh and Péhu).

Post-mortem examination has revealed enlargement of the liver and spleen as constant findings. Frequently, too, hypertrophy and enlargement of the heart have been noted. Except for slight jaundice or small haemorrhages, remarkable macroscopic changes in the other viscera have not been found as a rule, although the lungs were sometimes atalectatic. The lymph glands have not been found to be enlarged.

Microscopically widespread blood formation, mostly erythropoietic, has been found in extramedullary sites, chiefly in the liver and spleen, but also in the kidneys and lymph nodes and in rarer places, such as the thyroid, thymus and suprarenal glands, the heart and lungs, the subcutaneous tissues and skin.

Some authorities have noted that in a few cases there is evidence of the hypoplasia of the erythropoietic tissues, and in the main sites of extramedullary haematopoiesis, the liver and spleen, blood formation has been no more or even less active than in foetal tissues at the same stage of gestation (Salomonsen).

Less striking features have also been noted. Great displacement and breaking up of the liver cell columns, with degeneration of the liver cells, have been common features. In the spleen the Malpighian bodies have been rudimentary and inconspicuous. Similarly in the lymph glands the lymph nodes have been small. Phagocytosis in all these tissues has been prominent.

Varying reports on haemosiderosis have been made. In some cases iron deposition in the liver and spleen has been excessive (Péhu) and in others scanty or absent (Goormaghtigh).

Goormaghtigh made an interesting contribution to the knowledge of the condition, when he showed that enlargement of the placenta was not so much due to oedema as to proliferation of the villi, in the lumina of which he found very extensive erythropoiesis. Sometimes however, although the villi have been hyperplastic, there have not been any erythropoietic islets, and only an increase in the numbers of circulating nucleated red cells was found (Péhu).

In brief, hydrops foetalis has a close connection with other forms of neonatal anaemia. It starts during foetal life, and leads to death of the foetus before or shortly after birth. There is foetal anasarca, enlargement of the liver, spleen and placenta, and, microscopically, there is generally hyperplasia of the extramedullary sites of foetal blood formation.

SECTION B.

I. TREATMENT OF THE NEONATAL HAEMOLYTIC ANAEMIAS.

(i) Introduction.

In the previous pages only the condition of the child before transfusion has been discussed. The reason for this is that all the cases of which records of progress over a prolonged period are available, have been those cases in which transfusion has been done, and because transfusion produces such a great change in the blood picture that it is essential to consider progress and treatment together.

Detailed records have not been kept in cases of the 1921-33 series because it was not until the last year of this period that anaemia of the new-born became a widely recognised clinical entity in this country. During the three years, 1933 to 1936, those cases which were not transfused died within a short period of admission to hospital.

Because the blood findings and other features of both icterus gravis and haemolytic anaemia without jaundice are similar it will be convenient to discuss the course of these anaemias together.

Transfusion, which has come to be the accepted method of treatment in this country and America, was performed on seventeen out of twenty-four cases of icterus gravis (one from the 1921-33 series), and in five out of six cases of anaemia without jaundice. There were various reasons for failing to transfuse the eight cases of haemolytic anaemia. In two (Nos. 13 and 14) broncho-pneumonia was present and the children were obviously dying. In three cases (Nos. 18, 21 and 26) the children were either dead on admission or died very shortly after, and in another (Case 7) a history of convulsions suggested a cerebral haemorrhage. This suspicion was confirmed post-mortem. A seventh case (No. 9) was treated by intra-muscular injections of blood and the remaining case (No. 22) recovered spontaneously.

(ii) <u>The history of haemotherapy in the neonatal anaemias</u>.

Various methods of haemotherapy have been advocated by different authors. Pitfield (1912) who was under the impression that jaundice of the new-born was the same disease as haemorrhage of the new-born, was the first to use whole blood injections into the muscles. His case made a complete recovery.

Hampson (1929) recommended the use of small quantities of human blood serum in icterus gravis. Daily doses of five to ten cubic centimetres were injected intramuscularly until the bilirubin in the patient's serum began to decrease. By this method he claimed to have cured seventeen out of a series of eighteen cases. It seems however that his later experience was not so favourable (Hampson, 1933). In order to explain the action of serum he postulated the theory that human blood contains an anti-haemolytic factor and that the foetus in utero depends on the maternal blood for a supply of this agent. Shortly after birth the normal child acquires the ability to elaborate this anti-haemolysin, which then checks the normal post-natal blood destruction. Those cases in which haemolysis is excessive he explains as being due to failure to produce the anti-haemolytic factor. The curative action of serum is derived from its content of anti-haemolysin.

Kramsztyk (1931) reported a case in which subcutaneous injections of whole blood were successful. Hawksley and Lightwood (1934) failed to get satisfactory results with this method and Kleinschmidt (1930) had a similar experience. Péhu et al. (1934, 1935, 1936) however still advocate the early use of intra-muscular blood injections.

Buhrman and Sanford (1931) tried intraperitoneal injections of whole blood without success in two cases of icterus gravis. Ehrmann (1929) and Happ (1930) gave similar treatment in cases of haemolytic anaemia without jaundice. The cases recovered, but as many cases which have been published (Table IX) have recovered without any treatment, the value of these observations is lessened.

I have experience of only one case of icterus gravis (No. 9) treated exclusively with subcutaneous injections of whole blood. This child was first seen on the twenty-fifth day of life, and haemolysis was still very active, the red cell count being 1.44 millions per c.mm., 18% of these being reticulocytes, while even at this late stage of the disease 650 erythroblasts per c.mm. were still present. As will be seen from Chart V, three successive daily injections of 10 to

15 c.c. of blood failed to have any effect on the red cell count which continued to fall, and on the thirtieth day of life the red corpuscle numbers were reduced to 855,000 per c.mm. with a persistingly high reticulocytosis. The child died shortly afterwards.

It would seem that serum or whole blood injections frequently have little or no effect in arresting the haemolytic process, although they have a dramatic effect in haemorrhage of the new-born. Where success has been claimed in cases of icterus gravis it is possible that the cases would have recovered without treatment. For instance, Pitfield's case came from a family in which one child, and possibly two, had already recovered spontaneously. The high incidence of natural recovery in anaemia without jaundice makes the value of treatment doubtful.

It was natural that treatment by transfusion of blood intravenously should be tried, and this was first done in cases of anaemia without jaundice. Donnally published a successful case in 1924, and since then several similar results have been recorded (Table IX). In these cases however the same criticism of the efficacy of intra-muscular injections holds good for transfusion.

In 1925, Hart obtained a recovery in a transfused case of icterus gravis. Kleinschmidt (1930) thought that transfusion in these cases was beneficial. De Lange and Arntzenius (1929) published a successful result. Hampson (1933), although still recommending injection of serum in some cases of icterus gravis, was of the opinion that transfusion should be done in cases showing erythroblastaemia. In America, Clifford and Hertig (1932) and Diamond, Blackfan and Baty (1932) advised transfusion. In this country, Hawksley and Lightwood (1933 and 1934) claimed successes with repeated small transfusions and were of the opinion that if they were to be of value they should be started within forty-eight hours of the onset of jaundice. It was their aim to maintain the red cell count at a level of about four millions per c.mm. until haemolysis ceased.

(iii) Present practice in treatment.

It has been the practice in the Royal Hospital for Sick Children, Glasgow, to transfuse the child as soon as possible after admission. In each case the blood of the donor and recipient have been cross-matched. That is to say, not only has the more important test of compatibility of the recipient's serum with the donor's corpuscles been investigated but also that of the donor's serum with the patient's corpuscles. It has not been considered safe to omit matching of the blood of these young infants and the donors, because although it has been stated that agglutinins are not present in the infant's serum, Jones (1921) has shown that this is frequently not the case. In the transfusion of adult patients it is not usual to match the donor's serum with the patient's corpuscles, because even if they were incompatible the rapid dilution of the former renders the occurrence of agglutination unlikely. Nevertheless it is advisable to do this test when dealing with babies, as the proportion of transfused blood to the total circulating blood in the infant is much higher than in the adult.

As these transfusions were in the nature of emergencies, no time was available in which to do the donor's Wassermann reaction, but as a rule however, this was not of importance because the father's blood was used.

The choice of technique was of some importance because the transfusion of newly-born infants is a matter requiring some skill. Unfortunately as the child was usually well nourished, the superficial veins could not be seen or felt and consequently the injection of blood into them with a needle and syringe was often impossible. Injection after cutting down and inserting a cannula into a vein was excellent if only one or two transfusions were required, but if, as in some of my cases, three to six were necessary, it became difficult to find veins and would have left the child with rather too many wounds, which would be a source of danger, because in the event of infection occurring in them the child's life might be jeopardized.

A third method, that of injecting the blood into the superior longitudinal sinus in the region of the anterior fontanelle, carried the grave risk that some of the blood would be injected into the subdural space. Such an accident might be sufficient to cause speedy death, or serious damage to the brain from subsequent organisation of the extravasated blood. On the other hand the last method was undoubtedly the simplest. Where it was used in the present series of cases (1933-36) there was not any apparent tearing of the longitudinal sinus post-mortem, and meningeal haemorrhage when present was at some distance from the site of injection.

Apart from accident during injection, an objection to transfusion into the longitudinal sinus has been made on the grounds that it may increase the danger of intra-cranial haemorrhage, to which cases of neonatal haemolytic anaemia are susceptible (Hawksley and Lightwood, 1934). In practice however I have not found this to be the case. In Table X it is shown that there was actually a higher proportion of cerebral and meningeal haemorrhages in those cases transfused into a superficial vein or not transfused at all, than in the group in which the fontanelle route had been used.

Table X. Showing the incidence of intra-cranial haemorrhage in cases of icterus gravis, in relation to the route of transfusion.

	Total number	No. of cases of intracranial haemorrhage.
Cases transfused into longitudinal sinus.	11	3
" " " superficial vein.	3	2
" not transfused.	3	1

If blood is withdrawn into a closed sterile system, sufficient may be collected to do several transfusions, because if kept on ice the citrated blood will remain fit for use for seventy-two hours. This is of great value in giving repeated small transfusions.

A safe quantity of blood which can be given to an infant without fear of cardiac failure is 25 c.c. per kilogram of body weight. Smaller amounts are probably just as effective if they can be repeated at need. Larger transfusions of 30 c.c. or more per kilogram were sometimes given (Cases 8 and 23).

(iv) Other forms of treatment of neonatal anaemia.

Apart from haemotherapy, other forms of treatment have been advocated. Huenekens (1936) recently advised the administration of liver extract. He found a speedy reticulocyte crisis with a rise in the red cell count after the haemolytic period was over, in a case of anaemia without jaundice. I have, however, observed similar evidence of brisk erythropoiesis in the absence of haemolysis in a case of the same type (No. 28). Here there was a reticulocytosis of 15% of the red corpuscles at the beginning of the sixth week. The red cell numbers were rising at the time and continued to increase until eventually normal figures were reached. In this case iron had been given from the age of four weeks. Probably, however, the accelerated haematopoiesis was spontaneous because with similar treatment in other cases which were recovering I failed to obtain such a dramatic result.

Nevertheless, it is reasonable to supply the recovering child with extra haematopoietic materials, such as liver extract and iron. Although I have not given liver, I have made it a rule to supply iron to recovered cases. The dose given was 5 grains of ferri et ammonii citras daily, and it was continued for as long as possible throughout infancy. It is probable that babies who have been the victims of a neonatal haemolytic anaemia will be very liable to develop a nutritional anaemia which Mackay (1931) has shown to be frequent in normal infants, and which makes its appearance from the age of six months onwards.

Cathala (1926) was of the opinion that antisyphilitic treatment cured a case of icterus gravis. In the family described there was not any clinical or serological evidence of syphilis in the father and mother or in any of the children. Cathala based his assertion on the fact that several previous children had died of neonatal jaundice and that the only one which recovered had been treated with heavy metals. In view of the wide search for a syphilitic basis to the neonatal anaemias and the fact that the hypothesis has been generally rejected it is more than likely that Cathala's case was an example of spontaneous recovery.

Treatment other than transfusion was purely general. Where possible breast feeding was continued, and the child treated as an out-patient in order to lessen the chances of the acquisition of a ward infection. Sometimes in artificially fed children the fat in cow's milk was reduced by dilution with water because of the diminution of bile excreted into the intestines. Additional sugar, usually well tolerated by a damaged liver was given. Where diarrhoea with dehydration occurred intravenous glucose in saline was supplied.

II. THE EFFECT OF BLOOD TRANSFUSION ON THE HAEMATOLOGICAL FINDINGS AND THE COURSE OF THE ANAEMIA.

(i) The effect on the red cell count.

Following on transfusion there is an increase in the number of circulating red corpuscles. From a transfusion of 28 c.c. per kilogram of body weight a rise of 1.5 million red cells per c.mm. was found immediately after (Chart VII). Although in other cases the red corpuscles were not counted immediately after transfusion, it would appear that a donation of 25 c.c. of blood per kilogram of body weight causes a rise of one to one and a half million red corpuscles per c.mm. (Charts I, III and VI), and larger transfusions, for instance 33 c.c. per kilogram, an increase of about 2 millions per c.mm. (Chart IV).

(ii) The effect on haemolysis.

The new supply of blood however did not appear to have any effect on the haemolytic process, and subsequent diminution of the red cell numbers was frequently seen (Charts II, III, IV, VI, VIII, and XI). In many cases two or more transfusions were required to prevent grave relapse (Charts II, IV, VI, VIII, IX, X, and XI).

(iii) The effect on reticulocytosis and erythroblastaemia.

Transfusions, single or repeated, caused a diminution of the reticulocyte numbers. This could sometimes be explained by dilution, e.g. in Case 8 (Chart IV) where the red cell count was roughly doubled (1.7 millions to 3.7 millions per c.mm.) and the reticulocyte count halved (16% to 8%). In other cases however no, or only a trivial, decrease in reticulocyte numbers followed, and, as a rule, especially in the earlier stages of the anaemia, the reticulocyte count was high where there was a falling blood count and low when there was a rising count (Charts II, III, IV, VII and VIII). When blood loss was balanced by transfusion or spontaneous blood formation reticulocyte and red cell counts ran a parallel course (Charts X (first week), IX, XI).

Erythroblastaemia almost disappeared after the initial transfusion. A reappearance was sometimes seen when an exacerbation of haemolysis occurred, being most marked in Cases 5 and 17 where 444 and 300 per c.mm. respectively were present.

(iv) The rate of haemolysis.

The rate of destruction of red cells was variable in different cases and also during the course of individual cases. In Case 1 (Chart I), after one transfusion the increased number of corpuscles was maintained. Blood destruction was still occurring however, as was shown by the persistence of a reticulocytosis of from 5 to 7% without an appreciable rise in the red cell count. Any rise present did not exceed

200,000 red corpuscles per c.mm. and this figure is generally accepted as being within limit of experimental error.

Two transfusions within a week were required in Case 8 (Chart IV) to keep the red cell count above 3 millions per c.mm. Here again there was evidence of accelerated blood production in the raised reticulocyte count. Very active haemolysis was present in Case 23 (Chart X) where three transfusions of 100 c.c. each were necessary during one week to maintain a red cell count of 3.5 millions per c.mm. A considerable reticulocytosis was found during this period.

Remarkably severe and constant haemolysis associated with a high reticulocytosis was seen in a case of anaemia without jaundice, which required four transfusions in eight days (Chart XI). Thereafter although the reticulocytosis persisted there was not any appreciable improvement in the red cell count during the next four weeks, showing that haemolysis was still present but that the erythropoietic organs were able to balance the loss.

(v) Intermittent haemolysis.

In these cases to which reference has just been made it would appear that the destruction of red cells was more or less continuous. This was not always the case, and sometimes it was found that there were periods in which the haemolytic process was slight or in abeyance and that exacerbations or recrudescences occurred.

Case 5 (Chart II) illustrates this point. Transfusion was done on the ninth day, and on the thirteenth the red cell count was 1.5 millions per c.mm. above the pre-transfusion level, the reticulocytes having diminished considerably in numbers. During the following week there was a rapid fall in red cell numbers and at the same time an increase of reticulocytosis to a maximum of 15%.

Similarly in Case 17 (Chart VIII) transfusions raised the blood count from the eleventh to the thirteenth day, and there was a diminution of reticulocytosis. Between the fourteenth and seventeenth days there was a brisk haemolysis and increase of reticulocytosis to 22%. A third transfusion was then given but haemolysis persisted, as was shown by the persistence of reticulocytosis, and unaltered blood count between the eighteenth and twentieth days. Two further transfusions, making five in all, raised the red cell count to over 4 millions per c.mm. Haemolysis apparently ceased at this time because there was no increase of anaemia thereafter and the reticulocyte numbers quickly fell to within normal limits (0 to 1%).

Slight recrudescences of haemolysis were seen in three cases (Charts III, VII and X). In each case there was a fall in the red cell count accompanied by reticulocytosis. This occurred in fourth and fifth weeks in Case 23 (Chart X), in the sixth week in Case 6 (Chart III), and in the eighth week in Case 15 (Chart VII). Spontaneous recovery followed in all those instances.

When haemolysis became more severe or recurred there was an increase of jaundice and in Case 6, urobilinuria and splenomegaly which had been temporarily absent became again apparent.

(vi) The interpretation of the haematological findings.

From the above account it is evident that there was in neonatal anaemias an over-production and excessive destruction of red cells. Haematopoiesis was most active during times of brisk haemolysis. Both of these features were either continuous or remittent. It might either be that the erythropoiesis was a response by the haematopoietic tissues to replace destroyed red cells or that haemolysis was the result of the pouring out into the circulation of immature red cells with some fault in their construction which rendered them excessively liable to destruction by the reticulo-endothelial system. The former seems to be more likely, because it was obvious that the donated blood was equally susceptible to the haemolytic process. Further after transfusion, although reticulocytosis persisted, nucleated red cells more or less disappeared from the circulation, indicating that the erythropoietic tissues were somewhat less active. The fact that there were periods of remission of haemolysis also suggests that blood destruction was the primary factor, because if faulty red cell production were paramount, haemolysis should have been continuous, as it is unlikely that the haematopoietic tissues would at one time produce normal cells and at another defective corpuscles.

(vii) The duration of haemolysis.

It was striking in many of the fatal cases that death did not occur when haemolysis was active, and that in most cases which survived into the third week or later there was not a fatal degree of anaemia. This seems to imply that the haemolytic process was of limited duration.

In Table XI I have collected data from my cases of haemolytic anaemia with or without jaundice, with regard to the duration and degree of haemolysis. Five cases (Nos. 6, 11, 13, 22 and 27) have been excluded because the time of cessation of haemolysis could not be accurately ascertained or because of special features such as late onset of jaundice. The latter type of case will be discussed later.

Of the twenty three cases included, death occurred in ten cases within fifteen days of birth, destruction of blood being apparently still considerable. In two cases (Nos. 1 and 19) death was in the third week but haemolysis was by this time only of slight degree, and the anaemia had been considerably alleviated by transfusion. Including recrudescences haemolysis finally ceased in the fourth week in three cases, two of which recovered (Nos. 8, 17, 28). Cessation of excessive blood destruction was found in six cases in the fifth week; two recovered (Nos. 23 and 15), and two lived for

Table XI. Duration of haemolysis in the neo-natal haemolytic anaemias.

Case No.	Haemolysis.	Age at death.	Remarks.
2	Active at death.	8 days	
4	Active until death.	7 days	
7	Active until death.	7 days	
10	Active at death.	7 days	
12	Active at death.	13 days	
14	Active at death.	5 days	
18	Probably active; child very pale. Dead on admission.	12 days	
25	Active at death.	15 days	
26	Active at death.	10 days	
29	Active at death.	11 days	
1	Active at birth. Slight in third week.	19 days	
19	Still present in third week.	20 days	
8	Ceased in fourth week.	8 weeks	
17	Ceased in fourth week.		Recovered.
28	Active in first week. Recurrence in third and fourth weeks.		Recovered.
23	Ceased finally in fifth week.		Recovered.
15	Ceased in fourth week. Slight recurrence in fifth.		Recovered.
16	Ceased in fifth week.	3 months	
20	Ceased in fifth week.	6 weeks	
5	Diminishing greatly in fifth week.	33 days	
9	Very active in fifth week.	33 days	
3	Persisted into sixth week.	6 weeks	
24	Still active in eighth week.	8 weeks	

some time afterwards (Nos. 16 and 20). In one case (No. 5) haemolysis was very slight at the time of death, while the remaining case (No. 9) showed very severe progressive anaemia and persisting reticulocytosis right to the time of death.

A more prolonged duration of haemolysis was seen in two cases, where it extended into the sixth and eighth weeks (Nos. 3 and 24 respectively). Both cases died.

From this analysis it will be seen that in most cases of neonatal haemolytic anaemia, the process of excessive blood destruction is most active in the majority within the first two weeks, and that during this period the greater number of the fatalities occur. In children who survived longer than two weeks, the severity of the haemolysis usually lessened, and frequently ceased in the fourth or fifth weeks. On rare occasions (two out of 23 cases) a more prolonged, and even severe process of red cell destruction, occurred. Further in all cases which recovered, haemolysis had ended in the fourth or fifth weeks.

(viii) The value of transfusion.

It is obvious that transfusion was not a specific cure for the neonatal anaemias, and, even when given at an early date, it often failed to prevent a fatal outcome. Transfusion however did prolong life until haemolysis ceased in many cases, and anaemia was not the cause of death in unsuccessful cases. In the 1933-36 series of cases there were three outstanding examples of recovery which was only made possible by transfusion. The first of these (No. 17, Chart VIII) was a premature child admitted at the age of eleven days, having been jaundiced from birth. The red cell count was 1.48 millions per c.mm. The child was immediately transfused and again on the following day. A recrudescence of haemolysis occurred and at the age of sixteen days the red cells numbered 1.82 millions per c.mm. In the subsequent four days no less than three transfusions were required to maintain the blood count at about 2.5 millions per c.mm.

The next case (No. 23, Chart X) was a full-time child and was admitted deeply jaundiced when only one day old. At this time the red cells numbered 3.65 per c.mm. and three transfusions were required in three days to keep pace with the destruction of blood.

The third case (No.28, Chart XII) showed a gross anaemia without response from the haematopoietic tissues, and one transfusion was sufficient to enable the child to survive an exacerbation of haemolysis in the fourth and fifth weeks.

Without transfusion it will be obvious that these cases would have died of anaemia. It will be noted however that they were not of the most acute type of haemolytic anaemia. Two had survived without treatment for eleven and twelve days respectively, while the newly born infant did not have a gross erythroblastaemia. There is a considerable contrast between these cases and Case 1. This child had an extremely acute haemolysis with a great erythroblastaemia and although treatment was instituted within forty eight hours of birth the child eventually died.

Of the remainder of the transfused cases which recovered another of icterus gravis (No. 6, Chart III) was probably saved by transfusion. The onset of jaundice was delayed until the fifteenth day, and by the seventeenth a severe anaemia had been produced - 2.02 million red cells per c.mm. The child's general condition was good. After one transfusion haemolysis lessened (Chart III). Some days later there was a slight recrudescence but the child was not upset. A second and more severe exacerbation occurred and in the fifth week the child was again transfused. Erythroblastaemia was not present at any time. One cannot categorically claim that without transfusion this child would have died, although it would have been unwise to have withheld treatment.

Case 15 (Chart VII) would in all probability have made a spontaneous recovery. The child, jaundiced from birth, had survived for three weeks without treatment, and at that time the blood count showed 2.45 million red cells per c.mm., although a considerable reticulocytosis was still present as well as an abnormal number of erythroblasts. The stools gave a normal reaction for urobilin, and although bile pigments were still present in the urine, the van den Bergh reaction was not marked (6 units) and was chiefly indirect, there being some doubt if there were any delayed direct reaction. Obviously damage to the liver was slight, if at all present. After one transfusion the red cell count was maintained and in a few days a further appreciable rise of nearly 0.5 million per c.mm. was found.

The sixth successful case in which transfusion was done was one where spontaneous recovery was a distinct possibility although at the time this could not be forecast with certainty. The child (No. 13) was slightly premature and, although a considerable degree of jaundice was present, the anaemia was of slow progress and of moderate severity, while very little evidence of haematopoietic activity was found. Three transfusions were required between the seventh and twenty-sixth days to prevent the occurrence of a severe anaemia.

Whether or not transfusion was essential for the recovery of these cases is of secondary importance because, in any case, the donated blood relieved the strain on the haematopoietic organs, and at the same time considerably improved the child's general condition.

I conclude that intravenous blood transfusion is not a specific cure for the neonatal haemolytic anaemias, but is effective in preventing a fatal degree of anaemia by replacement of the destroyed red blood corpuscles. Its value is greatest in the first two weeks when haemolysis is most active. Transfusions should be given as often as is necessary to maintain the red cell count at a safe level.

Further, the value of transfusion is greater in icterus gravis neonatorum, in which the early mortality is much higher than in anaemia without jaundice.

From Table IX it will be seen that the great majority of published cases of anaemia without jaundice have recovered, and often spontaneously. The process, however, was slow and as many as seven months elapsed before the blood counts returned to figures above 4 million red corpuscles per c.mm. (Ecklin, 1919; Åckerrén, 1933), although the haemolytic process, as in most of my cases, had apparently ceased in three to five weeks from birth.

After recovery from excessive haemolysis the haematopoietic organs should be encouraged by the administration of iron over a long period. In some cases benefit may be derived from liver extract.

SECTION C.

THE PATHOLOGY OF ICTERUS GRAVIS NEONATORUM, AND HAEMOLYTIC ANAEMIA OF THE NEW-BORN WITHOUT OEDEMA OR JAUNDICE.

Introduction.

The following study has been derived from post mortem material from 17 cases of icterus gravis, and 4 of haemolytic anaemia without oedema or jaundice from the 1933-36 series. I personally attended the post mortem examinations of these cases, and, under the supervision of Professor Blacklock, assisted at certain stages. The staining, examination, and photographing of the sections was personally done by me, with the assistance in the last instance of the laboratory technicians.

Stored paraffin blocks of tissues from five cases of icterus gravis and two of anaemia without jaundice were available from the files in the Pathological Institute at the Hospital.

Professor Blacklock permitted me to examine these, and the findings have been included in the histological study.

For ease of description it has been found simpler to describe separately the morbid anatomy of the two types of anaemia, but the histology of the tissues has been reported under one heading.

I. MORBID ANATOMY OF ICTERUS GRAVIS.

(i) The abdomen.

The peritoneum was jaundiced. A small quantity of bile stained fluid was frequently present, but only in one case was there peritonitis, and in this instance it was associated with pneumonia and empyema. Petechial haemorrhages into both the parietal and visceral peritoneum were common.

(ii) The liver.

The liver was moderately enlarged in most of my cases of icterus gravis neonatorum, and was nearly always of a dark green colour and firm and smooth in consistence. In one or two cases where jaundice was fading at the time of death, a corresponding decrease in the jaundice of the internal organs was found and the liver in such cases was yellow or brown but with a distinctly red tinge. The gall-bladder and bile ducts were always normal, although the bile in the former was of a rather thick consistence. It could however be quite easily expressed into the duodenum by applying gentle pressure to the gall-bladder.

Change was absent from the capsule of Glisson.

On section, the cut surface of the liver did not show any gross change, other than jaundice. Where there had been recent transfusion some congestion was present. Occasionally there was a slight diffuse fatty degeneration, the tissues being a little more greasy than usual.

In the bile ducts which were of a sufficient size to be appreciable to the naked eye, bile thrombi were not found.

Evidence of fibrosis was absent on macroscopic examination.

(iii) The spleen.

Usually the spleen was enlarged to about twice the normal size. In one case (No. 19) the degree of enlargement was greater, and in four cases (Nos. 5, 10, 14, 16) the spleen was of practically normal size. In one of these cases (No. 16) however the child no longer had jaundice or anaemia and had died of gastro-enteritis.

The colour of the spleen was uniformly dark red, but the consistence was very variable, in some firm and in others soft. The latter state was chiefly found when there had been recent transfusion.

Occasionally accessory spleens were found in the gastro-lienal ligament.

On section, the cut surface of spleen showed merely congestion of the pulp, and as a rule the Malpighian bodies were indistinct. An increase of fibrous tissue was not noted.

(iv) The lymph glands.

Excepting jaundice, naked eye changes were absent from the lymph glands in any site. They were not enlarged.

(v) <u>The bone-marrow</u>.

It has been my custom to examine the marrow of the ribs and a femur. It is of little importance in infancy which bones are examined, because at this time all the bones, even in a normal child, contain actively haematopoietic tissue throughout the marrow cavity. Therefore an examination of the bone marrow throughout the entire skeleton, even if it were possible, would not be of great value. I have chosen to examine the ribs and a femur, because of the easy accessibility of the first, and because of the other long bones, the femur is the most convenient.

The most valuable accessible part of the rib was that part of the bone near the costo-chondral junction. In this site there is usually a moderate amount of marrow. It was impossible however by splitting the rib along its length to lift out a portion of marrow for fixing because the entire marrow cavity is traversed by bony trabeculae. Therefore, and this holds even for young babies who had not any anaemia at death, it was necessary to express the marrow.

The femur after removal was split longitudinally.

A comparison of the macroscopic characteristics of the bone marrow in icterus gravis, and in babies dying of other causes, e.g., cerebral haemorrhage, showed that in the latter the marrow was of a deep red colour, and of viscous consistence. This may be taken as the normal appearance in early infancy, because in cases of cerebral haemorrhage there was little anaemia and absence of infection which might have caused any marked change in the marrow.

In icterus gravis, however, the marrow was scantier, of a more fluid consistence, of a paler red colour, and jaundiced. These changes became more evident the longer the case had survived. Both in the femur and ribs a considerable degree of osteo-sclerosis was found in eleven out of fifteen cases examined. There was in these eleven cases, even in very young babies dying at the age of nine or ten days, evidence of absorption of bone at the periphery of the medullary cavity and of the extensive laying down of new osseous trabeculae throughout the cavity. Thus it was usually found impossible to lift out from the femur a piece of marrow of sufficient size for fixing, and as a result sections of the bone had to be decalcified. Frequently for the making of smears marrow had to be expressed.

(vi) The kidneys.

The kidneys were bile-stained to a varying degree, the capsule could be stripped off easily and on section gross pathological lesions were not found. The apices of the pyramids were frequently very deeply bile-stained, and uric acid infarcts, which are not uncommon in young babies, were sometimes seen.

(vii) The supra-renal glands.

These glands shared in the general jaundice but a lesion was not seen in any case. From the post-mortem record files at the Hospital, I found a record of a case of neonatal jaundice in which the cause of death was extensive bilateral suprarenal haemorrhages. Pritchard and Smith (1931) have described a suprarenal haemorrhage in a case of anaemia haemolytica neonatorum.

(viii) The gastro-intestinal tract.

The stomach, duodenum, and intestines did not show any characteristic lesion except in one case (No. 19), where there was considerable oozing of blood from the whole of the gastric mucosa and from extensive areas of the mucosa of the small bowel. This haemorrhage seemed to be of recent onset, and this was borne out by the fact that before death, melaena was not present. Because the infant died at the age of twenty days, and had previously been transfused several times it was obviously not a case of primary haemorrhagic disease of the new-born.

In two cases (Nos. 10 and 20) acute gastro-enteritis was present.

(ix) The thorax.

The pleura, and intra thoracic organs were jaundiced. Small haemorrhages into the visceral and parietal pleura were sometimes seen.

(x) The lungs.

Gross haemorrhages into all lobes of both lungs were noted in one case (No. 18). The bleeding was not due to rupture of larger vessels but to a generalised leakage from the capillaries. The anatomy of the lobes was completely destroyed.

Slight terminal hypostatic congestion of the lower lobes, was sometimes found, and in five cases there was acute broncho-pneumonia (Nos. 1, 3, 8, 14, 32) and in one (Case 3) where the right lung was affected there was an overlying empyema.

(xi) The heart and pericardium.

Icteric staining of pericardium, myocardium and endocardium was often seen. The heart was sometimes slightly dilated but not hypertrophied, and gross fatty degeneration or "tigering" was not seen.

The intima of the great vessels was considerably bile-stained, and even in cases where clinical jaundice had practically disappeared, it was still very obvious in the vessels, and the valves.

(xii) The thymus gland.

Enlargement and other abnormalities were absent, excepting numerous small haemorrhages in one case (No. 18).

(xiii) <u>The brain and meninges</u>.

Jaundice of the central nervous system will be described in the section on kernicterus.

The meninges and the ependymal linings of the ventricles were icteric in most cases, and petechial haemorrhages similar to those found in the peritoneum and pleura were found in the meninges in one case (No. 5). Larger meningeal haemorrhages were seen in four cases (Nos. 4, 11, 18, 19). In the first of these cases there was an extensive subdural haemorrhage apparently from the rupture of a large number of small vessels and covering nearly the whole of the right cerebral hemisphere. Longitudinal sinus thrombosis of recent onset was also present. A similar picture was presented by Case 11. Diffuse subarachnoid haemorrhage over both hemispheres but unassociated with any change in the longitudinal sinus was noted in Case 19, while in Case 18 the haemorrhage was basal --- the basal sinuses being intact.

Gross cerebral haemorrhage was found only once (Case 5). This child died very suddenly, and extensive haemorrhage into the pons and cerebellum, mostly on the right side, was found. The infant seemed to have survived a previous haemorrhage because both lateral ventricles were considerably distended. There was deep yellow staining of their walls in which some brownish granular material was embedded. Actual blood or blood-stained fluid was not contained in the ventricles.

A small haemorrhage into the substance of the left cerebral hemisphere was seen in Case 1.

II. MORBID ANATOMY OF HAEMOLYTIC ANAEMIA OF THE NEW-BORN WITHOUT OEDEMA OR JAUNDICE.

(i) General.

The tissues were remarkable for their pallor, even when a recent transfusion had been given.

(ii) The abdomen.

The peritoneum was normal.

(iii) The liver.

In all of the six cases which came to post mortem examination the liver was enlarged. The colour was light brown, and in cases where transfusion had not been done, congestion was absent. The capsule stripped off easily. The cut surface of the liver did not show any naked eye lesion, not even the slight fatty change which was sometimes evident in cases of icterus gravis. In cases which had been transfused a slight diffuse reddish tinge was added but the brown colour was still predominant.

The gall bladder and bile ducts were normal, thrombi were absent from the smaller ducts, visible to the naked eye, and bile of normal appearance was easily expressed from the gall bladder into the duodenum. Fibrosis of the liver substance was not evident.

(iv) The spleen.

This organ was in all cases enlarged and in four cases to a considerable degree, four or five times the normal size. A dark red colour, and soft, sometimes almost fluid, consistence were constant findings. The Malpighian bodies were inconspicuous.

(v) The lymph glands.

These, both in the abdomen and elsewhere, did not show any abnormality other than pallor.

(vi) The stomach, duodenum and intestines, although pale, did not show any other abnormality, except in one case where there was gastro-enteritis (Case 27).

(vii) The thorax.

The thoracic contents, excluding pallor, were normal in all cases.

(viii) The brain and meninges.

The pallor of the brain was remarkable, but otherwise the appearance of the tissues were normal, except for a small meningeal haemorrhage which was found in one case - No. 29.

III. HISTOLOGY IN ICTERUS GRAVIS AND HAEMOLYTIC ANAEMIA WITHOUT OEDEMA OR JAUNDICE.

Introduction.

Because of the usual distribution of the haematopoietic tissues in the foetus I have followed the routine practice of examining only the liver, spleen, lymph glands, kidneys and bone-marrow, although on rare occasions blood formation has been found in other sites in the erythroblastoses (p. 61).

(i) The histology of the liver.

(a) In the normal infant.

Normally in the newly born infant the arrangement of the liver cell columns is more irregular than in the adult or older child. The course of the cell columns is more tortuous and the sinusoids are wider, although the radiating pattern of the lobule is in the main already present. In the widened sinusoids islets of haematopoietic tissue are seen but by the end of the first week of life in the full time infant these have completely disappeared, or are very small and scanty (Parsons et al., 1933). Haematopoiesis persists for a longer period in premature infants (Ferguson, 1931).

(b) In the neonatal anaemics.

The liver cells. Stained sections of the liver from cases of icterus gravis revealed that the disarrangement of the cell columns and widening of the sinusoids were exaggerated to a varying degree, and the radiating structure of the lobule was more or less lost. In some cases the cell columns, however tortuous, were more or less complete (Plates 2, 3 and 4), but in others the columns were broken up, and the parts displaced, so that no definite pattern remained (Plates 5 and 6). The liver cells always showed evidence of damage. This was sometimes indicated merely by indistinctness of the cell outline or lack of detail in the nucleus (Plates 2 and 4). In other instances the cell outlines had vanished and syncitial formation occurred by the fusion of several cells, (Plates 5, 6 and 7), while in some places only a mass of débris remained (Plates 8 and 9). Not infrequently where the liver cells were severely damaged the nuclei became grouped together (Plates 8 and 9). Brown pigment was nearly always present in the liver cells (Plates 12 and 16) and bile thrombi were frequently seen in the smaller bile ducts (Plate 5). Degeneration and pigmentation were not equally intense throughout the sections, some lobules being more severely affected than others.

Occasionally slight fatty infiltration was present in the portal and mid-lobular regions (Plate 4) but this was not a characteristic change.

By contrast, the liver in cases of haemolytic anaemia without oedema or jaundice showed a practically normal anatomical arrangement of the lobule (Plate 10). Degeneration of the liver cells was absent or slight although a moderate amount of granular pigment was present (Plate 13). Bile thrombi were absent from the bile ducts (Plate 11).

In both types of anaemia, the sinusoids were always wide, and a moderate amount of congestion was sometimes present (Plates 3 and 16), especially if recent or frequent transfusions had been given.

Haematopoiesis. Hepatic erythropoiesis was found in eighteen out of twenty-two cases of icterus gravis and in all of the six cases of haemolytic anaemia without oedema or jaundice. The primitive red cells appeared in groups in the wider portions of the sinusoids. These groups varied in size and numbers, from small, scanty foci of four or five cells (Plate 5) to numerous and extensive collections of a score or more (Plate 2) which formed the most striking characteristic of even the low power field. Again, large erythropoietic islets sometimes occurred at infrequent intervals, most of the sinusoids being free of haematopoiesis (Plate 7) or the whole of the sinusoids were filled with strands and islets of erythroblastic cells (Plates 3, 4 and 10). Large numbers of circulating nucleated red cells were sometimes present in the portal and central veins (Plates 10 and 11).

In order to assess the extent of hepatic erythroblastosis I have adopted an arbitrary system, which, although only roughly accurate, is sufficient to show (Table XII) that, in cases dying during the first week of life, erythroblastosis was only moderate or even scanty, and that for the most part

Table XII. To show the relation between hepatic erythroblastosis and the age of the subject.

Case No.	Degree of Erythroblastosis.	Age at death (in weeks).
21	±	1
14	+	1
10	+	1
7	+	1
26	+++	2
29	+	2
37	+++	2
31	++	2
4	±	2
18	+	2
2	++	2
12	++	2
36	+++	3
25	+	3
1	±	3
19	+	3
34	+	3
35	+	3
30	-	3
11	±	4
5	++	5
8	-	5
9	+	5
20	+	6
3	±	6
27	±	15
32	-	15

- Erythroblastosis absent

± Scanty erythroblastosis. (Plate 5)

\+ Moderate " (Plates 3 & 4)

++ Abundant " (Plate 2)

+++ Very abundant " (Plate 10)

it was during the second and third weeks that erythroblastosis was chiefly present, there being thereafter a diminution of hepatic haematopoiesis although it might be present to a slight degree until as late as the fifteenth week.

Cells representing several stages in the evolution of the erythrocyte were found in the erythroblastic foci. In describing them I have used the classification of Doan, Cunningham and Sabin (1925), which was also supported by Peabody (1926). The megaloblast, (Plates 12, 13 and 14) the most primitive cell, was large, with dark basophile cytoplasm, and a pale staining nucleus, which occupied a major portion of the cell. This nucleus, rounded or oval, showed several nucleoli, up to five in number. The karyomitome was arranged in nodes, which were most frequently found towards the periphery of the nucleus, of which the limiting membrane was well defined. Rather smaller cells (Plate 15) with a coarser and more densely staining nucleus, indistinct nucleoli, and less basophile cytoplasm --- the early erythroblasts --- were also frequent. The late erythroblasts, still smaller, with even denser nuclei appeared in considerable numbers (Plate 16). The more mature cells showed a further diminution in size and increasing density of the nucleus (Plate 17). Some of these cells were of the normoblastic type with piknotic nucleus and cytoplasm containing haemoglobin. Among the earlier cells, however, chiefly the late, but occasionally in the early erythroblasts haemoglobin was evident, while in occasional megaloblasts the cytoplasm was less basophile than usual, suggesting that haemoglobin was being acquired (Plate 13). Active mitotic division of the nucleus of the erythroblasts was not infrequent (Plates 14 and 16).

The larger erythropoietic islets, of course, were composed of varying numbers of all the cells just described, although only the smaller foci with less variety of cell types could be photographed with a high magnification.

Quite frequently small islets of erythroblasts were apparently almost completely surrounded by Küpffer cells (Plates 17, 18, 19 and 20), suggesting, as has been maintained by Sabin that the origin of the red corpuscles is intravascular.

Leucopoietic depôts, although never numerous, were sometimes seen (Plates 21 and 22), and usually adjacent to the portal tracts, as has been previously noted by Hawksley and Lightwood (1934). The cells were as a rule myelocytes, while a few myeloblasts and metamyelocytes were also present. Fully segmented polymorpho-nuclear forms were rare. Occasional eosinophile cells were seen.

Rarely megakaryocytes (Plate 23) were seen lying in the sinusoids. From the literature, this observation seems to be unique, in the neonatal haemolytic anaemias, although it is known that megakaryocytes occur in the foetal liver in animals (Sharpey-Schafer, 1912). They were, in my cases, always single and of a primitive type with a nucleus arranged as an incomplete ring. They evidently lacked the property of phagocytosis.

It would appear therefore that in the neonatal haemolytic anaemias, the liver may retain all the forms of haematopoiesis, but that the only one of importance is the erythropoietic, which may persist for many weeks beyond the physiological period, being most active in the second week. The process of maturation from the more primitive cells is pathological, because of the premature acquisition of haemoglobin.

Phagocytosis. Coincident with the evidence of hepatic blood formation there was also considerable indication of increased blood destruction as well as removal of necrotic liver tissue by phagocytic Küpffer cells. This was more obtrusive in icterus gravis than in haemolytic anaemia without jaundice because in the former the damage to the liver cells was much greater and because the phagocytes were deeply bile-stained.

Many of the Küpffer cells were swollen although they still maintained their normal position lining the sinusoids (Plate 24). Others, more distended, although still roughly spindle-shaped were lying free in the sinusoids (Plate 24), while at a later stage the cells had lost all semblance of their original form (Plate 25). Ingested red corpuscles, erythroblasts and other material were present in the cytoplasm (Plates 24, 25 and 26). Finally, some cells were ruptured and disintegrating (Plate 26).

Haemosiderosis. Deposition of iron was found by the hydrochloric acid and potassium ferrocyanide method of staining, in all livers from cases of anaemia without jaundice, and in all but one of the cases of icterus gravis.

Haemosiderosis affecting the Küpffer cells almost exclusively was sometimes seen, (Plate 27) or moderate quantities might be found in both liver and Küpffer cells (Plates 28 and 29), although by its concentration into small space, in the Küpffer cells, these were more prominent. The haemosiderin showed a tendency to follow the course of the bile canaliculi throughout the liver cell columns (Plate 28), and was mostly concentrated in the portal and mid lobular regions (Plate 27), but where much haemosiderosis occurred, the central parts of the lobules were also impregnated. Some lobules or groups of lobules contained much more iron than others.

Where there was severe cell degeneration and heavy iron deposition, the granules of haemosiderin were frequently aggregated into large and dense masses (Plates 30, 31 and 32).

As a rule the ferroginous pigment occurred as deep blue, fine granules, but occasionally the chemical change into haemosiderin was not complete and a greenish crystalline appearance was found.

Theoretically, after haemolysis the iron should appear first in the Küpffer and later in the liver cells, it was usually found impossible in my experience to correlate the relative amount of iron in the two types of cells with the period which had elapsed since the previous transfusion or rapid fall in red cell numbers. For instance, although a case (No. 5) might have been transfused several times, iron might be found almost exclusively in the Küpffer cells (Plate 27). Again, iron was discovered in moderate quantities, in both liver and Küpffer cells (Plate 28) as long as seven weeks after transfusion, although during this period there had been no excessive haemolysis, but actually a rise in the red cell count. The explanation here, however, is probably that as the child failed to thrive, and put on very little weight during the seven weeks, the iron stored in the tissues had been in excess of the amount required for haematopoiesis.

A certain amount of haemosiderosis is normally present in the liver in young babies. Bunge (1898) was of the opinion that iron was stored in the liver during the last trimester of intra-uterine life, and that after birth there was a continual decrease in these deposits. Gladstone (1932) failed to find much pre-natal iron storage, but showed that in the early post-natal period there was a steady increase in the amount of iron in the liver and spleen, the maximum quantities being found between one and ten weeks of age. He attributed this to the iron obtained from the haemoglobin of the blood destroyed during the early weeks of life. It is obvious then that the mere presence of iron in the liver cannot be used as evidence of excessive or pathological haemolysis. Further it has also been shown by Gladstone (1932) that there is an increase in hepatic iron following transfusion, slight in the first two days, and greater between the third and seventh days.

Many of my cases had been transfused and doubtless much of the iron present in the liver was due to destruction of the foreign blood. Nevertheless, in some cases (Nos. 31, 21, 26) not treated by transfusion such large amounts of iron were found that the only explanation possible was the excessive breaking down of haemoglobin (cf. Plates 33 and 34 from untransfused cases with Plates 30, 31 and 32 from frequently transfused cases).

Fibrosis. Hawksley and Lightwood (1934) described a fine fibrosis of the liver in icterus gravis, and an apparent increase of connective tissue round some of the portal tracts in my cases of icterus gravis (Plate 5) led us to examine the liver from this point of view. Better results were obtained with Gallego's stain than with van Gieson's.

I found that in many instances, the amount of fibrous tissue round the vessels, and the appearance of the reticulum in the lobules was perfectly normal (Plates 35, 36 and 37), even although there was considerable degeneration of the liver cells. In others, however, a slight increase of young fibrous tissue particularly round the portal tracts occurred, and from this tissue, fine fibrous tendrils spread out along the sinusoids for a varying length throughout the lobule (Plate 38). In such cases, where small areas of necrosis were present, the fibres were more numerous (Plates 39, 40, 41, and 42). The liver in a few cases, while without fibrosis, showed an appreciable thickening of the reticulum (Plate 43).

In haemolytic anaemia without jaundice, hepatic fibrosis was not found, but reticular thickening was present in three cases.

The presence of fine fibrosis in the liver raises the question of the possibility of the development of cirrhosis in those cases which recover. The liver of one child (Case 16) who had recovered from icterus gravis, but failed to thrive, and died of gastro-enteritis at the age of ten weeks, did not show any greater increase of connective tissue than was seen in younger infants during the jaundiced period. In another case (No.22) which recovered perfectly, but died of pneumonia at the age of ten months, the liver revealed no lesion which is not commonly present in pneumonia. In this instance however anaemia and jaundice had been of comparatively mild degree and of late onset.

(ii) <u>The spleen</u>.

Histological examination of the spleen was made in seventeen cases of icterus gravis and six of haemolytic anaemia without oedema or jaundice.

(a) <u>Congestion</u>.

Congestion of the pulp spaces with blood was one of the main features in both types of anaemia. Previous transfusion however seemed to be the principal cause, as in cases where death had occurred a few hours afterwards the most remarkable pictures of engorgement were obtained, while in cases where transfusion had not been done, congestion was slight or absent.

As I have already indicated, engorgement occurred in the pulp spaces (Plates 44 and 45), the sinusoids remaining comparatively empty (Plate 45) and not infrequently compressed. The Malpighian bodies were scanty, small, ill-defined, and often fragmentary (Plate 44). They were always free of red blood cells. The central lymphoblasts were frequently diminished in numbers.

(b) Haematopoiesis.

Splenic erythropoiesis was found in nine out of seventeen cases of icterus gravis. Usually it was much less extensive than in the liver, but in one case (No. 11), which died at the end of the fourth week of life, the onset of jaundice having been on the fifteenth day, the splenic haematopoiesis was considerably in excess over the hepatic, and in another (Case 30), which died in the first week of life, and where erythropoiesis was absent from the liver, occasional small islets were seen in the spleen.

In anaemia without jaundice, there was extensive haematopoiesis in the spleen in three cases, all of which died during the second or early in the third week of life, moderate blood formation in one case, which died at the end of nine days, and scanty in a fifth in which death occurred in the fifteenth day. Occasional erythropoietic foci were found in a case which survived until the fifteenth week. In all instances the splenic haematopoiesis was less extensive than the hepatic.

Erythropoiesis occurred within dilated sinusoids in islets very similar to those described in the liver. Large (Plate 46) and small foci (Plate 47) were found. Some were chiefly megaloblastic (Plate 48) and again many showed various types of cells, from the more primitive down to the piknotic normoblasts with full content of haemoglobin in the cytoplasm (Plates 47, 49 and 50). Haemoglobinization of some of the erythroblasts was present, and the nuclei of others were dividing by mitosis, while many normoblasts were in the process of extruding parts of the nucleus (Plate 49). The lining cells of the sinusoids enclosing the islets were frequently complete or almost complete (Plates 46 and 50).

(c) Phagocytosis.

Again, as in the liver, large phagocytes containing ingested blood cells were frequently seen in the sinusoids (Plate 51). These along with similar cells in the pulp were often one of the most conspicuous features of the sections, especially in icterus gravis, when they were bile stained, but also in anaemia without jaundice. In the latter phagocytes seemed to be much more numerous in the spleen than in the liver.

(d) Haemosiderosis.

On staining for iron it was found that much larger deposits were present than had been found in the liver. Some of the intra-sinusoidal phagocytes were heavily loaded but most was contained in the large pulp phagocytes (Plates 52, 53, 54 and 55). The Malpighian bodies were invariably iron-free (Plate 55). Where abundant haemosiderosis occurred, a considerable iron staining reaction was obtained in the fibrous tissue of the capsule and trabeculae (Plate 56), and the connective tissue coat of the vessels (Plate 54) while a light deposit was sometimes seen in the sinusoidal walls (Plate 52). On rare occasions the whole reticular structure was revealed by its iron content (Plates 57 and 58).

Although it has been shown (Gladstone, 1932) that moderate haemosiderosis occurs in the spleen within forty-eight hours of transfusion, and although, as in the case of the liver, this explains part of the iron deposit in many of my cases, it was in the cases of anaemia without jaundice which had not been transfused, that some of the grossest examples of haemosiderosis occurred. This again points to the presence of excessive haemolysis in these anaemias.

(e) Fibrosis.

Slight increase of connective tissue was sometimes seen in the spleen. Normally in sections stained by Gallego's method, the adventitia of the vessels was composed of an open arrangement of fine fibrous tissue, and the reticulum of the pulp was of a very delicate type (Plate 59). In some cases of icterus gravis the density of the fibrous adventitia of the vessels was increased (Plate 60) and the trabeculae were somewhat coarser. Not infrequently quite an appreciable thickening of the pulp reticulum and supporting membrane of the sinusoids occurred (Plates 61 and 62).

(f) Summary of the histology of the spleen.

In brief the spleen in the neonatal haemolytic anaemias was chiefly characterised by the active phagocytosis of blood and deposition of iron, and to a less extent by persistence of the foetal depôts of blood formation.

(iii) The kidneys.

The glomeruli were often of the foetal type and in the younger babies some were as yet incompletely developed. The most frequent change in the kidneys was cloudy swelling indicated by poor cell outline or vacuolation of the cytoplasm, and indifferent nuclear staining, in the cells of the collecting tubules. Sometimes there appeared to be intertubular oedema (Cases 36, 37) with slight increase of fibrous tissue (Case 36) or thickening of the basal membranes of the tubules (Cases 20, 5). In one case (No. 3) the entire reticulum of the kidney was coarser than usual. Rarely, exudate in the capsule of Bowman was observed (Case 36).

These changes were undoubtedly due to damage sustained in the excretion of toxic products of excessive haemolysis, and other tissue destruction.

Erythropoiesis, excepting one or two negligible islets in Cases 25 and 36, was not seen and only occasionally were the tubular cells pigmented (Case 3).

Haemosiderosis occurred in a few cases (Nos. 36, 37 3 and 9) and was found in the cells lining the tubules in the glomerular region and intermediate zone (Plates 63 and 64). One case of icterus gravis and two cases of anaemia without jaundice, which showed iron deposition, had not been transfused, and it may be concluded that the presence of renal haemosiderosis indicated gross blood destruction.

(iv) The bone-marrow.

(a) Introduction.

The marrow was examined histologically in sixteen cases of icterus gravis. Smears and sections were obtained in twelve of these, and smears only in the remaining four. Both methods were used in the examination of the marrow in three cases of anaemia haemolytica sine icterus. Sections fixed in formol were stained by Jenner and Giemsa's stains, and smears by Leishman's.

(b) Amount of haematopoietic tissue.

Although as we have already seen there was considerable osteo-sclerosis of the medullary cavities of the ribs and femora, the marrow lying between the trabeculae was nearly always very cellular in both types of anaemia (Plates

65 and 66). Congestion of the sinusoids was not present.

One case (No. 29) showed definite hypoplasia and congestion (Plate 67), and even a few fat cells were present (Plate 67). This is most unusual in the infant marrow (Piney, 1931). The congestion in this instance is probably accounted for by the transfusions given. Death had occurred at a comparatively early age, seven days, and the extra-medullary haematopoiesis had been moderate. A hypoplastic marrow with appreciable extramedullary erythropoiesis was recorded by Susstrunk (1924) in a case of anaemia without jaundice.

(c) Erythropoiesis.

Irrespective of age at death, erythropoiesis was in all cases, even those showing extensive extramedullary blood formation, less prominent than might have been expected, except for one case (No. 2) where both hepatic and marrow erythropoiesis were intense. The impression was also received that from the third week onwards there was as a rule a further diminution in the number of erythropoietic foci.

These foci were easily discerned by the darker staining of the nuclei of the erythroblasts (Plates 66, 68, 69, 72) and intra-sinusoidal erythropoiesis was sometimes seen (Plates 70 and 71). The commonest cells were the erythroblasts, early and late, and their cytoplasm usually showed premature haemoglobinization (Plates 74, 75 and 76). Mitotic division was observed in the early erythroblasts (Plates 76 and 78), while in the late, extrusion of parts of the nucleus, single or multiple, was in progress (Plates 74, 75, and 76). Normoblasts with piknotic nuclei were commonly less frequent than erythroblasts but were always present (Plates 73 and 78).

(d) Leucopoiesis.

Leucopoiesis was active as a rule and even in the hypoplastic bone marrow (Case 29) the formative marrow present was largely of this type. Where terminal infection, such as pneumonia, was present, a brisk reaction on the part of the white cell-forming tissues occurred (Plate 65), and in one case this was true although there had been a leucopenia in the peripheral blood (Case 14).

In the leucopoietic foci the most numerous cells were the neutrophile myelocytes, although varying numbers of immature metamyelocytes were also present (Plates 65, 78, 79, 80). Myeloblasts (Plate 74) and premyelocytes, i.e., granular cells in which the nucleus still retained the nucleolated character of the myeloblast (Plate 78), were not infrequent.

Eosinophile cells, myelocytes and metamyelocytes were as a rule present in moderate numbers and in some cases (Nos. 5, 9, 25) were very numerous. Only very rare mast cells were seen. Buchan and Comrie (1909) mention their presence in their cases.

(e) Conclusions from (b), (c) and (d).

From the examination of my cases, I have concluded that the bone marrow as a rule showed evidence of an attempt to speed up blood formation to counteract the anaemia. This reaction was chiefly indicated by the haemoglobin content of the more primitive erythroblastic cells, and evidence of their active division or maturation. Quantitatively the reaction seemed to be temporary and commonly there was a progressive diminution in the number of erythropoietic cells and an increase in their immaturity, while an excessive growth of osseous tissue partially replaced the marrow in the medullary cavity. In one case the bone marrow may, from the first, have been hypoplastic or certainly became so early in the course.

The leucocyte forming mechanism was on the whole less affected, the myelocyte being as in the normal marrow the predominating cell, although numbers of earlier and later forms were present. When however a terminal infection occurred, it was found that the marrow was still capable of producing a marked neutrophile hyperplasia.

(f) Reports on the marrow in the literature.

Reports on the histology of the bone marrow in the neonatal anaemias are comparatively few, and owing to the various terminologies of the blood cells used, often confusing.

Buchan and Comrie (1909) described the marrow in icterus gravis as "megaloblastic." MacClure (1931) said that in his case the histology was "normal." Parsons et al. (1933) describing the cells called by me megaloblasts as pro-erythroblasts, record in their cases considerable marrow erythropoiesis. Hawksley and Lightwood (1934) using the terminology of Doan, Cunningham and Sabin have concluded that there was a shift to the left in both erythropoiesis and leucopoiesis.

Buhrman and Sanford (1931) described abundant erythropoiesis in the marrow of an icteric case, which died on the first day, and note that in another where death was on the thirty-second day, leucopoiesis was much more evident than erythropoiesis. Diamond et al. (1932) found very active marrow erythropoiesis in two cases of icterus gravis which died at twenty-eight and sixty-two hours respectively. In a case where death was at fifteen days the marrow erythropoiesis, although active, was much less marked.

In anaemia without jaundice there was sometimes a marked regenerative activity of the marrow (Abbot and Abbot, 1935, Case 2). Anomalous cases regarded as hypoplastic have been recorded however. Susstrunk (1924) found hypoplasia of the marrow with appreciable extramedullary haematopoiesis. Pasachoff and Wilson (1931) described a case in which the marrow showed "a myeloid reaction with practically no response in the erythropoietic system." Abbot and Abbot (1935) said of the marrow in one of their cases that the predominating cells were undifferentiated types with many eosinophilic myelocytes. Erythrocytes were few and chiefly mature. Fat was also present in this marrow. I conclude that there was erythropoietic hypoplasia. Excessive and primitive leucoblastosis without erythropoiesis was described by Brown et al. (1934).

We see therefore that in the literature my contention that the activity of erythropoiesis in the marrow diminishes from the late second or third weeks is supported, and that hypoplasia at all stages is in some cases a feature.

(g) Megakaryocytes.

Megakaryocytes in the bone marrow of cases of neonatal haemolytic anaemia have been variously described as normal in numbers (Abbot and Abbot, 1935) or decreased (Diamond et al., 1932). Certainly in some of my cases (Nos. 1, 2, 5 and 25) they were scanty, but in others (Nos. 8 and 20) they were quite plentiful. I was able to confirm an observation of Hawksley and Lightwood that there is sometimes an increase in the number of megakaryocytes after recovery from anaemia. This occurred in Case 16, where the child died at the age of ten weeks from gastro-enteritis, the jaundice and anaemia having departed (Plate 81).

(h) Phagocytosis and haemosiderosis.

Throughout the literature which has been available to me, there has been no mention of the occurrence of phagocytosis in the marrow, although numerous references to its occurrence in the liver and spleen have been made. Speaking of the marrow histology MacClure (1931) remarked that phagocytosis was not present in his case. This was my experience in all of my cases, except one of icterus gravis, where numerous large phagocytes deeply bile stained and containing ingested cells were found (Plate 82). Further reticulo-endothelial cells lining the sinusoids were somewhat swollen with yellow pigment (Plates 70 and 83), and one or two capillaries were actually occluded by bile thrombi (Plate 84). On staining for iron however a reaction was not obtained from the phagocytic cells, and an absence of haemosiderosis in the marrow was characteristic of the other cases.

The absence of haemosiderosis of the marrow in haemolytic anaemias of the new-born is very much different from what is found in adult anaemias in which haemolysis is a feature (e.g. in acholuric jaundice and pernicious anaemia). It may be that nearly all of the reticulo-endothelial cells in the young infant are occupied with blood formation.

The presence of bile staining of the marrow cells and bile thrombi in the capillaries in occasional cases of icterus gravis indicates that sometimes there may be a gross toxic action on the marrow by the bile in the blood.

SECTION D.

DEVIATIONS FROM THE TYPICAL CLINICAL PICTURE IN THE NEONATAL HAEMOLYTIC ANAEMIAS.

Introduction.

Strictly speaking these anomalies should have been discussed under the clinical or haematological headings earlier in this thesis. Because I wished to refer to pathological findings however it has been found necessary to hold over the discussion of certain anomalies until the pathology of typical cases had been considered.

(i) Absence of signs of active blood regeneration.

When describing the blood picture in icterus gravis, and anaemia without jaundice I showed that the most characteristic features were progressive anaemia with active blood regeneration as shown by reticulocytosis, and sometimes erythroblastaemia. Certain cases, however, were notable for the absence of marked indications of accelerated blood formation (Nos. 2, 3, 7, 10, 13, 11 and 22). In these, reticulocytes did not exceed 5% of the total red corpuscles. With the exception of cases 13 and 22, which recovered, there was not any opportunity for observation over any length of time because death occurred within a few days of admission to hospital. Nevertheless because of the poor reticulocyte response, it could be tentatively concluded that there was hypoplasia of the erythrocyte forming tissues.

In the pathological investigation of the haematopoietic system, we have seen that there was a tendency for extramedullary erythropoiesis to decrease from about the third week onwards, and that in the bones from quite early stages of the anaemia, a certain amount of osteosclerosis of the medulla occurred although the remaining marrow was actively haematopoietic in most cases.

Of the fatal cases of icterus gravis without brisk reticulocytosis, two died at four (Case 11) and six weeks (Case 3) respectively. In the former although there was moderate splenic erythroblastosis, the hepatic was scanty, and the medullary cavities of the femur and ribs were osteosclerotic, while the marrow showed more prominent leucopoiesis than erythropoiesis. The latter case showed very little extramedullary blood formation, but osteosclerosis in the femur was marked and, although the marrow was red and thick, smears showed a great excess of leucocyte forming elements over nucleated red cells. This was in accordance with the presence of a terminal infection which was penumonia, empyema and peritonitis.

The erythropoietic tissues were in these cases probably diminishing in amount and activity.

The three remaining fatal cases without reticulocytosis died on the fourth (Case 7), seventh (Case 10) and ninth days (Case 2). All were free of infection and showed kernicterus. The last case (No. 2) was outstanding because in spite of the absence of reticulocytosis thirty-six hours before death, there was extensive extramedullary haematopoiesis, and although there was considerable osteosclerosis in the bones with an apparent macroscopic diminution in the amount of marrow, what marrow was present was showing excessively active erythropoiesis. This case can therefore scarcely be described as hypoplastic.

Of the other two cases, one (Case 7) showed moderate erythroblastosis, and the other (Case 10) very little, while in both there was osteosclerosis of the medullary cavities of the bones and histologically a preponderance of leucoblastic elements. It may be concluded therefore that in these cases the erythropoietic tissues were diminished in amount.

Somewhat similar pictures were presented by three cases of haemolytic anaemia without jaundice where little or very slight reticulocytosis was present.

I have already referred (p. 47) to a prolonged case of anaemia without jaundice (No. 27) where in spite of transfusion in the ninth and eleventh weeks, and treatment with iron for a fortnight, blood regeneration failed to occur. Death was probably due to septicaemia. Very scanty, almost negligible, hepatic erythroblastosis persisted, and although the marrow was macroscopically normal, erythropoiesis was little in evidence, and leucopoiesis was more marked. The latter however was of a more primitive order than was customarily found in the neonatal anaemias, myeloblasts and premyelocytes being excessively numerous, myelocytes less frequent, and metamyelocytes scanty. This possibly explains the leucopenia found in the peripheral blood.

Another case of anaemia without jaundice (No. 28) had no reticulocytosis on the thirteenth day, although a moderate neutrophile leucocytosis was found. It seems that in this case the haemolytic process had practically ceased at this time and that the erythropoietic system had reached a stage of temporary exhaustion. Later a recurrence of haemolysis indicated by a rapid fall in the blood count (Chart XII) was observed. Reticulocytosis showing that the marrow had recovered was observed later in the course.

In Case 29, also without reticulocytosis, in spite of moderate erythroblastosis in the liver and spleen, the bone marrow of the femur was obviously hypoplastic (Plate 67), the red cell producing elements, particularly, being scanty.

It would seem from these examples that, with one possible exception (Case 2), the absence of evidence of blood regeneration, in the peripheral blood, corresponded with a diminution of erythropoiesis in the bone marrow, and that scanty or even moderately extensive extramedullary haematopoiesis was insufficient to compensate for an inactive marrow. Such cases may, therefore, as far as the red cell producing mechanism is concerned, be regarded as hypoplastic.

The two cases of icterus gravis which showed little reticulocytosis and which recovered spontaneously (Cases 13 and 22) had only a moderate anaemia of slow onset. The red corpuscles did not fall below 3.5 millions per c.mm. in numbers. Jaundice was marked and contributary signs of haemolysis, such as excessive urobilinuria, and strongly positive indirect van den Bergh reactions were found. It seems that in these cases the blood forming system was not greatly strained, and that probably a slightly increased rate of production over a long period was sufficient to counterbalance the loss. Fuller reference is made to these cases, when discussing delayed onset of jaundice and kernicterus.

(ii) Delayed onset of neonatal haemolytic anaemia.

The age at which jaundice appeared in icterus gravis was subject to considerable variation in my series of cases, and, although the highest incidence was seen in the first or second days, with a diminishing incidence in the subsequent days of the first week, eight instances of later onset of jaundice were recorded between 1921 and 1936.

Cases of haemolytic anaemia without jaundice, where the anaemia has occurred later than the first ten or fourteen days of life, have sometimes been classified in a special group, as haemolytic anaemias of the later neonatal period (Parsons et al., 1933). Josephs (1936) failed to come to any conclusion as to whether such cases should be regarded as identical with those which were more strictly neonatal.

While it was possible that in some cases of alleged late onset, the history was wrong and that mild degrees of jaundice had not been noticed by the parents, three cases where no doubt could arise were observed by me in the 1933-1936 series, jaundice first appearing on the fifteenth day in two cases (Nos. 6 and 11) and on the twenty-third day in the third (No. 22).

In Case 6, the child, a male, was said to have been born at full term. He was fed on the breast, supplemented by cow's milk, and was well until the fifteenth day when he became jaundiced. At the same time the urine became "brown" in colour. On admission to hospital on the sixteenth day, the baby was found to be markedly jaundiced with slight enlargement of the liver and spleen. The urine contained bile pigments and a moderate reaction for excess of urobilin was obtained. The general condition of the child was excellent; he did not appear to be upset and weighed 4.2 kilograms, the average weight at this age being 4.4 kilograms. Anaemia, however, was severe - 2.02 million red cells per c.mm., with a moderate reticulocytosis of 7.5%. The child improved after transfusion and jaundice quickly cleared. Then at the age of six weeks exactly the same thing happened. Quite suddenly jaundice reappeared, bile pigments with excess of urobilinuria occurred in the urine, and the blood count fell to under 3.0 million red cells per c.mm., 7% of these being reticulocytes. Another transfusion was given and the child recovered.

Whatever the cause, it seems that in this case there was on two occasions a sudden and appreciable destruction of blood without evidence of haemorrhage, and that the first occurred at the beginning of the third week of life.

The second case showing jaundice of late onset (No. 22) was a full-time child, which was breast fed and thriving until the twenty-third day of life when icterus first appeared. During the following week the jaundice deepened but the child was not greatly upset. Examination on the seventh day of illness showed a small but fairly well-nourished child weighing 3.62 kilograms. There was jaundice of the skin and sclerotics. The liver was enlarged but there was not any splenomegaly. The urine did not contain bile pigments, although a strong reaction for urobilin was obtained. The van den Bergh reaction of 32 units consisted of a slight delayed direct, and a strong indirect reaction. Wassermann's test was negative. Anaemia was slight, 4.02 million red cells per c.mm., but within the next five days the blood count fell to 3.5 million red cells per c.mm. without increase in the reticulocyte numbers.

Spontaneous recovery followed. Jaundice disappeared and the red corpuscle level was maintained at just over 4 millions per c.mm.

Here again is a case of late onset, showing a moderate but significant fall in the red corpuscle numbers within a short time, and evidence in the blood serum and urine of excessive blood destruction.

In the third case (No. 11), a male twin, the other child being healthy, jaundice appeared on the fifteenth day, and the child was admitted to hospital two days later. This baby had never thriven. At thirteen days he began to vomit. On the seventeenth day there was a series of generalized convulsions. The child was found to be small and greatly dehydrated. The weight was only 1.6 kilograms. There was deep jaundice, enlargement of both liver and spleen, and considerable urobilinuria with slight albuminuria. Bile pigments were not present in the urine. The anaemia was slight, 3.95 million red cells per c.mm. Dehydration however would cause a considerable apparent rise in red cell numbers (Paxton, 1935), and partially mask the true extent of the anaemia. There was not any reticulocytosis, and nucleated red cells were absent from the blood films, while leucocytes numbered only 11,000 per c.mm. After a day or two the child got gastro-enteritis with fever, and died on the twenty-second day of life.

Clinically, this case is more complicated than the preceding two. The child, a twin, was feeble from birth, and jaundice was preceded by gastric symptoms. The latter, as has been noted previously, are not infrequent in the neonatal anaemias. In spite of the absence of fever, leucocytosis, and umbilical infection, infection could not be definitely excluded. The post-mortem findings however clarified the issue. A focus of infection beyond the terminal gastro-enteritis was not found, and the bile ducts were normal. Histological examination showed the typical destruction of the liver cells so frequently found in icterus gravis, and although erythroblastosis was absent from the liver, moderate numbers of erythropoietic islets were found in the spleen. Phagocytosis of red cells was also present. The medullary cavity of the femur was osteo-sclerotic and microscopically there was diminution of the erythropoietic foci in the marrow. The convulsions were explained by the presence of a haemorrhage over the left cerebral cortex, a common finding in icterus gravis.

It would seem then that the onset of excessive haemolysis and jaundice may be delayed into the second or third weeks in occasional cases. Where this occurs in a previously healthy child the anaemia is of mild degree, and the prognosis good, although, as in the more typical cases of icterus gravis, a recrudescence of haemolysis may occur. If the child is weakly, the prognosis is not so favourable.

Toxic damage to the liver may be present, and a tendency to haemorrhage develop. Extramedullary depôts of blood formation and hypoplasia of the erythron may be found.

At present, and until the fundamental cause of the haemolysis is understood, there seems to be little cause to separate the cases of late onset of either icterus gravis or anaemia without jaundice from the strictly neonatal cases.

SECTION E.

STUDIES OF THE MECHANISM OF THE PRODUCTION OF ANAEMIA IN CASES OF ERYTHROBLASTOSIS FOETALIS.

Introduction.

How blood is destroyed in haemolytic anaemia of the new-born is not so far understood. Hampson (1929) was of the opinion that because injections of normal adult serum were sometimes effective in arresting the progress of anaemia, an antihaemolytic factor was absent from the child's serum. Abt (1933) thought that blood destruction occurred in the blood stream because he found that in cases of icterus gravis the content of iron in the serum was excessive.

I conducted the following series of experiments with a view to finding out something of the mechanism of red cell destruction.

(i) Method of obtaining serum and red corpuscles.

Blood was obtained from infants by puncture of the longitudinal sinus in the region of the anterior fontanelle, and from the median basilic vein in adults and older children.

In some of the experiments the red corpuscles were obtained by oxalating the blood with one drop of 15% potassium oxalate to 5 c.c. of blood. The cells were then separated from the plasma by spinning and pipetting off the supernatant fluid. Thereafter the corpuscles were washed three times with normal saline. In other instances the blood was defibrinated by shaking up vigorously with metal tacks, and the corpuscles separated and washed as before. I found by duplicating some of the experiments that it was immaterial which method was used, but the former had the advantage of being more suitable in dealing with small quantities of blood.

Serum was obtained by allowing the blood to clot in a sterile tube, and pipetting off the serum after spinning. It was then put up in quill tubing in quantities of about 1 c.c., and heated to 55°C. for half-an-hour to destroy complement. Adult serum was pooled.

(ii) Conventional signs.

The following conventions have been followed in the tables, and in the interpretation of the experiments which follow.

R.B.C.	=	red blood corpuscles.
dil.	=	dilution.
susp.	=	suspension.
0	=	lysis absent.
v.f.tr.	=	very faint trace of lysis.
f. tr.	=	faint trace of lysis.
tr.	=	trace of lysis.
d.	=	distinct lysis.
m.	=	marked lysis.
v.m.	=	very marked lysis.
a.c.	=	almost complete lysis.
j.c.	=	lysis just complete.
c.	=	complete lysis.
H.S.	=	anti-human red cell serum.
M.H.D.	=	minimum haemolytic dose.

In the following pages only the principles of, and conclusions from experiments, with a few detailed illustrative examples are given. The detailed findings of the entire series will be found in the Appendix (p. 274).

(iii) Is there a free lysin in the serum, or are the red corpuscles excessively fragile?

It was possible that in the neonatal haemolytic anaemias there was a free lysin in the circulating blood. I therefore put up washed red corpuscles from a normal subject with varying quantities of serum from a case of icterus gravis (No. 1). The tubes were incubated for one hour at 37°C. (Table 1). Lysis did not occur and I concluded that a free lytic agent was absent from the serum in icterus gravis.

Table 1. To find if there is a free lysin in the blood in icterus gravis (Case 1). (Blood citrated).

				Control
Packed corpuscles of a normal adult (c.c.)	0.5	0.5	0.5	0.5
Serum from a case of icterus gravis (c.c.)	0.125	0.25	0.5	0
Normal saline (c.c.)	0.375	0.25	0	0.5
Lysis	0	0	0	0

Goldbloom and Gottlieb (1929, a) found that in blood taken from the umbilical cord, and oxalated to prevent clotting, lysis occurred and that after a few days all or practically all of the corpuscles had lost their haemoglobin content. Blood taken from the median basilic vein of the mother, or from the longitudinal sinus of the child at birth, however, did not show this phenomenon. I found that in cases of icterus gravis oxalated blood could be kept under sterile conditions for three days without any haemoglobin being set free.

Also if oxalated red corpuscles from a case of icterus gravis were washed free of the oxalate, and then incubated with serum from the same case, lysis was not present.

If washed red corpuscles from a case of icterus gravis were incubated with varying quantities of pooled normal human serum, lysis did not occur. It seemed therefore that the red corpuscles in icterus gravis were not unduly fragile.

(iv) <u>The effect of an antihuman haemolytic serum on the red corpuscles.</u>

The effect of a lysin which was specific for human corpuscles was then studied. A haemolytic serum was made by injecting intraperitoneally a full-grown, young adult rabbit with 3 to 5 c.c. of washed, packed, red corpuscles from a normal adult. One injection per week was given for six weeks, the titre of the rabbit's serum being tested periodically. It was found that, even after six injections of red corpuscles had been given, the haemolytic effect of the rabbit serum on normal human red cells was very low, but that if complement were added the titre of the haemolytic serum was greatly raised.

In this respect the reaction of the rabbit to human corpuscles is very different from that to ox corpuscles, because in the latter case the haemolytic action of the serum is appreciable without the presence of added complement.

One week after the sixth injection of blood corpuscles, the rabbit was bled. The serum was separated, stored in quill tubing, and heated to 55°C. for half-an-hour on three successive days, to destroy complement and sterilise the serum. From time to time the titre of the serum was tested on the corpuscles with which the rabbit had been injected. It was found that eighteen months after the serum had been obtained, it was still actively haemolytic. By that time the investigations had been completed.

The haemolytic effect of complement (guinea pig serum) was found to be sufficiently slight to be negligible in the doses which I proposed to use.

I then investigated the minimum haemolytic dose of the rabbit serum in combination with 0.05 c.c. of complement on the red corpuscles of (1) adults and children of varying ages, (2) four cases of familial acholuric jaundice, (3) full-time and premature babies a few days old, and (4) cases of neonatal haemolytic anaemia.

For adults and children, the minimum haemolytic dose of rabbit serum was never less than 0.001 c.c. (Table 2). A similar minimum figure was obtained in investigating four full-time babies (two with icterus neonatorum) and varying between four and eight days old (Table 3), and one case of a premature baby aged four days (Table 4).

Six cases of icterus gravis varying in age between 24 hours and 3 weeks, and one case of haemolytic anaemia without jaundice were investigated. With the exception of one case of icterus gravis (Case 2) it was found that the red corpuscles were not more susceptible to lysis by the haemolytic serum, than the corpuscles of normal babies or of older subjects (Table 5). In the exception however it was found that the minimum haemolytic dose of the antihuman red cell serum was less than 0.0005 c.c. (Table 6).

An explanation for this anomalous result could not be found. The case was a typical one of icterus gravis with splenomegaly, a profound anaemia, and reticulocytosis. The blood was taken on the seventh day, but similar experiments done on older and younger cases with negative results ruled out the possibility of the age factor having any significance. That the prematurity of the child could have any bearing on the result was eliminated by the normal findings obtained on

Table 2. To find the M.H.D. of antihuman red cell serum for normal adult corpuscles.

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	0	v.f.tr.	f.tr.	d.	m.	0	0

						Controls	
R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	j.c.	c.	c.	c.	c.	0.	0

∴ M.H.D. of H.S. = 0.01 x 2 = 0.001 c.c.

Table 3. To find the M.H.D. of antihuman red cell serum for the corpuscles of a normal baby, aged 5 days.

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0	0	0.05
Lysis	m.	a.c.	a.c.	j.c.	c.	0	0

						Controls	
R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	a.c.	j.c.	c.	c.	c.	0.	0

M.H.D. of H.S. = 0.00125 c.c.

M.H.D. of H.S. = 0.0015

Average M.H.D. of H.S. = 0.00137 c.c.

Table 4. To find the M.H.D. of antihuman red cell serum for the corpuscles of a premature baby, aged 4 days.

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	v.f.tr.	v.f.tr.	f.tr.	d.	d.	0	0

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	d.	m.	m.	m.	a.c.	0	0

M.H.D. of H.S. > 0.003 c.c.

Table 5. To find the M.H.D. of antihuman red cell serum for the corpuscles of a case of icterus gravis, aged 24 hours.

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	tr.	m.	a.c.	j.c.	j.c.	0	0

∴ M.H.D. of H.S. = 0.00125 c.c.

						Controls	
R.B.C. (c.c.of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	j.c.	j.c.	c.	c.	c.	0	0

∴ M.H.D. of H.S. = 0.001 c.c.

Average M.H.D. of H.S. = 0.001125 c.c.

<u>Table 6.</u> To find the M.H.D. of antihuman red cell serum for the corpuscles of a case of icterus gravis, aged 7 days.

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	c.	c.	c.	c.	c.	0	0

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	c.	c.	c.	c.	c.	0	0

∴ M.H.D. of H.S. < 0.0005 c.c.

<u>Table 7.</u> To find the M.H.D. of antihuman red cell serum for the corpuscles of four cases of familial acholuric jaundice.

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis:							
Jean H.	v.f.tr.	tr.	tr.	d.	d.	0	0
Nancy H.	v.f.tr.	v.f.tr.	v.f.tr.	tr.	d.	0	0
John H.	v.f.tr.	v.f.tr.	v.f.tr.	f.tr.	d.	0	0
Jas. H.	v.f.tr.	tr.	tr.	tr.	d.	0	0

						Controls	
R.B.C. (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis:							
Jean H.	d.	d.	m.	a.c.	j.c.	0	0
Nancy H.	tr.	tr.	d.	a.c.	j.c.	0	0
John H.	v.f.tr.	f.tr.	d.	m.	a.c.	0	0
Jas. H.	v.f.tr.	d.	m.	v.m.	a.c.	0	0

M.H.D. of H.S.:- Jean - 0.003 c.c.; Nancy - 0.003 c.c.; John -> 0.003 c.c.; Jas. -> 0.003 c.c.

another premature baby which did not suffer from either anaemia or jaundice.

Unfortunately by the time the experiment was completed the child was dead and further investigation was not possible.

It seemed therefore that as a rule the red corpuscles of cases of erythroblastosis foetalis were not unduly susceptible to the action of a haemolytic serum which was specific for human blood.

It was interesting to note in the cases of acholuric jaundice, a chronic haemolytic anaemia, that although the corpuscles were excessively susceptible to lysis in hypotonic saline, they were not unduly affected by the haemolytic serum (Table 7).

(v) Is there a haemolytic agent in the liver or spleen?

The possibility of there being a haemolytic agent present in the liver and spleen in cases of neonatal haemolytic anaemia was now considered. These organs were chosen as the probable sites of this hypothetical agent because, as we have seen, it was in them that phagocytosis was chiefly found.

Saline and alcoholic extracts of liver and spleen from a typical case (No. 7) of icterus gravis, which at the time of death showed active destruction and regeneration of blood, were made according to the method described by Browning and Watson (1919). I used twice the amount of tissue (40 gm.)

advocated by these authors. The organs were obtained within 12 hours after death. Owing to the presence of large quantities of blood in the spleen it was found difficult to prepare a saline extract which was haemoglobin free, but by grinding up the splenic tissue with silver sand, a slightly turbid, yellowish saline extract was obtained. The saline extract of liver was also somewhat cloudy. The alcoholic extracts were practically clear. In the experiments which I shall describe, the saline extracts were diluted with equal parts of normal saline, and a dilution of one part of the alcoholic extracts with six parts of normal saline was used.

The effect of the extracts on washed red corpuscles from a case of icterus gravis was investigated, but after incubating 0.5 c.c. of a 3% suspension of the corpuscles with quantities of the extracts varying from 0.1 c.c. to 0.5 c.c., lysis did not occur, proving that a free haemolytic agent was absent.

(vi) Is there, in the liver or spleen, an antigenic principle, for which an antisubstance is absent in the serum of icterus gravis?

It was then thought that possibly in the liver and spleen there might be an antigenic principle of the nature of a haemolysin, to which in normal serum an antisubstance was present, but absent in cases of icterus gravis.

A series of experiments on the lines shown in Tables 8 and 9 was done.

The various extracts were put up with pooled normal human serum which had been heated to 55°C. for half-an-hour to destroy complement, the minimum haemolytic dose of which had been ascertained. The whole was then incubated at 37°C. for $1\frac{1}{4}$ hours, and frequently shaken up during this time.

Ox red cells, which had been put up as a 3% suspension in normal saline, and sensitized by the addition of 5 minimal haemolytic doses of antihuman red cell serum, were added, and a further incubation period of $1\frac{1}{2}$ hours allowed.

Table 8: (with 9). To find if in the liver and spleen (in icterus gravis) there is an antigenic principle present in normal human serum but lacking in icterus gravis.
M.H.D. of added complement = 0.0025 c.c.

Saline extract of organs from case of icterus gravis (dil. $\frac{1}{2}$; c.c.)	0.5	0.5	0.5	0.5	0	0
Normal adult serum. (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0.	c.

Similar experiments were then performed using serum from cases of icterus gravis, and heated to 55°C. for half-an-hour to destroy complement.

Table 9. Second part of experiment (1st part in Table 8).

Saline extract of organs from case of icterus gravis (dil. $\frac{1}{2}$; c.c.)	0.5	0.5	0.5	0.5	0.5	0
Serum from case of icterus gravis (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0	c.

Complete lysis with both splenic and liver extracts (saline and alcoholic) occurred, whether normal serum or serum from cases of icterus gravis were used, thus showing a haemolytic system was not present in the sera when in contact with tissue extracts, and, therefore, that evidence, in normal serum, of an antisubstance which was absent in the serum in icterus gravis, was lacking.

(vii) Is there an abnormality in the red corpuscles in icterus gravis?

A further possibility was then considered. In the neonatal haemolytic anaemias might not there be an abnormality of the red corpuscles which either in combination with the tissue extracts, or the serum, or both, could cause fixation of complement and thus produce lysis? I therefore sensitized red corpuscles from cases of icterus gravis by the addition of 5 minimum haemolytic doses of an antihuman red cell serum, obtained from a rabbit as previously described, to a 3% suspension of the corpuscles, and substituted this for sensitized ox cells (Table 10). With each extract, however, complete lysis was ultimately present, showing that complement had not been fixed and that a haemolytic principle was not present.

Table 10. Is there an abnormality of the red corpuscles in icterus gravis, which in conjunction with tissue extracts, and/or an abnormality of the serum, would fix complement, and cause lysis? M.H.D. of complement = 0.005 c.c.

Saline extract of organs from case of icterus gravis (dil. $\frac{1}{2}$; c.c.)	0.5	0.5	0.5	0.5	0.5	0
Serum from case of icterus gravis (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized r.b.c. from case of icterus gravis (c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0	c.

(viii) Conclusions.

From the foregoing experiments I have therefore made the following conclusions:-

(1) A free lysin is absent from the circulating blood in the neonatal haemolytic anaemias.

(2) The red cells do not show any tendency to autolysis in (a) normal human serum, (b) in their natural serum.

(3) A haemolytic serum, specific for human blood, does not have an excessive lytic effect on the corpuscles of (a) normal infants, (b) premature infants, (c) infants with neonatal haemolytic anaemia, (d) cases of acholuric jaundice --- a haemolytic anaemia.

The exceptional case of icterus gravis which broke this rule cannot be regarded as having any special significance as regards the neonatal haemolytic anaemias in general. Lack of opportunity for further study of the case excludes the possibility of further comment on it.

(4) Experiments were done to show that the liver and spleen in icterus gravis did not contain any free haemolytic agent, nor was there any factor capable of fixing complement in the presence of normal adult serum.

(5) Similarly the extracts did not combine with complement to form a haemolytic system in the presence of serum from cases of icterus gravis. Therefore the anaemia was not due to a product of the tissues and an abnormality of the serum acting conjointly.

(6) Anaemia was not due to any abnormality of the red corpuscles which by acting in conjunction with tissue extracts or serum of a similar case, or both, allowed the fixation of complement and consequent lysis.

The experiments showed that in erythroblastosis foetalis lysis does not occur in the circulation, and that there is not apparently any abnormality of the serum or red corpuscles, or any secretion of the liver and spleen, which by interaction could produce lysis.

It would therefore seem that the anaemia is directly due to the destruction of the red corpuscles by phagocytes. This, as we have seen, was a conspicuous feature in the liver and spleen. Any ferments which the phagocytes secreted were either destroyed after death, or, if present in the tissue extracts, were innocuous to the corpuscles in the absence of the phagocytes themselves.

SECTION F.

KERNICTERUS.

Introduction.

Only very rarely in the adult is the brain substance affected in generalised jaundice, but in the infant suffering from icterus gravis this is not infrequent, and for this phenomenon Schmorl (1904) invented the term kernicterus.

Strictly speaking kernicterus is a pathological entity, but certain nervous symptoms occurring in children who have recovered from icterus gravis have been attributed to this condition, although it is obvious that while the damage in the brain is permanent, the jaundice of the affected areas will be of transient duration.

(i) Incidence.

Schmorl found kernicterus in 6 (5%) out of 120 cases of icterus gravis. In my series (1933-36) the condition was somewhat more frequent, 5 cases (21.74%) out of 23, or, if two cases in which the condition was diagnosed clinically are accepted, 7 cases (30.43%).

(ii) Kernicterus and prematurity.

In two instances, proved post mortem (Cases 2 and 10) the child was premature, and one of the children, in which a clinical diagnosis was made (Case 13) was also premature. This is striking in view of the fact that icterus gravis has not been commonly found in premature babies (Diamond et al., 1932), although, as I have already noted, 10 of my 59 cases were in such children.

Descriptions of the pathological anatomy have been given by Schmorl (1904), Ylppö (1918), Zimmerman and Yannet (1933), Hawksley and Lightwood (1934), and Eckstein (1933).

(iii) Pathological anatomy.

It was commonly the basal nuclei, especially the caudate nucleus, and the putamen and globus pallidus of the lenticular nucleus which have been affected, but the yellow staining has also been noted in the columns of the fornix, the grey matter of the hippocampus and very markedly in the nuclei of Luys. Neither the fimbria hippocampi nor the alveus, which are composed of white matter, were stained (Hawksley and Lightwood, 1934). Schmorl noted that in his series the optic thalami were not involved but in one of my cases (No. 21) these were stained, and also in one case reported by Hawksley and Lightwood (1934).

The cerebellum has sometimes been affected and jaundice of the grey matter of the cortex, of the flocculi, and in the dentate nuclei has been observed. Staining of the nuclei of the brain stem has been frequently recorded, in the corpora mamillaria, the red nuclei, the basilar portion of the pons, and in the nuclei and olives of the medulla oblongata.

Small circumscribed areas of jaundice have been noted in the cerebral cortex, in the frontal, parietal and occipital regions, and in the neighbourhood of the Island of Reil.

In my series the most widespread kernicterus was seen in Case 10, where there was sharply demarcated jaundice of the caudate and lenticular nuclei, and in the dentate nuclei of the cerebellum. There were also areas of staining in the medulla oblongata. Unfortunately the brain at the time of autopsy was of a semi-fluid consistence and a detailed study of the anatomy was not possible.

In Case 21, the icterus was deep but confined to the basal nuclei, especially in the thalami and lenticular nuclei. Kernicterus affecting the caudate and lenticular nuclei, and the nuclei of the medulla oblongata was seen in Case 14, but again owing to the softness of the brain the details were obscured. The caudate and lenticular nuclei alone were affected in Case 7, and only slight staining of the lenticular nuclei was found in Case 2.

(iv) Histology.

Microscopically it has been found that in the affected areas many of the ganglion cells have been totally destroyed, and some partially, while a few usually escaped. The cytoplasm of the damaged cells was bile stained. Overgrowth of the neuroglia has been reported, as well as staining of the ground substance. Sometimes there was round cell infiltration especially round the vessels. Staining of the ground substance without apparent damage to the ganglion cells was reported in the inferior olives in one case (Hawksley and Lightwood, 1934).

(v) The nature of the pigment.

It has not yet been decided whether the injury to the nerve cells is caused by the modified bilirubin or whether pigment is deposited in cells already damaged by some other toxic agent. The concensus of opinion favours the latter view.

It was noted by Schmorl (1904) and confirmed by Hawksley and Lightwood (1934) that staining was apparently due to a modified bilirubin, because after exposure to formalin there was not any change of colour, which gradually faded, contrary to the findings in tissues in obstructive jaundice. A specimen of kernicterus mounted in formalin for museum purposes at the R.H.S.C., Glasgow, very quickly lost its colour.

(vi) Prognosis.

In my fatal cases death occurred early --- during the first or at the beginning of the second week, and in three there was no other apparent cause of death. Hawksley and Lightwood (1934) have remarked that kernicterus caused death early and that very rarely has the condition been found in cases of icterus gravis dying after the second week. Probably death has been due to involvement of the vital nuclei of the brain stem.

(vii) Clinical signs in the early stages.

It was impossible to diagnose kernicterus with any degree of certainty in the early weeks of life. Sometimes the children were excessively drowsy, that is to say, drowsiness was even more marked than in the ordinary case of icterus gravis. I found that in two of my cases there was extreme hypertonicity of the muscles of the limbs (Nos. 10 and 21) and in one (No. 10) head retraction was present. Convulsions were occasionally recorded, but on the other hand convulsions in cases without kernicterus were also noted, and were sometimes due to intra-cranial haemorrhage, and sometimes were unexplained at the post-mortem examination.

(viii) The cerebrospinal fluid.

The cerebrospinal fluid was examined in one case (No. 21) where kernicterus was found post mortem. The cell count and globulin content were normal but the fluid was quite yellow in colour. This colouration however was found in other cases of icterus gravis in which nuclear jaundice was absent.

(ix) The later clinical signs.

It is now universally accepted that some cases of icterus gravis have recovered, and subsequently presented symptoms indicative of an extrapyramidal lesion.

In 1915, Guthrie reported a child of one year and seven months, and who made a recovery from severe icterus gravis, which had been present throughout the first six weeks of life. The child was generally backward and could not talk, sit up or stand. The muscles especially of the legs were hypotonic, and there were generalised involuntary movements which he described as choreo-athetosis. The tendon reflexes were normal. The writer was of the opinion that there was a thalamic or cortico-thalamic lesion.

Spiller (1915) described four cases of children who had recovered from icterus gravis. In the first at the age of seven years there were generalised choreiform movements of the limbs, trunk and face. The limbs were rigid.

In a premature child of one year and four months, there was generalised rigidity of the limbs which were, however, moved freely. Rigidity was increased when the limbs were handled, for instance in attempting to elicit the tendon reflexes. Much inco-ordination of movements was noted, and the child could not sit up or hold up the head.

Another child aged two years, apparently intelligent, had marked hypotonicity of the neck muscles so that the head could be supported for only a short period. There was if anything slight spasticity of the limbs. Walking had been achieved late, and speech had not been learned.

In the fourth case, aged three years and ten months, and obviously mentally deficient, there had been convulsions at the age of five months. The child had never held the head up or sat up. There was gaping of the mouth, swallowing was difficult and the limbs were so spastic that the tendon jerks could not be elicited.

Paul (1924) also recorded generalised muscular rigidity and athetosis. Hoffmann and Hausmann (1926) described the clinical manifestations as being convulsions, especially of the upper extremities, paralysis and paresis of the muscles of swallowing and respiration, and opisthotonus.

Smallwood (1933) recorded generalised spasticity and convulsions in a case during the icteric period. After the jaundice had cleared and the child was four months old spasticity of the limbs and opisthotonus gradually increased and there was primary optic atrophy. The author thought that the symptoms indicated a cortical rather than a basal lesion.

I have seen two cases which showed severe extrapyramidal symptoms following icterus gravis.

One of these, Case 33, a girl, born at full time was jaundiced at birth and had a moderate anaemia of 2.45 million red cells per c.mm. at the fourth day. Anaemia was controlled by transfusions and in the third week jaundice began to diminish.

On the tenth day it was noted that there was a claw hand deformity on the right side. This was of transient duration.

By the age of eleven months it was obvious that the child was mentally deficient.

At the age of two years and four months, the child could hold up her head and sit up without support, but could neither walk nor talk. The muscular tone was normal but there were continual choreo-athetotic movements of the face, limbs and trunk. The cranial nerves were intact, the knee jerks present, the plantar responses flexor and the abdominal reflexes active. The fundi were normal. An X-ray plate of the skull did not reveal any abnormality, the cerebrospinal fluid was normal, nor was any abnormality shown by an encephalogram.

The second case (No. 13) was of a slightly premature female child who was jaundiced from birth. She was transfused and appeared to thrive from the age of four weeks, the jaundice by that time having cleared. From the fifth week onwards the red cell numbers remained above 4.0 millions per c.mm. At this time there was no apparent lesion of the central nervous system. When she reached the age of six months the mother complained that the child was always staring in a vacant manner although a nervous lesion was not found. When seen again at the age of one year it was obvious that the child was mentally deficient. There was generalised hypotonia of the muscles. The child could not sit up although she could support her head. No attempt at speaking was being made. Voluntary movements of the limbs could be made but the patient was very inactive. The head was of normal dimensions, the reflexes were normal, and swallowing was not affected. Athetosis or chorea were not present.

(x) Summary of the later clinical signs.

From the preceding description of cases it will be seen that the common feature has been an extrapyramidal lesion, characterised by excessive rigidity or hypotonus of the musculature. Frequently inco-ordinated or choreo-athetotic movements have been present. Convulsions have been noted. Bulbar symptoms such as difficulty in swallowing or breathing have been recorded. Mental retardation has been nearly always present. It would seem that the median nerve affection noted in one of my own cases indicated a temporary involvement of the spinal cord in the cervical enlargement. Unfortunately post-mortem examination of the spinal cord in cases of kernicterus has never been done, and consequently it is not known whether the grey matter inferior to the medulla has been affected.

It is obvious from the case reports that the lesions have always been extra-pyramidal and that their distribution has varied widely, apparently involving the cerebral cortex, the cerebellum and basal ganglia, and the nuclei of the brain stem. These clinical findings are in keeping with the pathological observations in cases which died before symptoms were obvious. It was nevertheless impossible to diagnose kernicterus with certainty because intracranial haemorrhage, to which jaundiced babies are so liable, might have produced any of the above clinical pictures.

(xi) Pathology of kernicterus in the older child.

The researches of Eckstein (1933) however have thrown some light on the later aspects of kernicterus. He found that in a case which died at the age of five months, and showed muscular rigidity and Parkinsonianism following on icterus gravis, there were severe changes in the ganglion cells of the basal nuclei, in the corpora Luysii and in the hippocampus major. The ganglion cells were extensively damaged although no longer jaundiced, and there was proliferation of the glia. The picture was that of a toxic encephalitis or encephalopathy.

This observation provided a link between the early fatal cases where there was absence of a definite clinical nervous upset, but post-mortem evidence of damage to the brain, and the later cases where the clinical picture suggested similar intracranial damage, but where pathological material was lacking.

Eckstein, however, made the interesting observation that the encephalitis or encephalopathy is not peculiar to icterus gravis, but has been found in cases of untreated diabetic coma in which idential microscopic appearance in the hippocampus and cerebral cortex were found.

(xii) Summary.

In brief, icterus gravis is sometimes complicated by toxic damage to nuclear elements of the brain. This has been estimated to occur in 5% of fatal cases, but as my series shows, is probably more frequent or about 20%, and if the cases which recover are included, even higher.

The toxin shows a selective action for the basal nuclei but may also affect the cortex cerebri, the cerebellum, the hippocampal region, the pons and medulla. Clinical evidence is given that the grey matter of the spinal cord may also be damaged. The ganglion cells of the grey matter are destroyed or damaged. The pyramidal tract is not affected.

The majority of the cases die in the first or second week of life but a few recover and show diverse extra-pyramidal symptoms, and, frequently, mental deficiency.

SECTION G.

MORTALITY AND PROGNOSIS IN ERYTHROBLASTOSIS FOETALIS.

Over a period of fifteen years (1921-36) the total number of cases of neonatal haemolytic anaemia admitted to the Royal Hospital for Sick Children, Glasgow, was 67. Only 38 of these occurred in the twelve year period between 1921 and 1933, while 29 were seen in the subsequent three years. There is no reason to believe that there has been a true increase in the frequency of the condition among the population as a whole. It may be that the increase of medical interest in the condition partially explains the discrepancy.

In both periods icterus gravis was by far the most frequent type of case encountered 94.74% and 79.3% respectively (Table XIII). Hydrops foetalis was not seen.

The total mortality in all cases for the 1921-36 period was 54 out of 67 cases (80.6%), but the figure for the 1921-33 series was slightly higher (84.21%) than in the later series (75.86%), the difference being 8.35% (Table XIV).

From 1921 to 1933 six cases (16.66%) of icterus gravis out of a total of 36 recovered, and in five instances the recovery was spontaneous while in the sixth the child was transfused. The mortality rate was therefore 83.33% (Table XV). Between 1933 and 1936 the mortality was 17 cases (73.91%) out of 23, six cases (20.69%) having recovered (Table XV).

Table XIII. Showing the relative incidence of two types of erythroblastosis foetalis.

	1921-33		1933-36		1921-36	
	No. of cases.	% of total cases of erythroblastosis (38).	No. of cases.	% of total cases of erythroblastosis (29).	No. of cases.	% of total cases of erythroblastosis (67).
Icterus gravis neonatorum	36	94.74	23	79.31	59	88.06
Anaemia haemolyticae neonatorum	2	5.26	6	20.69	8	11.94

Table XIV. Showing the incidence of erythroblastosis foetalis and the mortality.

	1921-33					1933-36					1921-36				
	TOTAL	RECOVERED		FATAL		TOTAL	RECOVERED		FATAL		TOTAL	RECOVERED		FATAL	
		Total	%	Total	%		Total	%	Total	%		Total	%	Total	%
Cases of erythroblastosis foetalis.	38	6	15.79	32	84.21	29	6	24.14	23	75.86	67	13	19.4	54	80.6

Table XV. Showing the mortality in icterus gravis neonatorum and anaemia haemolytica neonatorum.

	1921-33					1933-36					1921-36				
	TOTAL	RECOVERED		FATAL		TOTAL	RECOVERED		FATAL		TOTAL	RECOVERED		FATAL	
		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%
Icterus gravis neonatorum.	36	6	16.66	30	83.33	23	6	20.69	17	73.91	59	12	20.34	47	79.64
Anaemia haemolytica neonatorum.	2	0	0	2	100.0	6	1	16.66	5	83.33	8	1	12.5	7	87.5

The total mortality rate in anaemia without jaundice between 1921 and 1936 was given(87.5%) out of eight cases (Table XV).

The improvement in the mortality rate in the 1933-36 figures for all cases of neonatal haemolytic anaemia coincides with the period in which transfusion was practically a routine treatment. This treatment of these anaemias was introduced into the hospital in 1933 and actually only one case in my 1921-33 series was thus treated, whereas transfusion was done in 21 cases in the 1933-36 series.

In Table XVI, the mortality rates of all cases before and after the institution of transfusion therapy are compared, and it will be seen that a reduction in mortality from 86.49% in the former group to 73.33% in the latter occurred, the difference being 13.16%.

Because various cases in the more recent years were not transfused for reasons previously explained, it seems reasonable to compare the mortality rates of the total number of transfused cases with the total number not so treated. This is done in Table XVII, where it will be found that there were 45 cases which were not transfused, and, of these, 5 (11.11%) recovered spontaneously, while 40 (88.88%) died. Of the 22 cases which were transfused 8 (36.36%) recovered and 14 (63.64%) died.

Roughly speaking the recovery rate was more than trebled, although the mortality remained high.

Table XVI. Showing the mortality and recovery rates before and after the institution of transfusion as treatment in the erythroblastoses.

	Total No. of cases.	RECOVERIES.		FATALITIES.	
		No.	% of total cases.	No.	% of total cases.
Before treatment by transfusion	37	5	13.51	32	86.49
After treatment by transfusion	30	8	26.66	22	73.33

Table XVII. Showing the comparative mortality in cases of erythroblastosis according to whether treated by transfusion.

	Total No. of cases.	RECOVERIES.		FATALITIES.	
		No.	% of total cases.	No.	% of total cases.
Cases not treated by transfusion	45	5	11.11	40	88.88
Cases treated by transfusion	22	8	36.36	14	63.64

It has long been recognised that hydrops foetalis is invariably fatal while icterus gravis usually causes death. Rolleston (1920) in a series of 130 cases found a death rate of 77%. Fatal cases of haemolytic anaemia without oedema or jaundice have been recorded comparatively rarely. From Table IX it will be seen that of 40 cases only 6 (15%) died. In this respect our experience of this type of anaemia at the Royal Hospital for Sick Children, Glasgow, has been unfortunate. It is possible that milder cases with spontaneous recovery have not been brought to hospital, but it is certain that neither in the out-patient department nor in the wards, where a sharp lookout for them has been kept, have any such cases appeared.

It is noticeable, however, that of the few cases of anaemia without jaundice published in this country, Parsons et al. 2 cases (1933), Pritchard and Smith (1931) one case, and Mackay and O'Flynn (1933) one case, two have died, a rather higher proportion than in the American and continental literature.

On the whole then it will be seen that in spite of treatment the prognosis in icterus gravis remains bad, but that in anaemia without jaundice the prognosis is as a rule good, and that although treatment by transfusion may be of value on general grounds it is not essential to the majoriry of cases which recover.

SECTION H.

THE CAUSES OF DEATH IN THE NEONATAL HAEMOLYTIC ANAEMIAS.

(i) Icterus gravis.

Death was due to one or more of four causes unless in very exceptional circumstances. These causes were:-

(1) profound anaemia,

(2) haemorrhage,

(3) kernicterus,

(4) secondary infection.

In the 1933-36 series of cases of icterus gravis, 17 died.

Profound anaemia was the cause of death in one case (5.88%). Transfusion was not done in this instance, and two days before death, which occurred on the thirty-first day of life, the red cell count was reduced to 800,000 per c.mm.

Five cases (29.4%) died of cerebral or meningeal haemorrhage, one of the former and four of the latter. One case of meningeal haemorrhage was associated with extensive haemorrhage into all lobes of both lungs, and this was probably the immediate cause of death. Another case showed large haemorrhages into the stomach and bowels.

Kernicterus was apparently the sole cause of death in three cases (17.65%).

Secondary infection accounted for seven cases (41.18%), of which five died of acute broncho-pneumonia and two of gastro-enteritis. One of these cases with broncho-pneumonia also showed kernicterus. Infection was usually a late event and in six of the cases occurred no earlier than the third week of life. The seventh instance was the case showing a combination of kernicterus and broncho-pneumonia.

Unfortunately it is not possible to tabulate the cause of death in icterus gravis so accurately in the 1921-33 series because of lack of data in the records. An analysis of 22 cases from the 1921-33 series gives the following figures:-

Deaths due to	anaemia	- 11 or 50%
	haemorrhage	- 2 or 9.09%
	kernicterus	- 1 or 4.55%
	secondary infection	- 8 or 36.36%

One of the cases in the secondary infection group had also kernicterus.

In ascribing the cause of death to anaemia in 11 cases I am making a certain amount of assumption, because some of these cases may have died of intracranial haemorrhage or kernicterus about which I have no information. On the other hand the paucity of deaths from haemorrhage may also be partially due to the fact that the lives of these children had not been prolonged by transfusion and they had not lived long enough to develop the tendency to haemorrhages.

Even allowing for the inclusion of cases of intra-cranial mischief under the heading of profound anaemia it is obvious that there was a markedly higher incidence of death from this cause in the pre-transfusion period. It has already been seen that no case which had been transfused had a sufficiently profound anaemia at the time of death to produce a cardiac failure from anoxaemia per se, and that many were showing little or no evidence of acute haemolysis. From the reduction of the death rate from pure anaemia, then, we get confirmation of a point already made, namely, that although transfusion controls the anaemia, the prognosis depends on other factors.

(ii) Anaemia without oedema or jaundice.

There remain to be discussed the data of cause of death in anaemia without jaundice. These were similar to the findings in icterus gravis. Including the two cases from the 1921-33 series, three died of profound anaemia between the tenth and eighteenth days of life. None had been transfused, but those which had, lived considerably longer. Secondary infection was present in two cases. One (No. 24) died of ileo-colitis, and a second (No. 25) had acute broncho-pneumonia although the immediate cause of death seemed to have been an acute haemolysis following a transfusion. The child had previously been transfused with blood from the same donor and this seems to have been one of these rare and unexplained examples of haemolysis of properly matched blood which have been occasionally noted in haemolytic anaemias, especially in acholuric jaundice (Dawson, 1931)

One fatality was apparently from a small meningeal haemorrhage (Case 29) and another (Case 27) was probably from septicaemia, although unfortunately a splenic culture was not done.

(iii) Comment.

I have already pointed out that often where anaemia was controlled by transfusion and haemolysis was in abeyance, death was frequent. I have also already shown that kernicterus, where it occurs in icterus gravis, tends to cause early death. The tendency to haemorrhages, which occurs in the neonatal anaemias and which is peculiar to them, explains death in one group where anaemia may be absent.

The remaining group to be considered is that in which secondary infection proved fatal. This liability to infection, in the absence of anaemia, kernicterus and haemorrhage can, I think, be explained chiefly by the damage to the liver, which I have shown was a common post-mortem finding. From the illustrations given it will be obvious that when the liver was so severely affected the digestion and general metabolism of the child were greatly impaired, and consequently there was a general deterioration of the general condition, so that the infant, lacking resistance, became an easy prey to intercurrent infection.

SECTION I.

DIFFERENTIAL DIAGNOSIS.

(i) Physiological jaundice.

Icterus gravis has first of all to be distinguished from simple icterus neonatorum, or physiological jaundice of the new-born. In this condition, which is very common, a family history of deaths from neonatal jaundice is usually lacking, although in families where icterus gravis has occurred it is not infrequently found that some of the children have had simple icterus.

Simple icterus is rarely evident before the third day of life, the degree of jaundice is usually slight and not accompanied by pallor. The child's general appearance is good and there is no increase of drowsiness. There is neither hepatomegaly nor splenomegaly, the stools are normal in appearance, and although the urine shows excess of urobilin, bile pigments are not present. Nevertheless in a few cases the jaundice may be sufficiently marked to cause doubt. Furthermore the icterus may appear earlier than usual, e.g., on the first or second day of life (Case 39), or as in one case seen by me (No. 38) a history of slight icterus at birth may be given. Again as I have already indicated the appearance of jaundice in undoubted cases of icterus gravis may be delayed. In such circumstances only an investigation of the blood on repeated occasions is reliable for accurate diagnosis. Various workers (Mitchell, 1921; Martin and Evans, 1935) find that the blood count is, if not higher, frequently not lower than in the child without jaundice, and consequently red corpuscles counts which do not fall below five millions per c.mm. are common.

In exceptional cases of physiological jaundice in full-time children an appreciably greater fall in the red cell numbers may be found, and I have seen one case (No. 39) in a full-time child, where jaundice was said to have appeared within forty-eight hours of birth, and in which the blood count had fallen to 4.25 million red corpuscles per c.mm. by the sixth day. Reticulocytes were 2% and erythroblasts numbered 150 per c.mm., that is to say, within normal limits. In the following few days jaundice cleared, and the blood count did not show any further reduction.

The degree of erythroblastaemia is of some value. As we have seen, in simple icterus neonatorum 5,000 nucleated red cells per c.mm. may be taken as a safe upper limit of the normal in the first day or so, although such high numbers are rarely found.

The van den Bergh reaction in these cases is always a pure indirect reaction although it may be considerable as in Case 38, where it amounted to 48 units, and yet the lowest blood count was 4,860,000 per c.mm.

(ii) <u>Anaemia of prematurity</u>.

Considerable difficulty may be encountered in diagnosing erythroblastosis in premature children. These infants show a greater degree of blood destruction after birth than full-time babies (Abt and Nagel, 1932; Mackay, 1932; Merritt and Davidson, 1934), and some may acquire an anaemia of moderate severity, e.g. 3.5 millions per c.mm. with a reticulocyte and erythroblast count in excess of that found in mature infants (Josephs, 1934; van Creveld and Heybroek, 1932). Jaundice is more frequent in premature children and is then of considerable depth. As a rule the anaemia is of slower onset than in true icterus gravis, usually reaching its greatest extent at 8 to 10 weeks, and as there is little or no damage to the liver and the bile is excreted normally into the intestines, bile pigments do not appear in the urine, while the liver and spleen are not enlarged (Abt and Nagel, 1932).

If the child is under observation from birth and frequent blood examinations are made little difficulty is experienced in diagnosis. It is when the infant is first seen after the development of anaemia that considerable care is required, because one cannot afford to wait long lest a sudden exacerbation of haemolysis, which of course would verify a diagnosis of icterus gravis, jeopardizes the child's life by the production of a gross anaemia.

Furthermore, clinical features such as degree of jaundice, hepatomegaly and splenomegaly are sometimes misleading. For instance in my own series of six cases of icterus gravis in 5 premature children (1933-36 series), splenic enlargement occurred in only one, although in all cases there was some enlargement and increase of consistence of the liver.

Ferguson (1931) has shown that in premature children the extent of extramedullary haematopoiesis is greater in the early days of life than in full time babies, and doubtless in those infants developing the severer degrees of anaemia a corresponding increase in duration of the extramedullary depôts of blood formation happens and that all grades of anaemia and erythroblastosis occur in prematurity, there being no definite border-line between the severer types and true icterus gravis.

(iii) Congenital atresia or stenosis of the bile ducts.

This is a rare disease and doubtless many cases diagnosed as such before attention was focused on erythroblastosis were really examples of icterus gravis.

Typically in congenital abnormalities of the bile ducts, jaundice is of gradual onset and unremittent in character. The onset of icterus may be delayed into the second or third weeks. The stools are free of urobilin, although the outer surface of the motion may contain bile pigments which have been excreted by the intestinal mucosa (Hawksley and Lightwood, 1934). In cases where obstruction is not complete urobilin may be found in the faeces. The urine contains bile pigments. The liver becomes enlarged and firm but splenomegaly is a later occurrence. The characteristic anaemia of haemolytic jaundice is not present. The course of the disease is usually over a period of weeks or months, unless an intercurrent infection occurs.

From an examination of the records at the Royal Hospital for Sick Children, Glasgow, between 1921 and 1933, I find that congenital atresia or stenosis of the bile ducts was diagnosed in nineteen cases of neonatal jaundice. Gross abnormalities of the biliary channels were present, post mortem, in ten cases, but in one of these numerous "myeloid foci" were found in the liver on microscopic examination, and it would appear that haemolytic icterus gravis had occurred simultaneously. In another case the child had a considerable anaemia with splenomegaly and therefore was probably another example of coincidence of the diseases. This phenomenon has been recorded by Pasachoff and Wilson (1931) and Rupilius (1934).

Six cases were probably wrongly diagnosed. Jaundice in all of them was of early onset, one to four days after birth. One case had a gross anaemia (1.48 million red cells per c.mm. with 680 erythroblasts per c.mm. at the age of five weeks), and at post-mortem examination a gross lesion of the bile ducts was not found. In a case with similar post-mortem findings, erythroblastosis seems to have been found on section of the liver. The remaining four cases all showed considerable splenic enlargement, and such anomalies as variable jaundice, very little bile in the urine, and the occurrence of haemic cardiac murmurs indicating the presence of anaemia. One of these cases seems to have been practically recovered at the age of three months.

In three cases, where a post-mortem examination was not done, it is impossible to give an opinion from the clinical data available.

A very conservative estimate, therefore, is that one third of the cases diagnosed as congenital malformation of the bile ducts were suffering from haemolytic icterus.

(iv) <u>Congenital syphilis</u>.

This disease is now comparatively rare in the West of Scotland, and is certainly much less frequently seen than icterus gravis or anaemia of the new-born without jaundice. Jaundice is not common in congenital syphilis, and usually other signs such as a typical skin condition or osteochondritis, rhinitis with snuffles and collapse of the bridge of the nose, and rhagades suggest the correct diagnosis. Some cases of syphilis of the new-born, however, present a picture of anaemia with erythroblastaemia quite indistinguishable from that of a pure case of haemolytic anaemia (Hawksley and Lightwood, 1934; Haler, 1936). The Wassermann reaction is then the diagnostic criterion. Because this type of anaemia is not by any means common in syphilis it is an undecided point whether its occurrence is merely a coincidence or whether syphilis may on occasion be the cause of excessive haemolysis.

In my 1921-33 series the Wassermann reaction was done in nearly all cases and also in several of the 1933-36 series. The result was invariably negative.

(v) Neonatal sepsis with jaundice.

Umbilical sepsis is the common precursor of this disease, the infection passing to the liver by way of the umbilical vein. Usually a septico-pyaemia is present and multiple abscesses frequently occur throughout the body. There is considerable fever of the swinging type. Splenic enlargement may occur. A high leucocytosis is a common feature, and the typical anaemia of haemolytic icterus is absent.

(vi) Catarrhal jaundice.

Much doubt has been expressed as to whether catarrhal jaundice ever occurs in the new-born. The probability is that many cases published with this diagnosis were really examples of haemolytic icterus.

(vii) Congenital or familial acholuric jaundice.

Acholuric jaundice may be present from birth even although no other members of the family can be shown to be affected (Sutherland, 1933). The disease may be fatal within the neonatal period (Hichens, 1912-13). The diagnostic criterion lies in the excessive fragility of the red cells to hypotonic saline. This is probable present at birth and has certainly been demonstrated at the age of a few days (Hawksley and Bailey, 1934).

SECTION J.

PATHOGENESIS OF ERYTHROBLASTOSIS FOETALIS.

(i) Introduction.

Anaemia is the one constant clinical feature in the foetal erythroblastoses. Before discussing its possible or probable causes it is as well to summarise the details of the haemolytic process which occurs after birth in the normal child. Immediately after birth or shortly before it there starts a process of blood destruction, and the red cell count falls to about 5 millions per c.mm. in the first week or ten days (Lucas et al., 1921; Merritt and Davidson, 1933). There is a proportional fall in the haemoglobin level (Mackay, 1931). The haemolytic process does not apparently quite cease until about the third month (Lucas, Merritt and Davidson, and Mackay). In the earlier part of this haemolytic period the erythropoietic tissues are inactive, as is shown by the low reticulocyte counts, but about the third month there is evidence of an increase of haematopoietic activity. During the earlier period of the fall in blood count, there is secondary evidence of haemolysis in the presence of urobilinuria and increased bilirubinaemia (Lucas et al., 1921; Hampson, 1928; Goldbloom and Gottlieb, 1929, a).

This phenomenon of post-natal blood destruction has been attributed to the sudden change of environment at birth from one of low oxygen tension, that of placental blood, to one of high tension, that of alveolar air. Under the new conditions the large amounts of circulating haemoglobin of the foetus are no longer required, and consequently the excess is eliminated. Goldbloom and Gottlieb (1929, b) showed that by subjecting guinea-pigs to a low oxygen tension there was an increase of reticulocyte numbers, and eventually the development of a polycythaemia. When the normal oxygen tension was restored there was a period of blood destruction and decreased haematopoiesis with secondary signs of haemolysis such as increased bilirubinaemia.

It has also been shown that the haemolysis in premature children is more severe than in full time, and they may develop a moderate anaemia (van Creveld and Heybroek, 1932; Merritt and Davidson, 1934). The process is much more prolonged, however, and the degree and rate of fall in blood count is never so acute as in the neonatal anaemias.

It is therefore evident that in icterus gravis, and anaemia of the new-born without jaundice or oedema, there is what may be described as an exaggeration of the normal haemolysis. There is however much greater haematopoietic activity of the haematopoietic organs in the neonatal anaemias than in the normal or premature child. This is shown by the persistent reticulocytosis and by the erythroblastaemia.

(ii) Cause of anaemia in erythroblastosis foetalis.

That the anaemia is due to increased blood destruction is incontestible because of the rapidity of its occurrence, the clinical and pathological evidence of increased blood formation and the secondary signs such as increased bilirubinaemia and urobilinuria, and, pathologically, by the excessive haemosiderosis.

It is necessary to decide however whether the increased haemolysis is primary or secondary.

(iii, a). Is haemolysis secondary to abnormality of the red corpuscles?

Diamond et al. (1932), Abt (1933) and other American authors were of the opinion that the anaemia is secondary to qualitatively deficient red cells produced by haematopoietic tissues which are congenitally inefficient, and that the abnormal circulating erythrocytes are quickly removed by the reticulo-endothelial system. Péhu (1934) holds a similar view.

Against this attitude there are several important factors. One is that the onset of the anaemia may be delayed. Secondly haemolysis may cease for a time and recur. If the theory were true these findings would be unlikely because a congenitally deficient haematopoietic system would produce abnormal cells right from birth and continue to do so constantly. Further it is unlikely that a congenital defect or delay of development in the blood forming tissues, which have been developing in utero for five months, is going to right itself in the first few weeks of extra-uterine life because this could be the only explanation of the recovery of so many cases of anaemia without jaundice and for the cessation of haemolysis towards the fourth or fifth weeks of life in icterus gravis.

Immaturity of the red cells is certainly present, because, as we have seen, there is premature haemoglobinisation of the nucleated precursors of the erythrocytes. In the early stages of the anaemia there may be excessive megalocytosis - a further proof of immaturity. Later although the cells become more globular in shape there is no constant increase of the fragility in hypotonic saline. The change in shape however I believe to be due to an influence of the spleen, similar to that found in acholuric jaundice, and not due to a primary defect of the erythropoietic tissues.

Immaturity, then, is the only abnormality of the red cells attributable to the haematopoietic organs. It might be argued that the immaturity of the cells renders them excessively liable to rapid destruction by the reticulo-endothelial system, and while this is true, it is only to a very moderate degree, because in a disease such as pernicious anaemia where there is great immaturity of the red corpuscles, the anaemia produced is not of such an acute type, although the destruction of the red cells is accelerated.

Further the mature cells from healthy adults are apparently equally as susceptible to destruction as the immature corpuscles of the patient.

(iii, b) <u>Is haemolysis primary</u>?

In this country Parsons et al. (1933) and Hawksley and Lightwood (1934) support the view that haemolysis is primary, and that the erythroblastosis is due to an effort on the part of the erythropoietic system to replace lost cells.

If the latter theory is true, where is the blood destroyed? It can scarcely be by erythrophagocytosis in the blood stream because this has only rarely been seen in blood films. I have shown that there are not any demonstrable lysins in the blood stream, and further that the red corpuscles in icterus gravis and anaemia without jaundice are not more susceptible than normal infant or adult corpuscles to a haemolytic serum which I prepared from a rabbit by injecting it with my own blood.

Also, I have shown that a heamolytic agent was not produced in the liver and spleen, nor was haemolysis due to any factor derived from these organs, and acting in combination with abnormalities of the serum or red corpuscles.

The next factor to be considered is if there are signs of increased activity of the reticulo-endothelial system as a destructive agent. We have seen that, although there was very little in the bone-marrow, phagocytosis was exceedingly active in the liver and spleen.

It seems to me therefore that because only immaturity of the red corpuscles has been proved, that because destruction of the red corpuscles does not occur in the blood stream, and that because the transfused cells are also quickly destroyed, an abnormality of the reticulo-endothelial system must be accepted as the primary factor in haemolysis, although the immaturity of the red corpuscles doubtless plays a minor part. Erythroblastosis must therefore be a compensatory mechanism.

Extramedullary blood formation has also been found, although to a less extent, in the haemolytic anaemias of late infancy and childhood, (Luzet, 1891) and even in adults, in acholuric jaundice (Dawson, 1931), in pernicious anaemia, and in idiopathic haemolytic anaemia (Davidson, 1932). The marked degree of erythroblastosis in the newly-born infant is therefore probably due to the fact that at the time of onset of blood destruction the extramedullary depôts of blood formation were still present. Later in life when a similar stimulus to blood production may occur in anaemic states there is little and only rudimentary erythropoietic tissue remaining in the extramedullary sites.

The acceptance of blood destruction as a primary element has the advantage that it is not contra-indicated by the varying times of onset of haemolysis, --- during pregnancy in hydrops foetalis, shortly before or at birth in icterus gravis and anaemia without jaundice, and occasionally a few weeks after birth in icterus gravis.

Nor is the theory of an abnormally destructive reticulo-endothelial system contra-indicated by the familial incidence of the disease, because there is a somewhat similar phenomenon in acholuric jaundice which may be both familial and hereditary. Here the very definite abnormality of the red cells renders them easy prey for the reticulo-endothelial system. There is evidence, as I will show later, that the spleen has a considerable influence on the shape of the cell and it is not at all certain that the reticulo-endothelial system is not excessively destructive.

(iv) Comment on theory of maternal toxins.

This hypothesis of the causation of the haemolytic anaemias of the new-born has not included the consideration of the equally hypothetical conception of maternal and placental toxins previously discussed (p. 6). I think there is more likelihood of truth in an explanation of the anaemias which ignores the theory of toxins because of the fact that sometimes the anaemia apparently starts after separation of the child from the placenta, and because of the intermittent course which the haemolytic process sometimes shows.

Rolleston's (1910) suggestion that jaundice in icterus gravis was due to an ascending infection in the common bile duct is not supported by the pathological evidence.

(v) Comment on theory of infection.

Knoepfelmacher's theory (1910) that icterus gravis was a form of septicaemia of the new-born may also be dismissed because of the usual absence of fever, umbilical sepsis and high leucocytosis and also because blood cultures in the early stages have commonly been sterile. Further the association of icterus gravis with the other forms of erythroblastosis renders a theory of infection untenable.

(vi) Discussion of causes of different clinical features in the three types of erythroblastosis foetalis.

Apart from anaemia and splenomegaly, the clinical manifestations of the three types of erythroblastosis foetalis are widely different. In the first, hydrops foetalis, generalised oedema is the striking feature.

(a) Oedema.

In view of the low oxygen tension of the foetal blood, a possible cause of the oedema may lie in cardiac failure, because sometimes the heart has been found to be hypertrophied. The sequence of events would seem to be that with increasing anaemia there is cardiac hypertrophy in order to speed up the circulation and maintain oxygenation of the tissues, but as the anaemia progresses there is gradual cardiac failure and finally oexema is produced. The failure of the foetal circulation would in some cases cause increase of the amniotic fluid by back pressure on the placental circulation.

(b) Jaundice.

In icterus gravis jaundice is not purely haemolytic, as we have seen, but is also due to escape of bile from the bile canaliculi into the general blood circulation after damage to the liver cells and rupture of the canaliculi. Still (1927) suggested that the jaundice might be due to failure of the liver to deal with the greatly increased quantity of bilirubin, and it was also thought that undue viscosity of the bile (a quite definite finding) and bile thrombi in the ducts might be a factor. In many cases of anaemia without jaundice however the amount of blood haemolysed within a short time is nearly if not equally as great as in icterus gravis, and yet gross jaundice does not follow. MacClure (1931) has suggested that there is a modification of the bilirubin which acquires toxic properties and gravely damages the liver cells. No biochemical or other proof of this has been attempted but it is borne out by the toxic encephalitis which is not infrequently found in cases of icterus gravis. In the affected parts of the brain, as we have already seen, the bilirubin appears to be modified, and the toxin it contains has apparently a selective action on the basal ganglia. Beyond saying that the probable site of the elaboration of this hypothetical toxic is in the liver where the chief damage occurs, it is impossible to go further in suggesting the mode of origin.

The absence of oedema in cases of icterus gravis, and anaemia without jaundice may be due to the fact that theycommence about the time of or after birth when the circulation of blood is improved and the oxygenation more plentiful.

(c) Absence of oedema and jaundice.

Why cases of anaemia without jaundice fail to develop more than a slight and transient icteric tinge, that is to say, why the bile does not acquire very toxic properties, is inexplicable because often the amount of haemolysis is quite as great as in icterus gravis. It is possible, however, that in these cases the liver, which at the time of birth has not fully developed its metabolic properties, acquires its complete function more readily than in cases of icterus gravis, and that when excessive haemolysis starts there is less delay in the excretion of bilirubin, and consequently the chances of the acquisition of toxic properties by the bile are minimised.

(vii) Summary of pathogenesis.

The preceding tentative explanation of the erythroblastoses may be summarised as follows:-

(1) Anaemia is primarily due to the excessively destructive action of the reticulo-endothelial cells of the liver and spleen.

(2) A minor cause of the anaemia lies in the immaturity of the red corpuscles.

(3) The change of shape of the red cells, i.e. their globular form, is due to an influence of the pathological spleen.

(4) The theory of placental or maternal toxins appears to be unlikely.

(5) A basis of infection has been repeatedly disproved.

(6) The generalised oedema in one form of erythroblastosis foetalis is due to gradual foetal cardiac failure.

(7) The deep jaundice in the second type of erythroblastosis is due to failure of the immature liver to excrete the excess of bilirubin, which, probably in the liver, acquires toxic properties. The toxins not only destroy the liver cells, but have a selective action on certain brain cells.

(8) In erythroblastosis foetalis without oedema or jaundice the absence of the latter is possibly due to early maturation of the liver's excretory functions.

(9) In both icterus gravis and anaemia without jaundice the haemolysis starts either only shortly before birth or after, and the improved post-natal circulation and oxygenation of the blood probably prevent the slow onset of cardiac failure.

SECTION K.

ERYTHROBLASTOSIS FOETALIS: SUMMARY AND CONCLUSIONS.

(1) Erythroblastosis foetalis was defined as the hyperplasia or prolonged persistence of the extramedullary sites of foetal blood formation.

(2) Clinically, there were three manifestations of erythroblastosis foetalis -

(i) Icterus gravis neonatorum.

(ii) Haemolytic anaemia of the new-born, without oedema or jaundice.

(iii) Hydrops foetalis.

(3) There was sufficient evidence in the family histories to warrant the term familial to the condition.

A common basis for the three types of foetal erythroblastosis was indicated by the occurrence of two or more of them in the same family.

The high incidence of miscarriage and stillbirths in the affected families was striking.

(4) Icterus gravis: clinical and haematological.

The condition occurred chiefly in full-time infants, born after a normal pregnancy and of healthy mothers. A smaller proportion of cases was found among premature children.

Jaundice and anaemia were the first abnormal clinical features, and appeared usually within the first 48 hours of birth, and in a smaller proportion of cases at varying times during the first week of life. Rarely the onset of jaundice and anaemia was delayed into the second or third weeks, but the clinical, haematological, and pathological features of such cases, did not produce evidence that there was any fundamental difference from the more typical cases with early onset.

Excluding jaundice and pallor the main clinical feature was splenomegaly, except in those cases in premature infants, in whom it was typically absent. Slight hepatomegaly was a common feature.

The anaemia was of the haemolytic type, that is to say there was an excessive and speedy destruction of red corpuscles. This was usually accompanied by evidence of increased blood formation as shown by reticulocytosis and erythroblastaemia.

The process of haemolysis was most active in the first and second weeks of life, and was sometimes continuous and sometimes intermittent. From the second and third weeks onwards, the degree of blood destruction gradually decreased, and usually spontaneous cessation had occurred in the fourth or fifth weeks. This spontaneous limitation of haemolysis did not necessarily affect the prognosis.

Secondary signs of haemolysis were present, namely, increase of serum bilirubin which had not passed through the liver cells, and, sometimes, urobilinuria. The value of these signs in indicating excessive blood destruction was lessened by the presence of damage to the liver cells, so that sometimes bile was not excreted into the intestinal tract, and consequently urobilinuria could not occur. Further a delayed direct positive van den Bergh reaction was obtained from the serum.

In certain cases, from the lack of or the slight degree of reticulocytosis or erythroblastaemia, the erythropoietic mechanism was regarded as being somewhat hypoplastic. Unless anaemia was severe, this did not necessarily cause a fatal termination.

After an excessive initial megalocytosis the diameter of the red corpuscles became reduced to a greater extent than is found in normal infants. An influence of the spleen was regarded as being the cause.

The only other important change in the blood was the tendency to bleeding, which occurred after anaemia and jaundice had been well established, and was associated with prolonged bleeding time.

(5) Haemolytic anaemia without oedema or jaundice.

(i) Clinical and haematological.

As in icterus gravis the affected children were usually full time, healthy at birth and born of healthy mothers.

Slight and transient jaundice was sometimes present. Pallor became severe at the age of 7 to 10 days.

Splenomegaly and slight hepatomegaly were common.

Anaemia was severe, and identical with that in icterus gravis, being most rapidly produced in the first two weeks, and although cases were seen in which excessive blood destruction occurred for many weeks, the high percentage of recoveries observed in the literature, indicated a spontaneous limitation of the haemolytic process, similar to what was found in icterus gravis. Again haemolysis was sometimes intermittent, and the erythropoietic mechanism showed signs of exhaustion.

Haemorrhages were very rare.

In this type of erythroblastosis foetalis, signs of damage to the liver were slight or absent, and although the course of the condition was sometimes of considerable chronicity, recovery was the rule. This seemed to be associated with the absence of serious damage to the liver.

(ii) Prognosis and mortality.

(a) Icterus gravis. Prognosis was bad the mortality being 83.3% in the 1921-33 series of cases and 73.9% in the later series.

(b) Haemolytic anaemia without oedema or jaundice. Although in my short series of cases a very high death rate occurred, a study of the literature proved that this was exceptional, and that the great majority of cases (85%) recovered.

(6) Hydrops foetalis.

I did not have the opportunity to see a case of hydrops foetalis, but from the literature, it was shown that this condition began in foetal life, and was incompatible with independent life of the child for more than a few hours.

The outstanding clinical features were oedema and anaemia, the latter being very similar to what was found in cases of icterus gravis and anaemia without oedema or jaundice.

Hyperplasia of the foetal depôts of blood formation was usually present although, in a number of cases, these tissues were apparently hypoplastic.

(7) Treatment of the neonatal anaemics.

A specific treatment was not available. Transfusions, begun as early as possible and repeated at need, were beneficial and prolonged the life of many of the children, while some cases, which would otherwise have died, were tided over the period of haemolysis and recovery eventually occurred.

We have shown that routine transfusion trebled the recovery rate.

Transfused blood acted by lessening the deficiency of red corpuscles and prevented death from grave anaemia. It did not affect the progress of haemolysis, and indeed, the transfused corpuscles seemed to be destroyed along with the patients' own.

The routes for transfusion were discussed, and it was found that careful injection of blood into the longitudinal sinus was not associated with an abnormal incidence of intracranial haemorrhage, although the danger of injection of a quantity of blood into the subarachnoid space was always grave. Nevertheless, when numerous transfusions were required, this method was more convenient than cutting down on a number of superficial veins.

(8) Pathology of the neonatal haemolytic anaemias.

(a) Haematopoiesis.

The most striking feature of the neonatal haemolytic anaemias was the prolonged persistence and often hyperplasia of the foetal depôts of extramedullary blood formation, i.e. erythroblastosis. This was found chiefly in the liver and to a less extent in the spleen. It appeared to be most extensive in these sites in the second and third weeks of life, and thereafter to diminish, although in a few cases small and infrequent depôts were found as late as the sixth or fifteenth weeks.

The haematopoietic islets were intrasinusoidal in both organs, and composed of all intermediate forms of cell between the megaloblast and the normoblast. There was evidence of active cell division, and in the more primitive cells premature haemoglobinization was found.

Extremedullary leucopoiesis and thrombocytopoiesis (as indicated by the occurrence of megakaryocytes) was not found to any important extent.

Frequent osteosclerosis of the bone-marrow, even in the first week of life showed evidence of exhaustion of haematopoiesis. Microscopically erythropoiesis was less active than might have been expected, and there were signs that it was of an abnormally primitive type. From the late second and third weeks there was evidently a diminution of erythropoiesis, parallel with what was found in the liver and spleen.

It might be argued that the diminution of erythropoietic activity at this time, was due to a cessation of haemolysis, but I have shown that the latter did not occur until the fourth or fifth weeks. It is my opinion, because of the frequency of osteosclerosis, the excessive numbers of primitive erythroblasts, and the frequency of premature haemoglobinization, that the facts are better interpreted by assuming that the red cell producing mechanism was becoming exhausted.

In the marrow leucopoiesis was not greatly affected, there being a slight increase in the neutrophile and, sometimes, in the eosinophile forms. The onset of secondary infection caused a brisk response from the neutrophile leucocytes.

Megakaryocytes did not show any constant changes in numbers.

(b) Phagocytosis and haemosiderosis.

After haematopoiesis, phagocytosis seemed to be the chief feature. It was found to a very marked extent in the liver and spleen, and in the absence of other indications of low blood was destroyed in the neonatal anaemias, It seemed probable that this was due to the ingestion and destruction of red corpuscles by the reticulo-endothelial system.

Associated with phagocytosis was haemosiderosis of the tissues. While a certain amount of iron deposition is normal in the liver and spleen of infants, and while an increase would occur after transfusion it was shown that in spite of these facts, haemosiderosis was excessive in these organs, and sometimes occurred in the kidneys. This could only be explained on the grounds of increased blood destruction.

(c) Damage to tissues.

Degeneration of the liver cells was a striking feature of icterus gravis, but absent or slight in anaemia without oedema or jaundice. Its presence explained the extreme degree of jaundice in the former, the presence of the delayed direct positive van den Bergh reaction, and the absence of urobilin from the stools and urine.

The degree of damage was sufficient to elucidate the discrepancy between the mortality in icterus gravis and anaemia without jaundice.

Elsewhere toxic damage to the tissues was less marked. Lymph follicles were usually hypoplastic, and in the kidneys some damage to the tubular epithelium was present. Doubtless the latter was due to the excretion of abnormal metabolic products derived from increased haemolysis and degeneration of the liver.

Fibrosis of the liver was sometimes present, and was of a very fine nature. Thickening of the reticulum in the liver and spleen was seen more frequently.

Evidence that hepatic fibrosis might subsequently lead to cirrhosis was not found in children who died after recovery from anaemia and jaundice.

(9) Haemorrhage.

I have shown that clinically this was frequent in icterus gravis, and rare in anaemia without jaundice. These facts were borne out by the pathological investigations. In icterus gravis petechial haemorrhages into serous membranes were not infrequent, and larger haemorrhages into the subarachnoid space, the brain substance, the lungs, gastro-intestinal tract and suprarenal glands were found, while in anaemia without oedema or jaundice only a small meningeal haemorrhage was found in a single case.

Late haemorrhages in icterus gravis were shown to be associated with prolonged bleeding time.

(10) The nervous system in icterus gravis: kernicterus.

Kernicterus, i.e. toxic damage to the ganglion cells of the brain with icteric staining of the destroyed tissues, was shown to be considerably frequent, over 20% of my cases being affected as compared with 5% of Schmorl's (1904).

The site of kernicterus was commonly in the basal ganglia, although the grey matter of the cerebellum, cerebrum, mid-brain, pons, and medulla oblongata was sometimes affected.

The condition was as a rule quickly fatal. Evidence was led to show however that a number of cases might survive, and in these signs of an extra-pyramidal lesion, associated frequently with mental deficiency, were found.

(11) The mechanism of the production of anaemia in erythroblastosis foetalis.

It was shown that destruction of blood did not occur in the circulation, either from the presence of lysins in the serum, or from the undue fragility of the red corpuscles in the serum.

Nor was haemolysis due to any factor present in the liver or spleen, or to any combination of such a factor with abnormalities of the blood serum or the red corpuscles.

These facts seemed to show, as the histological investigation had done, that anaemia was due to the ingestion of the red corpuscles by an excessively destructive reticulo-endothelial system.

(12) The causes of death.

These were in icterus gravis, (i) profound anaemia, (ii) haemorrhage, (iii) kernicterus, (iv) secondary infection, and in anaemia without oedema or jaundice (i) profound anaemia, (ii) secondary infection, (iii) haemorrhage (rare).

Treatment by tranfsusion prevented death from anaemia, and as this usually occurred in the earlier stages, there was an increase of deaths from secondary infection or haemorrhage.

(13) The differential diagnosis of the neonatal anaemias was discussed.

(14) Pathogenesis of erythroblastosis foetalis.

(a) Cause of anaemia.

It was concluded that the anaemia, a feature common to all types of foetal erythroblastosis, was due to destruction of red corpuscles by an abnormally active reticulo-endothelial system, and that the chief sites of its activity were in the liver and spleen.

The theory that anaemia was due to a primary defect of the erythropoietic system in releasing qualitatively deficient red corpuscles which were destroyed by a normally functioning reticulo-endothelial system was discounted.

Erythroblastosis was therefore an attempt on the part of the haematopoietic tissues to counteract the anaemia.

(b) Maternal toxaemia.

Although the peculiar family history afforded indications that maternal toxaemia might be the cause of the erythroblastoses, this seemed unlikely because of the usual good health of the mothers during pregnancy, and because the affected child had often been living an independent existence for varying periods before the onset of anaemia, and also because in the case of twins only one child might suffer, the other being quite healthy.

(c) Cause of distinguishing characteristics of the three forms of erythroblastosis foetalis.

An attempt was made to explain the main distinguishing clinical characteristics of the erythroblastoses.

Oedema, in hydrops foetalis, was thought to be due to gradually increasing foetal cardiac failure in utero when the circulation is relatively poor.

Jaundice, in icterus gravis, was due to the failure of the immature liver to excrete the products of haemolysis. These assumed toxic properties and damaged the liver cells, leading to an increase of jaundice, and also had a selective noxious action on certain cells of the central nervous system.

Absence of oedema, or marked jaundice in anaemia haemolytica of the new-born, was perhaps explained by a greater degree of development of liver function in these children.

ADDITIONAL PAPERS:-

I. AN ANALYSIS OF NINE CASES OF ACHOLURIC JAUNDICE IN CHILDHOOD, WITH SPECIAL REFERENCE TO RED CELL MORPHOLOGY AND THE EFFECT OF SPLENECTOMY ON IT

II. THE RED CORPUSCLES IN ACIDOSIS AND ALKALOSIS.

AN ANALYSIS OF NINE CASES OF ACHOLURIC JAUNDICE IN CHILDHOOD, WITH SPECIAL REFERENCE TO RED CELL MORPHOLOGY AND THE EFFECT OF SPLENECTOMY ON IT.

Introduction.

With the awakening of interest in diseases of the blood in comparatively recent years, and the invention of new methods of investigation further points of interest in the mechanism of the production of anaemia have been discovered. In acholuric jaundice, although the treatment was accidentally discovered before the anaemia was recognized, there is a growing mass of new facts, many of them not fully or not at all explained.

The purpose of this paper is not to review the literature as a whole, but to deal in particular with some of the newer aspects of the anaemia, especially the peculiar morphology of the red corpuscles and how they are affected by splenectomy.

At the same time brief comments on the more outstanding clinical features will be made.

During the past few years we have observed nine cases of acholuric jaundice in children, at the Royal Hospital for Sick Children, Glasgow.

I. SUMMARIES OF THE CLINICAL AND HAEMATOLOGICAL FINDINGS IN THE CASES.

Family L.

The maternal grandmother of our patients was said to have been jaundiced all her life. The mother was never jaundiced except during pregnancy, although owing to the discovery of splenomegaly, subsequent investigation had shown that she was the subject of acholuric jaundice. She had persistently refused to have her spleen removed.

When first seen by me, her age was thirty years. She was stout and unhealthy in appearance. Anaemia was apparent, and there was very slight jaundice of the skin and sclerotics. At this time she was about five months pregnant. The spleen was enlarged and the lower pole was palpable three fingers' breadth below the costal margin. A blood count showed that a marked anaemia was present.

R.B.C. 2.53 millions per c.mm.; Hb. (Haldane) 55%; reticulocytes 10.3%; leucocytes 9.000 per c.mm.

A Price-Jones curve (Fig. VII) revealed a considerable apparent microcytosis and the mean corpuscular diameter was 6.20 μ. The fragility of the red cells was excessive, lysis being present in 0.60% saline, the lowest dilution used. The blood serum gave an indirect positive van den Bergh reaction of 4.5 units, and there was excess of urobilin in the urine.

The father was a healthy man of thirty-two years, quite without jaundice. He refused to permit any investigations.

There had been eight pregnancies.

(1) ♂ ; died aet. 4 months of meningitis; not known if jaundiced.

(2) ♀ , aet 9 years. Acholuric jaundice; splenectomy at 7 years.

(3) Miscarriage.

(4) Miscarriage.

(5) Premature still-birth.

(6) ♀ ; died aet. 11 months of pneumonia; not known if jaundiced.

(7) ♂ , aet. 4 years; acholuric jaundice.

(8) ♀ , aet. 1 year, 8 months; acholuric jaundice.

Following, are the histories and clinical findings in the three surviving children.

Case 1. Margaret L., aet 9 years.

Born at full time. Jaundice was not noticed by the parents. The general health was fair. An enlarged spleen had been removed at the age of seven years. Excluding the father, this child was the only healthy looking member of the family. She was well developed. There was absence of all jaundice. The skin and sclerotics were clear, and the mucous membranes well coloured. The liver was not palpable.

Otherwise physical examination failed to reveal any abnormality.

A blood count showed the following findings:-
R.B.C. 5.16 millions per c.mm.; Hb.(Haldane) 80%; colour index 0.79; reticulocytes $< 1\%$; leucocytes 11,600 per c.mm. Differential leucocyte count.

Neutrophile immature metamyelocytes	nil	81.5%
" mature "	10%	
" segmented forms.	71.5%	
Eosinophiles	1.5%	
Basophiles	0.5%	
Monocytes	4.0%	
Lymphocytes	12.5%	

Excessive fragility of the red corpuscles was found, lysis being present in 0.60% saline.

The red cells in stained films were well coloured, small and round. Nucleated forms were absent. A Price-Jones curve (Fig. VIII) revealed an apparent microcytosis, and the mean corpuscular diameter was 6.21 μ.

The increase of neutrophile cells was not due to any clinically apparent infection.

The serum van den Bergh reaction showed a slight indirect positive reaction of 1.5 units. Urobilinuria was not present.

Case 2. Roderick L., aet. 4 years and 10 months.

Pregnancy was uneventful and the child was born at full term. He was apparently healthy except for slight jaundice which was present at birth, and lasted for two weeks. He developed in a normal way. At the ages of eighteen months, four years and seven months, and four years and eight months, he had pneumonia. The earliest attack was followed by subacute gastro-enteritis which lasted for nine weeks. Since the last bout of pneumonia the boy had been much paler than previously although his colour had never been good. The parents had not noticed jaundice at any time.

The boy when first seen at the hospital was small, under-nourished and pale with a slight icteric tinge of the skin and sclerotics. The spleen was palpable, the lower pole being two fingers' breadth below the costal margin. The lower border of the liver was felt one-and-a-half fingers' breadth below the costal margin. Except for enlarged and inflamed tonsils, physical examination was otherwise negative.

A blood count gave the following figures:-

R.B.C. 3.17 millions per c.mm.; Hb.(Haldane) 60%; colour index 0.95; reticulocytes, 5.6%; leucocytes 17,200 per c.mm. As in the two previous cases, the fragility of the red cells was excessive.

The leucocytosis was almost certainly due to the tonsillitis.

The red corpuscles were well stained, small and round. Nucleated forms were absent. A Price-Jones curve (Fig. X) revealed an apparent microcytosis, the mean diameter of the corpuscles being 6.08 μ. The cell volume index however was 1.0.

The van den Bergh reaction was indirect positive (4.5 units) and there was excess of urobilin in the urine. Bile pigments were absent.

Case 3. Jean L., aet. 1 year and 8 months.

Pregnancy and labour were normal. The child was healthy and without jaundice at birth. Development was normal. At the age of fifteen months, the child had pneumonia, but was not very acutely ill. The parents had not noticed any jaundice or excessive pallor.

The girl was brought to hospital because she had been out of sorts for a few days, without definite symptoms.

On examination, the child was found to be small but moderately well nourished. There was neither pallor nor jaundice. The spleen and liver were both enlarged and the lower margins were palpable one finger-breadth below the costal margin. The tonsils were enlarged and inflamed. There was slight fever which lasted for a few days.

The data obtained by examination of the blood are shown in Table 1 and Chart XIV. It will be seen that there was a slight anaemia at the earliest date of examination,

<u>Table 1.</u> Blood counts - Jean L.

Date	3.4.34.	6.4.34.	28.4.34.	28.6.34.
R.B.C. per c.mm.	4,290,000	4,030,000	3,650,000	3,270,000
Haemoglobin %	75	75	64	66
Colour index	0.88	0.94	0.89	1.02
Reticulocytes %	3.0, 3.0	6.0, 5.6	7.4	5.8
Leucocytes per c.mm.	21,000	23,000	18,400	12,600
Neutrophiles	75.3%	79.2%	73.6%	48.5%
Myelocytes	0.4	0.4	nil	nil
Immature metamyelocytes	1.1	1.2	0.4	1.0
Mature metamyelocytes	30.5	33.6	29.2	28.5
Segmented forms	48.3	44.0	44.0	19.0
Eosinophiles	3.3%	2.0%	nil	6.5%
Basophiles	1.2%	0.8%	0.8%	nil
Monocytes	2.2%	1.6%	0.8%	6.5%
Lymphocytes	18.0%	16.4%	24.8%	38.5%
Normoblasts	1,200 per c.mm.	1 seen	180 per c.mm.	nil

but that this increased slightly later. The colour index varied between 0.88 and 1.02. Reticulocytes were persistently increased in numbers, although never to a great extent. The subnormal red cell numbers, and the reticulocytosis, however, indicated an excessive blood destruction and increased marrow activity.

The fragility of the red cells was increased as for the other members of the family. The stained red corpuscles were small, round and dark, and nucleated forms, normoblasts, were sometimes present, the maximum number being 1200 per c.mm. A Price-Jones curve (Fig. IX) revealed a shift to the left, with a mean corpuscular diameter of 5.77 μ. The cell volume index was 0.96.

The leucocyte count on three occasions showed an appreciable increase, this being due to increased numbers of neutrophile cells. Occasional myelocytes were sometimes present. When the first three blood counts shown on Table I and Chart XIV were done the child had undoubted subacute tonsillitis, but on the last occasion she was apparently free of infection. At that time the leucocytes were normal in numbers for the child's age, and the polymorphonuclear forms numbered 6.101 per c.mm., which is very little in excess of normal.

The van den Bergh reaction when the child was first seen was indirect positive (3.5 units), and a slight excess of urobilinuria was sometimes found in the urine at various later dates.

The Wassermann reactions of mother and children were negative.

Unfortunately the parents refused to permit removal of the spleen from any of the children. The family later changed their address, and although an attempt was made to find them, it was unsuccessful.

Case 4. Rena, B., aet. 10 years.

The child was the second of a family of four. There had been one miscarriage.

Pregnancy and labour were normal. The baby was considerably jaundiced at birth. This only partially cleared up, and ever since there had been slight icterus, which was sometimes more marked than at others. Except for whooping cough at the age of five years, the girl had not had any acute illnesses. In contrast to the other children of the family, she had never been robust however, and suffered from bronchitis every winter. The urine was always dark.

Seven days before admission, she complained of headache, shivered severely and became delirious. Next day she had improved, and although not well, attended school until the day of admission to hospital.

The child was found to be of fair height, but spare. There was pallor without jaundice although the skin was sallow. The temperature was 102°F., and the respiratory rate 28 per minute. Examination of the chest revealed signs of consolidation at the left base. There was a haemic cardiac murmur. The spleen was enlarged to four fingers' breadth below the costal margin, smooth and firm. The lower hepatic border was one finger's breadth below the costal margin. Glandular enlargement was absent except in the cervical region. The throat was excessively red.

Urinary examination showed marked urobilinuria. The van den Bergh reaction was indirect positive (4.0 units), and the Wassermann reaction negative.

Examination of the blood showed the presence of a gross anaemia (Chart XV, Table 2), with a colour index of 1.17, but without any increase in the reticulocyte numbers, although 500 normoblasts per c.mm. were present. There was a moderate leucocytosis (25,300 per c.mm.), the increase being largely due to increase of neutrophile leucocyte numbers, 4.3% of which were myeolocytes. The leucocytosis was doubtless due to the pneumonia.

The temperature fell by crisis on the first night in hospital, and although two days later there was a recurrence of intermittent fever, which lasted for three days, the child's general condition became markedly improved, while signs of consolidation in the chest partially cleared.

Coincident with this, there was a sharp fall in the leucocyte count, which nine days after admission was 7,000 per c.mm. More striking was the reticulocyte and erythroblastaemia crisis which followed immediately on the fall of

Table 2. Blood counts - Rena B.

Date	24.2.34	26.2.34	27.2.34	5.3.34	8.3.34	10.3.34	12.3.34	17.3.34	21.3.34
R.B.C. millions per c.mm.	1.24		1.57	2.67	2.38	3.21	3.54	3.69	3.77
Haemoglobin %	28		32	58	57	68	68	68	68
Colour index	1.17		1.03	1.09	1.21	1.06	0.97	0.93	0.91
Reticulocytes %	1.0	23.3	26	9.6	7.7	3.8	4.1	6.0	6.0
Platelets per c.mm.			208,810		190,400				211,120
Leucocytes per c.mm.	25,200	23,000	19,000	7,200	6,900	8,600	7,000	5,800	5,600
Neutrophiles: Myeloblasts	-	0.42	1.14	-	-	-	-	-	0.25
Neutrophiles: Myelocytes	4.33	3.8	2.86	-	0.66	-	-	-	0.25
Neutrophiles: Immature myelocytes	7.0	5.31	5.14	2.5	3.33	-	1.6	2.0	0.25
Neutrophiles: Mature do.	43.0	57.85	54.85	28.5	36.0	38	36.8	30.0	39.5
Neutrophiles: Segmented forms	30.0	19.53	23.0	42.5	24.0	36	32.0	29.0	21.75
Neutrophiles (total)	84.33%	86.91%	86.99%	73.5%	64%	74%	70.4%	61%	62%
Eosinophiles	0.66%	0.21%	-	1.5%	1.33%	3.0%	1.6%	5.0%	3.0%
Basophiles	0.66%	-	-	-	2.0%	-	0.8%	-	0.25%
Monocytes	2.0%	0.88%	4.0%	0.5%	2.66%	7.0%	1.6%	2.0%	4.0%
Lymphocytes	12.33%	11.98%	9.0%	24.5%	30.0%	21.0%	25.6%	32.0%	30.75%
Normoblasts per c.mm.	504	6,900	4,560	72	nil	occas.	-	-	-

Date	24.3.34	31.3.34	2.4.34	4.4.34	4.4.34	9.4.34	12.4.34	20.4.34	21.4.34	24.4.34
				Splenectomy 4.4.34						
R.B.C. millions per c.mm.	3.54	3.2	3.12	3.58	3.86	4.37	4.46	5.24		5.0
Haemoglobin %	67	64	66	71	83	85	87	90		88
Colour index	0.96	1.0	1.06	1.0	1.09	0.95	0.98	0.87		0.88
Reticulocytes %	7.8	3.2	5.0	5.6		0.8	0.8	<1		<1
Platelets per c.mm.							700,000	687,000	517,000	
Leucocytes per c.mm.	3,600	5,200	4,000	6,800	22,100	11,100	10,000	15,000		12,000
Neutrophiles: Myeloblasts	-	-	-	-		-	-	-		-
Neutrophiles: Myelocytes	-	-	0.5	-		-	-	-		-
Neutrophiles: Immature myelocytes	1.0	1.0	1.0	1.0		3.66	-	-		-
Neutrophiles: Mature do.	37.33	40.5	42.5	36.5		60.33	45.5	30		29
Neutrophiles: Segmented forms	25.66	26.0	15.0	14.5		17.66	34.5	43		37.5
Neutrophiles (total)	64%	67.5%	59%	52%		81.66%	80%	73%		66.5%
Eosinophiles	0.66%	-	1.5%	0.5%		-	0.5%	1.0%		0.5%
Basophiles	1.0%	1.0%	1.0%	-		-	-	-		1.0%
Monocytes	3.33%	2.0%	4.5%	3.5%		2.66%	3.5%	1.0%		3.0%
Lymphocytes	31.0%	29.5%	34.0%	44%		15.66%	16.0%	25.0%		29.0%
Normoblasts per c.mm.	-	-	-	-		-	-	-		-

Reticulocytes % - Feb. 28. 29.5 March 2 13.0, 6 6.2, 7 2.0, 9 2.6, 11 1.8, 13 5.0, 14 2.0, 15 3.2, 16 8.6, 18 7.0, 19 8.3, 20 9.5, 22 4.2, 23 7.3, 26 6.0, 28 7.4, 29 6.0 April 3. 5.8

Platelets per c.mm. - April 13, 800,000 14. 900,000 temperature. The peak of the reticulocytosis (29.5%) was reached four days after admission. The highest number of nucleated red cells, 6,900 per c.mm., was found two days previous. Coincident with and following on the reticulocyte crisis, the red cell numbers and haemoglobin level rose sharply, and except for a minor setback, steadily, until the red cells numbered 3.77 millions per c.mm., twenty-five days after admission to hospital. For some time the colour index remained above unity, but later the formation of red cells outstripped the haemoglobin output, and the colour index fell to slightly below 1.0 (minimum 0.93). During this period however the reticulocyte numbers remained increased, but never beyond 9.5%. From the ninth day after the crisis of the pneumonia nucleated red cells were absent from the peripheral blood. Urobilinuria persisted.

The leucocyte count continued to diminish and from the twenty-first to the twenty-third day after admission there was a true leucopenia, all forms of cells being affected.

With the passing of the acute symptoms the spleen became reduced in size, although the lower pole remained palpable two fingers' breadth below the costal margin.

From the twenty-fifth day after admission there was a slow but steady fall in red cell count, with a less marked diminution of the haemoglobin percentage, so that the colour index again exceeded 1.0. Reticulocytosis was still present throughout this period. The spleen became slightly larger, and the lower pole was palpable two and a half finger's breadth below the costal margin. Clinically there was not any infection or other apparent factor to explain the fall in blood count.

Meanwhile other methods of investigation had been performed. Tests done when the blood count was at its lowest and again when it had reached the maximum, showed great increase in the fragility of the red corpuscles to hypotonic saline. In the first instance lysis was present in the lowest saline dilution used, 0.60%, and was complete in 0.42%. In the second, lysis was present in 0.68% saline and complete in 0.48%, that is the fragility had increased after the blood crisis.

A Price-Jones curve (Fig. XI) constructed from blood taken on the third day after admission, showed a marked microcytosis, scanty megalocytosis and a mean diameter of 5.75 μ.

The child's condition was sufficiently good five weeks after admission to permit of splenectomy being done.

The spleen weighed 250 gm. Histologically there was marked distension of the pulp spaces with blood. Haemosiderosis was absent.

The operation was well tolerated, but during the following week there was fever, the cause being pneumonic consolidation at the bases of the lungs. The child was never acutely ill however and her condition did not cause alarm.

The effects of splenectomy on the blood picture were dramatic. Examination of the blood seven hours after operation showed that a slight rise of 280,000 red cells per c.mm. and 2% haemoglobin had occurred. The leucocytes had risen from their previous level of 6,800 per c.mm., to 22,100 per c.mm., an increase of 15,300 per c.mm. Although much of the subsequent leucocytosis was due to the pulmonary infection, it is likely that this very speedy increase was due to removal of the spleen because the temperature at the time the count was done was only 98.8°, and the pneumonia with high fever did not have its onset until over twenty-four hours after operation.

As will be seen from Table 2, the leucocytosis was chiefly due to increased numbers of neutrophile cells, and also, to a minor extent, to increase of lymphocyte and monocyte numbers.

In spite of the infection the red cell count continued to rise and seventeen days after operation numbered 5.24 millions per c.mm., while the haemoglobin level, although considerably improved (90%) showed a much less quick rise and consequently the colour index had fallen to 0.87. After operation the reticulocutosis disappeared permanently, urobilinuria was absent, and the van den Bergh reaction attained almost normal figures --- indirect positive 0.5 units.

The fragility of the red cells to hypotonic saline was slightly altered, there being a slight improvement in their resistance to the lower dilutions. Lysis began in 0.64% and was complete in 0.46%.

The behaviour of the platelet count was striking. Counts done in the pre-operative period showed numbers varying between 190,000 per c.mm., and 211,000 per c.mm. (Table 2). The first post-operative enumeration, eight days after splenectomy revealed a tremendous increase to 700,000 per c.mm., and four days later a further rise to 900,000 per c.mm. Eighteen days after operation the platelet count (517,000 per c.mm.) was approaching the upper limits of normal (450,000 per c.mm.).

The child has remained well ever since.

All the other members of the family were found to have a normal fragility test. None had splenic enlargement.

Family H.

The four children of this family were transferred from the Victoria Infirmary, Glasgow, to the Royal Hospital for Sick Children for splenectomy. Their earlier history has been published by Dr Angus Scott (1935). Acholuric jaundice had not been suspected in the family until serial haemolytic crises, occurring in these children led to investigation. It was then discovered that the grandfather of the children was noted for his extremely sallow complexion. He had a family of nine children, three of whom were found to be cases of acholuric jaundice. One of these was the father of my four cases. The mother of the children was healthy. The families of the other two affected persons were apparently in good health.

Case 5. Jean H., aet. 12 years.

The child was born at full time and developed normally. Before she was six years old she had had measles, whooping cough, chicken-pox and mumps, without undue upset. At the age of eight years she became jaundiced for the first time, and splenic enlargement was then noticed. The jaundice cleared after a fortnight and the child remained well until the age of eleven years. In February 1934, she had a sudden haemolytic crisis and was admitted to the Victoria Infirmary, after being ill for three days with headache, shivering, increasing pallor and slight jaundice. On admission to hospital, the red cells numbered 1.0 millions per c.mm., 2% of these being reticulocytes, the colour index was 1.0, the leucocytes numbered 7,600 per c.mm., and the fragility of the red corpuscles was slightly increased. Evidence of infection either clinical or haemotological was lacking. The spleen was enlarged. She was transfused and recovered slowly. During the recovery phase, the fragility of the red cells increased, and a moderate reticulocytosis occurred and was maintained. The red cell count did not rise beyond 3.0 millions per c.mm.

In September 1934, she was admitted to the Royal Hospital for Sick Children. The child was found to be under-sized and thin. Considerable pallor and slight jaundice of the skin were present, and sclerotic icterus was marked. The spleen was enlarged, the lower pole being palpable two-and-a-half fingers' breadth below the costal margin. The liver could not be felt. The urine contained urobilin in large amounts but bile pigments were absent.

A blood examination (Table 3, Chart XVI) revealed a moderate anaemia, 3.19 million red corpuscles per c.mm., and a haemoglobin level of 75%, giving a colour index of 1.19. Reticulocytes numbered 13.7% of the red corpuscles. The leucocyte count was 6,600 per c.mm., and the differential count was normal. Platelets numbered 127,600 per c.mm.

The blood films showed the typical well coloured, small, round red corpuscles, and an occasional normoblast. The fragility of the red corpuscles was markedly increased, lysis being present in 0.62% saline and complete in 0.44%. Van den Bergh's reaction gave an indirect positive reaction of 9.5 units.

The spleen was removed on 17:9:34. It weighed 340 gm., and was firmly adherent to the abdominal wall and also to the stomach. Microscopically the pulp was hyperplastic and the spaces engorged with blood, while the sinusoids were empty and flattened. The Malpighian bodies were scanty and compressed. Only small traces of iron were

Table 3. Blood counts - Jean H.

Date	13.9.34	25.9.34	1.10.34	8.10.34	22.10.34	30.10.34
R.B.C. per c.mm.	3,190,000	3,920,000	4,500,000	4,410,000	4,920,000	510,000
Haemoglobin %	75	85	88	85	96	96
Colour index	1.19		0.98		0.98	0.94
Reticulocytes %	13.2, 16.2	2.2	<1		<1	<1
Platelets per c.mm.	127,600	768,320	468,000		270,000	312,000
Leucocytes per c.mm.	6,600	4,800	7,700	8,800	5,700	10,200
Neutrophiles: Immature metamyelocytes	nil	0.5	nil		nil	nil
Neutrophiles: Mature do.	21.25 68%	13.5 54%	10 44%		10.75 46.25%	14.25 47.25%
Neutrophiles: Segmented forms	46.75	40.0	34		35.5	33.25
Eosinophiles	0.5%	1.75%	3.0%		3.25%	3.25%
Basophiles	0.5%	2.25%	0.5%		0.5%	nil
Monocytes	3.25%	5.25%	6.0%		4.25%	2.25%
Lymphocytes	27.75%	36.75%	46.5%		45.75%	47.0%
Normoblasts	1 seen	nil	nil		nil	nil

Splenectomy 17.9.34

found in the pulp phagocytes, which were, however, numerous.

Following on splenectomy recovery was uncomplicated. The jaundice disappeared rapidly.

Thirty-three days after operation, the red cells numbered 5.10 millions per c.mm., and although the haemoglobin level had risen considerably (96%) it had not progressed so quickly as the red cell count, and consequently the colour index had fallen slightly below unity (0.96).

After operation there was a speedy reduction of reticulocyte numbers to less than 1% of the red corpuscles.

Urobilinuria was absent. The van den Bergh reaction showed an indirect reaction of only 0.5 units five weeks after splenectomy.

Leucocyte numbers varied between 4,800 and 10,200 per c.mm., in the post operative period of observation.

The platelet count rose sharply to 768,000 per c.mm., one week after splenectomy, but had returned to within normal limits (250,000 to 450,000) a week later.

A slight improvement in the resistance of the red corpuscles in hypotonic saline was found, lysis starting at 0.58% saline, and being complete in 0.40%.

Case 6. Nancy H., aet. 10 years.

This child except for the usual infectious diseases of childhood had always been well until she had an acute haemolytic crisis at the age of nine years. This occurred twelve days after her sister had been taken ill. The symptoms, clinical and haematological findings, treatment and recovery were similar to thosefound in the older girl. Again evidence of infection was lacking.

She was admitted to the Royal Hospital for Sick Children, in September 1934. She was found to be pale and thin, with a sallow skin, and sclerotic icterus. The spleen was palpable, two and a half fingers' breadth below the costal margin. The liver was not felt. The urine contained excess of urobilin, and a positive indirect van den Bergh reaction of 9.5 units was obtained from the blood serum.

The blood examination (Table 4, Chart XVII) showed a moderate anaemia, the colour index being above unity, a moderate reticulocytosis (12.8%) and 11,000 leucocytes per c.mm. The differential leucocyte count was practically normal there being however a slight increase of the numbers of neutrophile polymorphs (7232 per c.mm.). The proportion of mature metamyelocytes was excessive, 17.75%. In doing this count I did not see any nucleated red cells. The platelet count was somewhat subnormal, 151,000 per c.mm.

Considerable increase of the fragility of the red cells in hypotonic saline was found, haemolysis being present in 0.60% saline and complete in 0.46%.

Splenectomy was performed on 17:9:34. The spleen weighed 300 gm., and its microscopical appearance was similar to that of the previous case, but haemosiderosis was slightly more evident.

Table 4. Blood counts - Nancy H.

Date	13.9.34	Splenectomy 17.9.34	29.9.34	1.10.34	8.10.34	22.10.34	30.10.34
R.B.C. per c.mm.	2,980,000		4,470,000	4,760,000	4,800,000	4,850,000	4,830,000
Haemoglobin %	64		80	82	82	82	82
Reticulocytes	1.08		0.90	0.86		0.82	0.82
Reticulocytes %	12.8, 16.4		3.2	<1	<1	<1	<1
Platelets per c.mm.	150,980		759,000	400,000		213,000	367,000
Leucocytes per c.mm.	11,000		9,000	11,300	10,400	10,800	10,400
Neutrophiles: Immature metamyelocytes	-		0.25	-		-	-
Neutrophiles: Mature do.	17.75 } 65.75%		34.0 } 83.5%	19 } 69%		12.25 } 33.5%	15.25 } 57.75%
Neutrophiles: Segmented forms	48.0		49.25	50		21.25	42.25
Eosinophiles	1.75%		1.75%	1.0%		5.0%	2.0%
Basophiles	1.0%		0.25%	0.5%		0.75%	-
Monocytes	2.75%		2.5%	4.5%		2.25%	4.25%
Lymphocytes	28.75%		12.0%	25.0%		58.5%	36.0%
Normoblasts	-		-	-		-	-

Convalescence was uninterrupted. The sclerotic icterus rapidly cleared.

After operation the red cell count rose rapidly, and in twelve days had increased by 1.49 millions per c.mm., to 4.47 millions per c.mm. Thereafter there was a further and smaller increase but the red cell level did not quite reach 5.0 millions per c.mm. The haemoglobin percentage showed a marked elevation but the colour index was reduced to 0.82.

The reticulocyte count dropped within a few days after operation and remained, thereafter, normal.

The leucocyte count throughout showed little alteration, although towards the end of my period of observation, the percentage of neutrophile cells was reduced to within normal limits.

Platelet numbers increased remarkably after splenectomy and twelve days later the count was 759,000 per c.mm. After a further week, the numbers became normal.

Urobilinuria was absent in the post-operative period and five weeks after operation the van den Bergh reaction was negative.

The resistance of the red cells was slightly improved, although lysis occurred in 0.58% saline.

Case 7. John H., aet. 6 years.

The early history of the child was normal except for chicken-pox, whooping cough and measles, none of which had been severe, until he had a haemolytic crisis which occurred fifteen days after the eldest child's. Because of the crises which his sisters had experienced he had been admitted to hospital although his general health was good. Nevertheless the blood count fell rapidly to 850,000 red cells per c.mm., within three days of the onset of fever and general malaise. Infection was apparently absent. At the height of the crisis the fragility of the red corpuscles was within normal limits. He was transfused and recovered. At the same time the fragility of his corpuscles increased, and there was an increase of reticulocyte numbers, which had been practically normal during the period of extreme anaemia.

The boy was admitted to the Royal Hospital for Sick Children in September 1934. He was thin and weakly in appearance with considerable pallor and a definite icteric tinge of the skin and sclerotics. The spleen was palpable, its lower pole being two fingers' breadth below the costal margin. The liver was not palpable. Excess of urobilinuria was present, and the van den Bergh reaction of the blood was indirect positive (4.5 units).

A blood examination showed (Table 5, Chart XVIII):- Hb.(Haldane) 48%; red corpuscles 2.81 millions per c.mm.;

Table 5. *Blood counts - John H.*

Date	22.9.34	24.9.34	1.10.34	9.10.34	22.10.34	30.10.34
R.B.C. per c.mm.	2,810,000	4,080,000	4,450,000	4,500,000	4,780,000	4,860,000
Haemoglobin %	48	66	68	75	80	80
Colour index	0.86	0.82	0.80	0.83	0.84	0.82
Reticulocytes %	16.4	10.0	<1	<1	<1	<1
Platelets per c.mm.		155,000		842,000	809,000	576,000
Leucocytes per c.mm.	7,700		8,700	12,200	6,000	10,600
Neutrophiles: Immature metamyelocytes	-		-		-	0.5
Neutrophiles: Mature do.	29.75		14		12.75	8.5
Neutrophiles: Segmented forms	47.75		48		46.0	53.0
Neutrophiles	77.5%		62%		58.75%	62%
Eosinophiles	-		4.75%		3.75%	2.25%
Basophiles	1.0%		-		0.75%	1.75%
Monocytes	1.0%		2.25%		3.25%	4.75%
Lymphocytes	20.5%		31.0%		33.25%	29.25%
Normoblasts per c.mm.	77		-		-	-

Transfused 23.9.34

Splenectomy 25.9.34

colour index 0.86. He was the only member of the family to have an initial colour index of less than unity. A reticulocytosis of 16.4% was present. Leucocytes numbered 7,700 per c.mm. The polymorphonuclear forms showed a somewhat high figure, 5967 per c.mm., and the incompletely segmented forms were excessive in numbers. A few normoblasts, 77 per c.mm., were found.

The fragility of the red cells was increased, lysis being present in 0.62% saline and complete in 0.42%.

A transfusion of 450 c.c. of whole blood was given two days before operation. This raised the blood count to 4.08 million red cells per c.mm., and 66% haemoglobin, the colour index being 0.82. The reticulocytosis persisted.

The spleen was removed on 25:9:34. It weighed 240 gm. Congestion of the pulp spaces was found and the Malpighian bodies were scanty and small. Phagocytosis was abundant. The deposit of iron was larger than in any of the other cases of acholuric jaundice and was found chiefly in the pulp phagocytes and to a less extent in the reticulum. The distribution of the iron containing tissues was chiefly round the Malpighian bodies.

Following on operation, the jaundice cleared quickly. There was a steady increase in the red cell count which reached 4.86 millions per c.mm. in five weeks. Haemoglobin formation was less active and the percentage did not exceed 80. A slight fall in colour index to 0.82 was therefore present. The reticulocyte count rapidly fell to within normal limits after operation. Urobilinuria disappeared at the same time. Five weeks after operation the van den Bergh reaction was negative.

The post operative fluctuations of the leucocyte counts were unimportant, the lowest count being 6,000, and the highest 10,600 per c.mm. The percentage of neutrophile cells fell to within normal limits and fewer incompletely segmented forms were present.

The platelet count rose from the preoperative subnormal level to 842,000 per c.mm. a fortnight after operation. Three weeks later the count was much reduced, although still excessive (576,000 per c.mm.).

The fragility of the red corpuscles five weeks after splenectomy was slightly reduced, the earliest lysis being found in 0.60% and complete lysis in 0.38%.

Case 8. James H., aet. 8 years.

Except for measles, whooping cough and chicken-pox, the boy had been healthy and free from jaundice until the onset of a haemolytic crisis. This occurred nineteen days after the crisis in the eldest child. The red cell count fell to 1.45 millions per c.mm. in a few days. Reticulocytosis was absent and the fragility of the red cells was only slightly increased. A transfusion was given. Recovery with reticulocytosis and greatly increased corpuscular fragility, followed slowly. There was an interruption and a slight fall in the blood count caused by an attack of acute tonsillitis. During the initial crisis however evidence of infection was not found.

The boy was admitted to the Royal Hospital for Sick Children in September 1934. He was moderately well nourished but pale and with definite icterus. The spleen was palpable its lower pole being two fingers' breadth below the costal margin. The liver could not be felt. Urobilinuria was present, and the serum van den Bergh reaction was positive indirect (4.5 units).

Examination of the blood (Table 6, Chart XIX) showed:- R.B.C. 3.09 millions per c.mm.; Hb. 66%; colour index 1.08; reticulocytes 16.2%; platelets 131,000 per c.mm. and leucocytes 8,200 per c.mm. there being a slightly raised proportion of mature metamyelocytes, although the total numbers of neutrophiles (5309 per c.mm.) was practically normal. Occasional normoblasts were found in the films.

The fragility of the red cells was excessive, lysis being present in 0.62% saline and complete in 0.42%.

A blood transfusion of 350 c.c. was given and two days later the red corpuscles numbered 3.64 millions per c.mm. The colour index had fallen below unity, (0.97). Reticulocytosis persisted (9.0%).

The spleen was removed on 25:9:34. It weighed 280 gm. Histologically there was engorgement of the pulp

<u>*Table 6.*</u> *Blood counts - James H.*

Date	22.9.34	24.9.34	1.10.34	9.10.34	22.10.34	30.10.34
R.B.C. per c.mm.	3,090,000	3,640,000	4,100,000	4,520,000	4,630,000	4,890,000
Haemoglobin %	66	70	74	80	86	86
Colour index	1.08	0.97	0.90	0.89	0.93	0.88
Reticulocytes %	16.2	9.0	1.5	<1	<1	<1
Platelets per c.mm.		131,000	598,000		712,000	502,000
Leucocytes per c.mm.	8,200		8,000	10,800	9,000	8,200
Neutrophiles: Immature metamyelocytes	-		-		-	-
Neutrophiles: Mature do.	22 } 64.75%		7.75 } 54%		13.5 } 57.25%	10.25 } 47.75%
Neutrophiles: Segmented forms	42.75		46.25		43.75	10.75
Eosinophiles	1.0%		11.75%		2.75%	1.25%
Basophiles	1.5%		0.25%		0.5%	-
Monocytes	1.0%		5.0%		2.25%	3.25%
Lymphocytes	31.75%		29.0%		37.25%	47.75%
Normoblasts (W.B.C.)	1/400		-		-	-

Transfused 22.9.34.

Splenectomy 25.9.34.

spaces. Increase of fibrous tissue was more obvious in this spleen than in any of the others. There was a diffuse haemosiderosis, again more marked in the pulp phagocytes in the neighbourhood of the Malpighian bodies.

After operation the jaundice cleared rapidly. The red cell count reached a maximum of 4.89 millions per c.mm. and the haemoglobin level of 86% in five weeks. The colour index had by this time fallen to 0.88.

Six days after operation the reticulocyte numbers were slightly increased (3%), but thereafter they were reduced to within normal limits.

Except for a reduction in the number of incompletely segmented forms, there was not any marked alteration in the leucocyte picture.

The platelet count rose to 598,000 per c.mm., six days after splenectomy, and a further rise to 712,000 per c.mm., was noted a fortnight later. Thereafter the numbers decreased although five weeks after operation they were still slightly raised (502,000 per c.mm.).

The fragility of the red corpuscles was practically unchanged. Urobilinuria was absent from within a few days after operation, and a van den Bergh reaction done after five weeks was negative.

Case 9. Millicent M., aet 6 years and 9 months.

Family History.

The father, aet, 40 years, was in good health. He was neither anaemic, nor jaundiced, and the fragility of his red corpuscles was normal. The mother, aet. 39 years, was anaemic, but jaundicé, splenomegaly, and urobilinuria were absent. A blood examination showed that the anaemia was of the microcytic hypochromic type:- R.B.C. 5.44 millions per c.mm.; haemoglobin 55%; colour index 0.54; reticulocytes 0.4%; leucocytes 7,900 per c.mm. The fragility of the red corpuscles was within normal limits.

There was a family of seven children. All the others were healthy, but I was not permitted to do blood examinations on them. Certainly, none were jaundiced and anaemia was not apparent.

History of the patient.

The patient was the fifth child. Pregnancy and labour were normal, and the child was born at full time. She was apparently healthy. Breast feeding was given for ten months, and the child throve. She was in good health until she had scarlet fever at five years of age. Since that time, that is for nearly two years up to the time of admission to hospital, she had been listless and was not thriving. For three months she had been having attacks of abdominal pain, but this was not associated with diarrhoea or vomiting or apparent increase of pallor or jaundice.

On examination the child was found to be small and pale. A faint yellowish tinge was present in the skin and the sclerotics were very slightly jaundiced. The spleen was palpable the lower pole being two fingers' breadth below the costal margin. The lower border of the liver was felt one finger's breadth below the costal margin. Enlargement of the superficial lymphatic glands was absent, and the only remaining positive physical finding was a haemic cardiac murmur.

The urine contained excess of urobilin but was otherwise normal. The van den Bergh reaction was indirect positive (5 units). A Wassermann reaction was reported positive, but in the absence of any stigmata of congenital syphilis and because the parents gave a negative reaction it did not seem that the report on the child's serum could be correct. The blood was investigated in the Clinical Pathology Department of the Western Infirmary and it was reported that the Wassermann reaction was undoubtedly negative.

Examination of the blood revealed the presence of a moderate anaemia, with a normal colour index (Table 7, Chart XX). The leucocytes were slightly increased in numbers, and this was due to increase of neutrophile polymorphs, which showed many incompletely segmented forms. Occasional primitive leucocytes, myelocytes and myeloblasts, were present. During the first ten days in hospital there

Table 7. Blood counts - Millicent M.

Date	3.6.34	11.6.34	20.8.34	26.8.34	4.9.34	10.9.34	17.9.34	24.9.34	1.10.34
R.B.C. millions per c.mm.	3.0	3.15	3.93	3.70	3.79	4.0	3.93	3.75	3.94
Haemoglobin %	50	60	68	70	75	75	74	75	76
Colour index	0.83	0.95	0.87	0.93	1.0	0.94	0.95	1.0	0.97
Reticulocytes %	10.3	12.4 *	8.8	10.0	7.0	11.6	7.6	10.0	11.4
Platelets per c.mm.		273,000		283,000					
Leucocytes per c.mm.	12,600	5,600	7,600	9,000	6,200	9,200	7,800	5,800	7,200
Neutrophiles: Myeloblasts	0.25		Measles -	-	-	-	-	-	-
Myelocytes	0.5		-	-	-	-	-	-	-
Immature meta-myelocytes	0.75		1	-	0.5	-	0.25	1.0	0.25
Mature do.	36.25		8	16	14.5	10	12.25	18.5	9.75
Segmented forms	39.25		39	52	37.0	60	43.0	47.5	46.35
Neutrophiles (total)	77%		48%	68%	52%	70%	55.5%	67.0%	56.25%
Eosinophiles	2.25%		9%	5%	5%	2%	3.5%	2.25%	2.0%
Basophiles	0.75%		-	-	0.5%	-	-	-	-
Monocytes	1.25%		2%	2%	2.5%	2%	5.5%	0.75%	4.25%
Lymphocytes	18.75%		41%	25%	40.0%	26%	35.5%	30.5%	37.5%
Normoblasts (W.B.C.)	2/400		-	-	-	-	-	-	-

Date	8.10.34	15.10.34	12.10.34	29.10.34	5.11.34	12.11.34	19.11.34	26.11.34	10.12.34	24.12.34
R.B.C. millions per c.mm.	4.21	3.96	3.57	3.83	3.97	4.00	3.87	3.93	3.45	4.5
Haemoglobin %	80	78	82	78	78	80	80	76	70	84
Colour index	0.95	0.98	1.06	1.02	0.99	1.0	1.04	0.97	1.02	0.93
Reticulocytes %	9.2	7.0	7.0	12.4	5.8	19.4	12.6	8.8	15.6	9.6
Platelets per c.mm.										
Leucocytes per c.mm.	11,000	5,400	7,000	7,700	7,000	7,000	6,600	6,600	7,800	8,600
Neutrophiles: Myeloblasts				-	-	-	-	-	-	-
Myelocytes				-	-	-	-	-	-	-
Immature meta-myelocytes				-	1.5	1.0	1	0.5	0.5	-
Mature do.				26	14.5	15.5	18	15.5	22.5	19.5
Segmented forms				46	41.0	37.5	35	34.0	21.0	46.5
Neutrophiles (total)				72%	57%	54%	54%	50%	44%	66%
Eosinophiles				2.0%	1.5%	1.0%	2.0%	2.0%	0.5%	nil
Basophiles				0.5%	-	-	0.5%	0.5%	0.5%	0.5%
Monocytes				1.5%	2.0%	3.5%	5.0%	2.5%	1.5%	6.0%
Lymphocytes				24.0%	39.5%	41.5%	38.5%	45.0%	48.0%	27.5%
Normoblasts (W.B.C.)				-	-	-	-	-	-	-

Date	10.5.34	23.5.35	27.5.35	29.5.35		11.6.35	21.6.35	3.7.35
R.B.C. millions per c.mm.	3.86	3.94	3.6	3.02	Splenectomy 4.6.35	4.59	4.91	5.39
Haemoglobin %	72	72	64	58		78	90	96
Colour index	0.94	0.92	0.89	0.96		0.86	0.92	0.89
Reticulocytes %	8.2	11.8	11.6	6.5		<1	<1	<1
Platelets per c.mm.		380,000		422,000		688,000	914,000	802,000
Leucocytes per c.mm.	6,300	6,800	5,700	7,500		7,000	8,700	8,200
Neutrophiles: Myeloblasts	-	-	-	-		-	-	-
Myelocytes	-	-	-	-		-	-	-
Immature meta-myelocytes	-	0.5	0.5	0.5		-	0.5	0.5
Mature do.	13.5	15.0	17.0	21.0		12.0	10.5	13.0
Segmented forms	35.5	41.0	25.0	32.5		41.0	44.5	48.0
Neutrophiles (total)	49%	56.5%	47.5%	54%		53%	55.5%	61.5%
Eosinophiles	2.5%	2.0%	1.5%	2.0%		3.0%	2.5%	1.5%
Basophiles	0.5%	0.5%	1.0%	0.5%		0.5%	1.0%	0.5%
Monocytes	2.5%	3.0%	4.5%	2.5%		4.5%	3.5%	3.5%
Lymphocytes	45.5%	38.0%	45.5%	41.0%		39.0%	37.5%	33.0%
Normoblasts (W.B.C.)	-	-	-	-		-	-	-

* Reticulocytes % - June 3 10.3, 4 12.8, 5 10.8, 6 13.8, 7 12.4, 8 12.0, 9 12.0, 11 12.4, 12 10.3, 13 8.5. Aug. 28. 9.3. Sept. 1 11.8, 7 9.4, 8. 8. Oct. 19 6.0, 20 5.4 Nov. 1 11.8

was a persistent reticulocytosis of between 8.5% and 13.8%, and occasional normoblasts were present in the films. Towards the end of this time the leucocyte count fell to 5,600 per c.mm. This was explained by the occurrence of a measles rash two days later. The child was removed to a hospital for infectious diseases.

Meanwhile a fragility test had shown that there was a very slight lowering of the resistance of the red cells in hypotonic saline, lysis beginning in 0.50% saline and being complete in 0.40%. The mean diameter of the red corpuscles (Fig. XVI) was within normal limits (7.04 μ) as was also the cell volume index (0.99).

In August 1934, the child was readmitted to hospital. Slight jaundice, urobilinuria and splenomegaly were still present. The van den Bergh reaction was indirect positive (4.5 units).

Anaemia was slightly less marked (Table 7, Chart XX) and between August and December, 1934, the red cell count varied between 3.45 and 4.5 millions per c.mm., with a colour index of between 0.87 and 1.06. During all this time there was a persistent reticulocytosis varying between 5.4 amd 19.6%.

The leucocyte numbers and differential counts did not show any marked abnormality.

Corpuscular fragility was estimated three times between August and December, and it was found on each occasion to be slightly increased. Twice haemolysis started in 0.48% saline and once in 0.54%.

The spleen varied in size and in October it was for a short time impalpable. Urobilinuria was intermittent but more often present than not.

In December, between the 5th and 11th there was a sharp attack of diarrhoea with fever, and the red cell count fell to 3.45 millions per c.mm., while there was a slight increase of reticulocytosis to 15.6%. At that time the spleen became slightly larger. It seems that the infection had caused a slight increase of haemolysis. The illness was of short duration and the leeway was soon made up after recovery.

In December 1934, the child went to a convalescent home where she remained for a few months.

She was readmitted on 10th May, 1935. A loss of weight had occurred. Jaundice was very faint. The tip of the spleen was palpable. Urobilinuria and an indirect positive van den Bergh reaction (6.0 units) were present. The red cells count varied between 3.02 and 3.94 millions per c.mm. in the next nineteen days. The colour index lay between 0.86 and 0.96 per c.mm. There was a tendency to slight leucopenia.

The fragility of the red cells still showed a slight increase, lysis starting in 0.50% saline. A Price-Jones curve (Fig. XVI) showed that the mean corpuscular diameter

(6.81 μ) lay within normal limits.

On June 4, 1935, splenectomy was performed. The spleen weighed 160 gm. Histologically the pulp spaces showed marked distension with blood, the sinusoids being comparatively empty. Phagocytosis was active. A slight degree of excessive fibrosis was present, and small deposits of haemosiderin in the pulp phagocytes were found. In other words, the spleen showed the characteristics commonly associated with acholuric jaundice.

After splenectomy, the girl made an uninterrupted recovery.

A steady rise in the red cell count was noted, and four weeks after operation had reached 5.39 millions per c.mm. The haemoglobin level had also made a remarkable recovery to 96% and the colour index remained normal.

The leucopenia passed off and the differential leucocyte count became normal.

Reticulocyte numbers fell to less than 1% of the red corpuscules. The fragility of the red corpuscles was unchanged.

A remarkable increase in the platelet count was recorded, the highest count being 914,000 per c.mm., seventeen days after operation.

Urobilinuria was not present from a few days after the spleen was removed.

II. ANALYSIS AND DISCUSSION.

A. Congenital acholuric jaundice.

(1) The family history.

Eight of the preceding cases were of the congenital type of acholuric jaundice, in which the haemolytic tendency may be transmitted as a Mendelian dominant factor (Campbell and Warner, 1926). One example of the acquired type was encountered (Case 9). It has been doubted whether such a condition as acquired acholuric jaundice exists, and Dawson (1931) was of the opinion that all the cases were congenital, and those that were apparently acquired had been missed. This possibility is well illustrated in family H, in which the disease had been present unsuspected in two previous generations. In the case of Rena B. (No. 4) the condition was congenital but not apparently hereditary. Whether any members of collatoral branches of the family were affected I did not have the opportunity of ascertaining. On the other hand Millicent M. (Case 9) was in all probability not congenital and the haemolytic phenomenon had started at five years of age when the child had scarlet fever.

(2) Clinical findings.

In my series of cases the main characteristics of congenital acholuric jaundice in the child are well exemplified. Two were jaundiced at birth, one severely. In one instance the icterus lasted only a fortnight and in the other, although the degree of jaundice diminished, a slight icterus, varying in depth from time to time, was constantly present. The remaining cases had been free of jaundice sufficiently marked to attract the attention of the parents. From my observations however I was able to detect jaundice of the skin, or at least of the sclerotics, in all except one case, in periods during which there was no haemolytic crisis.

The general health of the children was variable. In Family L. the surviving children had been in rather poor health, and two of them had suffered from pneumonia, one on three occasions. Similarly Rena B. (Case 4), the only affected member of the family, had been conspicuous for her poor general condition. On the other hand the four children of Family H. had comparatively good health, and the common infectious diseases of childhood had not upset them unduly. There is no doubt however that the home conditions in this family were superior to those of the others, and probably this had a great deal to do with their better general condition.

The development of the children was not delayed, but none of them, with the exception of the child whose spleen had been removed some years before, were robust.

Splenomegaly was a constant finding, and the lower pole of the spleen was palpable about two fingers' breadth below the costal margin in the absence of exacerbations of haemolysis. During a crisis however the spleen became further enlarged and even when only a comparatively small fall in blood count occurred there was a slight increase in the size of the spleen (Case 4). Gansslen (1922, 1925) however has shown that splenomegaly is not always found.

The liver was slightly enlarged in three of my cases. Unfortunately there was not an opportunity for a histological examination. It is probable however that there was not any great hepatic mischief.

Excepting secondary findings such as enlarged tonsils, and haemic cardiac murmurs physical examination revealed little further of importance.

The urine of all cases contained excess of urobilin and increased amounts were present during exacerbations of haemolysis. Bile pigments were constantly absent.

Van den Bergh's reaction invariably showed an excess of bilirubinaemia. This finding might either indicate the presence of excessive blood destruction or failure of the liver to excrete the products of the normal removal of effete blood corpuscles from the circulation. As will be seen in discussing the type of anaemia present, the former explanation is doubtless the correct one. Support for this conclusion is given by the frequency of urobilinuria, because this can only occur if considerable quantities of bile pigments are excreted into the intestines (Astrachan, 1937).

(3) The blood picture.

(a) The red cells.

(i) Numbers. Anaemia was a constant finding except in the case (No. 1) where the spleen had already been removed. Excluding crises, the red corpuscle counts varied between 2.81 and 4.29 millions per c.mm. In the latter instance the comparatively high red cell numbers were temporary and later a reduction to 3.27 millions per c.mm. occurred.

These figures are very similar to those found by Dawson (1931) and Cheney (1934) whose cases were chiefly adults. It would appear then that quite early in childhood (our youngest case was under two years old) the extent of red cell deficiency is similar to that in the adult.

(ii) Haemoglobin. The haemoglobin level was reduced to a similar extent as the red cells and the colour index was in most cases on the upper limits of normal, between 1.0 and 1.2. In untreated cases however the colour index was sometimes less than unity but still above the lower limits of normal, 0.80.

(iii) Reticulotyctes. Reticulocyte counts were constantly raised, varying from 3% during periods when anaemia was slight to 16% when it was more severe. Nucleated red cells (normoblasts) were present in several cases but never in large numbers.

The permanent increase in the number of immature red cells indicates the constant hyperactivity on the part of the bone marrow. The failure of the blood count to reach normal limits in consequence, proves that excessive destruction of the red cells was occurring. Acholuric jaundice may therefore be considered as a chronic haemolytic anaemia.

(iv) Fragility. Chauffard (1909) made the interesting discovery that in acholuric jaundice the fragility of the red cells in hypotonic saline was increased. Since then this has been the diagnostic criterion. In all the above congenital cases the resistance of the red corpuscles was greatly decreased, and lysis started in one case in 0.68% saline, while complete lysis was found in higher dilutions than the normal 0.38%.

(v) Morphology. Price-Jones curves from the cases of congenital acholuric jaundice, including the mother of family L, showed that there was a considerable degree of apparent microcytosis (Figs VII to XV). The extent of microcytosis varied between 16.8% (Fig. XII) to 63.6% (Fig. IX). The mean corpuscular diameter (M.C.D. in the figures) showed limits of 5.75 μ and 6.52 μ, the smallest normal mean diameter according to Price-Jones being 6.686 μ.

The coefficients of variation (V in the figures) were always greater than the normal 5.64 to 7.26. The lowest figure was 14.35 (Fig. XII) and the largest 24.1 (Fig. XI). The coefficient of variation is the most delicate indication of a departure from the normal in red cell diameters, and is even more valuable than the mean diameter (Price-Jones, 1920). In these cases of acholuric jaundice then, the variability of cell distribution was at least twice and sometimes more than three times greater than in the normal subject.

Further, Fig. XI shows that during a haemolytic crisis in the course of pneumonia, the bone marrow was releasing appreciable numbers of cells of greater than the largest normal diameter. This phenomenon was not noted in any of the congenital cases which were not in the throes of a crisis at the time when the curves were constructed. It would appear then that in times of great stress the bone marrow is so overtaxed that a partial reversion to the more primitive megalocytic type of blood formation occurs. It was of course impossible to measure the thickness of the megalocytes but it was unlikely that they had the globular form which, as we shall see, characterised most of the cells, and it is striking that some of the red corpuscles produced by an overtaxed marrow should lack one of the main characteristics of the condition. The probable explanation is that not all of the erythropoietic mechanism produces faulty cells, and that those parts of it which do not show the customary tendency to release corpuscles at a premature stage of development in times of stress.

That microcytosis was more apparent than real was indicated by the cell volume index. This was done by the method described by Whitby and Britton (1935 b). In two cases (Nos. 2 and 3) the cell volume index was 1.0 and 0.96, i.e. within normal limits. The cells, then, although they showed a diminished mean diameter had a normal volume, and the inference is that they had become thicker and somewhat spherical. Haden (1934) was of the opinion that excessive fragility was a result of the spherical shape of the corpuscle.

The cell volume index however is not a particularly good method of measuring cell volume and like the colour index is only a rough measurement, because it depends on the acceptance of a "normal" volume of packed red corpuscles after centrifugalizing blood, just as in estimating colour index one accepts a "normal" value of 5.0 millions cells per c.mm., and 100% haemoglobin.

Wintrobe (1932) elaborated a more accurate method of measurement of cell volume. It had the further advantage of giving information about the haemoglobin content of the corpuscle as well as doing away with the necessity for a fixed volume of packed red cells in centrifugalized blood.

With the exception that blood was spun in graduated centrifuge tubes instead of in the haematocrit, my method was the same as Wintrobe's. I used a Haldane's haemoglobinometer of which the standard tube corresponded with blood containing 13.8 gm. of haemoglobin per 100 c.c.

I compared various data of four cases of acholuric jaundice (Nos. 5, 6, 7 and 8) with those of normal children and one case of microcytic hypochromic anaemia. The results are given in Table 8.

Table 8. Comparison of the cell volume and haemoglobin content in acholuric jaundice, with those in normal children, and in microcytic hypochromic anaemia.

	R.B.C. millions per c.mm.	Hb. gm. %	Vol. of packed red cells %	M.C.V. c.μ	M.C.H. γγ	M.C.H.C. %	M.C.D. μ
Average of 5 normal children	5.17	13.02	40.60	78.84	25.17	32.06	
Average of 4 cases of acholuric jaundice	3.02	8.52	23.95	79.08	28.2	35.46	6.41
One case of microcytic hypochromic anaemia	3.76	4.83	21.67	57.63	12.84	22.24	

M.C.V. = mean corpuscular volume.

M.C.H. = mean corpuscular haemoglobin.

M.C.H.C. = mean corpuscular haemoglobin concentration.

M.C.D. = mean corpuscular diameter.

Briefly mean corpuscular volume (M.C.V.) is the average volume of the red corpuscle and is expressed in cubic μ. Mean corpuscular haemoglobin (M.C.H.) is the average amount of haemoglobin by weight in the corpuscle, and is given as micro-micrograms (yy). Mean corpuscular haemoglobin concentration (M.C.H.C.) gives as a percentage the amount of haemoglobin per unit volume of red cell.

In acholuric jaundice there was a low mean corpuscular diameter, with a normal cell volume, a slightly increased content of haemoglobin, and increased concentration of haemoglobin. Actually in Cases 5 and 6, the M.C.V. (84.6 and 85.2 c.μ respectively) was just above the upper limits of the normal series (82.13 c.μ). In Case 8, M.C.V. fell within normal limits, but in Case 7 M.C.V. (70.21 c.μ) was slightly below the lower limits (74.21 c.μ) The haemoglobin content varied directly with the size of the corpuscle, being rather higher in Cases 5 and 6 (32.2 yy and 29.5 yy) in which there was definite enlargement of the cells, only very slightly increased in Case 8, and slightly below normal in Case 7 (23.56 yy). This is of course what would be expected, the larger cells being able to hold more haemoglobin. To show the small extent of the variations of cell volume and haemoglobin from the normal, the figures from a case of microcytic hypochromic anaemia in a child of three and a half years are added.

In all four examples of acholuric jaundice, however, the mean corpuscular haemoglobin concentration was higher than in the normal series, i.e. the amount of haemoglobin per unit volume of red cell was high.

These results compare favourably with the findings of Vaughan and Goddard (1934).

The conclusions are that in congenital acholuric jaundice the mean corpuscular diameter of the red corpuscles is much diminished, while the volume is increased, normal or slightly decreased, the cell having apparently assumed a globular shape. The haemoglobin content is slightly raised, normal or diminished, according to the change in the volume of the cell. The haemoglobin concentration in the corpuscle is however always raised. Although this may mean that the size of the haemoglobin molecule is different in acholuric jaundice from that in the normal or hypochromic blood, it is not possible to make a definite conclusion, because it is not known as yet how the haemoglobin is contained in the corpuscle.

(b) The leucocytes.

Beyond a slightly increased number of incompletely segmented neutrophile cells, and neutrophile leucocytosis obviously due to infection there was little abnormality of the leucocytes.

(c) Platelets.

The platelet counts were either normal, i.e. between

250,000 and 450,000 per c.mm., (Mackay, 1931) or slightly reduced, e.g. 150,000 per c.mm.

(d) Haemolytic crises.

The conditions under which haemolytic crises occurred were remarkable. In two children of family L., pneumonia did not apparently cause a dangerous destruction of blood. In none of the children in any of the families did children's troubles such as measles or whooping cough lead to an obvious or sudden increase of anaemia. On the other hand Rena B. (Case 4) during a sharp attack of lobar pneumonia, destroyed so much blood that a gross anaemia resulted within a week of the onset of infection. Again the serial crises in the children of family H. were unassociated with any discernible foci of infection, and acute tonsillitis in one of them during convalescence did not cause a serious haemolysis. The theory that infection is usually the exciting factor is more attractive than, for instance, the suggestion that emotional upset may be the cause (Piney, 1931). Salomonsen (1926) who described serial crises in two children, a brother and sister, concluded that occult infection underlay the phenomenon. Similarly the simultaneous occurrence of haemolytic crises in a father and son, mentioned by Vaughan (1934) looks remarkably like a reaction to infection. It would appear however from the histories of our patients that infection cannot be regarded as more than a contributory factor in the causation of the haemolytic crises. There is no evidence to show what is the immediate cause.

The clinical findings during the acute phases of acholuric jaundice in our series were vomiting, malaise, fever, and fainting attacks, with increase of pallor and the appearance of or increase of jaundice. In one localising signs of infection were present in the chest (Case 4). The urine became dark brown in colour, due to excess of urobilin. Bile pigments were not present. The spleen increased in size. The red cell counts fell to the neighbourhood of 1.0 millions per c.mm., the colour index remaining normal. A few megalocytes were present (Case 4). Reticulocyte numbers were diminished to within normal or almost normal limits. The fragility of the red corpuscles lessened greatly in four cases (5, 6, 7 and 8) but remained considerably increased in another (Case 4) although even in this case the fragility was not so excessive as after recovery from the crisis.

Recovery whether spontaneous as in Case 4 or aided by transfusion as in Cases 5, 6, 7 and 8, was accompanied by the cessation of general symptoms, and fever, decrease of jaundice, and reduction of the size of the spleen. Urobilinuria lessened but did not disappear. There was a sharp reticulocytosis which did not ultimately disappear but persisted at a lower level. Simultaneous with the increase of reticulocyte numbers, a marked erythroblastaemia occurred for a few days. The red cell count rose to about 3 millions per c.mm. in a few weeks.

During the acute anaemia there was absence of leucocytosis in those cases where a focus of infection could not be found, and a moderate leucocyte response, with the appearance of a few primitive cells, in the case with pneumonia.

These crises were remarkably similar to the acute haemolytic anaemia described by Lederer (1925 and 1930). This rare type of anaemia is often associated with a preceding infection, usually gastro-intestinal, but sometimes evidence of any previous illness is lacking. Not infrequently the crises in acholuric jaundice have been mistaken for Lederer's anaemia, which of course occurs in people who have not any demonstrable abnormality of the erythropoietic system such as occurs in acholuric jaundice.

Such cases have been recently reported by Findlay and Dobbs (1934) and Murray-Lyon (1935). The initial mistake occurred because the fragility of the red corpuscles at the height of the haemolytic process was normal, the inference being that all the fragile cells had been destroyed leaving only those with a normal resistance to hypotonic saline.

With the exception that in the acute haemolytic anaemia described by Lederer there was always a leucocytosis, the clinical and haematological findings are remarkably similar to those in the crises of acholuric jaundice.

B. Acquired acholuric jaundice.

The example of acquired acholuric jaundice presented above (Case 9) raised some interesting points. The child had been healthy until she had an acute infection - scarlet fever. After that her general condition had been poor and nearly two years later, when first seen by me, she was anaemic and slightly jaundiced. There was splenomegaly, urobilinuria and an indirect positive van den Bergh reaction. The anaemia which I observed over a long period, was similar to that in the cases of congenital acholuric jaundice, showing a red cell count varying between 3.0 and 4.5 millions red cells per c.mm., with a persistent reticulocytosis of moderate degree.

The great difference from the congenital type lay in the fragility and morphology of the red corpuscles. The resistance of the red cells to hypotonic saline was on several occasions only very slightly diminished, lysis beginning in 0.48% saline, and on one occasion moderately reduced, lysis starting in 0.45% saline. The mean diameter of the corpuscles was within normal limits. On the first occasion when the Price-Jones curve was constructed, the anaemia was rather more severe then usual (3.01 millions red cells per c.mm.) and there was a slight erythroblastaemia. The bone marrow seems to have been somewhat overtaxed at this period, and a few megalocytes were seen (Fig. XVI).

Later when the anaemia was less marked, the mean diameter was slightly less (Fig. XVI). This was not due to a tendency for microcytosis to occur but was the result of the disappearance of the megalocytes from the circulating blood. In both curves however a few microcytes were present and the coefficient of variation was above Price-Jones upper normal limit 7.4, indicating that there was a disturbance of the variability of cell diameters due to an abnormal functioning of the bone-marrow.

Such a case indicates that the developmental error of the red corpuscles in the majority of cases of acholuric jaundice is not the primary factor in producing the chronic excessive destruction of blood.

C. Treatment.

Dawson (1932) points out that splenectomy in acholuric jaundice was first performed by Spencer-Wells in 1887. The disease of course was not recognised as a haemolytic anaemia at that time, and the removal of the spleen was a pure speculation. The case however recovered and apparently did well thereafter. Of late years splenectomy has been the universal treatment of the condition.

Seven of my patients underwent the operation, one before she came under my notice. The operation was well tolerated by all but it is striking that even at the age of twelve years, there were in one case (No. 5) considerable adhesions to the abdominal wall and also to the stomach, which was opened in freeing the spleen. This child had had an attack of perisplenitis some months before. As the patients of six, seven and eight years (Cases 7, 1 and 8) stood the operation quite as well as did the older children on ten and twelve years, I think it is advisable to operate before the twelfth year, as Dawson (1931) advised. His reason was that until beyond the age of twelve adhesions were rare, but as adhesions cannot be diagnosed clinically, I think it inadvisable to wait, but to remove the spleen as soon as the diagnosis is definite. At an early age the operation is one of reasonable simplicity.

D. Pathology.

The enlargement of the six spleens was considerable, and their weights varied between 160 gm. and 340 gm. Except in one case perisplenitis was absent. The substance of the spleen was firm and dark red in colour, the Malpighian bodies being indistinct. Histologically, the most striking feature was the engorgement of the pulp spaces with blood, the sinuses being as Turnbull (1934) has emhpasized comparatively empty and often compressed. Phagocytosis of red corpuscles and unrecognisable material was a feature in the pulp. The Malpighian bodies were scattered and showed a deficiency of lymphoblasts. Haemosiderosis was never a marked characteristic and it would appear that the large iron deposits described by Dawson (1931) are only found in older subjects. Such haemosiderin as was present was found in the pulp phagocytes, and in one instance in the reticulum. The phagocytes in the neigbourhood of the Malpighian bodies were most heavily impregnated. Possibly in these young subjects the iron which was obtained from the splitting of haemoglobin was quickly used again by the marrow in making fresh haemoglobin.

The largest splenic iron deposits were seen in the two children who had been transfused a day or so before operation. This suggests that a moderate quantity of the transfused blood had been destroyed. The conclusion was borne out by the blood counts in only one case, where the increase in red cell count on the day after transfusion of 350 c.c. of blood was only 0.55 millions per c.mm. The other case showed a reasonable increase of red cell numbers (1.27 millions per c.mm.) after a transfusion of 450 c.c. of blood.

Slight increase of the fibrous tissue of the splenic trabeculae was present.

Metaplasia of erythropoietic tissues was not present. Extramedullary red cell formation has been described however in the spleen (Davidson,,1932) and in the costo-vertebral angles and kidneys (Dawson, 1931).

The pathological picture of the spleen was exactly similar in both acquired and congenital cases. Nor indeed was there much difference between the picture in acholuric jaundice and erythroblastosis foetalis, with the exception that in the latter erythropoiesis was not infrequent and haemosiderosis was usually more marked.

Engorgement of the pulp spaces, empty sinusoids and phagocytosis would therefore appear to be common to the haemolytic anaemias.

I did not have the opportunity of examining the bone-marrow from any of my patients and as the surgeon favoured the extraperitoneal route of operation, I have no information as to the presence or absence of pigment stones in the gall-bladder. It is unlikely that any were present in such young children.

Turnbull, writing the sections on pathology in Vaughan's "Anaemia" (1934) described the marrow as hyper-plastic.

E. The effect of splenetcomy on the blood picture.

(1) The red corpuscles and haemoglobin.

After operation there was a steady rise of the red cell count as was shown in the blood charts. Figures in the neigbourhood of five millions red cells per c.mm. were reached from an initial level of about three millions per c.mm. in periods varying from fifteen days (Case 4) to six weeks (Case 5). In none of the cases did a polycythaemia, i.e. a count of six millions or more per c.mm. occur, as happened in one of Dawson's cases (1931). Within a few days of operation the reticulocyte numbers fell to within normal limits, where they remained. Coincident with the disappearance of anaemia was the cessation of urobilinuria and the return of the bilirubin content of the blood to within normal or nearly normal limits.

The haemoglobin content of the blood rose in almost parallel fashion with the red cell count, but there was usually a slight fall in colour index although never to less than 0.8 which is the lower limit of normal.

In all cases whether congenital or acquired splenectomy had been successful in lowering the amount of blood destruction to normal dimensions, and in releasing the strain on the haematopoietic organs which were then able to restore a normal blood count and also to function to a less active degree, as was shown by the absence of reticulocytosis.

(2) <u>Fragility</u>.

Little alteration however was found in the red cell fragility in any of the cases. In five there was a slight increase of the resistance of the cells. The case in which splenectomy had been done two years before I saw her, had equally as fragile cells as the rest of the family who still retained their spleens.

Dawson (1932) noted a case in which fragility was still increased twenty-seven years after splenectomy, and

Thursfield (1913) and Whitcher (1925) each recorded one in which fragility remained normal after operation.

Reynolds (1930) had a case of acquired haemolytic jaundice which showed normal fragility before operation, but afterwards the resistance of the cells became subnormal. This did not occur in my case in which the fragility remained unchanged after removal of the spleen.

Thus it would appear that the influence of the spleen on the fragility of the red corpuscles is not constant. In some cases it would appear to have none, in others it appears to increase fragility. Sometimes it appears to be the cause of excessive fragility, and sometimes, doubtless by quickly destroying the fragile cells, it appears to mask the defect.

(3) Morphology of the red corpuscles.

Whitcher (1913) by rough measurements of the cells in acholuric jaundice with microcytosis came to the conclusion that the shape of the cell returned to normal after splenectomy.

I investigated this point in four cases of congenital acholuric jaundice (Nos. 5, 6, 7 and 8) by constructing Price-Jones curves just before operation, one week after and again six weeks from operation. The most striking results were obtained in Cases 5 and 6, and are shown in Figs. XII and XIII. It will be seen that the preoperative curves showed a shift to the left with a microcytosis of 16.8% and

24.8% respectively, the mean diameters being 6.52 μ and 6.47 μ. The coefficients of variation were considerably increased above the upper limit of normal, 7.4 to 14.35 and 16.39 respectively.

One week after operation the curves had shifted to the right, and the mean diameters were within normal limits, 6.95 μ and 6.84 μ, while the microcytosis was reduced to 7.0% and 9.4%. Although the curves had wider bases and lower peaks, the coefficients of variation were reduced but not quite to normal figures, being 10.57 and 12.81 respectively, showing that there was still some disturbance of the function of the bone marrow.

Six weeks after operation a shift to the left had again occurred. In Case 5 however the mean diameter (6.71 μ) was still within normal limits, the curve had a narrower base and higher peak and was practically normal in shape. The more delicate indication of abnormal variation of cell diameter however, was still high (10.63). A small degree of microcycosis persisted (5.6%).

In Case 6 the curve had shifted to practically the preoperative state, with a mean diameter of 6.33 μ, a coefficient of variation of 16.22 and microcytosis of 27.2%.

In Cases 7 and 8 (Figs. XIV and XV), a slight shift to the right had occurred one week after splenectomy, but this might have been partially due to the presence of normal cells which had remained from the blood transfusion given before operation. Six weeks after splenectomy, however, when the third curves were constructed all the transfused cells would be absent because Ashby (1919) and Escobar and Baldwin (1934) have shown that the transfused cells last from eighteen to thirty days. At this time the curves had reverted to the preoperative state although microcytosis was less extensive.

The Price-Jones curve of another case (Fig. VIII) two years after splenectomy showed a marked shift to the left and was indistinguished either by the mean diameter or the coefficient of variation from the curves of others of the family from none of whom was the spleen removed.

Although it was unlikely that splenectomy would have any effect on cells of normal size, a series of Price-Jones curves were constructed in a case of Banti's disease in a girl of 10 years (Fig. XVII). As will be seen, the curves on the third and sixth days after splenectomy were practically identical with that done on the day before operation.

Coincident with this investigation into cell diameter before and after splenectomy, Hawksley and Bailey (1934) published the results of a similar investigation in which they also noted the increase in cell diameter after splenectomy in acholuric jaundice, and the tendency to revert to the original subnormal dimensions.

From these findings it would appear that the spleen plays a part in the production of the globular shape of the red corpuscle in acholuric jaundice. The recurrence of microcytosis may be due to the assumption of the remainder of the reticulo-endothelial system of the functions of the removed spleen, although if this is the case it is difficult to understand why there is not a return of excessive blood destruction.

There are probably two factors. One, a developmental defect of the red cell which is shown by the excessive fragility and spheroidal shape. Two, a further distortion of the abnormal cells by the pathological spleen which is active in removing fragile cells from circulation.

(4) Similar morphological features in other anaemias.

This effect of the spleen on the abnormal cells is not peculiar to acholuric jaundice. In Cooley von Jaksch's disease, which is chiefly found in America and was extensively investigated by Cooley and his colleagues (1927, a and b, 1928, 1929, 1932 a and b) there are many points of resemblance to acholuric jaundice. Cooley found that the condition was familial and occurred in children of Mediterranean stock. Apparently similar cases are also found in Mediterranean countries and elsewhere on the continent (Lehndorff, 1936).

The main clinical characteristis are pallor, splenomegaly, often great, and widening of the marrow cavities of the bones, with rarefaction of the cancellous bone, and a typical spiculation of the outer table of the skull. The anaemia, often severe, is of the haemolytic type, with reticulocytosis and marked erythroblastanaemia, and microcytosis. The colour index is usually low. Excessive fragility of the red corpuscles is not a feature.

Recently Whipple and Bradford (1936) have shown that following on splenectomy there is a temporary increase of cell diameter. Contrary to what occurs in acholuric jaundice the haemolysis does not cease, and immediately after splenectomy there is a great increase in the numbers of reticulocytes and nucleated red cells in the circulation. Although the anaemia may improve for a time it eventually becomes worse and a fatal result is usual.

The similarities between congenital acholuric jaundice and Cooley von Jaksch's disease are striking. Both are familial, and show a developmental defect of the red corpuscles, hyperactivity of the marrow, excessive destruction of blood and enlargement of the spleen. In both the spleen has apparently an influence in decreasing the cell diameter. Removal of the spleen in one condition causes cessation of excessive haemolysis but in the other this effect is slight or absent.

A similar splenic influence on red cell diameter may also be present in the erythroblastosis foetalis, where as we have seen there is also severe haemolysis, splenic enlargement, and diminution of red cell diameter.

The production of diminished red cell diameter by the spleen is apparently confined to the haemolytic type of anaemia and as I have shown in Banti's disease, where there is also splenic enlargement, corpuscular diameter is unchanged.

The absence of microcytosis in some cases of the acquired type of acholuric jaundice is probably due to the fact that there is little if any inherent abnormality of red cell formation, and that the effect of the spleen is not sufficiently powerful in the absence of this to produce any marked change.

(5) The leucocytes after splenectomy.

Following on splenectomy the changes in the leucocytes are not nearly so striking as those in the red cells. In Case 4, a sharp leucocytosis occurred immediately after operation and was chiefly due to an increase in the numbers of neutrophile cells. Little change was seen in the other cases where blood counts were first done about a week after operation. There was however in Cases 5, 6, 7 and 8 a reduction of the less mature neutrophile polymorphs to within normal limits.

(6) The platelets after splenectomy.

The remarkable increase of platelet numbers after removal of the spleen was a constant feature, and lasted for some weeks. This however is not peculiar to acholuric jaundice, and I have noted the same thing in Banti's disease, and in purpura, where there was a pre-operative thrombocytopenia. Similar findings are recorded by Dawson (1932).

It seems reasonable to infer that excessive numbers of platelets, like red corpuscles, are destroyed by the spleen. After removal of that organ the temporary rise of platelet numbers indicates that probably platelet destruction elsewhere in the body is not of great extent by that after a time other parts of the reticulo-endothelial system may acquire the function.

From the practical point of view it is of interest to note that in none of the cases did post-operative embolism or thrombosis occur, even although the platelet count was very high. The relation between the platelet count and embolism is not known well, although there seems to be a clinical relationship (Evans, 1928). Possibly the absence of thrombosis and embolism lies in the fact that in ligating vessels at operation the endothelium was undamaged.

F. Summary and conclusions.

(1) Eight cases of congenital and one of acquired acholuric jaundice were analysed. The cases were from four different families. In two of the three families where the condition was congenital a history of acholuric jaundice was present in two previous generations.

The case of acquired acholuric jaundice did not appear to have been a congenital case, missed because of the mildness of the symptoms in the earlier years of life. The haemolytic process seemed to have begun during or following on an attack of scarlet fever. The rest of the family were not affected.

(2) Jaundice was variable. Sometimes it was present at birth and sometimes it made its appearance later. It was never marked except during crises, and was usually missed by the parents. A slight icterus was nearly always evident to the practised eye.

(3) On the whole the children were in poorer general condition than normal children.

(4) Splenomegaly was present in all cases, and varied directly with the severity of the anaemia. Slight enlargement of the liver was sometimes present, but this was not thought to have any grave significance.

(5) The anaemia was of the haemolytic type. Except during crisis the red cell count varied between 2.81 and 4.29 millions per c.mm., and was accompanied by a reticulocytosis, which was greater when anaemia was more severe. Occasional nucleated red cells were found. Secondary signs of haemolysis were constantly present. They were excess of urobilin in the urine and an indirect positive van den Bergh reaction. Bile pigments were invariably absent from the urine.

(6) In all the congenital cases the fragility of the red corpuscles was greatly increased, but in the acquired case only a slight and variable diminution of resistance was observed.

(7) The shape of the red cells was investigated. In congenital acholuric jaundice there was constantly an apparent microcytosis with a normal cell volume index. Consequently it was concluded that the cells were of a globular shape. More accurate measurement of cell volume by Wintrobe's method showed that the cell volume might be increased, normal, or slightly decreased. The haemoglobin content varied directly with the size of the cell. The concentration of haemoglobin per unit volume of red cell was significantly raised. This appeared to be peculiar to acholuric jaundice.

In the example of acquired type of acholuric jaundice microcytosis was absent but there was considerable increase of the variability of cell diameter indicating a disturbance of bone marrow function. The absence of globularity might be associated with the comparatively normal fragility.

(8) Leucocytes and platelets were not greatly affected. In the absence of infection there was evidence of slight hyperactivity of the marrow in producing neutrophile cells.

(9) Haemolytic crises were discussed. It was concluded that infection was a contributory factor in their

production, but that infection did not necessarily cause a severe haemolysis.

The striking parallel between the haemolytic crises in acholuric jaundice and Lederer's anaemia, and the fact that the former may temporarily be mistaken for the latter was considered.

(10) In discussing treatment early splenectomy without delaying till the twelfth year was advocated.

(11) Reference was made to the similarity of the histological picture in the spleen in both types of acholuric jaundice and in the haemolytic anaemias of the new-born. Comment was made on the small quantities of haemosiderin in the spleen in children with acholuric jaundice.

(12) The effect of splenectomy on the blood picture was dealt with in detail. It was seen that there was cessation of excessive blood destruction, a return of the blood count to normal, and evidence that the bone marrow was no longer required to function at a high level of blood production. Evidence was led to show that the spleen had a considerable influence in maintaining the globularity of the red cell, but that on removal of that organ the improvement in the shape of the cell was often temporary, and that a return to the preoperative condition sometimes occurred. It was suggested that there were two factors, a developmental

abnormality of the red corpuscle which was rendered liable to further distortion by the pathological influence of the spleen. A parallel was seen in Cooley von Jaksch's anaemia, and something similar was found in the neonatal anaemias.

(13) The influence of the spleen on fragility was extremely variable. Here again the developmental abnormality of the cells was probably the prime factor.

(14) After splenectomy there was probably a very transient polymorpho-nuclear leucocytosis, and the permanent effect appeared to be the cessation of the slight hyperactivity of the marrow leucopoietic tissues.

(15) A remarkable increase of platelet numbers followed splenectomy, but this was shown to occur in other diseases after similar treatment. The remarkable absence of post operative embolism in spite of the high platelet count was noted and it was suggested that the explanation lay in the absence of damage to the endothelial linings of ligated vessels.

(16) In brief, acholuric jaundice is a chronic haemolytic anaemia, which may be hereditary, congenital or acquired. It is characterised usually by a deformity of the circulating red corpuscles but this may be absent or slight, especially in the acquired type. The spleen is excessively active in destroying red corpuscles, and has also an influence in

increasing the abnormality of shape of the red corpuscles. The bone marrow is constantly stimulated to increase the output of red corpuscles to balance the destruction.

A similar condition occurs in Cooley von Jaksch's anaemia.

If the spleen is removed in acholuric jaundice however, the faulty construction of the red corpuscles is not sufficiently serious to prevent cure of the anaemia, whereas in Cooley von Jaksch's anaemia the defect of the erythron is of greater importance in the production of the anaemia than the destructive properties of the spleen, and splenectomy fails to alleviate the condition more than temporarily.

(1) THE RED CORPUSCLES IN ACIDOSIS AND ALKALOSIS.

Price-Jones (1929) in his investigations into the diameter of the red corpuscles found that there is a diurnal variation, the cells being smaller in the morning and larger later in the day, when the alkalinity is less. He further noted that violent exercise, by increasing the accumulation of lactic acid, and thereby causing lessened alkalinity of the blood, is accompanied by increase of cell diameter, and that in the converse state of increased blood alkalinity, produced by hyperventilation, which drives off carbon dioxide from the blood, there is a diminution of cell diameter. Experiments in vitro, blood being made more or less alkaline, produced corresponding results.

In these instances the change in acid-base equilibrium was transient. Similar findings, however, have been described when the disturbance was of longer duration. Földes (1924), Holler and Kulka (1927) noted an increase in the volume of the red corpuscles in diabetic coma, the first showing that treatment with alkali caused a return to normal. Wiechmann (1925) observed an increase in cell volume with return to normal during treatment with insulin. Increased cell volume, with decreased cell diameter, was however described by Holler and Kulka (1927), the cells apparently becoming globular in shape, and Horwitz made similar observations in experimental acidosis produced by calcium chloride. Increase of cell diameter occurs in the deep sleep induced by the administration of morphine (Wiechmann and Shürmeyer, 1925). The same workers describe diminution of mean diameter of the red corpuscles after administration of sodium bicarbonate.

An increase in red cell count in diabetic coma is described by Grawitz (quoted by Horwitz, 1930) and by Földes, the latter being of the opinion that the erythrocytosis is directly proportional to the severity of the acidosis. Detre and Zárday (1930) found erythrocytosis in experimental acidosis produced in dogs. The former observed an increase in the red cell count after strenuous work. This did not occur when alkali was given.

In the following study, Price-Jones curves were constructed from cases of acidosis and alkalosis occurring in children. The blood of ten children was examined. Four of these were examples of acidosis, two with diabetic coma and two with acidosis produced by calcium chloride. Six were examples of alkalosis and included two of subacute nephritis undergoing treatment with massive doses of sodium bicarbonate, and four of congenital hypertrophic pyloric stenosis. The technique followed in construction of the curves was that described by Price-Jones. Blood films, made on slides and dried by air, were fixed with Jenner's stain and counter-stained with watery eosin. A projection apparatus was used in drawing the cells, the magnification being 1,000 diameters.

The cells were measured in two directions at right angles, the square root of the product to the nearest 0.25 μ being taken as the mean diameter of the corpuscle. In the construction of a curve five hundred cells were measured.

(2) THE BLOOD IN ACIDOSIS.

Table 1 summarizes the results found in acidosis. It will be seen that in all four cases there was an increase in corpuscular diameter (shift to the right), but that this was not proportional to the diminution of blood carbon dioxide. Furthermore, the shape of the curves was not changed, the whole being moved *en masse* to the right in each case. Examples of this are shown in Figs. XVIII, XIX, XX, XXI. In three of the four cases it was found that the coefficient of variation was decreased during acidosis. Erythrocytosis occurred in diabetic coma, but in neither of the cases of calcium chloride acidosis.

Table 1. The effect of acidosis on the red corpuscles.

Case No.	Nature of Case	Hb. per cent.		R.B.C. in millions per c.mm.		M.C.D.* (μ)		Shift to right (μ)	Blood CO_2 (Vol. per cent.)
		During Acidosis	On Recovery	During Acidosis	On Recovery	During Acidosis	On Recovery		
1	Diabetic coma	106	95	5.64	4.96	7.459	6.745	0.714	27.8
2	Diabetic coma	-	-	-	-	7.752	7.146	0.606	-
3	Calcium chloride acidosis	78	76	4.46	4.38	7.468	7.079	0.389	11.8
4	Calcium chloride acidosis	50	50	4.00	3.94	6.731	6.099	0.632	21.5

*M.C.D. = Mean corpuscular diameter.

(3) THE BLOOD IN ALKALOSIS.

The findings in alkalosis produced by the administration of sodium bicarbonate in nephritis are found in Table 2 and Figs. I and II. Here there was a diminution in red cell diameter during alkalosis, though the general characters of the Price-Jones curve were unchanged, as shown in Figs. XXII and XXIII. In these cases the coefficient of variation was increased in the alkalotic period.

No change in the erythrocyte count was noted in either case.

Cell volume was estimated in Case 6. Blood, oxalated by the method described by Wintrobe (1932) in America and by Vaughan and Goddard (1934) in England, was centrifugalized in graduated centrifuge tubes, and not in the haematocrit as was done by these workers. Mean corpuscular volume (M.C.V.) of the red cells is given in cubic microns, mean corpuscular haemoglobin content (M.C.H.) in micro-microgrammes of haemoglobin ($\gamma\gamma$), and mean corpuscular haemoglobin concentration (M.C.H.C.), i.e., the haemoglobin per unit volume of red corpuscle, as a percentage. Table 3 shows that the volume of the red cells in alkalosis was reduced and, though the total amount of haemoglobin remained unchanged, its concentration was increased, i.e., fluid had been removed from the cells.

In pyloric stenosis it is well recognized that there is a state of alkalosis. Clinically this is manifested by the slow respiratory rate, which has been shown to bear a

Table 2. The effect of alkalosis on the red corpuscles.

Case No.	Nature of Case	Hb. per cent.		R.B.C. in millions per c.mm.		M.C.D.* (μ)		Shift to left (μ)	Blood CO_2 (Vol. per cent.)
		During alkalosis	On recovery	During alkalosis	On recovery	During alkalosis	On recovery		
5	Alkali administration in subacute nephritis	80	76	4.14	4.12	6.041	7.242	1.201	82
6	Alkali administration in subacute nephritis	90	85	4.87	4.61	6.706	7.381	0.675	-

*M.C.D. = Mean corpuscular diameter.

Table 3. The influence of alkalosis on the volume and the haemoglobin of the red cells.

	R.B.C.in millions per c.mm.	Hb. in gm. per cent.	Vol. of packed R.B.C. per cent.	M.C.V. in c.μ	M.C.H. in $\gamma\gamma$	M.C.H.C. per cent.
Average for five normal children	5.17	13.02	40.60	78.54	25.17	32.07
Case 6 (During alkalosis)	4.87	12.69	33.5	68.78	26.05	37.88
Case 7 (On recovery)	4.61	11.59	39.0	84.59	25.14	29.72

M.C.V. = Mean corpuscular volume.
M.C.H. = Mean corpuscular haemoglobin.
M.C.H.C. = Mean corpuscular haemoglobin concentration.

Table 4. The influence of the alkalosis of pyloric stenosis on the red corpuscles.

Case No.	M.C.D. (μ)	Normal M.C.D. for age (μ)	Shift to left (μ)	Respiratory rate per min.	Blood count.
7	7.28	7.94	0.66	32	Hb. 100 per cent. R.B.C. 4,800,000.
8	7.68	7.94	0.26	28	Hb. 105 per cent. R.B.C. 5,300,000.
9	6.68	7.94	1.26	22	Hb. 135 per cent. R.B.C. 6,800,000.
10	7.33	7.72	0.39	28	Hb. 110 per cent. R.B.C. 5,500,000.

M.C.D. = Mean corpuscular diameter.

close relationship to the blood carbon dioxide (Graham and Morris, 1933). In the four cases examined there was a definite diminution in the mean cell diameter but this did not appear to be proportional to the degree of alkalosis as gauged by the respiratory rate. The data are given in Table 4, where the normal cell diameter for the age, as determined by van Creveld (1932) is also shown. The Price-Jones curves have the characteristic broad base of curves at this age (Figs. XXIV, XXV, XXVI, XXVII). Marked erythrocytosis was present in all four cases.

(4) DISCUSSION.

Detre was of the opinion that acid stimulated erythropoiesis, but Horwitz (1930) points out that there is no evidence of this, as the number of reticulocytes is normal throughout acidosis and states that though on account of the reduction of blood volume in diabetic coma there may be an apparent erythrocytosis, the total number of red cells in the body is the same as when acidosis is absent. The increased red cell count in acidosis is therefore probably due to blood concentration, but erythrocytosis is not an invariable finding, as it occurred in neither of our cases of acidosis due to calcium chloride. In diabetes progressing to coma there is always great loss of fluid, while this is not a notable feature in cases of acidosis produced by calcium chloride, a fact which would explain the absence of erythrocytosis in our two cases of the latter.

All the results obtained by separating corpuscles from plasma, by centrifugalizing oxalated blood, are in agreement that in acidosis the ratio of red cells to plasma is increased. Horwitz, and Holler and Kulka, who hold that there is no increase in the mean diameter, maintain on the basis of this finding that the individual cell undergoes a change of size and shape, becoming more globular. The data on which Horwitz based this conclusion are more readily explained by the presence of anhydraemia due to loss of fluid. It would be necessary before concluding that the red cells enlarge by assuming a globular shape without increase in diameter to prove that the increase of the volume of packed cells in the centrifugalized blood was greater than could be explained by decreased plasma volume.

In my series, however, an increase of cell diameter occurred in both diabetic coma and acidosis from calcium chloride administration, and in the latter no change in red cell count was present, proving that while the blood plasma may or may not be decreased, there is certainly an increase of corpuscular size, though the shape of the cell is not apparently changed.

These results are parallel with the findings of Price-Jones in transient non-gaseous acidosis, and Wiechmann and

Shürmeyer in gaseous acidosis, and show in contrast to the results of other workers, that in prolonged pathological non-gaseous acidosis the same changes occur. It may be that in addition to the anhydraemia the increased size of the corpuscle increases the viscosity of the blood, and so plays a part in the circulatory failure which so frequently accompanies grave acidosis.

I have been unable to demonstrate any correlation between the extent of increase of cell diameter and the diminution of blood carbon dioxide, although the latter is not necessarily an exact measurement of the degree of acidosis.

It is of interest to note that Case 4 had a marked microcytic, hypochromic anaemia, which did not modify the behaviour of the cells in acidosis.

Alkalosis has the reverse effect on the red corpuscle, causing a reduction in its volume, and its mean diameter. The haemoglobin content remains unchanged, but the concentration of haemoglobin per unit volume of red cell rises. This effect is doubtless due to the removal of water from the corpuscle. There is no change in the erythrocyte count in alkalosis produced by sodium bicarbonate.

Price-Jones found that the coefficient of variation was increased in acidosis and suggested that possibly some cells were more sensitive to change in acid-base equilibrium than others. In three of our four cases the coefficient of variation was decreased in acidosis. These apparently conflicting results might be explained for, if, as Price-Jones suggests, some cells are more sensitive than others it would take longer for the less sensitive cells to be affected and it would only be when they had been subjected to acidosis for some time that an increase in size would occur.

In the two cases of alkalosis produced by sodium bicarbonate the variability was increased. This may be due to unequal sensitivity of the cells, and to the fact that conditions necessitating withdrawal of cellular fluid were not so urgent as those demanding the converse movement, which occurs in acidosis. For lack of data regarding the normal variability in the distribution of the cells of young infants, it is impossible to say whether there was any variation from normal in the cases of pyloric stenosis examined. In pyloric stenosis there is increase in the cell count from blood concentration, due to vomiting and inadequate intake of fluid.

In early infancy the diameter of the red cells is much greater than in older children and adults. The red cells probably attain normal size about the sixth month and certainly in the first year. In spite of these differences, a microcytosis occurs in alkalosis, but there again there is little correlation between the degree of alkalosis as estimated by the respiratory rate, which is recognized to be proportional to the amount of carbon dioxide in the blood.

Price-Jones has suggested that carbon dioxide is the immediate factor in producing changes in cell size. Since increase in cell diameter occurs both in gaseous and in non-gaseous acidosis, and decrease both in gaseous and non-gaseous alkalosis, it cannot be either the free or the total carbon dioxide which is the factor, but rather the ratio of the free to the combined carbon dioxide, i.e., the cell size varies as the pH of the blood, increasing with decreased pH and vice versa.

(5) SUMMARY AND CONCLUSIONS.

In acidosis, whether occurring in disease or induced by drugs, the red corpuscles are enlarged, the cell apparently retaining its normal shape. This may be accompanied by an increase in the red cell count, which, when it occurs, is probably due to blood concentration.

It is suggested that the increase in cell size in addition to the anhydraemia may be a further cause of increased blood viscosity, and may contribute to the tendency to circulatory failure in acidosis.

Alkalosis causes a reduction in the size of the corpuscles, without change in shape, or haemoglobin content. There is probably no change in the red cell count due to alkalosis per se.

The corpuscles in early infancy, in spite of their different features, react to alkalosis in the same way as those of the older individual.

APPENDIX.

APPENDIX.

The detailed experiments on the mechanism of the production of anaemia in erythroblastosis foetalis (Section E).

A. TO FIND IF THERE IS A FREE LYSIN IN THE BLOOD IN ICTERUS GRAVIS.

Table 1. Using defibrinated corpuscles. Incubation time - 1 hour.

				Control
Packed corpuscles of a normal adult (c.c.)	0.5	0.5	0.5	0.5
Serum from a case of icterus gravis (c.c.)	0.125	0.25	0.5	0
Normal saline (c.c.)	0.375	0.25	0	0.5
Lysis	0	0	0	0

Table 2. Using citrated blood. Incubation time - 1 hour.

Packed corpuscles of a normal adult (c.c.)	0.5	0.5	0.5	0.5
Serum from a case of icterus gravis (c.c.)	0.125	0.25	0.5	0
Normal saline	0.375	0.25	0	0.5
Lysis	0	0	0	0

B. TO FIND IF THE CORPUSCLES IN ICTERUS GRAVIS ARE UNDULY FRAGILE IN THEIR NATURAL OR IN NORMAL ADULT SERUM.

Table 3. Using defibrinated corpuscles. Incubation time - 1 hour.

				Control
Packed R.B.C. from a case of icterus gravis (c.c.)	0.5	0.5	0.5	0.5
Fresh adult serum (c.c.)	0.125	0.25	0.5	0
Normal saline (c.c.)	0.375	0.25	0	0.5
Lysis	0	0	0	0

A similar negative result was obtained with citrated blood.

C. TO FIND THE EFFECT OF AN ANTIHUMAN RED CELL SERUM ON THE RED CORPUSCLES OF THE NEONATAL ANAEMIAS.

(1) The preparation of the serum has already been described (p.130). It was found to have a negligible effect on human red corpuscles unless complement were added.

(2) To show that complement had a negligible haemolytic action on human red cells. The complement was obtained from guinea-pig serum.

Table 4. To find if complement has any haemolytic action on human red corpuscles.

3% suspension of R.B.C. (c.c.)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Complement (c.c.)	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10	Normal saline 0.1
Lysis	0	0	0	0	0	0	0	v.f.tr-	v.f.tr-	v.f.tr-	0

(3) To find the minimum haemolytic dose of the anti-human red cell serum on normal human corpuscles.

Table 5.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S. (c.c.of 1/1,000 dilution)	0.02	0.04	0.06	0.08	0.10	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	0
Complement (c.c.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lysis	0	0	v.f.tr.	v.f.tr.	v.f.tr.	f.tr.	d.	v.m.	v.m+	j.c.	j.c.	j.c.	j.c.	j.c.	j.c.	0

H.S. = antihuman red cell serum. M.H.D. = 0.002 c.c.

It was found convenient to use dilutions of 1/20 and 1/40 of the antihuman red cell serum as in Table 6.

Table 6. To find the minimum haemolytic dose of antihuman red cell serum for normal adult red corpuscles. (Incubation time - 1 hour).

						Controls	
R.B.C.(c.c.3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c.of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.3	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	0	v.f.tr.	f.tr.	d.	m.	0	0

						Controls	
R.B.C.(c.c.of 3% suspension).	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	j.c.	c.	c.	c.	c.	0	0

$\therefore$ M.H.D. of I.B. $= \frac{0.01 \times 2}{20} = 0.001$ c.c.

N.B. Defibrinated corpuscles were used.

Table 7. To find the minimum haemolytic dose of anti-human red cell serum for normal adult red corpuscles, using citrated blood. (Incubation time - 1 hour).

						Controls	
R.B.C.(c.c.3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c.of 1/40 diln.)	0.01	0.015	0.02	0.25	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	m.	v.m.	a.c.	a.c.	c.	0	0

M.H.D. of I.B. $= \frac{0.03 \times 2}{40} = 0.0015$ c.c.

						Controls	
R.B.C.(c.c. of 3% suspension).	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	j.c.	c.	c.	c.	c.	0	0

M.H.D. of I.B. $= \frac{0.01 \times 2}{20} = 0.001$ c.c.

Average M.H.D. of I.B. = 0.00125 c.c.

(4) To find the effect of the anti-human red cell serum on the corpuscles of normal babies (Tables 8, 9, 10, 11) and a premature infant (Table 12).

Table 8. Baby C. Age: 5 days.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	tr.	a.c.	a.c.	j.c.	c.	0	0

M.H.D. of I.B. = .00125 c.c.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	a.c.	j.c.	c.	c.	c.	0	0

M.H.D. of I.B. = 0.0015 c.c.

Average M.H.D. of I.B. = 0.00137 c.c.

Table 9. Baby B. Age: 4 days.

						Controls	
R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	v.f.tr.	v.f.tr.	m.	j.c.	c.	0	0

M.H.D. of I.B. = 0.00125 c.c.

						Controls	
R.B.C.(c.c.of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	j.c.	c.	c.	c.	c.	0	0

M.H.D. of I.B. = 0.001 c.c.

Average M.H.D. of I.B. = 0.00112 c.c.

<u>Table 10.</u> Baby W. Age: 8 days.

R.B.C.(c.c.of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	d.	m.	v.m.	a.c.	a.c.	0	0

M.H.D. of I.B. = > 0.0015 c.c.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	m.	v.m.	a.c.	j.c.	c.	0	0

M.H.D. of I.B. = 0.0025 c.c.

<u>Table 11.</u> Baby McC. Age: 6 days: Icterus neonatorum.

						Controls	
R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	f.tr.	f.tr.	d.	d.	m.	0	0

M.H.D. of I.B. = > 0.0015 c.c.

						Controls	
R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	d.	m.	v.m.	j.c.	c.	0	0

M.H.D. of I.B. = 0.0025 c.c.

Table 12. Baby R. Age: 4 days. Prematurity.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	v.f.tr.	v.f.tr.	f.tr.	d.	d.	0	0

R.B.C.(c.c.of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	d.	m.	m.	v.m.	a.c.	0	0

M.H.D. of I.B. > 0.003 c.c.

(5) To find the effect of the antihuman red cell serum on the corpuscles of cases of neonatal haemolytic anaemia.

In each experiment the tubes were incubated at 37°C. for 1 hour.

Table 13. Case of icterus gravis. Age: 24 hours.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	tr.	m.	a.c.	j.c.	j.c.	0	0

M.H.D. of I.B. = 0.00125.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	j.c.	j.c.	c.	c.	c.	0	0.

M.H.D. of I.B. = 0.001 c.c.

Average M.H.D. of I.B. = 0.001125 c.c.

Table 14. Case of icterus gravis. Age: 7 days.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	c.	c.	c.	c.	c.	0	0

R.B.C.(c.c. of 3% suspension	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	c.	c.	c.	c.	c.	0	0

M.H.D. of I.B. = > 0.0005 c.c.

Table 15. Case of anaemia without oedema or jaundice. Age: 9 days.

R.B.C.(c.c.of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	v.f.tr.	v.f.tr.	f.tr.	d.	d.	0	0

R.B.C.(c.c.of 3% suspension	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	d.	m.	m.	a.c.	j.c.	0	0

M.H.D. of I.B. = 0.003 c.c.

Table 16. Case of icterus gravis. Age: 12 days.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	v.f.tr.	tr.	tr.	tr.	d.	0	0

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	tr.	d.	m.	j.c.	c.	0	0

M.H.D. of I.B. = 0.00125 c.c.

Table 17. Case of icterus gravis. Age: 3 weeks.

R.B.C.(c.c.of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	tr.	d.	d.	m.	v.m.	0	0

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	m.	v.m.	a.c.	j.c.	c.	0	0

M.H.D. of I.B. = 0.00125 c.c.

Table 18. Case of icterus gravis. Age: 3 weeks.

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/40 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	m.	v.m.	v.m.	v.m.	v.m.	0	0

R.B.C.(c.c. of 3% suspension)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
H.S.(c.c. of 1/20 diln.)	0.01	0.015	0.02	0.025	0.03	0.03	0
Complement (c.c.)	0.05	0.05	0.05	0.05	0.05	0	0.05
Lysis	m.	v.m.	j.c.	c.	c.	0	0

M.H.D. of I.B. = 0.002 c.c.

D. TO FIND IF THERE WAS A HAEMOLYTIC AGENT IN THE LIVER OR SPLEEN, USING SALINE AND ALCOHOLIC EXTRACTS AS DESCRIBED IN SECTION E. (p. 136).

In the following experiments with tissue extracts, the saline extracts were diluted with an equal quantity of normal saline, and one part of the alcoholic extracts to 6 parts of normal saline was used.

Table 19. Saline liver extract: dil. $\frac{1}{2}$. Incubation time - 1 hour.

Extract (c.c.)	.1	.2	.3	.4	.5	0
R.B.C. from icterus gravis (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	0	0	0	0	0	0

Identical results were found in similar experiments done with alcoholic extract of liver, and saline and alcoholic extracts of spleen.

E. TO FIND IF THERE WAS AN ANTIGENIC HAEMOLYTIC PRINCIPLE IN THE LIVER OR SPLEEN IN ICTERUS GRAVIS TO WHICH AN ANTI-HAEMOLYSIN WAS PRESENT (1) IN NORMAL SERUM BUT (2) ABSENT IN ICTERUS GRAVIS.

(1) (a) Using normal adult serum with liver extracts.

Table 20. Saline extract of liver. Complement of serum destroyed.

M.H.D. of added complement = 0.005 c.c.

					Controls	
Saline extract of liver (c.c. of $\frac{1}{2}$ dil.)	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c.(c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0	c.

As in all the following experiments in this appendix, the tubes containing the tissue extract, serum, and complement were incubated for $1\frac{1}{4}$ hours at 37°C., and after sensitized red corpuscles had been added for a further $1\frac{1}{2}$ hours.

Table 21. Alcoholic extract of liver. Complement of serum destroyed. M.H.D. of added complement = 0.005 c.c.

					Controls	
Alcoholic extract (dilution $^1/7$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c.(c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	a.c.	c.	c.	c.	0.	c.

(1) (b) Using normal adult serum with splenic extracts.

Table 22. Saline extract of spleen. Complement of serum destroyed by heating. M.H.D. of added complement = 0.0025 c.c.

					Controls	
Saline extract ($\frac{1}{2}$ dilution) c.c.	0.5	0.5	0.5	0.5	0.5	0
Normal serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0.	c.

<u>Table 23</u>. Alcoholic extract of liver. Complement of serum destroyed by heat. M.H.D. added of complement = 0.0025 c.c.

					Controls	
Alcoholic extract (dilution $^{1}/7$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0.	c.

(2) (a) Using serum from cases of icterus gravis with liver extracts.

<u>Table 24</u>. Saline extract of liver. Serum from Case 7 (icterus gravis). Complement destroyed. M.H.D. of added complement = 0.0025 c.c.

					Controls	
Saline extract (dilution $\frac{1}{2}$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0.	c.

Table 25. Alcoholic extract of liver. Serum from Case 7 (icterus gravis). Complement destroyed. M.H.D. of added complement = 0.005 c.c.

					Controls	
Alcoholic extract (dilution 1/7) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	m.k.	c.	c.	c.	0	c.

Table 26. Saline extract of liver. Serum from Case 17 (icterus gravis) - complement destroyed. M.H.D. of added complement = 0.0025 c.c.

					Controls	
Saline extract (dilution ½) c.c.	0.5	0.5	0.5	0.5	0.5	0.5
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	m.k.	c.	c.	c.	0	c.

Table 27. Alcoholic extract of liver. Serum from Case 17 (icterus gravis) - complement destroyed. M.H.D. of added complement = 0.0025 c.c.

					Controls	
Alcoholic extract (dilution 1/7) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0	c.

(2) (b) Using serum from cases of icterus gravis with extracts of spleen.

Table 28. Saline extract of spleen. Serum from Case 7 (icterus gravis) - complement destroyed. M.H.D. of added complement = 0.005 c.c.

					Controls	
Alcoholic extract (dilution 1/7) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	m.k.	c.	c.	c.	0	c.

Table 29. Alcoholic extract of spleen. Serum from Case 7 (icterus gravis) - complement destroyed. M.H.D. of added complement = 0.005 c.c.

					Controls	
Alcoholic extract (dilution $^1/7$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	j.c.	c.	c.	c.	0	c.

Table 30. Saline extract of spleen. Serum from Case 17 (icterus gravis) - complement destroyed. M.H.D. of added complement = 0.0025 c.c.

Saline extract (c.c. of $\frac{1}{2}$ dilution)	0.5	0.5	0.5	0.5	0.5	0
Serum (icterus gravis) c.c.	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0	c.

Table 31. Alcoholic extract of spleen. Serum from Case 17 (icterus gravis) - complement destroyed. M.H.D. of added complement = 0.0025 c.c.

Alcoholic extract (dil. 1/7) c.c.	0.5	0.5	0.5	0.5	0.5	0.5
Serum (icterus gravis) c.c.	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized ox r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	d.	c.	c.	c.	0	c.

F. TO FIND IF IN ICTERUS GRAVIS THERE WAS AN ABNORMALITY OF THE RED CORPUSCLES WHICH BY ACTING IN COMBINATION WITH ABNORMALITIES OF THE TISSUES OR BOTH COULD FIX COMPLEMENT AND CAUSE LYSIS.

The experiments were performed as in the previous section using the tissue extracts and serum from cases of icterus gravis, but substituting sensitized red corpuscles from cases of icterus gravis for sensitized ox red corpuscles. The cells were sensitized by the addition of 5 minimum haemolytic doses of antihuman red cell serum.

(1) Liver extracts.

Table 32. Saline extract of liver. Serum from Case 20 (icterus gravis) - complement destroyed. R.B.C. from Case 1 (icterus gravis). M.H.D. of added complement = 0.005 c.c.

					Controls	
Saline extract (dilution $\frac{1}{2}$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0.	c.

Table 33. Alcoholic extract of liver. Serum from Case 20 (icterus gravis) - complement destroyed. R.B.C. from Case 1 (icterus gravis). M.H.D. of added complement = 0.005 c.c.

					Controls	
Alcoholic extract (dilution $^1/7$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	d.	c.	c.	c.	0.	c.

(2) Splenic extracts.

Table 34. Saline extract of spleen. Serum from Case 25 (icterus gravis) - complement destroyed. R.B.C. from Case 1 (icterus gravis). M.H.D. of added complement = 0.005 c.c.

					Controls	
Saline extract (dilution $\frac{1}{2}$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0.	c.

Table 35. Alcoholic extract of spleen. Serum from Case 20 (icterus gravis) - complement destroyed. R.B.C. from Case 1 (icterus gravis). M.H.D. of added complement = 0.005 c.c.

					Controls	
Alcoholic extract (dilution $^1/7$) c.c.	0.5	0.5	0.5	0.5	0.5	0
Serum (c.c.)	0.05	0.05	0.05	0.05	0	0.05
Doses of complement	2	4	6	8	2	2
Sensitized r.b.c. (c.c. of 3% susp.)	0.5	0.5	0.5	0.5	0.5	0.5
Lysis	c.	c.	c.	c.	0.	c.

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CASE HISTORIES.*

A. Icterus Gravis. 1933-36 Series.

Case I. Baby B., male.

Family History.

Father aged 30 years, mother aged 29 years; both healthy. 4 pregnancies - 1st child healthy; 2nd died of jaundice of the new-born; 3rd stillborn; 4th Patient.

Case History.

Full-time child. Jaundice first noticed at the age of 24 hours. Jaundice progressive. Breast-fed child, and lusty.

Condition on admission, aged 36 hours.

Very jaundiced, but vigorous when roused. Umbilicus healthy. Liver enlarged - lower border 2 fingers-breadth below the costal margin. Spleen enlarged - lower pole 2 fingers-breadth below the costal margin.

Urine: bile pigments ++; urobilin not found; urobilin absent from stools in 3rd week.

Course.

Child transfused (70 c.c. of blood) on admission, and on the 15th day.

Treated as an out-patient after the first transfusion. Jaundice did not clear, although the child maintained its general condition until the 19th day, when it developed gastro-enteritis and broncho-pneumonia on the 16th day. He died on the 19th day of life.

The spleen became smaller, but remained palpable until death (½-finger-breadth).

Course afebrile until the 16th day.

Blood count: see Table I.

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*As the leucocyte and differential counts before treatment have been given in the text, differential counts have been omitted from these summaries.

Post-mortem Findings.

The body was that of a small deeply jaundiced male infant.

Thorax: The thymus and thoracic glands small. Both lungs show scattered foci of early broncho-pneumonic consolidation, most marked at the left base. Heart nil.

Abdomen: Peritoneal sac and mesenteric glands nil. Liver of increased size and of deep green colour, showed no evidence of cirrhosis on surface or in substance. Main bile-ducts were widely patent in both lobes, and the gall-bladder was large and thin-walled. Spleen slightly enlarged and soft. Kidneys rather swollen and congested although the latter appearance was partly masked by the deep bile-staining.

Head: Bile-staining of the brain substance was noted. Cerebral haemorrhage of rather diffuse but not massive type as if from a number of small bleeding vessels was present in the substance of the left cerebral hemisphere. The brain substance was markedly softened throughout and it was not possible to define the limits of the haemorrhage. The superior longitudinal sinus, like the great venous sinuses, contained only a small amount of red thrombus.

Bone-Marrow: The marrow of the left femur was of the red formative type.

Histology.

Liver: Sections deeply bile-stained; green. Great derangement and damage to liver cells which contain large amounts of brown pigment. Frequent small syncitia formed by degenerated cells. Phagocytosis abundant. Küpffer cells swollen. Erythroblastosis scanty. Haemosiderosis abundant in liver and Küpffer cells. Fine fibrosis, especially near portal tracts.

Spleen: Marked congestion. Malpighian bodies small and ill-defined. Erythropoiesis absent. Active phagocytosis. Heavy iron deposit in phagocytes and also in fibrous tissue and reticulum.

Kidneys: Cloudy swelling. No erythropoiesis or haemosiderosis. No fibrosis.

Lymph Glands: Of the haemal type. Congested. Marked phagocytosis. Lymph follicles deficient in lymphoblasts. Small iron deposit in phagocytes.

Bone-Marrow: Erythropoiesis diminished. Shift to the left and premature haemoglobinization of erythroblasts. Leucopoiesis active. Chiefly neutrophile myelocytes and premyelocytes. Eosinophiles present. Megakaryocytes scanty. No iron or phagocytes.

TABLE I.

Case 1. Blood Counts.

Age in days.	2		4	9	13	15		16	18
R.B.C. (millions per c.mm.)	2.5	Transfused.	3.66	3.57	3.75	3.88	Transfused.	5.08	4.67
Haemoglobin %	60								
Reticulocytes %	12.2		14.0	4.3	6.4	7.0		6.0	2.5
Leucocytes per c.mm.	20,500		12,200	9,300	10,300	29,000		28,600	46,200
Nucleated red cells per c.mm.	20,500		1,098	1 seen	206	380		572	2 seen
Bleeding time	-		-	10 mins.	-	-		-	-

Case 2. Alistair B., male, aged 8 days.

Family History.

Father aged 32 years, alive and well; mother aged 28 years, alive and well. 2 pregnancies - (1) Male, aged 5 years, alive and well; (2) the patient.

Case History.

Surgical induction became necessary at 7½ months, because of toxaemia of pregnancy. Child healthy and vigorous at birth. Jaundice noticed shortly after; gradually increased. Child artificially fed.

Condition on admission.

Small premature child; weight 1.87 Kilo. Sutures of skull overlapping. Child drowsy.

Heart and lungs nil.

Abdomen: Liver - lower border palpable 1 finger-breadth below the costal margin. Spleen just palpable.

Urine: bile pigments ++.

Blood count: R.B.C. 1.5 millions per c.mm; Hb. 45% (Sahli); reticulocytes 3%; leucocytes 9,300 per c.mm.

Van den Bergh reaction: direct + biphasic; indirect, 88 units.

Course.

Transfused (70 c.c. of blood) on admission. Died 36 hours later.

Post-mortem Findings.

Skin and mucous membranes deeply jaundiced.

Thorax: Organs fairly deeply bile-stained. Heart, lungs and thymus nil.

Abdomen: Peritoneum nil.
Liver of deep greenish-brown colour. Bile can be readily expressed from the gall-bladder into the duodenum. No suggestion of cirrhosis of the liver.
Spleen enlarged and slightly soft. Malpighian bodies not prominent.
Kidneys small and deeply bile-stained.
Mesenteric glands, stomach and bowel nil.
Suprarenal glands and pancreas nil.

Head: Bones and great venous sinuses nil.
Middle ears nil.
Meninges deeply bile-stained. Slight bile-staining in lenticulate nucleus.

Bone-Marrow: Very little marrow in femur and ribs. Marked osteosclerosis.

Histology.

Liver: Some degeneration of liver cells. Sinusoids wide. Congested. Erythropoiesis abundant. Phagocytosis marked. Heavy iron deposit, portal in distribution. Thickening of reticulum.

Spleen: Congested. Scanty Malpighian bodies. Erythropoiesis present. Phagocytosis active. Heavy iron deposit. Thickening of reticulum.

Kidneys: Cloudy swelling of tubules.

Lymph Glands: Haemal type. Phagocytosis present. Lymph-nodes deficient. No erythropoiesis.

Bone Marrow: Active erythropoiesis. Premature haemoglobinization and active division of erythroblasts. Megaloblasts fairly numerous. Megakaryocytes scanty. Neutrophile myelocytes very numerous. Eosinophiles moderately frequent. No iron or phagocytes.

Case 3. Annie C., female.

Family History.

Father aged 45 years, healthy; mother aged 37 years, healthy. 10 pregnancies; 8 healthy children; 1 stillbirth.

Case History.

Pregnancy normal. Labour at full-time. Jaundice of baby first noticed at 3rd day. Pemphigus at 4 weeks. Pneumonia at 6 weeks.

Condition on admission.

Child very ill (pneumonia). Markedly jaundiced. Liver and spleen enlarged.
Urine: bile pigments ++; urobilin nil.
Blood count (8 weeks of age): R.B.C. 4.0 millions per c.mm; Hb. 88% reticulocytes 4½% leucocytes 21,000 per c.mm.

Course.

The child died at the age of 8 weeks. Urobilin in stools variable.

Post-mortem Findings.

	Poorly-nourished child. Jaundiced.
Thorax:	Right lung - pneumonia with empyema.
Abdomen:	Localised acute peritonitis of upper abdomen above the liver and spleen.
	Liver enlarged and deeply bile-stained.
	Gall-bladder normal.
	Spleen enlarged and congested.
	Kidneys pale and deeply bile-stained.
Head:	No abnormality.
Bone Marrow:	Osteo-sclerosis of femur marrow cavity. Marrow fairly abundant at lower end of shaft.

Histology.

Liver: Very marked degeneration and disarrangement of liver cells. Areas of necrosis. Pigment containing phagocytes abundant. Scanty erythropoiesis. Some leucopoiesis near portal tracts. Scanty iron deposit of greenish crystalline character. Slight increase of fibrous tissue round portal tracts and central vessels. Thickened reticulum.

Spleen: Congested. Ill-defined Malpighian bodies. Erythropoiesis absent. Phagocytosis abundant. Heavy greenish crystalline iron deposit. Slight thickening of connective tissue of trabeculae and vessels, and definite thickening of reticulum.

Kidneys: Nephrogenic tissue present. One or two small foci of erythropoiesis in arterioles near glomeruli. Pigment containing cells in wall of proximal tubules. Degeneration of tubular walls with intertubular oedema. Scanty haemosiderosis in walls of proximal tubules. No fibrosis.

Bone-Marrow: Active leucopoiesis; chiefly neutrophile myelocytes and premyelocytes. Erythropoiesis less prominent. Preponderance of prematurely haemoglobinised erythroblasts. Megakaryocytes present. No phagocytosis or iron.

Case 4. David D., male.

Family History.

Father aged 32 years, healthy; mother aged 27 years, healthy. 6 pregnancies - (1) Male, aged 6½ years, alive and well; (2) Twins, one stillborn, the other now aged 5 years, and well; (3) Male, aged 4 years, well; (4) Female, aged 2½ years, well; (5) Male, died of icterus gravis and intra-cranial haemorrhage (Case 5); (6) Patient.

Case History.

Pregnancy and labour normal. Child lusty at birth. Feet and hands oedematous (transient). On 4th day jaundice of hands and feet first seen. Colour gradually spread to face and trunk, and deepened. No vomiting. Child breast-fed.

Condition on admission on 7th day.

Well-developed child - 3.38 Kilo. Deep yellow jaundice, slight tinge of green. Afebrile. No oedema or haemorrhages. Liver + 3 fs.b. below costal margin. Spleen + 2 fs.b. Chest, heart and nervous system nil.

Urine: bile pigments +; urobilin nil. No urobilin in stools.

Blood count: R.B.C. 1.63 millions per c.mm; Hb. 45% (Sahli); colour index 1.4; reticulocytes 11.1%; leucocytes 29,000 per c.mm.

Course.

Transfused (60 c.c. of father's blood) on admission. Died suddenly 9 hours later while feeding.

Post-mortem Findings.

	Fairly well-nourished child. Deeply jaundiced.
Thorax:	Nothing abnormal except jaundice.
Abdomen:	Liver enlarged and of a green colour.
	Spleen enlarged and soft; congested.
	Kidneys jaundiced.
	Suprarenals, pancreas, stomach, intestines and mediastinal glands nil.
Head:	Longitudinal sinus thrombosis. Right sub-dural haemorrhage. Brain nil.
Bone-Marrow:	Extensive sclerosis of marrow cavity of femur. Little marrow tissue. Marrow could not be expressed from the ribs.

Histology.

Liver:	Degeneration of liver cells; contain brown pigment. Diffuse fatty infiltration. Phagocytosis moderate. Erythropoiesis scanty. Slight fine fibrosis. Haemosiderosis scanty.
Spleen:	Hyperplasia of lymph tissue. Congestion of pulp. No erythropoiesis. Abundant phagocytes. Excess of fibrous tissue. Scanty haemosiderosis of phagocytes.
Kidneys:	Cloudy swelling of tubules.
Bone-Marrow:	Erythropoiesis hypoplastic. Marked shift to the left. Leucopoiesis unaffected; numerous neutrophile myelocytes. Phagocytosis and haemosiderosis absent.

Case 5. Frederick D., male.

Family History.

As for Case 4.

Case History.

Normal pregnancy and labour. Slight jaundice at birth. Breast-fed and took well. Meconium normal. Stools thereafter yellow until the 3rd day when they became pale. Urine dark. Child became drowsy on 3rd day. Jaundice became steadily deeper. Refused feeds on 5th day.

Condition on admission, aged 6 days.

Very deeply jaundiced infant. Heart and lungs nil. Umbilicus clean. Nervous system nil. Liver enlarged - 2 fs.b. below costal margin. Spleen not palpable. Stools not acholic but pale.

Urine: bile pigments ++; urobilin nil.

Wassermann reaction: negative.

Van den Bergh reaction: direct biphasic; indirect positive, 30 units.

For blood counts on admission and subsequently see Table 2.

Course.

First transfused when 10 days old (100 c.c. of blood). At 18 days, jaundice was deeper. Liver palpable 3 fs.b. below the costal margin, spleen 1 f.b. below the costal margin.

Urine: bile pigments +; urobilin nil.

21 days old: jaundice still very deep; greenish tinge.

22 " " : transfused 100 c.c. of blood.

Started to vomit on 32nd day and died next day.

Post-mortem Findings.

Marked jaundice of skin and internal organs except the brain.

Thorax: Nothing abnormal except jaundice.

Abdomen: Liver enlarged and deeply jaundiced. Gall-bladder contracted and containing a small quantity of concentrated bile. No obstruction of bile-ducts.
Spleen not enlarged, but red and soft.
Kidneys nil except jaundice.
Stomach, bowel, pancreas, suprarenal and mesenteric glands nil.

Brain: Petechial haemorrhages into dura mater. On section of the brain both lateral ventricles were distended, with deep yellow staining of their walls in which some granular yellowish pigment was embedded - the result of previous haemorrhage. Actual blood or blood-stained material was not present in the ventricles. A recent extensive haemorrhage had occurred into the substance of the pons and cerebellum, chiefly towards the right side, where the brain substance was destroyed by red thrombus.
Basal ganglia normal.

Bone-Marrow: Osteo-sclerosis of medullary cavities of femur and ribs.

Histology.

Liver: Marked degeneration with small areas of necrosis of liver cells. Erythropoiesis abundant. Phagocytosis marked. Moderate iron deposit, chiefly in Küpffer cells. Bile thrombi in ducts. Slight thickening of connective tissue round central veins.

Spleen: Congested. Erythropoiesis marked. Abundant phagocytosis. Malpighian bodies practically normal. Heavy iron deposit in phagocytes, fibrous tissue and reticulum. No fibrosis.

Kidney: Cloudy swelling of tubular epithelium and exudate in capsule of Bowman. Thickening of basement membranes.

Lymph Glands: Lymphoid tissue normal. Phagocytosis present, but no erythropoiesis. Small iron deposit in phagocytes.

Bone-Marrow: Leucopoiesis active. Chiefly neutrophile myelocytes. Erythropoiesis less marked. Megaloblasts and primitive haemoglobinized erythroblasts frequent. Abundant eosinophile myelocytes. Megakaryocytes scanty. No phagocytosis or haemosiderosis.

TABLE 2.

Case 5. Blood Counts.

Age in days.	6	7	8	9	Transfused.	13	15	16	18
R.B.C. (millions per c.mm.)	2.8	2.38	1.94	2.07		3.42	2.63	2.57	2.29
Haemoglobin %			43	60		68			
Reticulocytes %	15		18	17		5.2	7.8	16.0	8.0
Leucocytes per c.mm.	22,600		14,000				14,800	12,400	10,000
Nucleated red cells per c.mm.	2,812		Occa-sional				444	165	195
Bleeding time									

Age in days.	20	Transfused.	23	25	26	27	29
R.B.C. (millions per c.mm.)	2.07		3.83	3.48	3.86	3.32	3.25
Haemoglobin %							
Reticulocytes %	7.8		7.5	6.0	2.0	1.5	2.5
Leucocytes per c.mm.	13,000		10,800	10,400		13,900	14,200
Nucleated red cells per c.mm.	Nil		Nil	Nil	Nil	Nil	Nil
Bleeding time							12 mins.

Case 6. Simon D., male.

Family History.

Father and mother aged 32, and well. 7 pregnancies - (1) Male, aged 9 years, well; (2) Female, died at 4 years of measles and whooping cough; (3) Miscarriage at 3 months; (4) Female, aged 5 years, well; (5) Male, aged 3 years, well; (6) Female, aged 1½ years, well; (7) Patient.

Case History.

Normal pregnancy and labour. Child born at full-time. Neonatal jaundice absent. Child apparently healthy. On the 15th day of life, he became jaundiced. General condition good and fed well. Urine dark brown and stools pale.

Condition on admission, aged 16 days.

Child in good general condition; weight 4.2 Kilo. Jaundice marked. Umbilicus slightly septic. Liver not enlarged. Spleen - tip just palpable.

Urine: bile pigments ++; urobilin +; urobilin in stools.

For blood count on day of admission and thereafter see Table 3. Van den Bergh reaction: biphasic 18 units.

Course.

Transfused (90 c.c. of blood) on 17th day, and again on the 18th day. Next day child not nearly so jaundiced. Still some bile pigments in the urine.

On the 29th day the child was well although pale. Jaundice absent. Bile pigments absent from the urine. Spleen not palpable.

On the 42nd day the baby became jaundiced again. Bile pigments present in urine. Spleen palpable.

46th day: still jaundiced, but bile pigments absent from urine. Jaundice cleared within the next 4 days.

Course afebrile. Child recovered.

Subsequent History.

The child was well until 7 months old when he died of an acute illness associated with fever and convulsions. Physical examination was negative. The blood count was: R.B.C. 4.63 millions per c.mm; Hb. 80% (Haldane); reticulocytes $<1\%$; leucocytes 14,200 per c.mm.

A definite diagnosis was not made, and a post-mortem examination was not permitted.

TABLE 3.

Case 6. Blood Counts.

Age in days.	16		18		20	22	24	26	29	31	36	42
R.B.C. (millions per c.mm.)	2.02	Transfused.	3.34	Transfused.	4.48	3.0	3.16	3.25	3.18	3.13	3.35	2.45
Haemoglobin %	55		72		76		70	69	70	65	60	47
Reticulocytes %	7.5		4.3		<1		2.6	1	1	2.5	3.4	7.0
Leucocytes per c.mm.	12,930											
Nucleated red cells per c.mm.	Nil											

Age in days.	46	50	57	65	79	90
R.B.C. (millions per c.mm.)	3.18	4.29	4.15	3.6	3.62	3.7
Haemoglobin %	75	75	73	74	70	72
Reticulocytes %	2.6	<1	<1	<1	<1	<1
Leucotytes per c.mm.						
Nucleated red cells per c.mm.						

Case 7. Edmund G., male.

Family History.

Father and mother healthy. 7 pregnancies - (1) Child well; (2) Child well; (3) Icterus gravis - died in 1st week; (4) Icterus gravis - died in 1st week; (5) Healthy child - died of pneumonia in 1st year; (6) Icterus gravis - died in 2nd week; (7) Patient.

Case History.

Full-time child. Jaundiced on 1st day. This progressive; otherwise child well.

Admitted on 3rd day. Markedly jaundiced child. Liver and spleen enlarged. Urine: bile pigments present, but no urobilin. Very drowsy. Examination of nervous system negative.

Blood count: R.B.C. 2.02 millions per c.mm; Hb. 49%; reticulocytes 4%; W.B.C. 14,000 per c.mm.

Course.

Died on 4th day.

Post-mortem Findings.

Nothing of importance except jaundice in Thorax.

Abdomen: Liver green in colour; no obstruction to bile-ducts. Spleen enlarged, red and soft. No gross abnormalities of other organs.

Head: Kernicterus; caudate and lenticular nuclei deeply bile-stained.

Bone-Marrow: Marked osteosclerosis of medullary cavity of femur. Little marrow in ribs.

Histology.

Liver: Degeneration of liver cells considerable. Erythropoiesis moderate. Pigment in cells and bile thrombi in ducts. Moderate iron deposit in liver cells and phagocytes which are abundant. No fibrosis.

Spleen: Congested. Some erythropoiesis. Malpighian bodies ill-defined. Phagocytes present. Small iron deposit. Thickening of fibrous tissue.

Kidneys: Cloudy swelling of tubules.

Bone-Marrow: Shift to the left of erythropoietic cells. Fer normoblasts. Neutrophile myelocytes abundant. Leucopoiesis more active than erythropoiesis.

Case 8. Ralph J., male.

Family History.

Father aged 27 years, mother aged 26 years; both well. 4 pregnancies - (1) Female, aged 5 years, well; (2) Male, aged 3 years, well; (3) Female, aged 2 years, well (transient icterus after birth); (4) Patient.

Case History.

Normal pregnancy. Labour at full-time. Child normal at birth. Jaundice first noticed after 24 hours, and thereafter gradually deepened. Vomited. Urine dark.

Condition on admission, aged 24 days.

Intensely jaundiced child - definite green tinge in skin. Anaemia obvious from pallor of mucous membranes. Liver palpable 2 fs.b. below the costal margin. Spleen palpable 1 f.b. below costal margin.

Urine: albumen +; bile pigments ++; urobilin +. Urobilin in stools.

Heart and lungs - nothing abnormal. Haemorrhages into skin and mucous membranes absent.

Van den Bergh reaction: direct biphasic; indirect positive, 14 units.

For blood counts: see Table 4.

Course.

Transfused (90 c.c. into median basilic vein) on day of admission, and again on the 31st day of life. About this time diarrhoea started and continued until death. Intravenous and intraperitoneal salines were given frequently, and a further transfusion was performed on the 43rd day. From the 48th day onwards fever and signs of broncho-pneumonia were present. By that time jaundice was slight. The child died on the 56th day of life.

Post-mortem Findings.

Very little jaundice.

Thorax: Heart - no lesion of note. Moderate bile-staining of the intima of the great vessels and endocardium.
Lungs - red broncho-pneumonic consolidation, chiefly in the right lung and in left lower lobe.
Thymus and thoracic glands - nothing abnormal.

Abdomen:	Peritoneal sac healthy. Liver congested and slightly green. Atresia or stenosis of bile-ducts absent. Spleen slightly enlarged, dark red and congested. Kidneys pale, with slight bile-staining. Suprarenal and mesenteric glands and stomach normal. Mucosa of small intestines and to a less extent of large intestine, congested and oedematous.
Head:	Bones and great venous sinuses normal. Purulent exudate in both middle ears. Kernicterus or other lesion of brain absent.
Bone-Marrow:	The cavity of the right femur was sclerotic and the marrow tissue scanty.

Histology.

Liver:	Moderate degeneration of cells. Phagocytosis abundant. Bile thrombi numerous. Erythropoiesis absent. Moderate iron deposit. Fine pericellular fibrosis.
Spleen:	Congested. Phagocytosis abundant. Malpighian bodies small and ill-defined, with deficient lymphoblasts. No erythropoiesis. Heavy iron deposit in phagocytes. Fibrous tissue slightly impregnated. Thickening of reticulum.
Lymph Glands:	Lymphoid tissue deficient. Phagocytes numerous. Contain iron.
Kidneys:	Some cloudy swelling of tubular epithelium.
Bone-Marrow:	Definite diminution of erythropoietic tissues. Only very scanty islets of erythroblasts. Leucopoiesis active. Eosinophiles present. Megakaryocytes very frequent. No phagocytosis or haemosiderosis.

TABLE 4.

Case 8. Blood Counts.

Age in days.	24		26	27	30	31		32	36	39		42	
R.B.C. (millions per c.mm.)	1.7	Transfused.	3.7	3.2	3.09	2.68	Transfused.	3.76	3.01	3.18	Transfused.	2.87	Transfused.
Haemoglobin %	38		92		75	65		87	75	72		70	
Reticulocytes %	16.4			5.0		4.4			7.0	2.5		2.0	
Leucocytes per c.mm.	17,000		7,600		7,000	7,200		8,400	8,950	9,600			
Nucleated red cells per c.mm.	Nil		Nil										
Bleeding time													

Age in days.	44	52
R.B.C. (millions per c.mm.)	5.09	4.27
Haemoglobin %	100	78
Reticulocytes %	<1	<1
Leucocytes per c.mm.		19,560
Nucleated red cells per c.mm.		Nil
Bleeding time	9.5 mins.	

Case 9. Maurice M., male.

Family history.

Both parents healthy. First 4 children alive and well. Absence of history of miscarriages, or jaundice of earlier infants.

Case History.

Pregnancy normal. Labour at full-time. Spontaneous delivery. Child jaundiced by 2nd day; green in colour by 7th day. Vomiting started in 4th week. Stools always pale.

Condition on admission on 25th day of life.

Small, thin feeble baby, grass green in colour. Liver and spleen palpable 3 fs.b. below costal margin.

Urine: bile pigments absent; urobilin present. Stools contain urobilin.

Blood count on 25th day: R.B.C. 1.44 millions per c.mm; Hb. 34% reticulocytes 18%; leucocytes 28,000 per c.mm; 560 nucleated red cells per c.mm. Treated by daily intramuscular blood injections of 10 to 15 c.c.

21st day: R.B.C. 1.25 millions per c.mm; Hb. 29%; retic. 20.5%.
23rd " : " 0.855 " " " " " 16%; " 19%;
leucocytes 44,000 per c.mm.

Course.

Afebrile. Child died on the 31st day of life.

Post-mortem Findings.

Poorly-nourished infant. Skin and mucous membranes deeply bile-stained.

Thorax: Heart and pericardium - nothing abnormal except jaundice.
Lungs - slight hypostatic congestion and oedema.
Thymus and thoracic glands not enlarged.

Abdomen: Peritoneal sac healthy.
Liver enlarged and of a deep olive green colour, but there was not any evidence of fibrosis. Thick dark bile could be expressed from the gall-bladder into the duodenum.
Spleen enlarged and congested.
Kidneys deeply bile-stained.

Stomach, bowel, suprarenal and mesenteric glands and pancreas without gross lesion.

Head: Bones, middle ears and great venous sinuses healthy. Meninges slightly yellow. Kernicterus absent.

Bone-Marrow: Slight sclerosis of medullary cavity of left femur. Very little marrow in ribs.

Histology.

Liver: Moderate cell degeneration. Pigmentation of cells. Numerous phagocytes. Erythropoiesis moderate. Appreciable haemosiderosis. Slight peri-central fibrosis.

Spleen: Congested. Small indistinct Malpighian bodies, deficient in lymphoblasts. Erythropoiesis more frequent than in liver. Numerous phagocytes. Moderate haemosiderosis. Increase of fibrous tissue round vessels and thickening of sinusoidal basement membranes.

Kidney: Haemosiderosis and cloudy swelling of tubules.

Bone-Marrow: Leucopoiesis more abundant than erythropoiesis. Marked shift to the left in the latter. Very numerous eosinophile myelocytes. Megakaryocytes normal in numbers. No phagocytosis or haemosiderosis.

Case 10. James L., male.

Family History.

Father aged 40, mother aged 35 years; well. 4 pregnancies - (1) Male, aged 5 years, well; (2) Female, aged 3 years, well; jaundiced on 2nd day; cleared on 14th day; (3) Female, aged 2 years, well; (4) Patient.

Case History.

Born 14 days before term. Vomiting and jaundice began on the 2nd day. Child became exceedingly drowsy. Diarrhoea from 3rd day.

Condition on admission.

Admitted to hospital on 5th day. Intensely jaundiced. Liver + 1 finger-breadth. Spleen not palpable. Very drowsy. Limbs remarkably rigid in flexion. Moved very little. Some head retraction and nuchal rigidity. Reflexes normal for age. Respirations slow - 30 per minute. Heart rate - 140 per minute.

Urine: bile pigments +; urobilin - a trace. Stools: urobilin present.

Blood count: Hb. 70%; R.B.C. 3,270,000 per c.mm; reticulocytes 3%; leucocytes 11,900 per c.mm.

Course.

Transfused on 5th day. Immediately after the red cells numbered 4,310,000 per c.mm. Child died two days after admission, having remained comatose all the time.

Post-mortem Findings.

Thorax:	Nothing of importance except jaundice.
Abdomen:	Liver enlarged; deep olive green colour. Bile-ducts patent. Bile thick. Spleen small, red and firm. Kidneys jaundiced. Other organs normal, except for gastro-enteritis.
Head:	Bile-staining of meninges. Kernicterus. Basal nuclei and denticulate nuclei of cerebellum deeply bile-stained.
Bone-Marrow:	Osteo-sclerosis of medulla of femur. Very little marrow in femur and ribs.

Histology.

Liver: Moderate degeneration of cells. Some small necrotic foci. Moderate erythropoiesis. Some leucopoiesis near portal tracts. Moderate haemosiderosis, chiefly portal in distribution. Phagocytes present. No fibrosis.

Spleen: Congested. No erythropoiesis. Numerous phagocytes. Malpighian bodies indistinct. Few lymphoblasts. Heavy iron deposit in phagocytes. No fibrosis.

Kidneys: Cloudy swelling of tubules.

Lymph Glands: Deficient lymphoid tissue. Iron containing phagocytes.

Bone-Marrow: Chiefly leucopoietic; neutrophile myelocytes numerous. Eosinophiles abundant. Earlier erythroblasts more frequent than normoblasts. Premature haemoglobinization. Numerous megaloblasts. Megakaryocytes present. Reticulo-endothelial cells lining the sinusoids swollen with yellow pigment. Phagocytes containing ingested erythrocytes not infrequent. Pale thrombi in a few sinusoids. No haemosiderosis.

Case 11. John McI., male.

Family history.

Father and mother young and in good health. 2 pregnancies - (1) Female, died in second year of unknown cause; (2) Twins - a. Male, alive and well; b. Patient.

Case History.

The mother vomited a great deal during pregnancy. Child born at full-time and apparently healthy. Fed on Ostermilk, but did not thrive. Constipated. Started to vomit on the 13th day. Jaundice first noticed on 15th day and increased quickly. Urine became greenish on the 17th day. On the same day there was a series of convulsions (10 in 3 hours).

Condition on admission - 17th day of life.

Small feeble baby, moribund. Deeply jaundiced. Weight 1.6 Kilo. Buttocks excoriated, suggesting that there had been diarrhoea. Heart and lungs clinically normal. Liver palpable 1½ fingers-breadth below the costal margin. Spleen palpable 2 fingers-breadth below the costal margin.

Urine: albumen +; bile pigments absent; urobilin ++.

Blood count: (15th day) R.B.C. 4.61 millions per c.mm; leucocytes 10,000 per c.mm; reticulocytes.
20th day: R.B.C. 3.95 millions per c.mm; Hb.65%; leucocytes 11,000 per c.mm; reticulocytes 1.8%.

Course.

The child developed fever which was clinically inexplicable. He was transfused into scalp veins on the 20th and 21st days, but died on the 22nd day.

Post-mortem Findings.

Thorax: Absence of abnormality except jaundice, and slight congestion of the lungs.

Abdomen: Liver congested.
Spleen enlarged and congested.
Kidneys pale: otherwise abnormalities absent in the abdomen.

Head: Bones, great venous sinuses and middle ears healthy. Early thrombosis of longitudinal sinus. Some haemorrhage in subdural space.

Bone-Marrow: Slight osteo-sclerosis of medullary cavity of femur. Very little marrow in ribs.

Histology.

Liver: Some degeneration of cells. Moderate pigmentation. Pigmented phagocytes present. Erythropoiesis scanty. Occasional megakaryocytes in sinusoids. Moderate iron deposit. Slight reticular thickening.

Spleen: Intense congestion. Many Malpighian bodies compressed and deficient in lymphoblasts. Frequent small foci of erythropoiesis. Numerous phagocytes. Heavy iron deposit. Reticulum affected. No fibrosis.

Kidneys: No gross abnormality.

Bone-Marrow: More leucopoietic than erythropoietic. Chiefly neutrophile myelocytes. Numerous eosinophiles. Normoblasts scanty. Moderate numbers of megaloblasts and erythroblasts. Premature haemoglobinization. Megakaryocytes present. No phagocytosis or haemosiderosis.

Case 12. Sarah McI., female.

Family History.

Father aged 46, mother aged 42 years; both well. 4 pregnancies - (1) Female, aged 13 years, well; (2) Female, aged 11 years, well; (3) Male, aged 4 years, well; (4) Patient.

Case History.

Mother healthy during pregnancy. Child born 3 weeks prematurely. Lusty at birth. Slight jaundice noticed 6 hours after birth. Gradually deepened. Stools yellow after meconium evacuated. Brisk umbilical haemorrhage started on the 7th day and continued until the 9th, when 30 c.c. of blood were injected subcutaneously by the doctor in attendance.

The child was admitted to hospital shortly after the injection of blood.

Condition on admission - 9th day of life.

Small baby. Weight 3.8 Kilo. Intensely jaundiced. Marked sclerotic icterus. Haemorrhage from umbilicus had ceased by the time the child was admitted to hospital. Heart: basal V.S. murmur. Lower border of liver 2 fingers-breadth below the costal margin. Spleen not palpable. Stools yellow; contained urobilin.

Urine: bile pigments +++; urobilin +. Wassermann reaction negative. Van den Bergh reaction: direct biphasic; indirect positive, 28 units.

Blood count: R.B.C. 1.07 millions per c.mm; Hb. 20% reticulocytes 8%; leucocytes 20,700 per c.mm; nucleated red corpuscles 2,070 per c.mm.

Course.

Transfused on 9th and 10th days. Blood count after 2nd transfusion: R.B.C. 2.21 millions per c.mm; Hb. 40%; leucocytes 13,400 per c.mm.

Urobilin absent from stools on the 10th day, when the child died.

Post-mortem Findings:

Thorax: No gross abnormality of heart and pericardium.
Bile-staining of endocardium and intima of great vessels marked.
Lungs bile-stained but otherwise normal.
Thymus and thoracic glands and pleural sacs normal.

<u>Abdomen</u>: Peritoneal sac healthy. Umbilicus healthy. Liver enlarged and of a deep olive green colour. Fibrosis not apparent. Obstruction in bile-ducts absent. Spleen slightly enlarged and soft. Kidneys deeply bile-stained. Uric acid infarcts. Stomach, intestines, pancreas, suprarenal and mesenteric glands normal.

<u>Head</u>: Bones, venous sinuses and middle ears normal. Substance and surface of brain slightly bile-stained, but Kernicterus absent.

<u>Bone-Marrow</u>: Osteo-sclerosis of medullary cavities of femur and ribs.

<u>Histology</u>.

<u>Liver</u>: Great degeneration with some necrosis of liver cells. Abundant phagocytosis and erythropoiesis. Leucopoiesis near portal tracts. Heavy iron deposit, in a greenish crystalline form in the liver cells and Küpffer cells. Many of the phagocytes although pigmented, do not contain iron. Slight increase of fibrous tissue round central veins.

Case 13. Alison M., female.

Family History.

Father aged 37, mother aged 37; both healthy. 3 pregnancies - (1) Female, aged 4 years, well; (2) Tubal mole; (3) Patient.

Case History.

Hyperemesis during pregnancy. Child born 2½ weeks prematurely. Slightly jaundiced at birth. Colour rapidly deepened. Slight vomiting for a few days, starting on the 3rd.

Condition on admission when 7 days old.

Small, deeply jaundiced child. Liver and spleen not palpable. Haemorrhages absent.

Urine: bile pigments absent; urobilin +.

Van den Bergh reaction: direct biphasic; indirect positive, 20 units.

Blood counts: see Table 5.

Course.

Transfused on 7th day. Jaundice slowly faded. Uraemia increased in the 4th week. Further transfusions on 23rd and 26th days. Thereafter the red cell count was maintained at over 4.0 millions per c.mm. Jaundice disappeared entirely and the child throve.

The later nervous symptoms have been fully described in the text.

TABLE 5.

Case 13. Blood Counts.

Age in days.	7		8	9	12	15	19	22	24		24
R.B.C. (millions per c.mm.)	3.44	Transfused	4.14	3.96	3.97	3.97	3.49	2.73	2.94	Transfused.	3.64
Haemoglobin %	70						70	65			
Reticulocytes %	3.0		2.6	1.5	1.6	1.5	1.5	1.5	<1		<1
Leucocytes per c.mm.	10,000		8,700	17,800	11,000	8,800	8,600	11,000	17,000		
Nucleated red cells per c.mm.	Nil		Nil	Nil	Nil	Nil	Nil		Nil		

Age in days.	25		28	30	31	34	37	45	52
R.B.C. (millions per per c.mm.)	3.2	Transfused.	4.1	4.08	4.1	4.27	4.19	4.38	4.25
Haemoglobin %	66		82	87	90	95	88	88	88
Reticulocytes %	<1		1.4	1.6	<1	1.4	<1	1.0	2.2
Leucocytes per c.mm.	7,000		5,100	9,300	10,600	17,600	13,700	8,900	12,100
Nucleated red cells per c.mm.	Nil		Nil	Nil	Nil	Nil	Nil	Nil	Nil

Case 14. Samuel McL., male.

Family History.

Father aged 32, mother aged 33 years; both healthy. 10 pregnancies - (1) Female, aged 14 years, well; (2) Male, died at birth; (3) Male, aged 12 years, jaundiced at birth but recovered; (4) Male, aged 9 years, jaundiced at birth but recovered; (5) Male, died aged 9 months, spina bifida; early jaundice; (6) Female, died aged 13 months, pneumonia; (7) Female, died aged 11 months, pneumonia; (8) Male, aged 13 months, well; jaundiced early but recovered; (9) Female, died aged 3 days of icterus gravis; (10) Patient.

Case History.

Pregnancy uneventful. Labour at full-time. Healthy at birth. Jaundice noticed one day after birth. Steadily deepened. The child rapidly became feeble and drowsy, and could not suck. A little red blood was vomited on the 4th day. Urine dark.

Condition on admission on the 4th day of life.

Well-nourished child. Intensely jaundiced. Drowsy. Cyanosed. Frequent respirations. Umbilicus slightly septic. Spasticity of limbs and neck absent. Chest full of moist râles. Inspiratory indrawing of the costal margin. Heart: no abnormality detected. Bloody vomit in mouth, but lesion in mouth or throat not found. Liver palpable 2 fingers-breadth below the costal margin. Spleen not palpable. Reflexes normal.

Urine: bile pigments +; urobilin absent.

Van den Bergh reaction: direct biphasic; indirect positive, 46 units.

Blood count: R.B.C. 3.50 millions per c.mm; Hb. 75%; reticulocytes 8.6%; leucocytes 5,600 per c.mm; normoblasts 1,176 per c.mm.

Course.

The child died on the 5th day.

Post-mortem Findings.

Thorax: Thymus and thoracic glands not enlarged. Both lungs were the seat of extensive broncho-pneumonic consolidation which involved all the lobes. There was not any gross lesion of the heart and pericardium.

<u>Abdomen</u>: Peritoneal sac healthy.
Mesenteric glands small.
Liver deeply jaundiced. Cirrhosis not apparent; Gall-bladder normal in size and appearance. Bile expressed into duodenum easily.
Spleen not enlarged.
Kidneys well-marked foetal lobulation. Uric acid infarcts present at apices of the papillae.
Fairly intense congestion of stomach and bowel.

<u>Brain</u>: Slight jaundice throughout. Kernicterus.

<u>Bone-Marrow</u>: Osteo-sclerosis of medullary cavity of femur absent. Marrow abundant in femur and ribs.

<u>Histology</u>.

<u>Liver</u>: Slight degeneration of cells. Erythropoiesis moderate. Numerous phagocytes. Moderate iron deposit. No fibrosis. Occasional megakaryocytes in sinusoids.

<u>Spleen</u>: Congested. Malpighian bodies scanty, rudimentary and deficient in lymphoblasts. Phagocytosis abundant. Erythroblastosis absent. Moderate haemosiderosis. Thickening of reticulum.

<u>Kidneys</u>: Congested. Cloudy swelling of tubular epithelium.

<u>Lymph Glands</u>: Deficient in lymphoblasts; congested; numerous phagocytes.

<u>Bone-Marrow</u>: Both leucopoiesis and erythropoiesis active, but the former predominates. The neutrophile myelocyte is the most frequent cell. Eosinophiles present. Fairly frequent megaloblasts; very frequent prematurely haemoglobinized and actively dividing erythroblasts, but by comparison few normoblasts. Megakaryocytes present. No phagocytosis or haemosiderin.

Case 15. Harriet N., female.

Family History.

Father aged 30, mother aged 25 years; both healthy. 4 pregnancies - (1) Male, aged 7 years, well; (2) Stillbirth; (3) Stillbirth; (4) Patient.

Case History.

Pregnancy uneventful. Child born at full-time. Jaundiced and cyanosed at birth. The former increased during the first week, then remained unchanged for about a week, but increased again in the 3rd week. Small abscesses in the thighs were incised in the 3rd week and the wounds healed well.

Condition on admission when 21 days old.

Child markedly jaundiced but fairly well-nourished. Weight 3.53 Kilo. Liver palpable 3 fingers-breadth and spleen palpable 2 fingers-breadth below the costal margin. Nervous system normal.

Urine: bile pigments +; urobilin +.

Van den Bergh reaction: ? delayed direct positive; indirect positive, 6 units.

Blood count: see Table 6.

Course.

Transfused (100 c.c.) on 21st day. Jaundice slowly cleared and bile pigments disappeared from the urine. The spleen and liver remained palpable although they progressively diminished in size until the 6th week.

The red cell count remained near 4.0 millions per c.mm. for some months, but later reached nearly 5 millions, with nearly 90% haemoglobin. The child developed in a satisfactory fashion.

TABLE 6.

Case 15. Blood Counts.

Age in days.	21	Transfused on 21st day.	21	24	26	29	32	39	43
R.B.C. (millions per c.mm.	2.44		3.88	4.05	4.35	4.26	4.26	3.60	3.94
Haemoglobin %	55			80	85	80	90	75	80
Reticulocytes %	12.2			10.0	1.0	1.5	2.0	1.8	2.0
Leucocytes per c.mm.	14,200			13,600	7,500	15,700	19,000	14,000	11,000
Nucleated red cells per c.mm.	317			Nil	Nil	Nil	2 seen	Nil	Nil

Age in days.	50	59	65	69
R.B.C. (millions per c.mm.)	4.20	3.53	3.57	3.63
Haemoglobin %	80	70	76	80
Reticulocytes %	0.8	1.0	2.8	3.6
Leucocytes per c.mm.	8,200	10,000	11,000	8,700
Nucleated red cells per c.mm.	Nil	Nil	Nil	Nil

Case 16. Robert P., male.

Family History.

Father aged 21, mother aged 25 years; both healthy. 2 pregnancies - (1) Male, aged 14 months, well; (2) Patient.

Case History.

Pregnancy and labour normal. Child healthy at birth. Breast-fed. Jaundice was first seen on the 5th day, and gradually increased in depth. The movements of the child became slow and listless.

Condition on admission on 15th day.

Child deeply jaundiced - olive green in colour. Weight 2.5 Kilo. Liver and spleen palpable 2 fingers-breadth below the costal margin.

Urine: bile pigments ++; urobilin nil; no urobilin in stools.

Van den Bergh reaction: direct biphasic; indirect positive, 34 units.

Blood counts: see Table 7.

Course.

3 Blood transfusions were given within a week and the jaundice slowly disappeared, being practically absent by the age of 6 weeks, when the spleen was no longer felt. There was an attack of gastro-enteritis in the 4th week. Urobilin returned to the stools.

The child, however, failed to thrive, and died during a second attack of gastro-enteritis at the age of 10 weeks.

Post-mortem Findings.

Jaundice absent.

Thorax: Thymus and thoracic glands small. No lesion in heart or lungs. Intima of aorta slightly bile-stained.

Abdomen: Peritoneal sac healthy.
Mesenteric glands small.
Liver jaundiced. No evidence of increased fibrous tissue. Atresia or stenosis of ducts absent.
Gall-bladder normal.
Spleen not enlarged; no gross lesion.
Kidneys no gross lesion.
Stomach and small bowel: areas of intense congestion

of mucosa, most marked in the upper parts of the small bowel. Colon healthy.

Head: Bones of vault and base of skull show bile-staining - greenish in colour.
Middle ears nil.
Brain nil.

Histology.

Liver: Great cell degeneration and some necrosis. Numerous phagocytes. No erythropoiesis. Moderate iron deposit. No increase of fibrous tissue.

Spleen: Congested. Malpighian bodies normal. Phagocytes numerous in pulp. No erythropoiesis. Slight increase of fibrous tissue round vessels. Moderate haemosiderosis.

Kidneys: Cloudy swelling of tubules.

Bone-Marrow: Erythropoiesis equally as prominent as leucopoiesis. Numerous normoblasts - more frequent than erythroblasts. Premature haemoglobinization not seen. Myelocytes numerous. Eosinophiles frequent. Very numerous megakaryocytes. No phagocytosis or haemosiderosis.

TABLE 7.

Case 16. Blood Counts.

Age in days.	15		16	18		19	20	23	24		25
R.B.C. (millions per c.mm.	0.57		2.32	2.19		3.65			2.53		3.29
Haemoglobin %	?20										
Reticulocytes %	6.4	Transfused.	3.8	10.0	Transfused.	21.4	24.4	7.6	6.0	Transfused.	2.2
Leucocytes per c.mm.	23,100		11,600	9,900		8,000		9,900	8,200		9,000
Nucleated red cells per c.mm.	5082		1044	1089		320	Nil	Nil	Nil		Nil

Age in days.	27	29	34	10 weeks
R.B.C. (millions per c.mm.)	3.23	3.33	3.22	4.08
Haemoglobin %				
Reticulocytes %	4.8	2.8	3.0	2.5
Leucocytes per c.mm.	8,200	6,100	8,800	7,800
Nucleated red cells per c.mm.	1 seen	Nil	Nil	Nil

Case 17. William P., male.

Family History.

Father aged 35, mother aged 35 years; both healthy. 3 pregnancies - (1) Female, aged 4 years, well; (2) Miscarriage at 2 months; (3) Twins - a. died at birth, ? cause; b. Patient.

Case History.

Parturition 2 weeks premature. The mother had albuminuria and was rather unwell in the later months of pregnancy. Child jaundiced at birth.

Condition on admission on the 11th day.

Moderately jaundiced. Chest and heart nil. Liver slightly enlarged. Spleen not palpable. Nervous system nil.

Urine: bile pigments absent; urobilin +; stools - urobilin +.

Blood counts: see Table 8.

Course.

Transfused on 11th and 13th days. Recurrence of haemolysis required transfusion on the 17th and 20th days. Thereafter child progressed, and jaundice cleared. The spleen became palpable in the 3rd week, but subsided in the 4th.

At the age of one year, the child was in excellent health, the red cell count being just over 5 millions per c.mm. and the haemoglobin 90%.

TABLE 8.

Case 17. Blood Counts.

Age in days.	11		12		14	16	17		18	19	20		21		21	22
R.B.C. (millions per c.mm.)	1.48	Transfused.	3.28	Transfused.	3.36	1.82	1.95	Transfused.	2.42	2.5	2.56	Transfused.	3.71	Transfused.	4.38	4.16
Haemoglobin %	32					46	44		54	50	48		86		98	96
Reticulocytes %	8.4		2.8			23.0	17.0		4.7	8.6	9.1		6.5			3.5
Leucocytes per c.mm.	12,000					10,000	10,500		6,700	7,000	8,400		9,100			10,700
Nucleated red cells per c.mm.	Nil		Nil		Nil	Nil	300		Nil	Nil	Nil		Nil		Nil	Nil

Age in days.	23	24	26	31	34	38	7 weeks	10 weeks	13 weeks	18 weeks
R.B.C. (millions per c.mm.)		4.29	4.04	4.33	4.31	3.44	2.94	4.32	3.98	4,80
Haemoglobin %		92	96	94	90	68	70	74	84	78
Reticulocytes %	3.9	2.4	<1	<1	<1	<1	6.8	<1		
Leucocytes per c.mm.		10,000	13,600	11,800	11,400	10,300	9,000	7,000		
Nucleated red cells per c.mm.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil		

Case 18. Baby R., male.

Family History.

Unimportant.

Case History.

Jaundice appeared on the 8th day (?). Child apparently well. Died very suddenly.
Dead on admission to hospital on the 12th day.

Post-mortem Findings.

	Well-nourished child. Jaundiced.
Thorax:	Lungs - extensive haemorrhage. All lobes of both lungs involved. Small haemorrhages into thymus.
Abdomen:	Liver bile-stained. Spleen enlarged, soft and congested. Kidneys jaundiced. Uric acid infarcts. Stomach, intestines, suprarenals and pancreas nil.
Head:	Basal meningeal haemorrhage. Brain substance generally slightly bile-stained.
Bone-Marrow:	Osteo-sclerosis of medulla of femur. Very little marrow in either femur or ribs.

Histology.

Liver:	Slight congestion. Some degeneration of liver cells. Phagocytosis present. Pigmentation of cells moderate. Erythropoiesis moderate. Moderate haemosiderosis. No fibrosis.
Spleen:	Congested. Moderate erythropoiesis. Phagocytes present. Considerable haemosiderosis. Malpighian bodies scanty; deficient in lymphoblasts. No fibrosis.
Kidneys:	Cloudy swelling of tubules.
Lymph Glands:	Lymphoid tissue deficient. Some phagocytosis.
Bone-Marrow:	Actively erythropoietic and leucopoietic. Numerous prematurely haemoglobinized erythroblasts. Numerous normoblasts. Myelocytes (neutrophile) are the chief white cells. Megakaryocytes present. No haemosiderosis.

Case 19. Leo. R., male.

Family History.

Father aged 31, mother aged 28 years; both healthy. 5 pregnancies - (1) Female, aged 7 years, well; (2) Male twins - a. died of pallor preceded by jaundice; b. 6 years, well; (3) Female, aged 4 years, well; (4) Female, died aged 7 months of meningitis; (5) Patient.

Case History.

Born at full-time. Jaundiced but lusty at birth. Jaundice partially cleared,but child became increasingly pale up to admission to hospital on the 13th day.

Condition on admission - 13th day.

On admission, the child was thin but active, and was slightly jaundiced. Liver + 2 fingers-breadth and spleen + 2½ fingers-breadth below the costal margin. Urobilin present in stools.

Urine: bile pigments absent; urobilin present. Occasionally became cyanosed and dyspnoeic.

Wassermann reaction: negative.

Blood counts - 13th day: R.B.C. 1.48 millions per c.mm; Hb. 41%; W.B.C. 32,000 per c.mm; Reticulocytes 36%. 15th day: R.B.C. 3.34 millions per c.mm; Hb. 77%; Reticulocytes 1.7%; W.B.C. 18,400 per c.mm. 16th day: R.B.C. 4.05 millions per c.mm; Hb. 80%; Reticulocytes 8.0%; W.B.C. 6,400 per c.mm.

Course.

Transfused (60 c.c.) on 14th, 15th and 16th days. On the 15th day jaundice became much worse, and on the 19th day, when jaundice was severe, purpura appeared over the lower abdominal wall. Death occurred on the 20th day.

Post-mortem Findings.

Body jaundiced.

Thorax: Lungs - hypostasis. Haemorrhages under pleura.

Abdomen: Liver deeply bile-stained. Spleen enlarged to about 4 times the normal size, soft and congested. Kidneys pale. Suprarenals and pancreas nil. Copious diffuse haemorrhage into stomach and intestines.

Head — Diffuse haemorrhage over surface of brain. Kericterus absent.

Histology.

Liver: Moderate degeneration with considerable pigmentation of the liver cells. Numerous phagocytes. Erythroblastosis moderate. Considerable haemosiderosis. Thickening of reticulum.

Spleen: Very congested Malpighian bodies fragmentary and deficient in lymphoblasts. Fairly numerous small islets of erythropoiesis. Phagocytes numerous. Heavy iron deposit in pulp phagocytes and fibrous tissue. Thickened reticulum with increase of fibrous tissue round some of the vessels.

Kidneys: Cloudy swelling of tubules.

Lymph Glands: Congested. Phagocytosis present. No erythropoiesis. Scanty lymphoblasts in lymph nodes.

Bone-Marrow: Leucopoiesis much more evident than erythropoiesis. Normoblasts are scanty and the early and late erythroblasts which are the most frequent red cell elements, are prematurely haemoglobinized. Myelocytes and premyelocytes are numerous. Phagocytosis and haemosiderosis absent. Megakaryocytes present. No phagocytosis or haemosiderosis.

Case 20. William S., male.

Family History.

Father aged 26, mother aged 26 years; both healthy. 4 pregnancies - (1) Female, aged 6 years, well; (2) Female, aged 4 years, well; (3) Female, lived only 2 hours; (4) Patient.

Case History.

Full-time infant. Jaundiced at birth. Occasional vomiting.

Condition on admission - 10th day.

Well-nourished. Liver enlarged - 2 fingers-breadth below the costal margin. Spleen palpable 1 finger-breadth below the costal margin.

Bile pigments absent; urobilin abundant in urine; urobilin in stools.

Blood counts: see Table 9.

Course.

Transfused on the 11th, 12th and 14th days. Jaundice and bile pigments in the urine thereafter lessened. Increase of anaemia in 4th week. Transfused on 26th day. Developed gastro-enteritis and died on the 44th day. Jaundice by this time had faded considerably and the spleen was not palpable.

Post-mortem Findings.

Child poorly-nourished. Moderate jaundice.

Thorax: Heart and lungs nil except hypostasis in the latter.

Abdomen: Liver enlarged and deeply jaundiced. No obstruction in bile-ducts. Spleen slightly enlarged and soft. Suprarenals and pancreas nil.
Mesenteric glands nil. Enteritis of small bowel.

Head: No abnormality.

Bone-Marrow: Little marrow in femur. Osteo-sclerosis absent. Similarly in ribs.

Histology.

Liver: In some parts of the sections the degeneration of the liver cells is moderate, but in others it is of severe degree, especially in central and mid-lobular regions. Pigmentation marked. Phagocytosis is considerable. Erythropoietic islets are moderate in numbers but of considerable size. Bile thrombi absent. Haemosiderosis marked; chiefly in liver cells. In the more degenerate areas excess of fine fibrous tissue is present.

Spleen: Not congested. Exceedingly large numbers of phagocytes present, often collected into large groups. Erythropoiesis absent. Scanty mother cells in Malpighian bodies. Very heavy iron deposit in phagocytes and also in reticulum, basement membranes of sinusoids and fibrous trabeculae. Generalised thickening of reticulum and basement membranes of sinusoids.

Kidney: Very marked cloudy swelling in tubules. Exudation in capsule of Bowman of glomeruli. Thickening of basement membranes of tubules. No haemosiderosis.

Bone-Marrow: Erythropoiesis comparatively inactive. Shift to the left. Normoblasts infrequent. Neutrophile leucopoiesis active. Megakaryocytes frequent. Phagocytosis and haemosiderosis absent.

TABLE 9.

Case 20. Blood Counts.

Age in days.	10		11		13	14		15	17	19	21	22	23	24	26	
R.B.C. (millions per c.mm.)	0.81	Transfused.		Transfused.	2.95	2.07	Transfused.	2.78	2.91	2.60	3.39	2.69	2.54	2.69	2.03	Transfused.
Haemoglobin %	30									55						
Reticulocytes %	8.0		10.0		9.0	4.0		3.0	2.0	3.0	2.0	<1	<1	<1	2.0	
Leucocytes per c.mm.	21,400									4,600						

Age in days.	27	30	33	35	37	40	42
R.B.C. (millions per c.mm.)	4.56	4.28	3.92	4.30	4.31	4.27	4.01
Haemoglobin %							
Reticulocytes %	<1	<1	<1	0.5	0.25	0.5	
Leucocytes per c.mm.							

Van den Bergh reaction: 3 units indirect positive

Case 21. John T., male.

Family History.

Father aged 43, mother aged 36 years; both healthy. 11 pregnancies - (1) Female, aged 17 years, well; (2) Male, aged 15 years, well; (3) Female, died aged 6 years of meningitis; (4) Male, aged 10 years, well; (5) Male, aged 9 years, well; (6) Miscarriage at 3 months; (7) Male, aged 7 years, well; (8) Female, died aged 6 months of meningitis; (9) Male, died aged 12 days of icterus gravis; (10) Female, aged 2 years, well; (11) Patient.

Case History.

Mother in rather poor health during pregnancy. Labour at full-time and normal. Baby slightly jaundiced at birth. Appeared first in face and later spread to body and limbs. Breast-fed and taking well until 3rd day.

Condition on admission on 4th day.

Small child. Deeply jaundiced. Umbilicus healthy. Heart, lungs and abdomen nil.

Urine: bile pigments ++; urobilin absent.

Cerebro-spinal fluid: yellow spinal fluid; cell count normal; no excess of globulin. Muscles of limbs hypertonic.

Course.

Child died on the 5th day.

Post-mortem Findings.

Thorax: Heart normal. Endocardium and intima of great vessels yellow.
Lungs - patchy collapse. Bile-stained.

Abdomen: Liver deeply bile-stained. Bile-ducts not obstructed. No cirrhosis.
Spleen enlarged, soft and congested.
Kidneys bile-stained.
Other organs normal.

Head: Brain slightly bile-stained. Kernicterus of basal nuclei, especially the thalamus and lenticular nuclei.

Histology.

Liver: Considerable degeneration and disarrangement of liver cells, with small necrotic foci. Phagocytes present. Scanty small nests of erythropoiesis. Some leucocyte formation near portal tracts. Considerable haemosiderosis, chiefly in liver cells but also in phagocytes. Iron deposit slightly heavier in portal regions. No excess of fibrous tissue.

Case 22. T.W., male.

Family History.

Father aged 22, mother aged 27 years; both healthy. 3 pregnancies - (1) Miscarriage at 3 months. (2) Male, aged 17 months, well; physiological jaundice; (3) Patient.

Case History.

Full-time birth. Instrumental delivery. Child well at birth. No jaundice. Breast-fed and throve until the 23rd day when jaundice started and steadily increased. Child thereafter irritable. Vomited occasionally.

Condition on admission, aged 4 weeks.

Well-nourished. Jaundiced. Spleen not palpable. Liver slightly enlarged.

Urine: bile pigments absent; urobilin - strong reaction.

Wassermann reaction: negative.

Van den Bergh reaction: delayed direct reaction ?+; indirect +, 32 units. Stools: urobilin present.

Blood counts: see Table 10.

Course.

The child made a spontaneous recovery.

At the age of 10 months, the child died of acute broncho-pneumonia.

Histology.

Liver: The cell columns have a radial arrangement. Except for slight fatty infiltration they are normal. There is no increase of fibrous tissue.

TABLE 10.

Case 22. Blood Counts.

Age in weeks.	4	5	6	6½
R.B.C. (millions per c.mm.)	4.02	3.5	3.98	4.07
Haemoglobin %	100	80	85	86
Reticulocytes %	0.5	1.0	<1	<1
Leucocytes per c.mm.	13,600	8,700	9,700	12,600
Nucleated red cells per c.mm.				

Case 23. Baby W., male.

Family History.

Father aged 40, mother aged 36 years; both healthy. 7 pregnancies - (1) Male, aged 14 years, well; (2) Twins, born prematurely (5 months), died; (3) Male, aged 10 years, well; (4) ? sex, died of icterus gravis on 4th day; (5) ? sex, died of icterus gravis on 7th day; (6) ? sex, died of icterus gravis on 2nd day; (7) Patient.

Case History.

Full-time birth. Child jaundiced a few hours later.

Condition on admission on 1st day of life.

Well-nourished. Deeply jaundiced. Umbilicus healthy. Liver considerably enlarged, to 4 fingers-breadth below the costal margin. Spleen slightly enlarged.

Urine: bile pigments +; urobilin absent; no urobilin in stools.

Blood counts: see Table 11.

Course.

Transfused immediately after admission, and again on the 6th day. The child recovered.

TABLE 11.

Case 23. Blood Counts.

Age in days.	2		6		11	18	32	46	57
R.B.C. (millions per c.mm.	3.65	Transfused (2nd). Transfused (5th).	3.52	Transfused.	3.68	4.34	3.12	3.82	4.10
Haemoglobin %	100		94		95	101	68	75	76
Reticulocytes %	16.6		15.5		3	2.2	4.0	3.0	3.0
Leucocytes per c.mm.									
Nucleated red cells per c.mm.	3,737		Nil		Nil	Nil	Nil	Nil	Nil

B. Haemolytic Anaemia of the New-born, without Oedema or Jaundice.

Case 24. R.B., male.

Family History.

Unimportant.

Case History.

Full-time child. Slightly jaundiced from 2nd to 4th days. Thereafter became gradually paler. Brought to hospital on 13th day because of recurrence of slight jaundice.

Condition on admission - 13th day.

The child was extremely pale and slightly jaundiced. The jaundice was of a lemon yellow colour.

Urine: bile pigments absent; strong reaction for urobilin.

Liver and spleen palpable $1\frac{1}{2}$ fingers-breadth below the costal margin.

Blood counts: see Table 12.

Course.

The child was transfused on the 13th, 14th, 18th and 21st days. In spite of this the haemolytic anaemia persisted, although it was of a less acute nature. Later ileo-colitis developed. Two further transfusions were given on the 51st and 52nd days, but the child, who was being treated as an out-patient, died a few days later.

Post-mortem examination not permitted.

TABLE 12.

Case 24. Blood Counts.

Age in days.	13		14		15	16	17	18		19	20	21		22
R.B.C. (millions per c.mm.)	1.55	Transfused	2.0	Transfused	2.85	2.15	1.57	1.65	Transfused		2.5	2.05	Transfused	2.65
Reticulocytes %	21		19		14	11.5	12	11.5		13.0	13	17		20

Age in days.	23	28	35	38	42	46	50		52		52
R.B.C. (millions per c.mm.)	2.25	2.5	2.7	2.3	2.5	2.65	2.2	Transfused	2.85	Transfused	3.4
Reticulocytes %	19	23	18		18	17	11		8		5.5

Case 25. James D., male.

Family History.

Father aged 40 years, mother aged 39 years; well. 11 pregnancies - (1) Female, aged 17 years, well; (2) Female, aged 15 years, well; (3) Male, died of scalds; (4) Male, aged 11 years, well; (5) Female, died of anaemia, aged 1 week; (6) Stillbirth; (7) Stillbirth; (8) Male, died of anaemia, aged 8 weeks; (9) Stillbirth; (10) Male, died aged 6 hours; (11) Patient.

Case History.

Full-time child. Jaundiced at birth. Very slight jaundice thereafter. Became increasingly pale.

Condition on admission on 13th day.

Extreme pallor with very slight lemon yellow jaundice. Liver and spleen enlarged.

Urine: bile pigments absent; urobilin present.

Course.

Transfused on 13th and 15th days. Haemoglobinuria shortly after 2nd transfusion. Died within a few hours.

Blood counts: 13th day - R.B.C. 1.20 millions per c.mm; Hb. 32%; Reticulocytes 5.0%; W.B.C. 10,000 per c.mm. 14th day - R.B.C. 2.8 millions per c.mm; Hb. 47%; Reticulocytes 12.0%.

Post-mortem Findings.

Anaemia; very slight jaundice.

Thorax: Diffuse broncho-pneumonia of both lower lobes.

Abdomen: Liver very slightly bile-stained; cloudy swelling.
Gall-bladder and ducts normal.
Spleen considerably enlarged and congested.
Kidneys very pale.
Other organs normal.

Head: Nothing except pallor of the brain.

Bone-Marrow: Slight sclerosis of femur medullary cavities.

Histology.

Liver:	Liver cells show negligible degeneration. Moderate erythropoiesis. No bile thrombi. Small amounts of pigment in liver cells. Moderate haemosiderosis. Slight increase of fibrous tissue.
Spleen:	Congested. Scanty erythropoiesis. Malpighian bodies small and deficient in mother cells. Marked phagocytosis. Heavy iron deposit chiefly in phagocytes, but also in fibrous tissue. Excess of fibrous tissue round vessels. Thickening of reticulum.
Kidneys:	Small scanty islets of erythropoiesis. Cloudy swelling of tubules. No haemosiderosis.
Lymph Glands:	Congested. Lymphoid tissue deficient in lymphoblasts. Phagocytes present. No erythropoiesis.
Bone-Marrow:	Erythropoiesis more active than leucopoiesis. Numerous megaloblasts and abundant erythroblasts, showing premature haemoglobinization. Normoblasts infrequent comparatively. Megakaryocytes scanty. Neutrophile myelocytes numerous. Very numerous eosinophile myelocytes. No phagocytes or haemosiderin.

Case 26. Matthew H., male.

Family History.

Father and mother healthy. 8 pregnancies - (1) Alive and well; (2) Died of icterus gravis in 1st week; (3) Died of icterus gravis in 1st week; (4) Died of icterus gravis in 1st week; (5) Alive and well; (6) Alive and well; (7) Alive and well; (8) Patient.

Case History.

Full-time child. Slightly jaundiced from the 3rd to the 6th day. Increasing pallor. Died on admission on the 14th day.

Post-mortem Findings.

Thorax:	Heart and pericardium normal except for pallor. Lungs pale; extensive oedema; Thymus pale; Glands not enlarged.
Abdomen:	Liver enlarged; café au lait colour. Spleen enlarged, soft and congested. Kidneys pale.
Head:	Brain extraordinarily pale; no lesion.
Bone-Marrow:	Red in femur and ribs. No osteo-sclerosis.

Histology.

Liver:	Radial arrangement of liver cell columns well preserved. No degeneration and little pigmentation of cells. Very abundant erythropoiesis extending practically all along the sinusoids. Numerous nucleated cells in vessels. Phagocytosis marked. Heavy iron deposit in liver cells, phagocytes and in adventitious coats of vessels. No fibrosis.
Spleen:	Not congested. Malpighian bodies scanty and deficient in mother cells. Marked erythropoiesis. Phagocytosis active. Abundant haemosiderosis. No increase of fibrous tissue or thickening of reticulum.
Kidneys:	Slight cloudy swelling. Some intertubular oedema.

Case 27. C. McA., female.

Family History.

Father aged 28, mother aged 25 years; both healthy.

Case History.

First child. Prematurely born; weighed 4½ lbs. at birth. Pallor first noticed by mother at 5 weeks, but according to the grandmother, the child had always been very pale.

Condition on admission at 9 weeks old.

Very pale child. Somewhat underweight - 77% of average weight for age. Slight café au lait tinge of skin. No sclerotic icterus. Chest and heart nil. Liver palpable 1 finger-breadth below the costal margin. Spleen palpable 2 fingers-breadth below the costal margin. Nervous system normal. Bile pigments and urobilin absent from the urine.

Course.

The child was transfused on two occasions. She failed to thrive and signs of blood regeneration were absent. After transfusion urobilinuria was present and the spleen became palpable. Death occurred at 15 weeks from ? septicaemia.

Blood counts: see Table 13.

Post-mortem Findings.

Thorax:	Nothing of importance.
Abdomen:	Liver - light brown in colour. Spleen small, red and soft. Kidneys pale. Slight congestion of upper part of small intestine.
Head:	Left otitis media. Brain - pallor only.
Bone-Marrow:	Red, soft and diffuse. No osteo-sclerosis.

Histology.

Liver:	Radial arrangement of cell columns very little disturbed; cells healthy but containing small amounts of pigment. Very scanty erythropoiesis. Small deposit of iron. No fibrosis.
Spleen:	Moderately congested. Malpighian bodies normal. Very scanty erythropoiesis. No thickening of reticulum. Slight haemosiderosis.
Kidneys:	No gross lesion.

Lymph Glands: Deficient in lymphoid tissue. Slight congestion.

Bone-Marrow: Leucopoiesis very active. Considerable shift to the left. Very numerous myeloblasts and premyelocytes. Erythropoiesis deficient. Shift to the left and premature haemoglobinization. Megakaryocytes present. No phagocytosis or haemosiderosis.

TABLE 13.

Case 27. Blood Counts.

<u>Age in weeks</u>.	9	Transfused on 3 successive days.	10	11	12	13	15
R.B.C. (millions per c.mm.	1.07		3.41	3.33	3.74	2.09	2.03
Haemoglobin %	19.0		60.0	60.0	32	39	37
Reticulocytes %	none seen		<1	<1	<1	<1	<1
Leucocytes per c.mm.	6,600		19,200	7,100	9,000	6,900	3,200

Case 28. Margaret M., female.

Family History.

Father aged 44, mother aged 39 years; both healthy. 4 pregnancies - (1) Male, aged 20 years, well; (2) Female, died aged 2 years, of measles; (3) Twins, aged 10 years, well; one was very pale in the early weeks of life; (4) Patient.

Case History.

Full-time child. Slight jaundice on the 8th day. Rapidly cleared. Progressive pallor since birth. Occasional vomiting.

Condition on admission on the 12th day.

Moderately well-nourished. Extremely pale. Slight yellow tinge of skin. Spleen palpable 1½ fingers-breadth below the costal margin. Liver just palpable. Nervous system nil. Bile and urobilin absent from urine.

Blood counts: see Table 14.

Course.

Transfused on 13th and 14th days. Recurrence of anaemia and reticulocytosis in the 3rd and 4th weeks.

Thereafter the child gradually recovered, and at the age of 10 months the blood count was: R.B.C. 5,100,000 per c.mm. and Hb. 92%.

TABLE 14.

Case 28. Blood Counts.

Age.	13 days		14 days		15 days	16 days	18 days	23 days	5 weeks	9 weeks
R.B.C. (millions per c.mm.	1.20	Transfused.	2.10	Transfused.	3.35		3.85	2.21	2.78	3.48
Haemoglobin %	20+		52		72		66	46	48	64
Reticulocytes %	<1				4.4	6.1	7.0	<1	15.6	3.8
Leucocytes per c.mm.	22,300				24,900		22,200	17,000	16,700	14,600
Nucleated red cells per c.mm.	666		Nil		3,569		Nil	Nil	Nil	Nil

Age.	13 weeks	6 months
R.B.C. (millions per c.mm.	4.0	5.28
Haemoglobin %	78	92
Reticulocytes %	<1	<1
Leucocytes per c.mm.	10,300	11,300
Nucleated red cells per c.mm.	Nil	Nil

Case 29. W. S., male.

Family History.

Unimportant.

Case History.

6th child. Normal pregnancy. Labour ? 2 weeks premature. Child weighed 6 lbs. 11 ozs. at birth. Slight jaundice on 2nd day. Anaemia first noticed on 7th day.

Condition on admission on 9th day.

Child very pale. Very slight jaundice. Liver and spleen considerably enlarged - 2 and 2½ fingers-breadth respectively. Urobilinuria present.

Blood count: R.B.C. 1.12 millions per c.mm; Hb.24%; Reticulocytes 15.0%; W.B.C. 19,000 per c.mm.

Course.

Transfused on 9th and 10th days, but died on the 11th. After 1st transfusion: R.B.C. 1.84 millions per c.mm.

Post-mortem Findings.

Thorax: Hypostatic congestion of lungs.
Abdomen: Liver light brown in colour and enlarged.
Spleen enlarged, soft and red.
Other organs normal except for pallor.
Head: Small meningeal haemorrhage over vertex of brain.
Bone-Marrow: Femur: osteo-sclerosis of medullary cavity. Marrow scanty in both ribs and femur.

Histology.

Liver: Slight disarrangement of cell columns. Sinusoids wide and congested. Erythropoiesis moderate. Phagocytes numerous. Numerous nucleated cells in blood vessels. No fibrosis. Moderate haemosiderosis.
Spleen: Congested. Considerable erythropoiesis. Numerous phagocytes. Malpighian bodies normal. Haemosiderosis marked. Present in reticulum and trabeculae as well as in phagocytes. Reticulum thickened.

<u>Kidney</u>: Of foetal type; exudate into capsules of Bowman. Oedema of tubular epithelium. No haemosiderosis.

<u>Bone-Marrow</u>: Definitely hypoplastic. Very little erythropoiesis present and that of a primitive type. Moderate numbers of neutrophile leucopoietic islets. Marrow congested. Very occasional megakaryocytes. No phagocytosis or haemosiderosis.

C. Icterus Gravis. 1921-33 Series.

Only summaries of cases used for pathological investigation are given.

Case 30. G. C., male.

Family History.

Father aged 29, mother aged 27 years. The mother was said to have pulmonary tuberculosis. 4 pregnancies - (1) Male, well; (2) Male, prematurely born, died aged 3 days; (3) Male, died of gastro-enteritis at 3 months; (4) Patient.

Case History.

The child was probably premature as the birth-weight was only 5½ lbs. Jaundiced at birth. Convulsions on the 10th and 13th days. Diarrhoea and vomiting on the 12th and 13th days.

Condition on admission - 13th day.

Child dehydrated and deeply jaundiced. Liver enlarged - 2 fingers-breadth. Spleen not palpable.

Course.

Died on the 15th day.

Post-mortem Findings.

Thorax: Nothing of importance, except patent foramen ovale in the heart, and hypostasis in the lungs.

Abdomen: Liver deeply jaundiced. Bile-ducts patent. Spleen slightly enlarged and congested. Kidneys pale and jaundiced.

Head: Bile-staining of the meninges. Kernicterus absent.

Histology.

Liver: Cell columns disarranged but liver cells healthy and containing only small amounts of pigment. Some phagocytosis. No erythropoiesis. No haemosiderosis. Slight increase of fibrous tissue round central veins and portal tracts.

<u>Spleen</u>: Congested. Malpighian bodies normal. Phagocytes present. Very scanty erythropoiesis. Small amount of haemosiderin. Slight increase of fibrous tissue round vessels.

Case 31. P. C., male.

Family History.

Unimportant.

Case History.

Full-time child. Jaundiced at birth. Purpura of lower abdomen and genitalia on 5th and 6th days.

Condition on admission on 6th day.

Deeply jaundiced. Purpura as above. Spleen and liver each palpable 3 fingers-breadth below the costal margin.
Urine: bile pigments present.
Wassermann reaction of mother and child: negative.
Van den Bergh: Biphasic, 25 units.

Post-mortem Findings.

Thorax: Thymus and thoracic glands not enlarged.
Lungs jaundiced. Small haemorrhages into their substance.
Heart - no abnormality.

Abdomen: Small haemorrhages into parietal peritoneum.
Mesenteric glands normal.
Liver enlarged. Green in colour. Bile-ducts patent.
Spleen considerably enlarged. Soft and red.
Kidneys - no gross abnormality.
Stomach and bowels normal.

Head: Bilateral otitis media. Kernicterus absent.

Bone-Marrow: The femur contains red marrow.

Histology.

Liver: Great degeneration of liver cells, with small areas of necrosis. Marked pigmentation of cells. Fatty infiltration in mid-lobular and central regions.
Phagocytosis abundant. Erythropoiesis abundant.
Haemosiderosis marked; chiefly periportal.
No fibrosis.

Case 32. R. H., female.

Family History.

Unimportant.

Case History.

Full-time child. Jaundiced at birth. Cleared quickly. The child throve thereafter until 6 weeks of age when jaundice recurred. During the next fortnight the jaundice was variable.

Condition on admission, aged 8 weeks.

Child intensely jaundiced. Bile pigments in urine. Liver palpable 1 finger-breadth below the costal margin. Spleen not palpable.

Van den Bergh reaction: biphasic, 18 units.

Wassermann reaction: negative.

Blood count (at 8 weeks): Hb. 55%; R.B.C. 2,930,000 per c.mm; leucocytes 15,400 per c.mm.

Course.

Jaundice was variable and the child died of pneumonia at the age of 15 weeks. There were convulsions towards the end. The cerebro-spinal fluid at this time was yellow, but there was no increase of cell count, or globulin.

Post-mortem Findings.

Thorax: Suppurating broncho-pneumonia of both lungs.
Heart and pericardium normal.
Thymus gland small.

Abdomen: Liver dark olive green. Bile-ducts patent.
Spleen enlarged and soft.
Kidneys bile-stained.

Brain: No abnormality.

Histology.

Liver: Sinusoids widely dilated; cell columns disarranged. Gross degeneration of liver cells with areas of necrosis. Bile thrombi present. Phagocytosis marked. No erythropoiesis. Small amounts of haemosiderin in Küpffer cells. Slight increase of fibrous tissue, chiefly in central regions of lobules.

Case 33. A. O., female.

Family History.

Unimportant.

Case History.

Full-time child. Jaundiced at birth.

Condition on admission on 4th day.

Deeply jaundiced. Heart and respiratory rates low. Spleen + 2 fingers-breadth. Liver enlarged.

Van den Bergh reaction: biphasic, 3 units.

Urine: bile pigments.

Nervous system normal. Wassermann reaction: negative.

Blood count: R.B.C. 2,450,000 per c.mm; Hb. 44%; leucocytes 10,320 per c.mm.

Course.

Transfused. Steady improvement and eventual recovery. The subsequent nervous symptoms have been fully described in the text.

Case 34. W. S., male.

Family History.

Father aged 39, mother aged 38; healthy. 7 pregnancies - 4 children alive and well, 2 stillbirths, and the patient. One of the surviving children (the 6th) apparently recovered from icterus gravis, because he was considerably jaundiced during the first 14 days of life.

Case History.

Child born at full-time. Jaundiced at birth. Purpura of face and limbs on the 14th day.

Condition on admission on 16th day.

Deeply jaundiced. Purpura as above. Liver greatly enlarged - 3 fingers-breadth. Spleen not palpable. Bile pigments in urine. Umbilicus oozing blood. 20 c.c. of father's blood injected subcutaneously. Child taken suddenly ill and died in the middle of a feed shortly after admission.

Post-mortem Findings.

Well-developed child; extremely jaundiced. All internal organs deeply bile-stained.

Thorax: Nil except petechiae in heart muscle and in parietal pleura.

Abdomen: Petechiae in visceral peritoneum.
Liver enlarged and deeply bile-stained. Petechiae under capsule. Bile-ducts patent.
Spleen greatly enlarged and firm.
Kidneys - small haemorrhages in substance.

Head: Extensive sub-arachnoid haemorrhage over right occipital and temporo-sphenoidal lobes. Kernicterus absent.

Histology.

Liver: Marked degeneration of liver cells. Fatty infiltration in mid-lobular and central regions. Pigment in liver cells. Bile thrombi in some of the smaller ducts. Moderate erythropoiesis. Phagocytosis marked. Small amount of haemosiderin. No fibrosis.

Spleen: Congested. Malpighian bodies ill-defined. Small islets of erythropoiesis present. Phagocytes numerous Very little haemosiderin. Slight increase of fibrous tissue round small vessels and radiating from trabeculae. Some thickening of basement membranes of sinusoids.

Case 35. H. T., male.

Family History.

The patient was a twin, the other being alive and well. Otherwise family details not important.

Case History.

Full-time child. Jaundiced from 2nd day. Hare-lip and cleft-palate. Diarrhoea for some days.

Condition on admission on 14th day.

Deeply jaundiced. Dehydrated. Liver palpable 2 fingersbreadth below the costal margin. Spleen not palpable.
Wassermann reaction: negative.

Course.

Died, aged 16 days.

Post-mortem Findings.

Thorax: Hypostatic congestion of lungs.
Abdomen: Liver enlarged and deeply bile-stained. Bile-ducts patent.
Spleen red and soft.
Kidneys jaundiced.

Histology.

Liver: Marked degeneration and pigmentation of the liver cells. Sinusoids congested. Fatty infiltration in some places. Erythropoiesis moderate. Bile thrombi present. Phagocytes very numerous. Very marked haemosiderosis. Slight increase of fibrous tissue.

C. Anaemia without Oedema or Jaundice. 1921-33 Series.

Case 36. J. P., male.

Family History.

Unimportant.

Case History.

Normal delivery at full-time. Jaundiced at birth, but this passed off quickly. Extremely pale by 7th day. Vomited occasionally every day.

Condition on admission when 16 days old.

Skin and mucous membranes very pale. Jaundice absent. Umbilicus healthy. Systolic murmur all over praecordium. Liver 2 fingers-breadth and spleen 3 fingers-breadth below the costal margin. Bile absent from urine.

Blood count: R.B.C. 1,330,000 per c.mm; Hb. 30%; leucocytes 28,000 per c.mm; numerous nucleated red cells seen in films.

Course.

Died on the 18th day.

Post-mortem Findings.

Well-nourished child. Great pallor.

Thorax: The pericardial sac contained a small amount of blood-stained fluid. Petechial haemorrhages in heart muscle.
Lungs pale; numerous small areas of collapse in both lungs.

Abdomen: Liver enlarged and brown in colour.
Spleen greatly enlarged and soft.

Bone-Marrow: Osteo-sclerosis of femoral medullary cavity. Only a few nucleated red cells seen in sections.

Histology.

Liver: The sinusoids are wide and slightly disarranged but there is no degeneration of the liver cells. The entire sinusoidal system is packed with erythropoietic cells. The blood in the vessels shows very numerous nucleated cells. Numerous phagocytes. Moderate haemosiderosis chiefly in liver cells. Slight thickening of fibrous tissue round central veins and portal tracts.

Spleen: Not congested. Abundant erythropoiesis. Phagocytes numerous but not pigmented. Scanty Malpighian bodies. Heavy iron deposit. No change in fibrous tissue or reticulum.

Kidneys: Slight increase of interstitial tissue with oedema. Exudate in capsules of Bowman. Cells of tubules contain small amounts of haemosiderin. Unusually large numbers of nucleated cells in circulating blood.

Case 37. M. T., female.

Family History.

Father aged 30, mother aged 28 years; both healthy. 5 pregnancies - (1) Female, well; (2) Female, well; (3) Died aged 10 days of unknown cause; (4) Miscarriage at 3 months; (5) Patient.

Case History.

Full-time child. Obstetrician's note: "Child jaundiced at birth. Spleen enlarged. Haemorrhages into both retinae." The jaundice cleared, but the child became progressively pale.

Condition on admission on 10th day.

Very pale and drowsy. Jaundice absent. Spleen enlarged - 2 fingers-breadth. Liver palpable.
Blood count: R.B.C. 860,000 per c.mm; Hb. 12%; leucocytes 32,000 per c.mm.

Course.

Died shortly after admission.

Post-mortem Findings.

Thorax: Excepting pallor, no striking abnormality.
Abdomen: Liver - café au lait in colour.
Spleen enlarged and soft; pulp almost diffluent.
Kidneys pale.
Head: Brain very pale.

Splenic culture: No growth.

Histology.

Liver: Slight pigmentation but very little degeneration of liver cells. Sinusoids wide. Very abundant erythropoiesis. Circulating blood contains very numerous nucleated cells. Phagocytosis marked. Moderate haemosiderosis. Slight increase of fibrous tissue.

Spleen: Considerable erythropoiesis. Not congested. Phagocytes frequent. Small scattered Malpighian bodies. Heavy iron deposit in phagocytes and in fibrous tissue. Thickening of reticulum near vessels.

<u>Kidneys</u>: Cloudy swelling and oedema of tubules. No erythropoiesis. Considerable haemosiderosis of cells of tubules in glomerular region and less in the medullary region. No fibrosis.

E. Cases of Physiological Jaundice.

Case 38. A. E., female.

Family History.

Unimportant.

Case History.

Labour at full-time. Healthy child. Slightly jaundiced at birth. Jaundice gradually increased for 4 days.

Condition on admission, aged 4 days.

Child well-nourished. Severe jaundice. Vomiting. Cord clean. Spleen not palpable. Liver 2 fingers-breadth below the costal margin.
Van den Bergh reaction: Indirect +, 48 units.
Urine: bile pigments absent; urobilin +.

Blood Counts.

	4 days.	5 days.	6 days.	7 days.	8 days.	12 days.
R.B.C. per c.mm.	5800000	5410000	5050000	4860000	4940000	4880000
Hb.%	130	130	120	105	100	118
Colour index	1+	1+	1+	1+	1+	1.22
Reticulocytes %	2.0	1.8	0.2	Nil	0.2	0.4
Leucocytes	8,900		7,900	11,300	10,400	12,200
Nucleated red cells	155/c.mm	Nil	Nil	Nil	Nil	Nil

Case 39. Baby McC., female.

Family History.

Unimportant.

Case History.

Healthy, full-time child. Jaundiced on 2nd day; became quite deep by 6th day. Otherwise well.

Condition on admission.

Spleen not palpable.

Blood count (6th day): R.B.C. 4.25 millions per c.mm; Hb. 95%; Reticulocytes 2%; no erythroblastaemia.

Course.

After the 6th day, the jaundice quickly faded. The child remained well.

Case 40. Baby K., male.

Case History.

Full-time child. Precipitate labour after pituitrin. Child required resuscitation. Convulsions continuous from a few hours after birth.

Course.

Died when 24 hours old.

Post-mortem Findings.

Extensive subdural haemorrhage and subtentorial tear. Cephalhaematoma.

Histology.

Liver: Cells healthy. No pigmentation. Some erythropoiesis. No phagocytosis. Negligible haemosiderosis. No fibrosis.

Spleen: Congested; otherwise normal. No erythropoiesis and negligible haemosiderosis.

Kidneys: Congested; otherwise normal.

Bone-Marrow: Active erythropoiesis and leucopoiesis. Normoblasts and late erythroblasts very frequent. Neutrophile myelocytes numerous. Megakaryocytes present. No phagocytosis or haemosiderosis.

ERYTHROBLASTOSIS FOETALIS

and

ADDITIONAL PAPERS

by

William T.W. Paxton, M.B. Ch.B., F.R.F.P.S.(Glas.).

Volume II.

Volume II.

C O N T E N T S.

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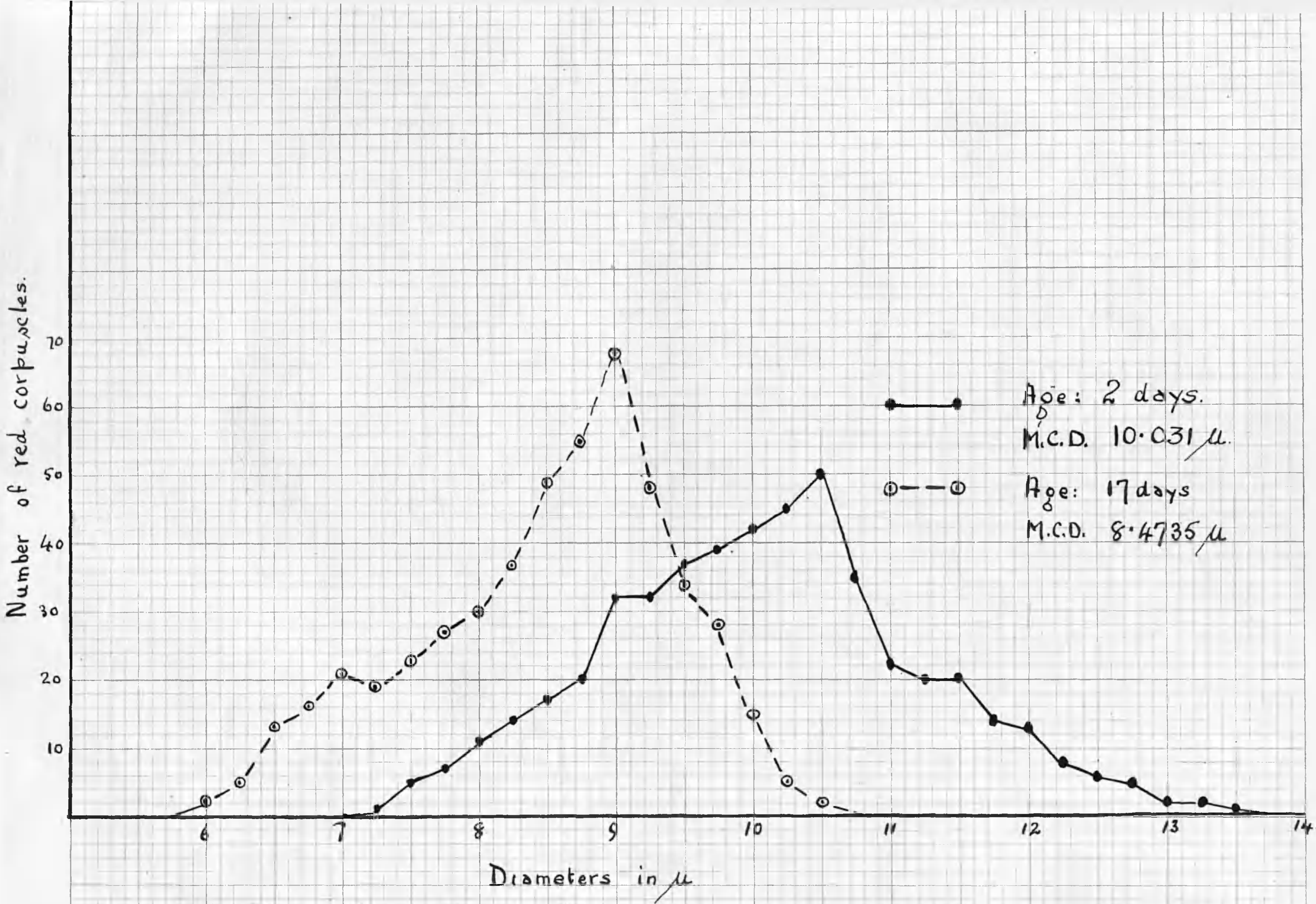

Fig. I Price Jones Curves in a case of Icterus Gravis

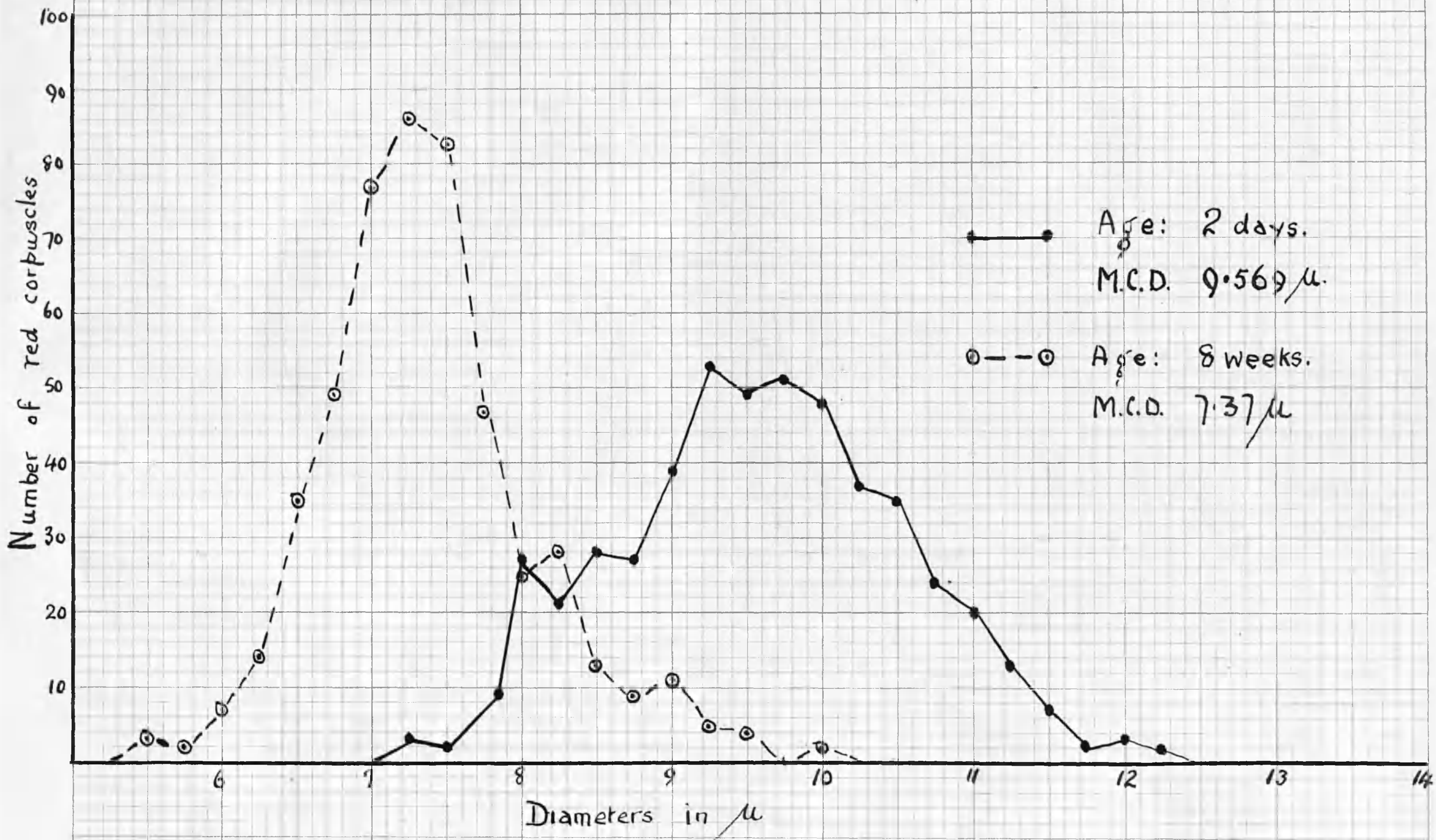

Fig II. Price-Jones Curves in a case of Icterus Gravis

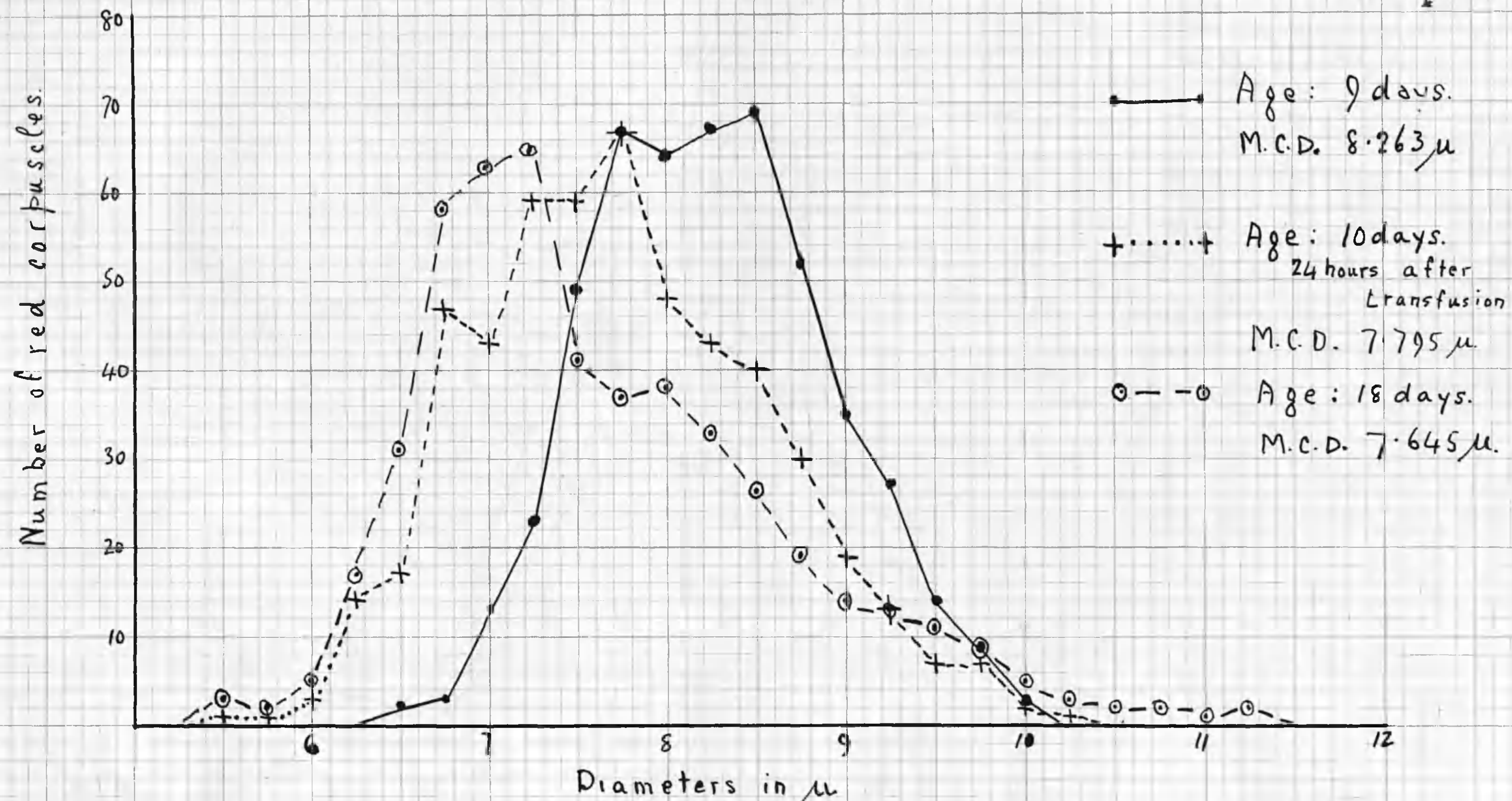

Fig. III. Price Jones Curves in a case of Icterus Gravis.

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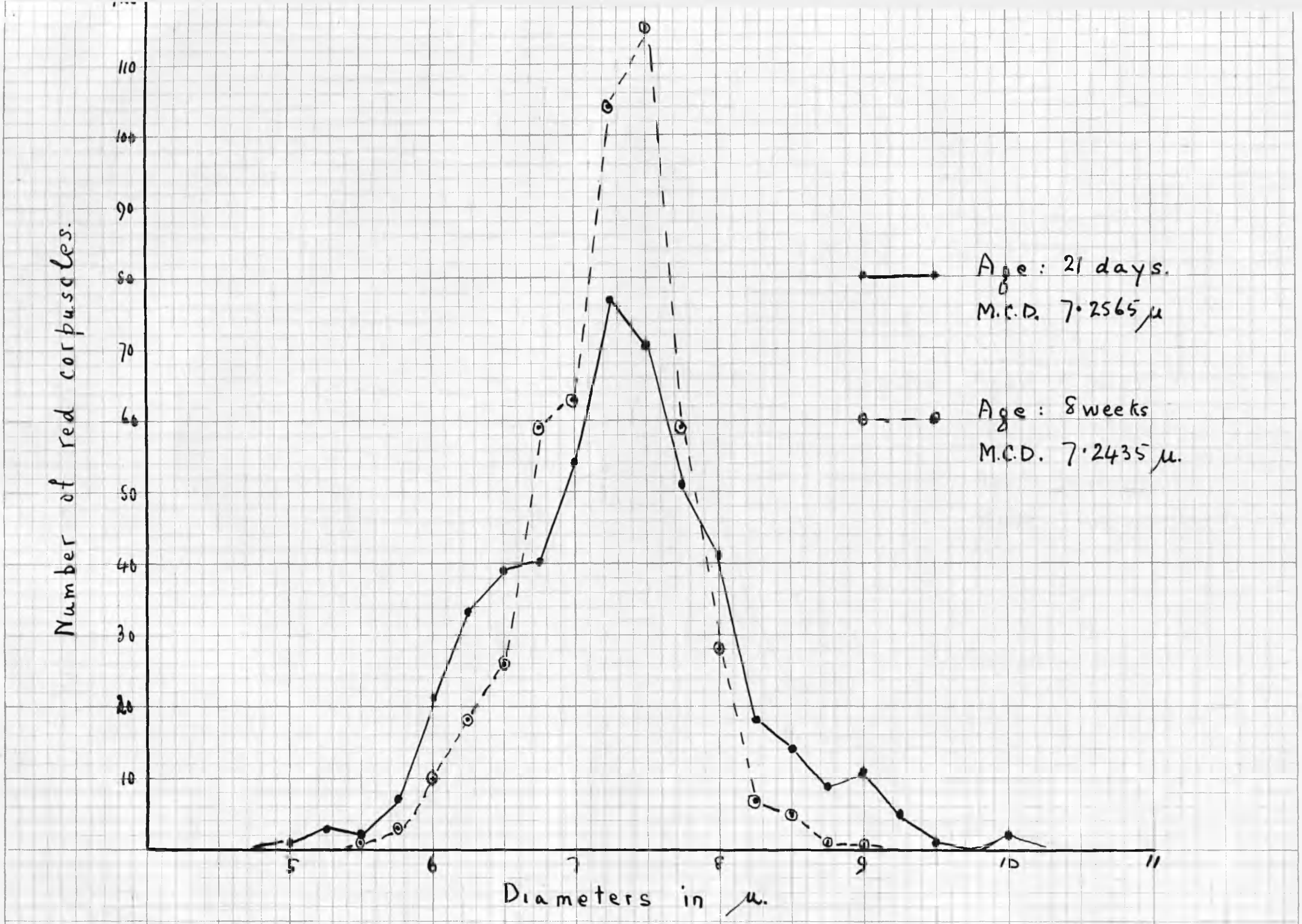


Fig. IV. Price-Jones Curves in a case of Icterus Gravis.

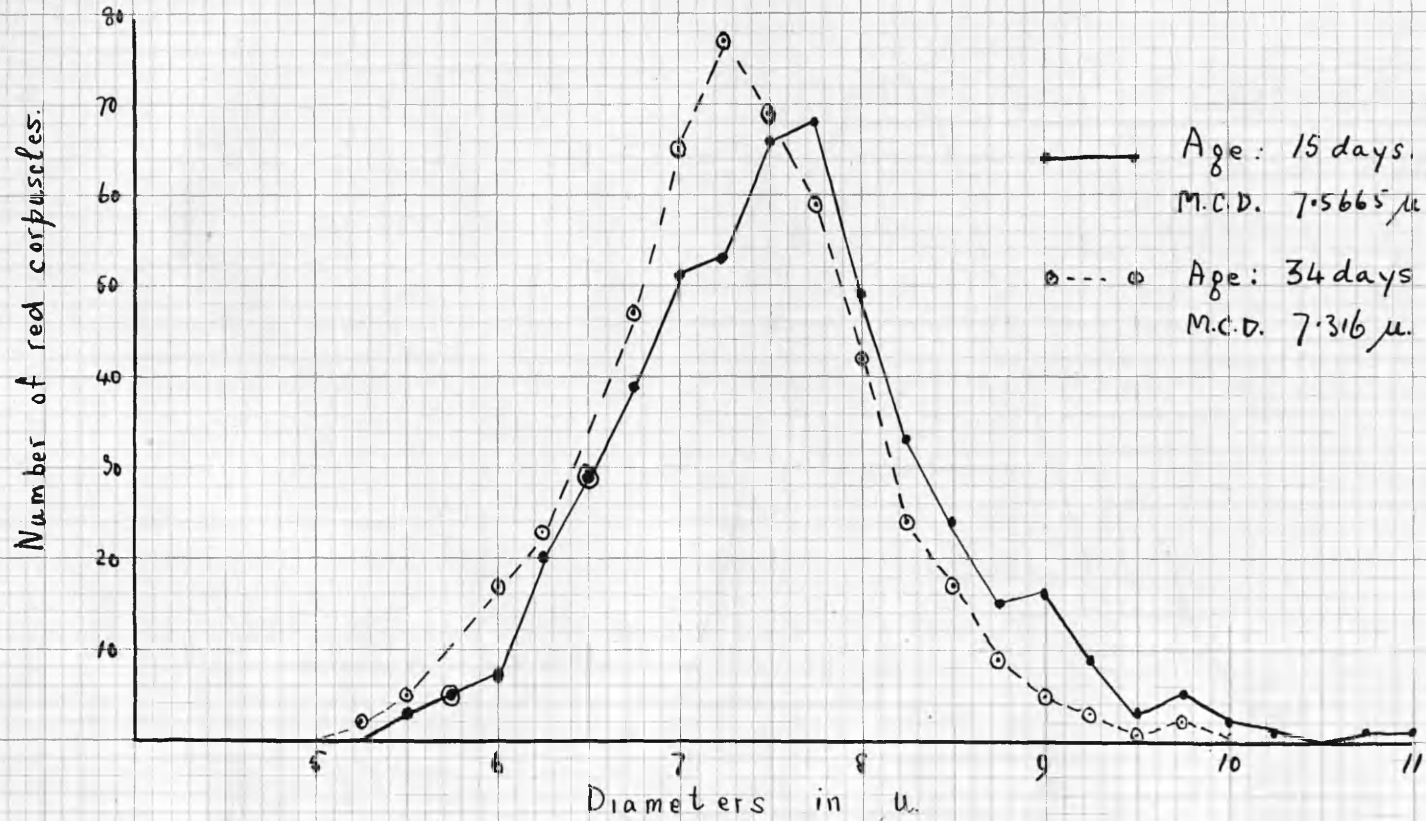


Fig. V. Price Jones Curves in a case of Icterus Gravis.

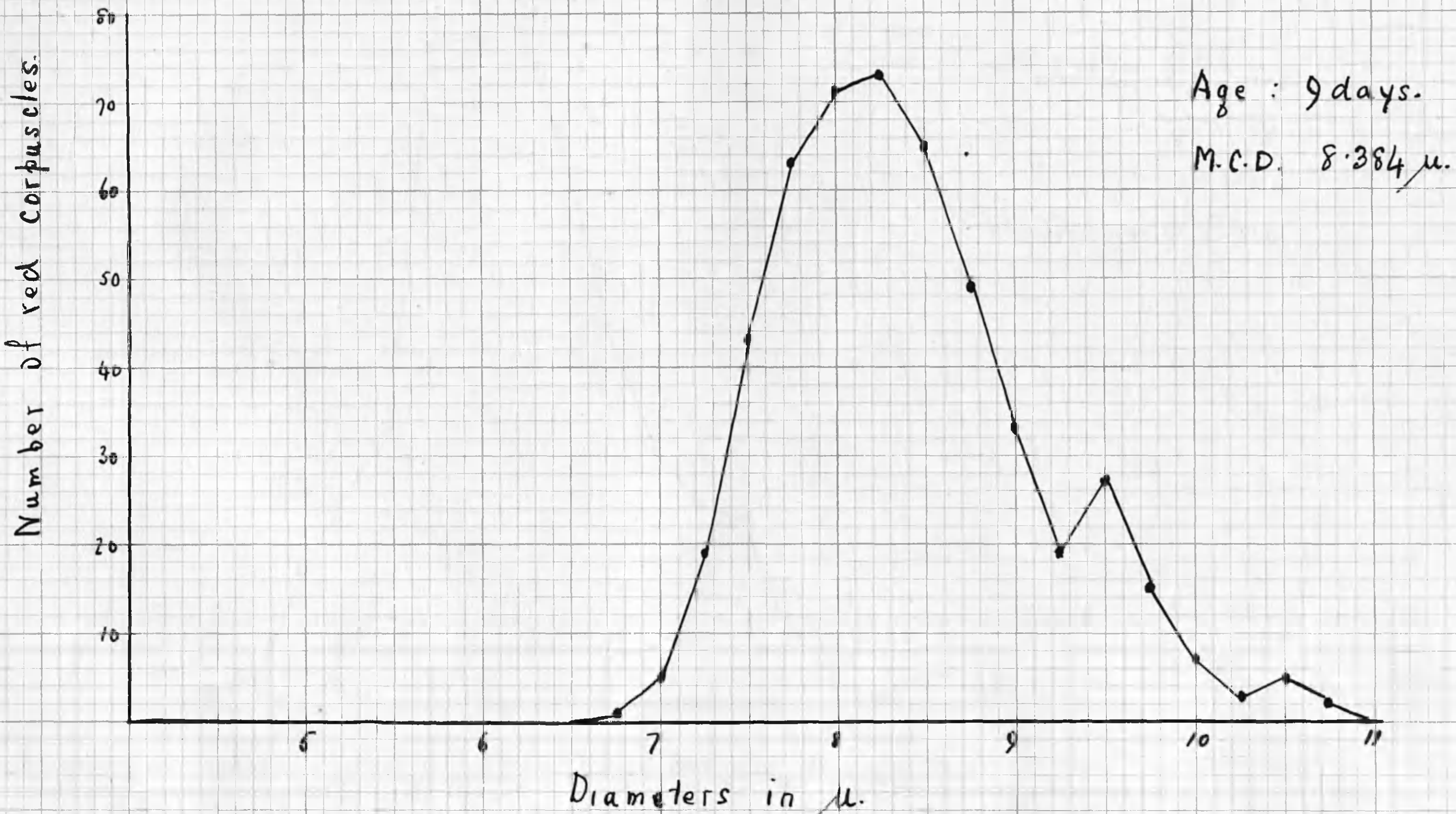


Fig. 6 VI. Price-Jones Curves in a case of haemolytic anaemia without jaundice.

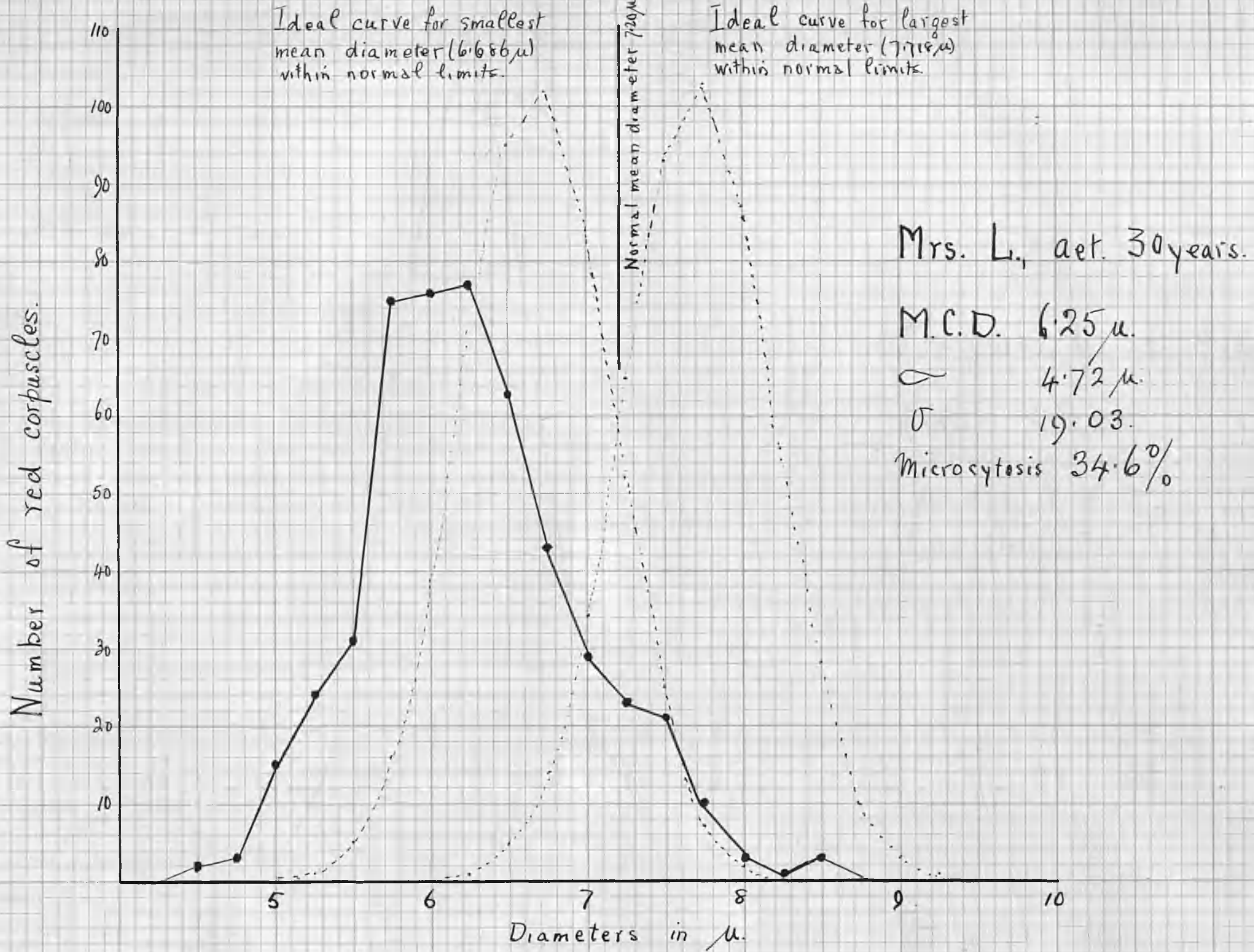


Fig. VII. Price-Jones Curves in a case of familial acholuric Jaundice.

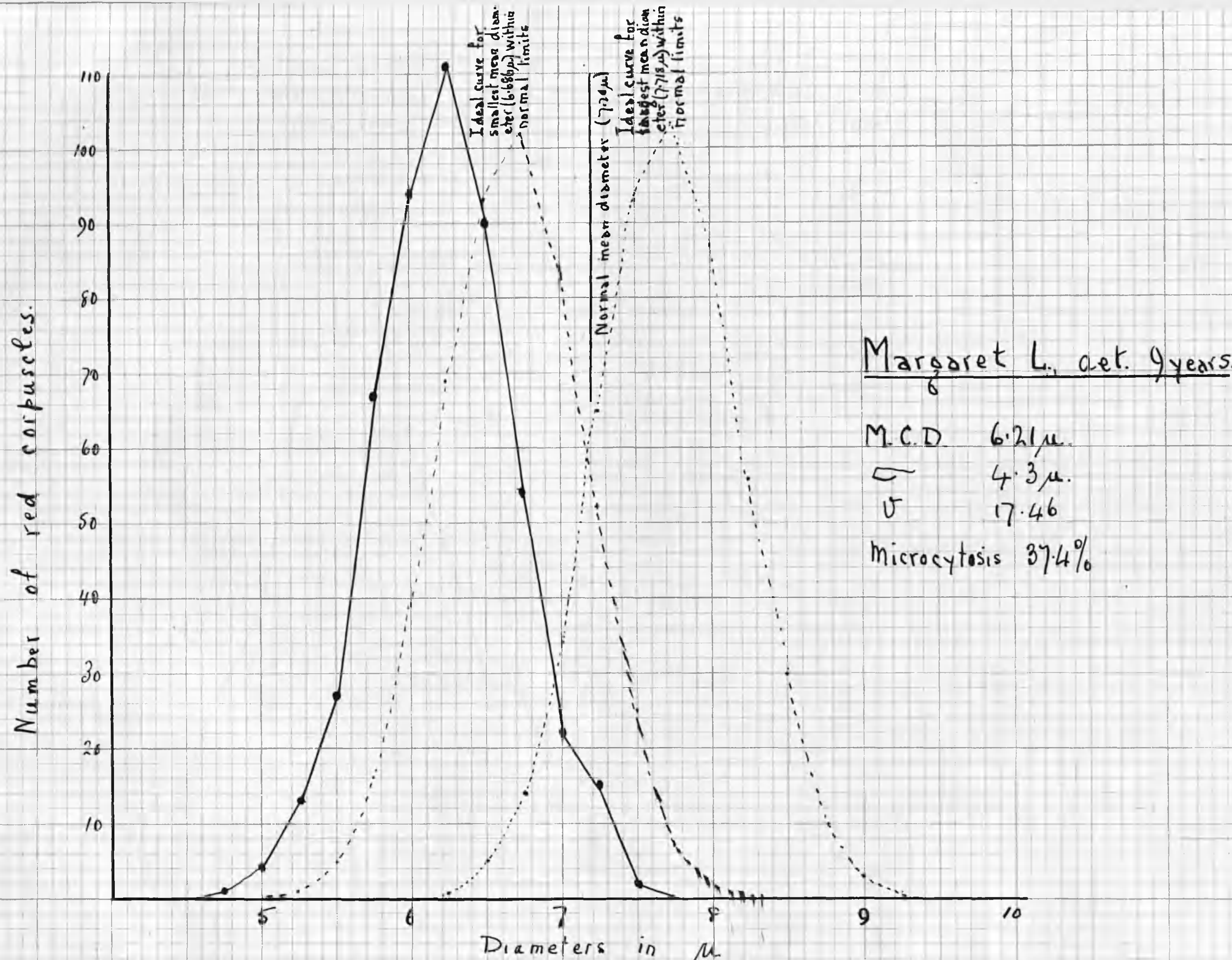


Fig. VIII. Price-Jones Curves in a case of familial acholuric jaundice two years after splenectomy.

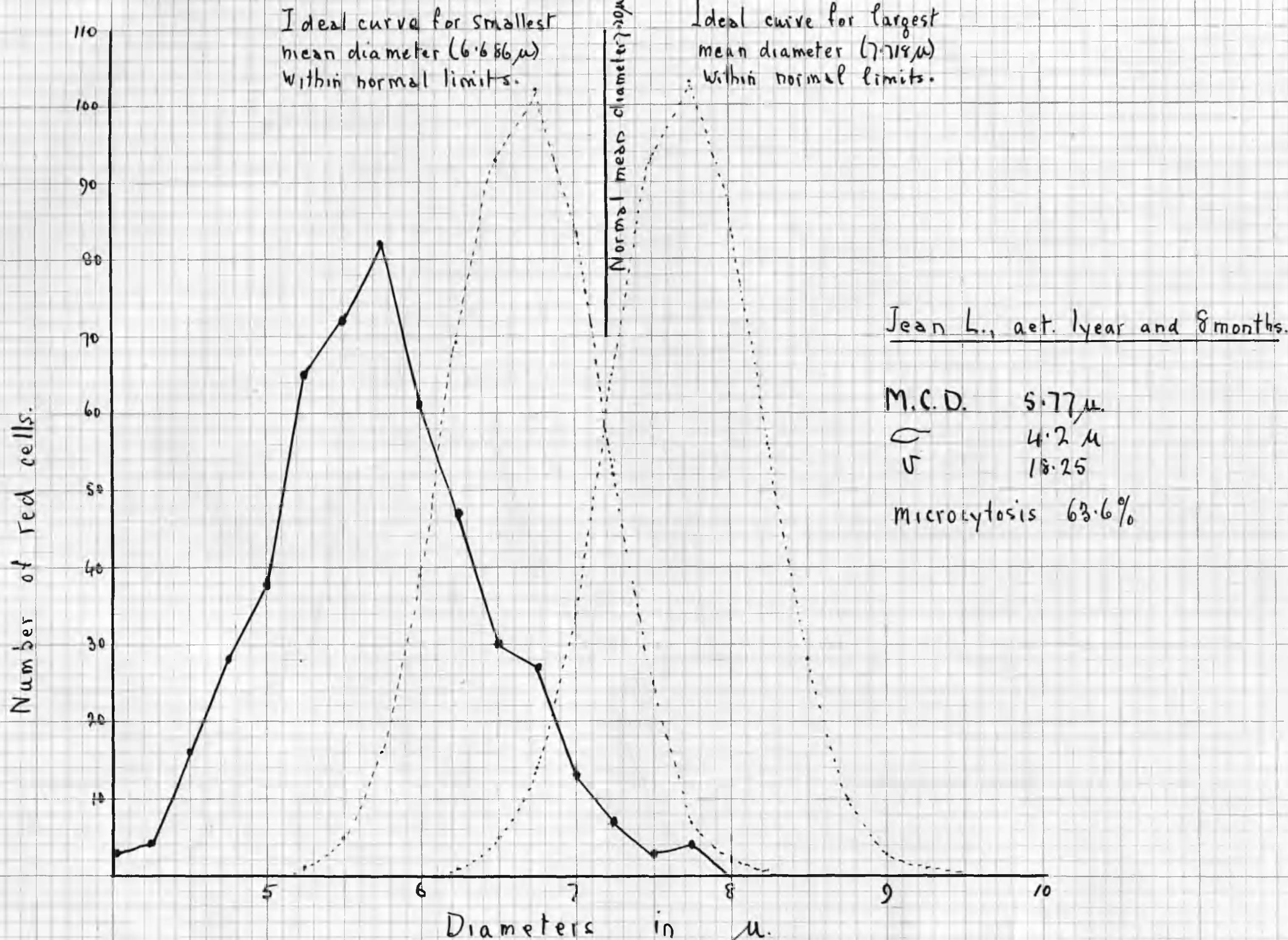


Fig IX Price-Jones Curves in a case of familial acholuric jaundice.

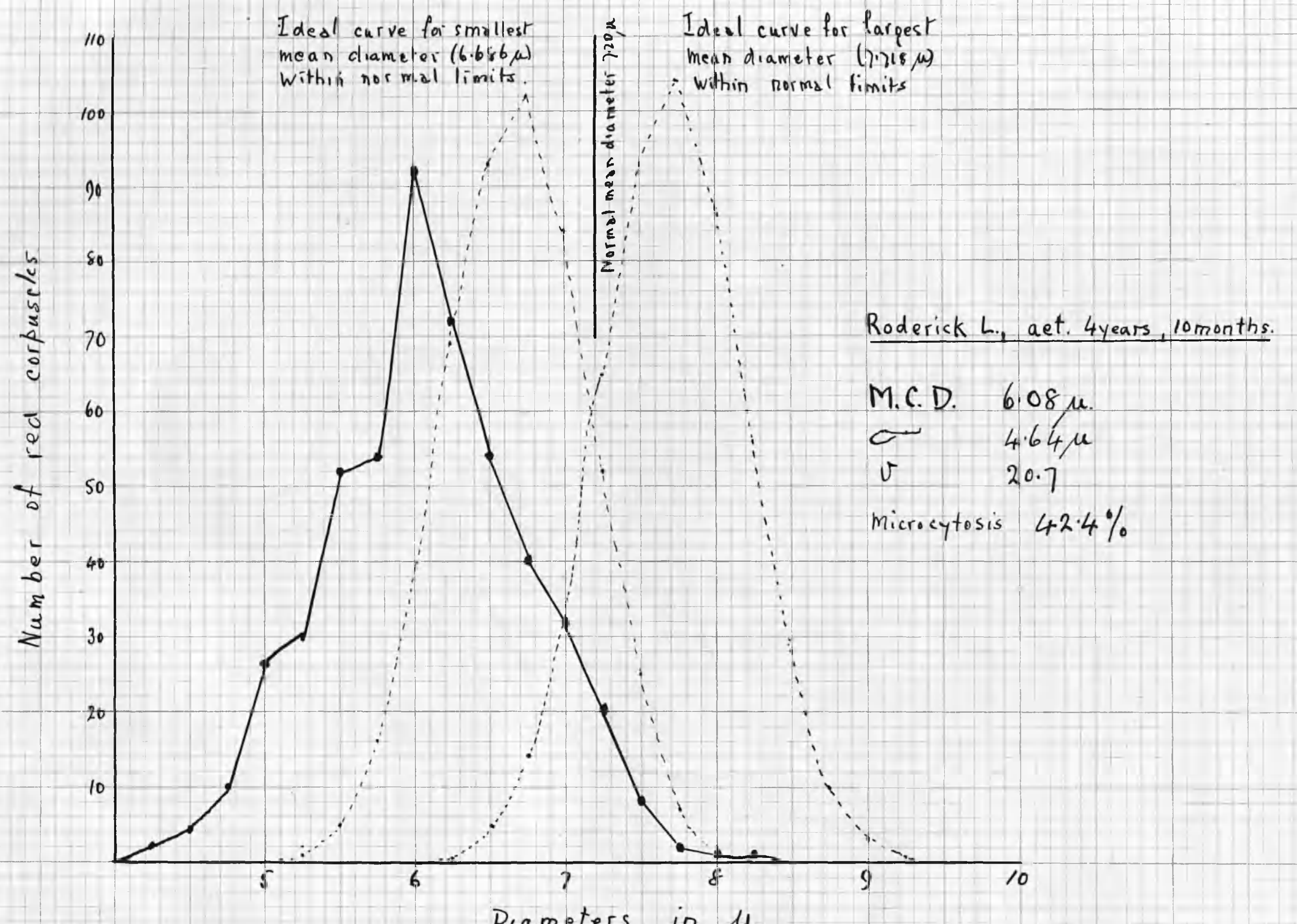


Fig. X Price-Jones Curves in a case of familial acholuric jaundice.

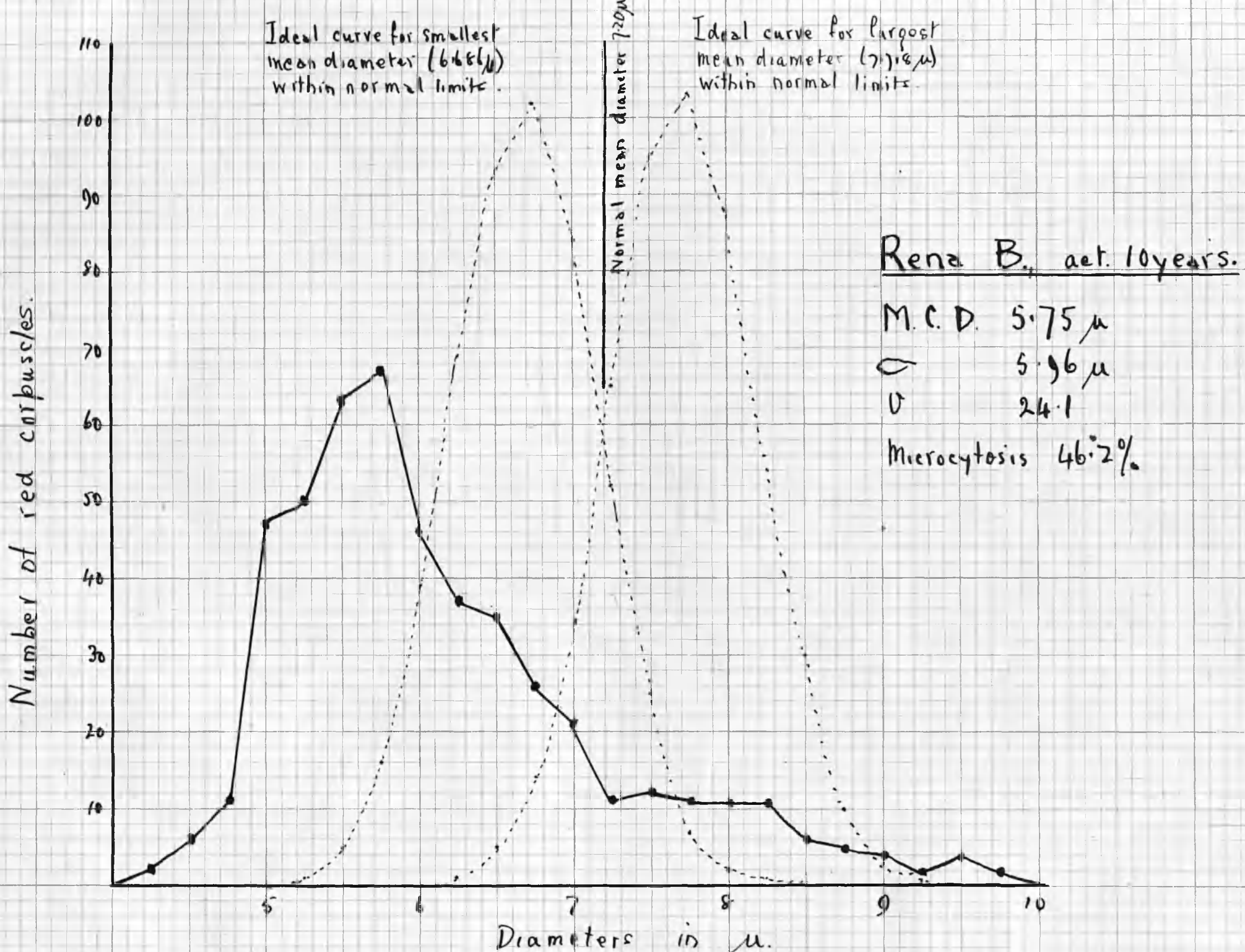


Fig. XI. Price-Jones Curves in a case of acholuric jaundice.

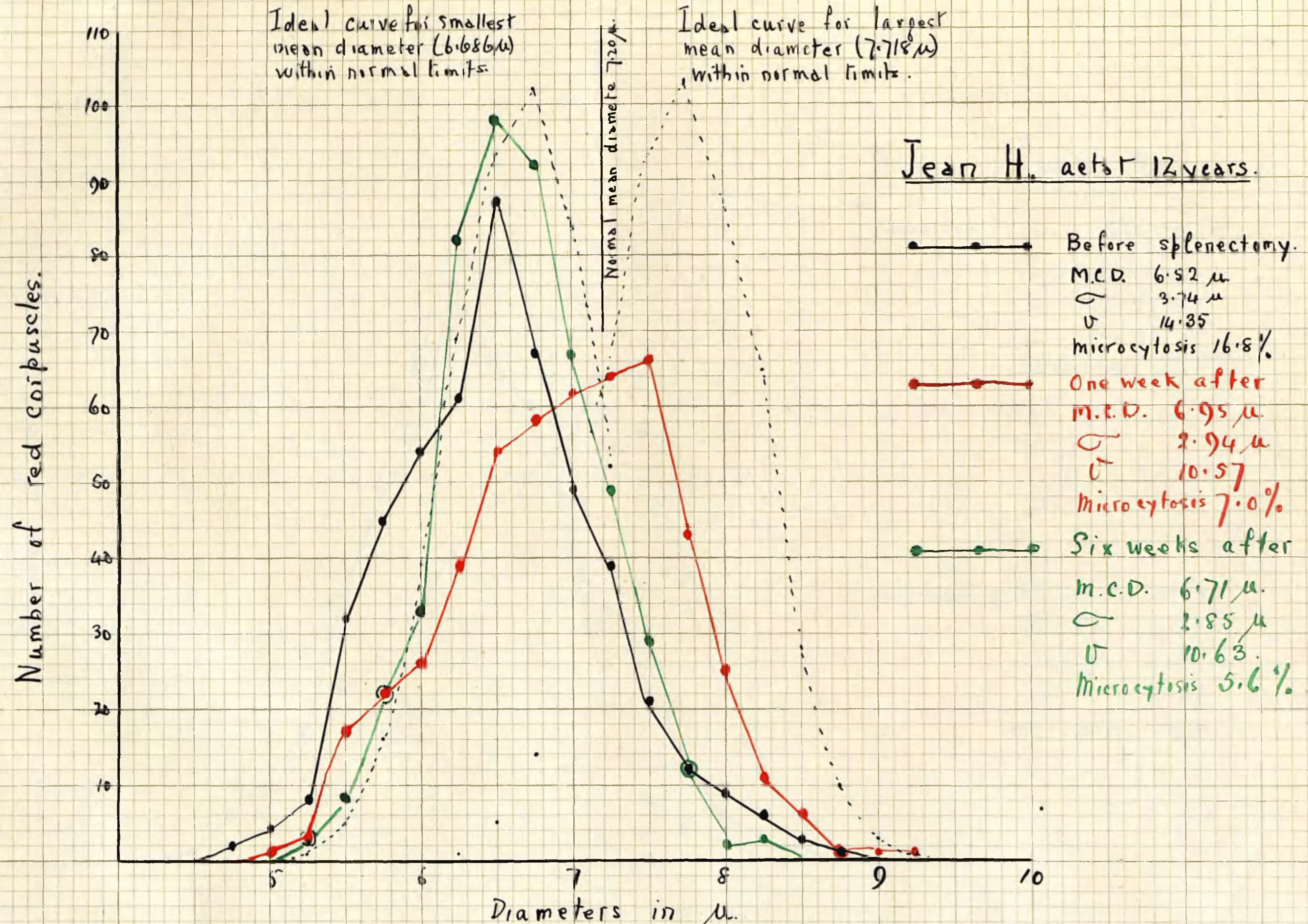


Fig XII. Price-Jones Curves in a case of familial acholuric jaundice before and after splenectomy.

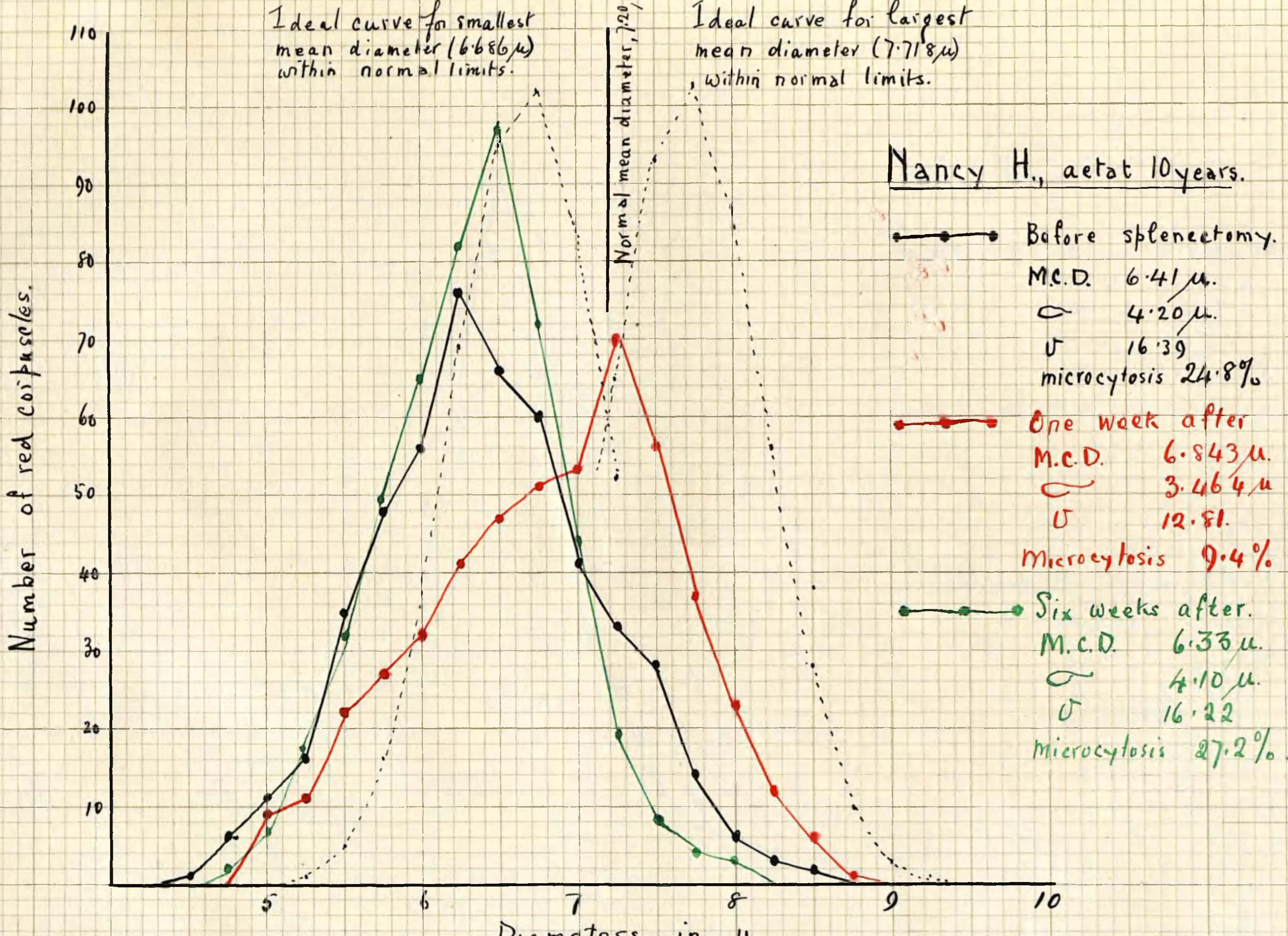


Fig. XIII, Price-Jones Curves in a case of familial acholuric jaundice, before and after splenectomy.

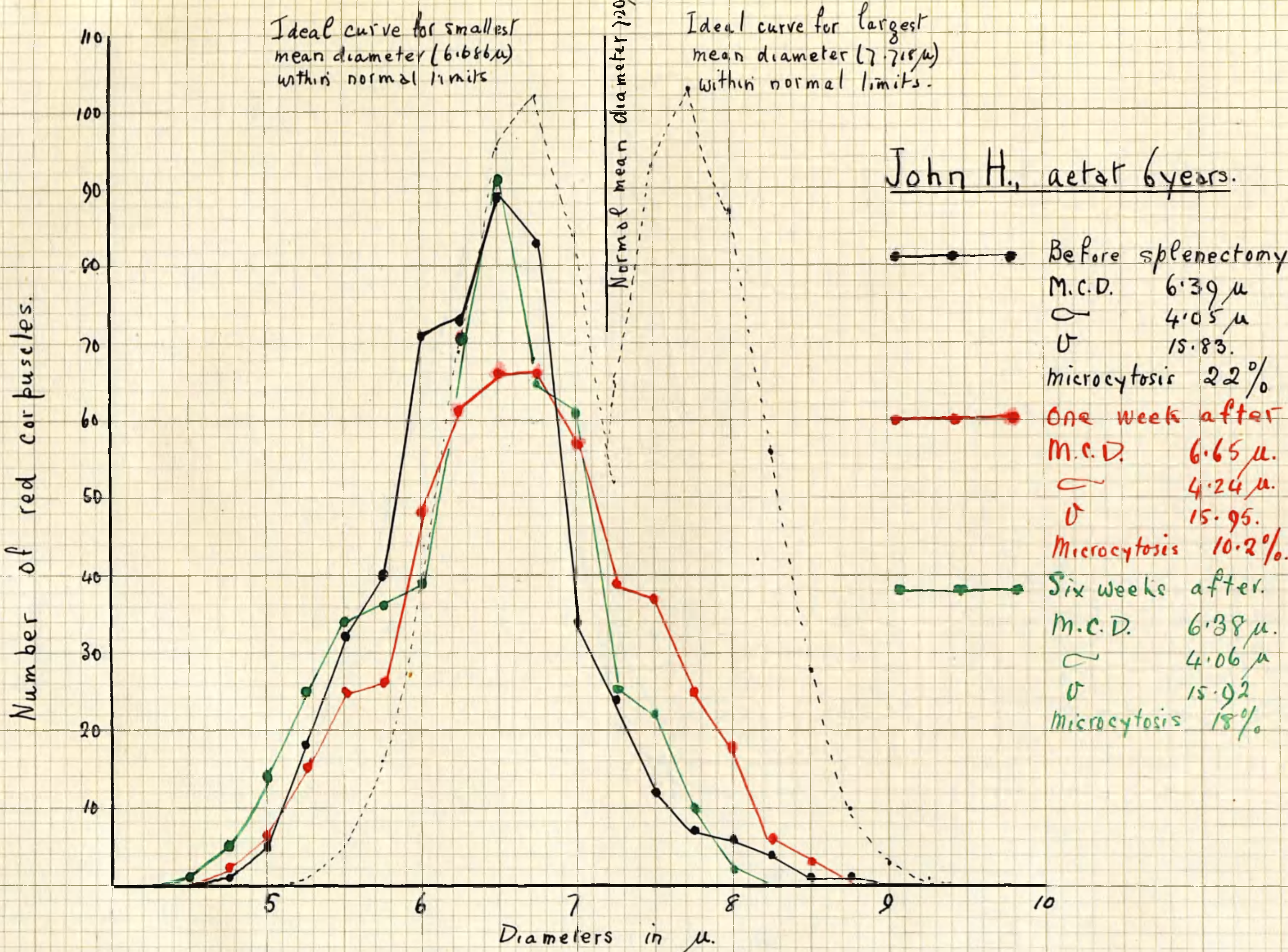


Fig. XIV. Price-Jones Curves in a case of familial acholuric jaundice, before and after splenectomy.

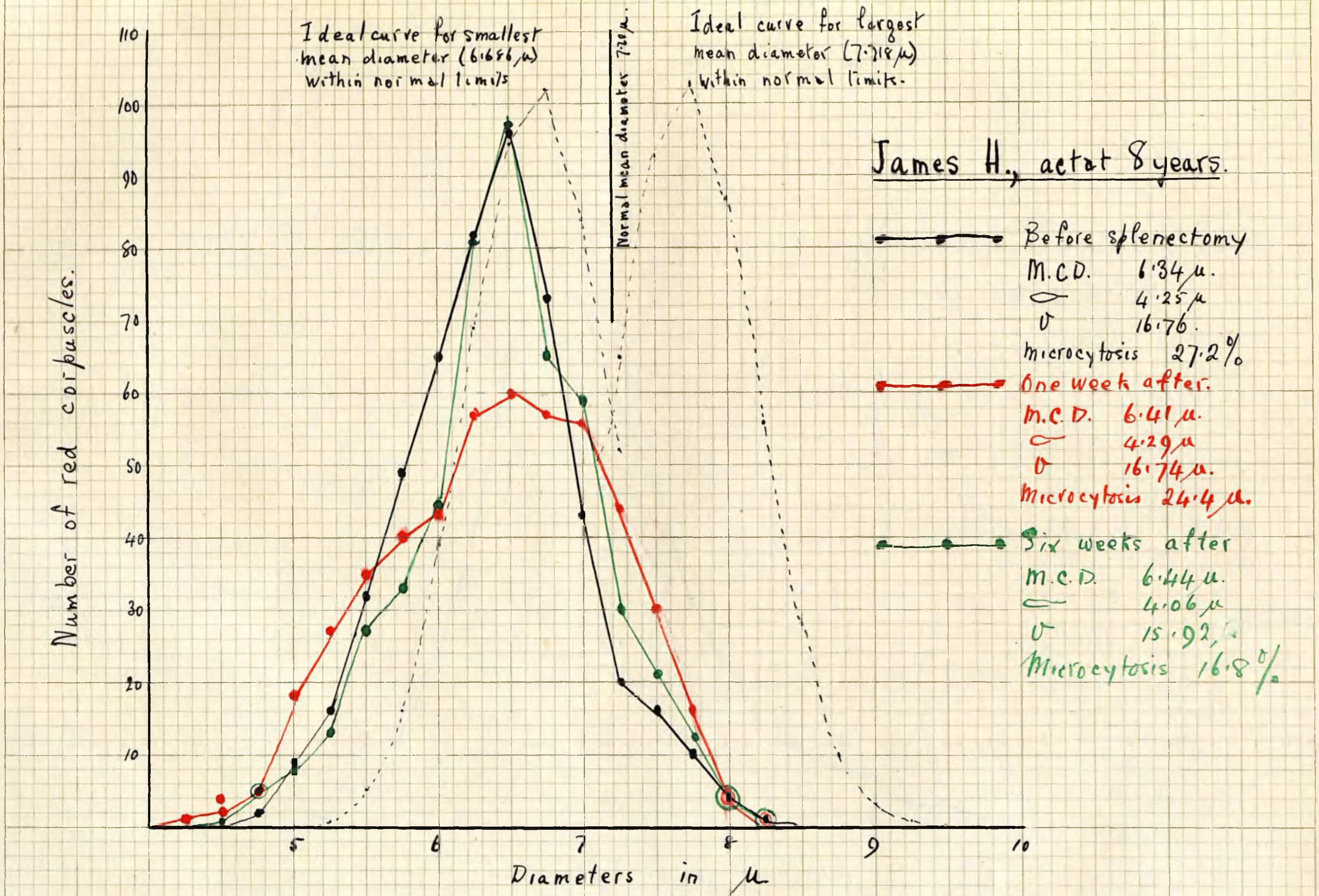


Fig. XV. Price-Jones Curves in a case of familial acholuric jaundice, before and after splenectomy.

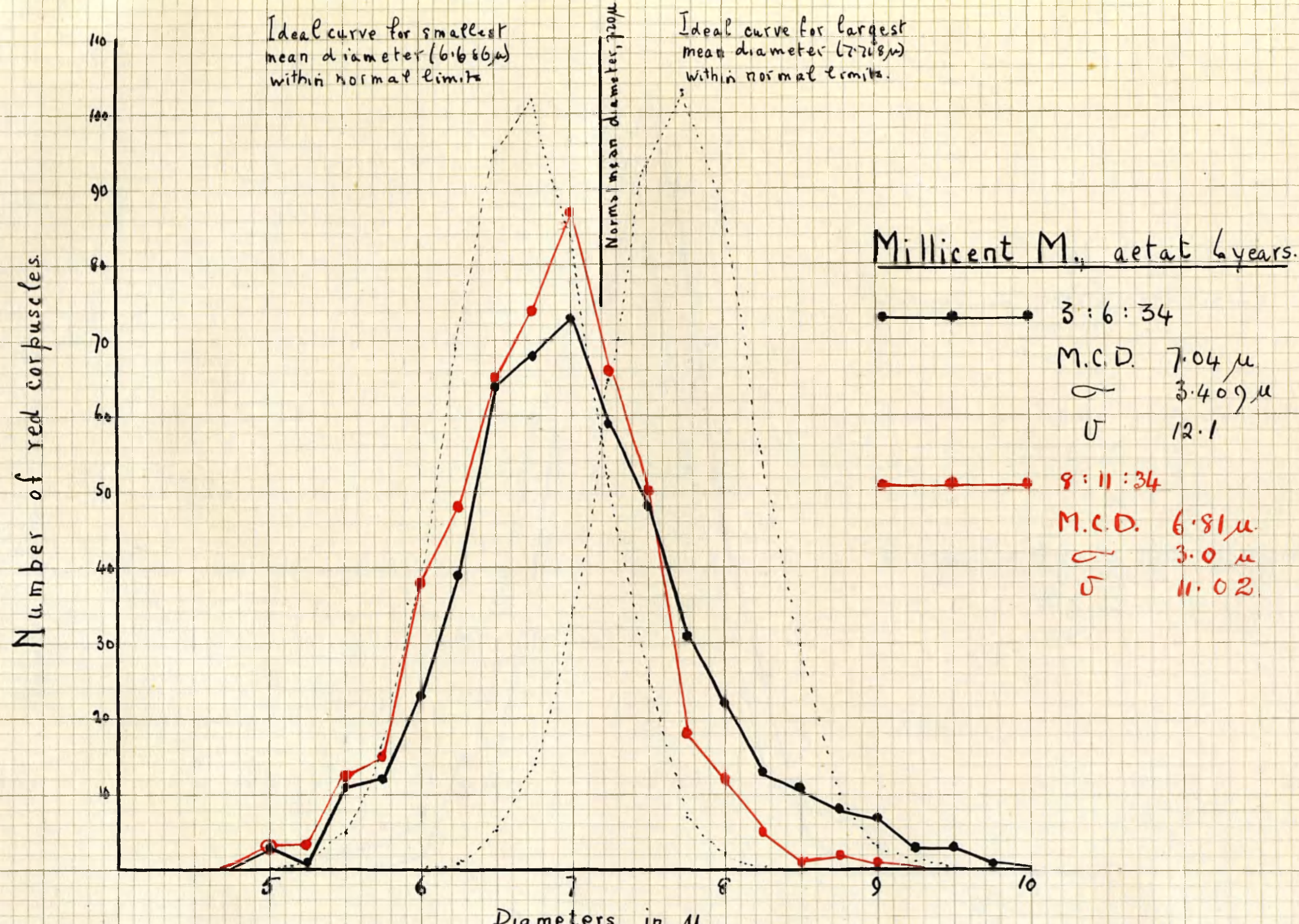


Fig XVI. Price-Jones Curves in a case of acquired acholuric jaundice.

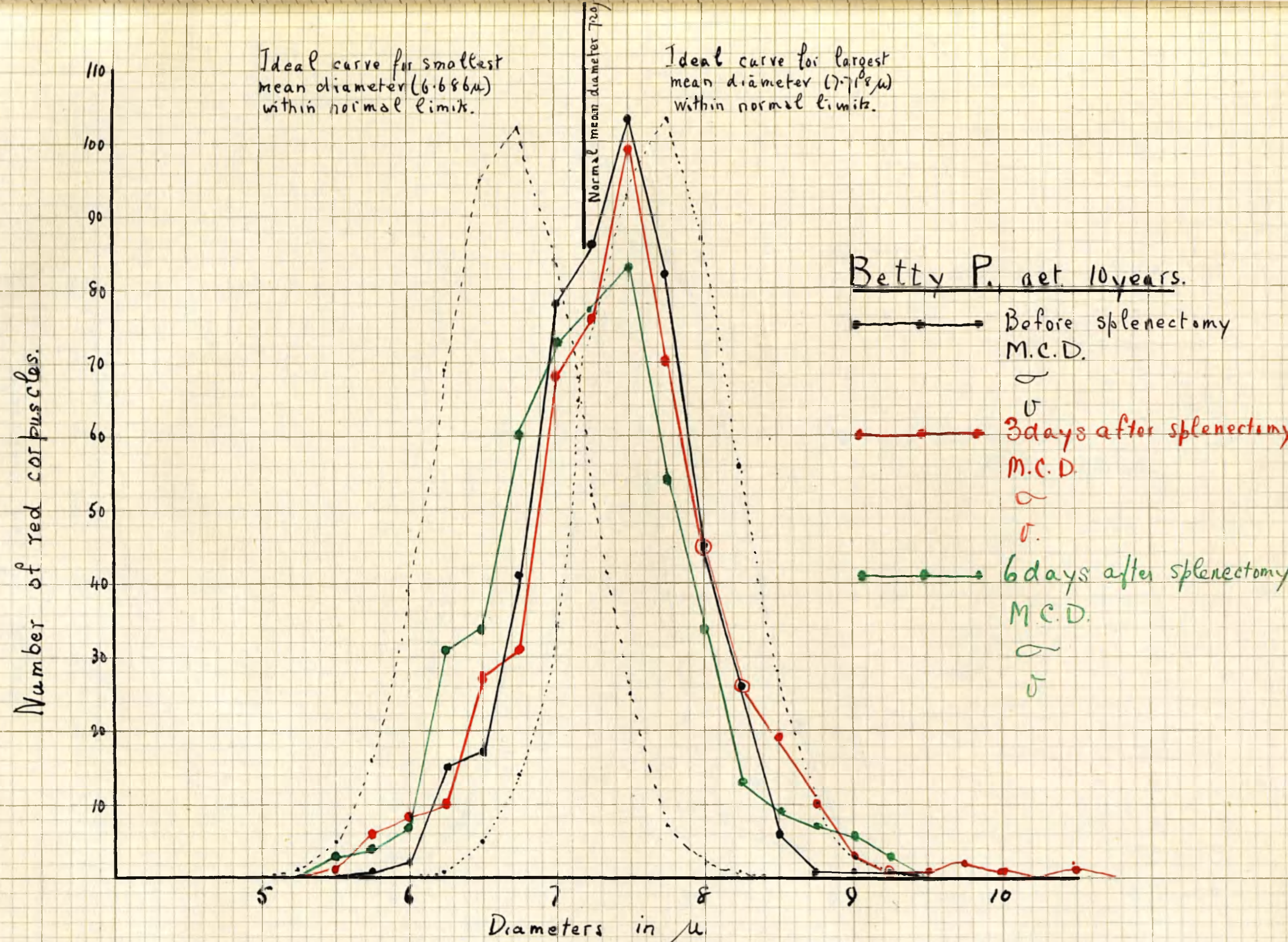


Fig. XVII. Price-Jones Curves in a case of Banti's Disease, before and after splenectomy.

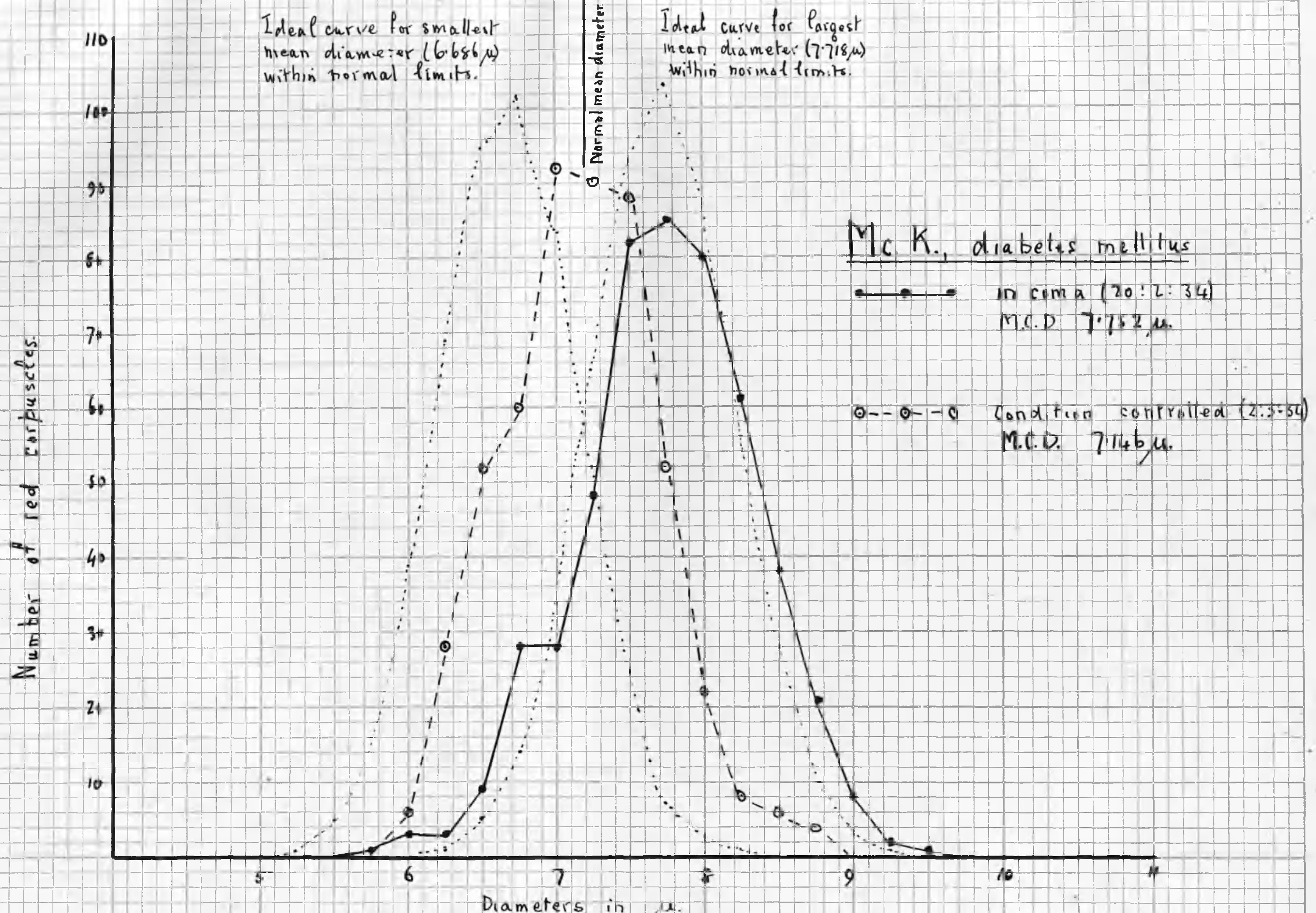


Fig. XVIII. Price-Jones Curves in a case of Acidosis from Diabetic Coma before and after control by treatment.

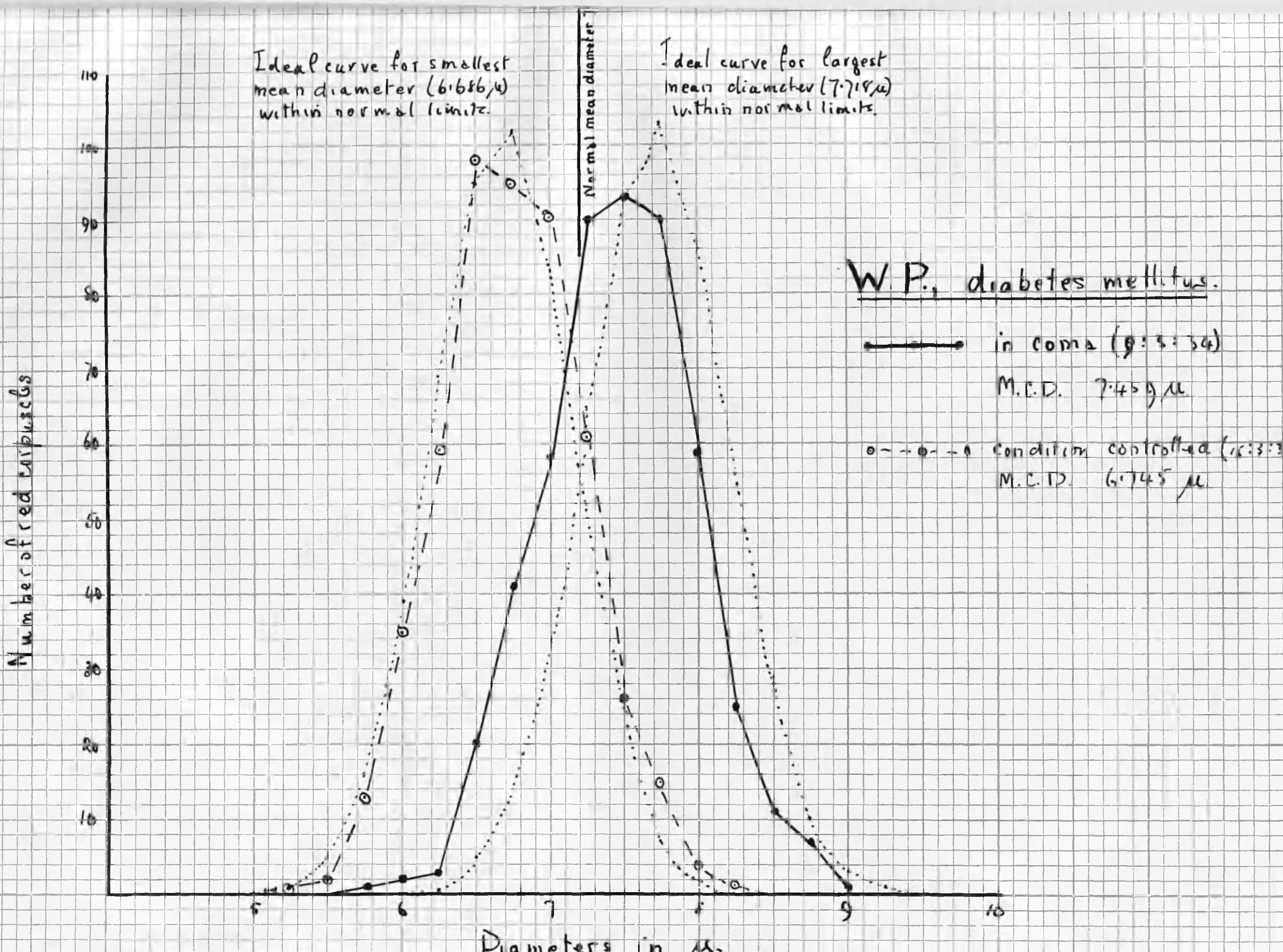


Fig. XIX. Price-Jones Curves in a case of Acidosis from Diabetic Coma, before and after control by treatment

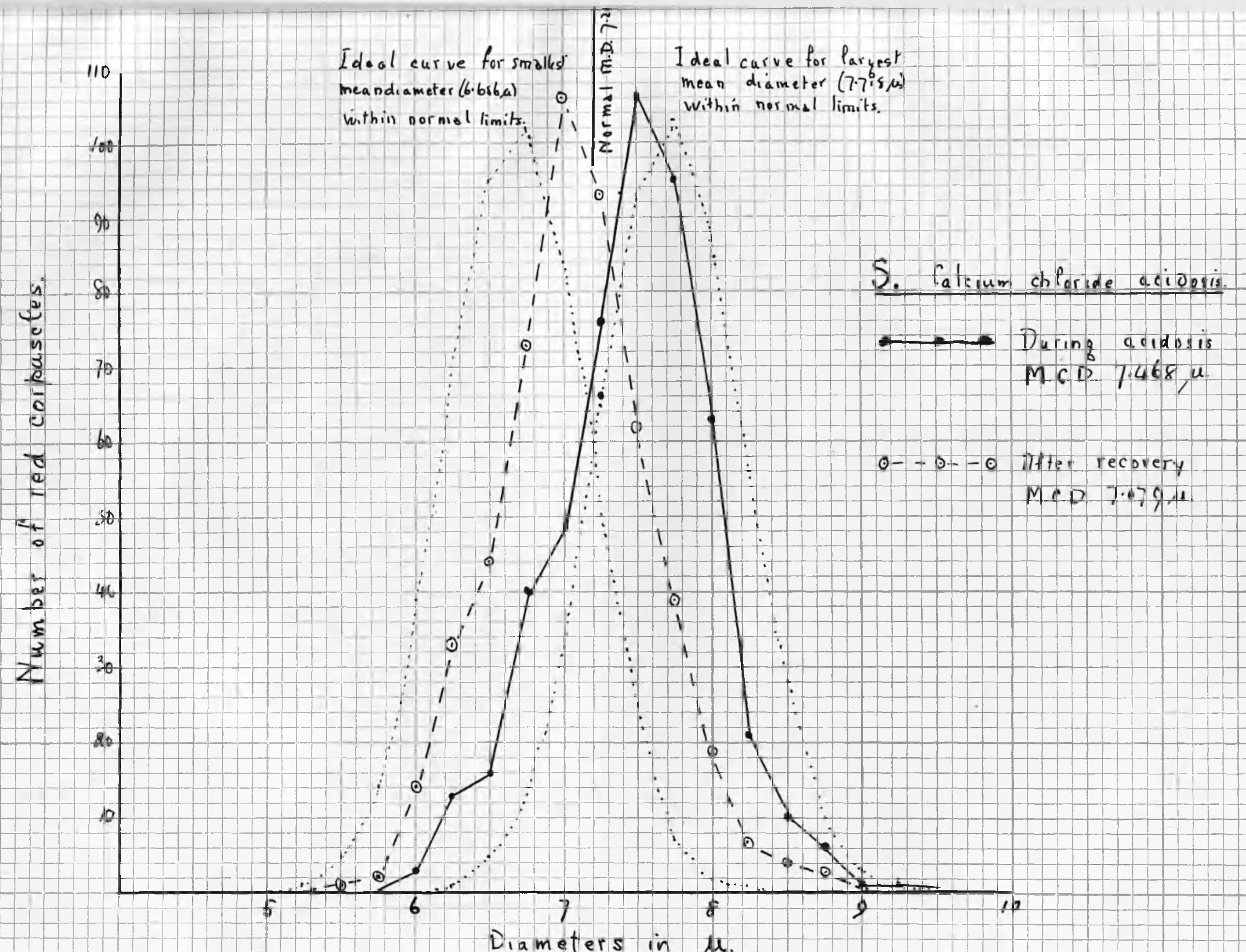


Fig. XX The effect of Acidosis on Red Cell Diameter.
(Acidosis produced by calcium chloride)

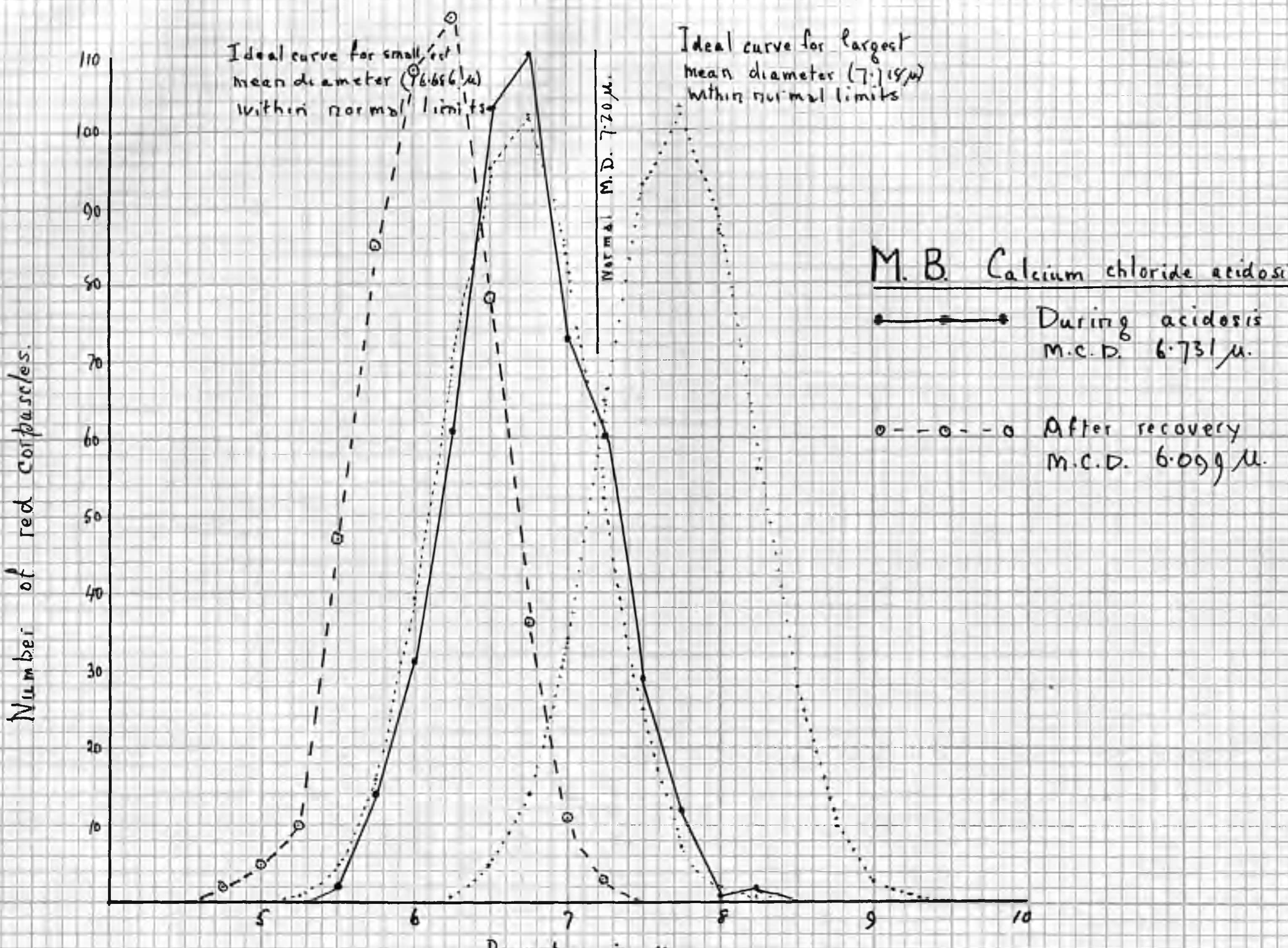


Fig. XXI. The effect of Acidosis on Red Cell Diameter
(Acidosis produced by calcium chloride.)

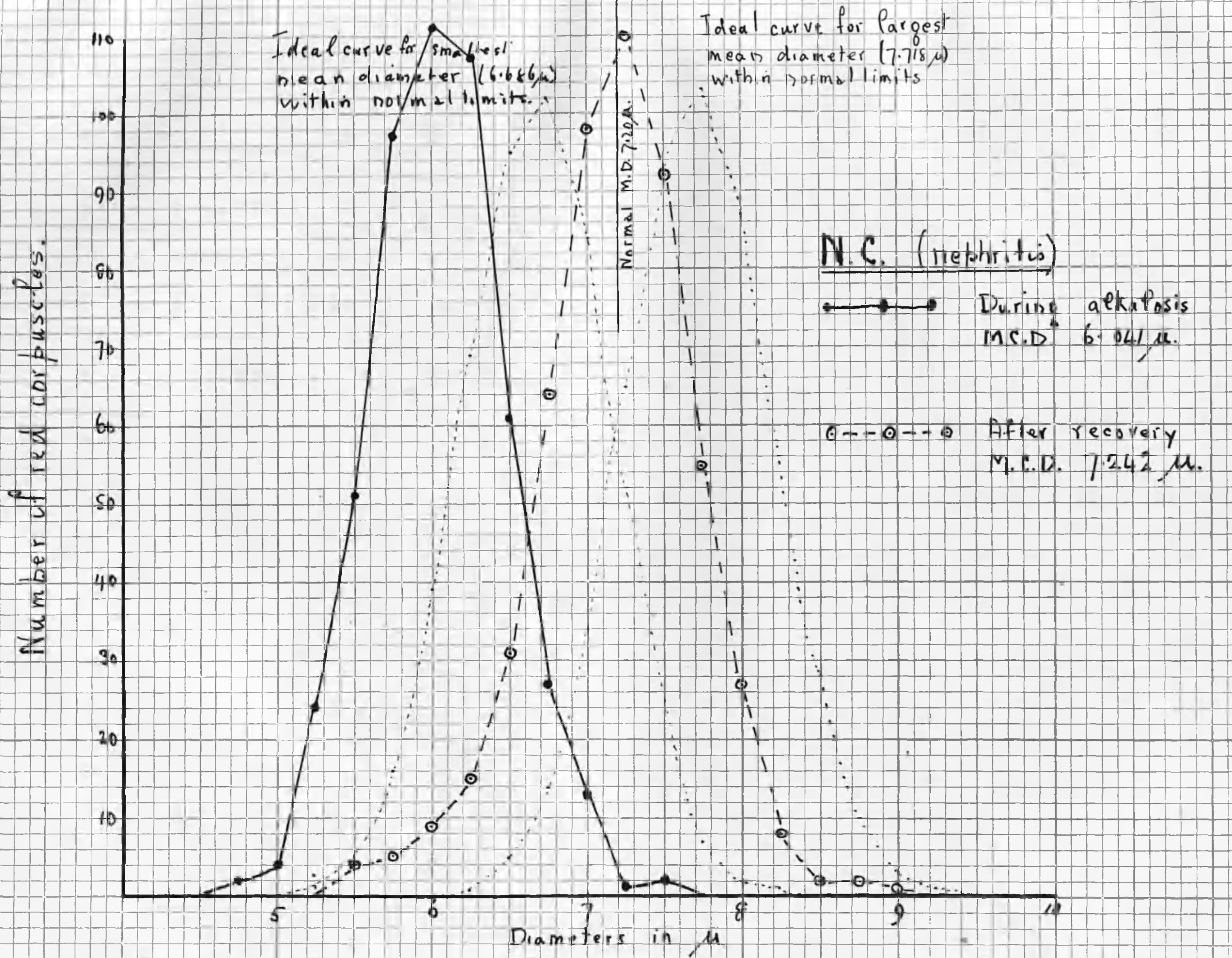


Fig. XXII. The effect of alkalosis on Red Cell Diameter
(Alkalosis produced by treatment with large doses of sodium bicarbonate.)

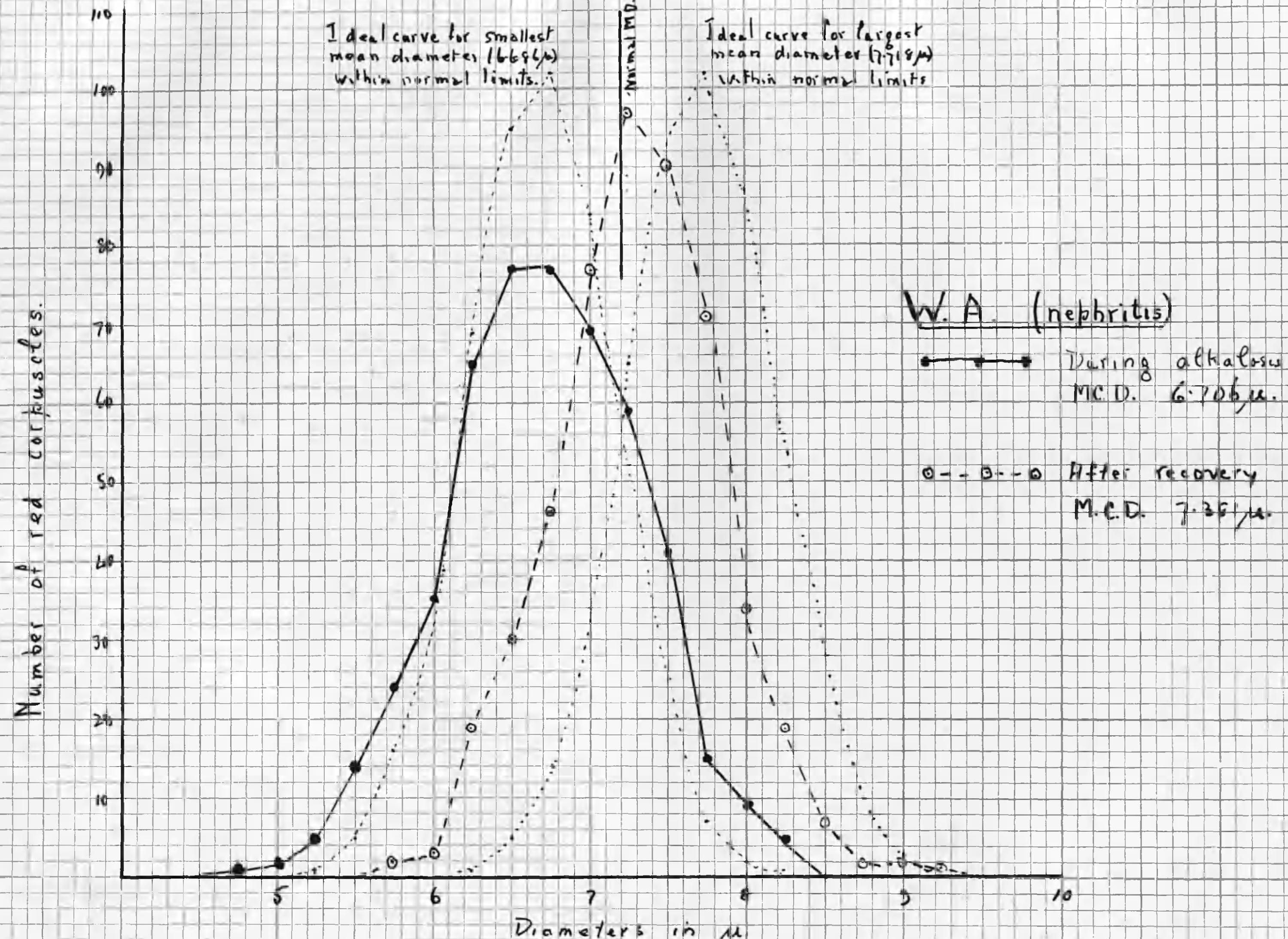


Fig. XXIII The effect of Alkalosis on Red Cell Diameter.
(Alkalosis produced by treatment with large doses of sodium bicarbonate.)

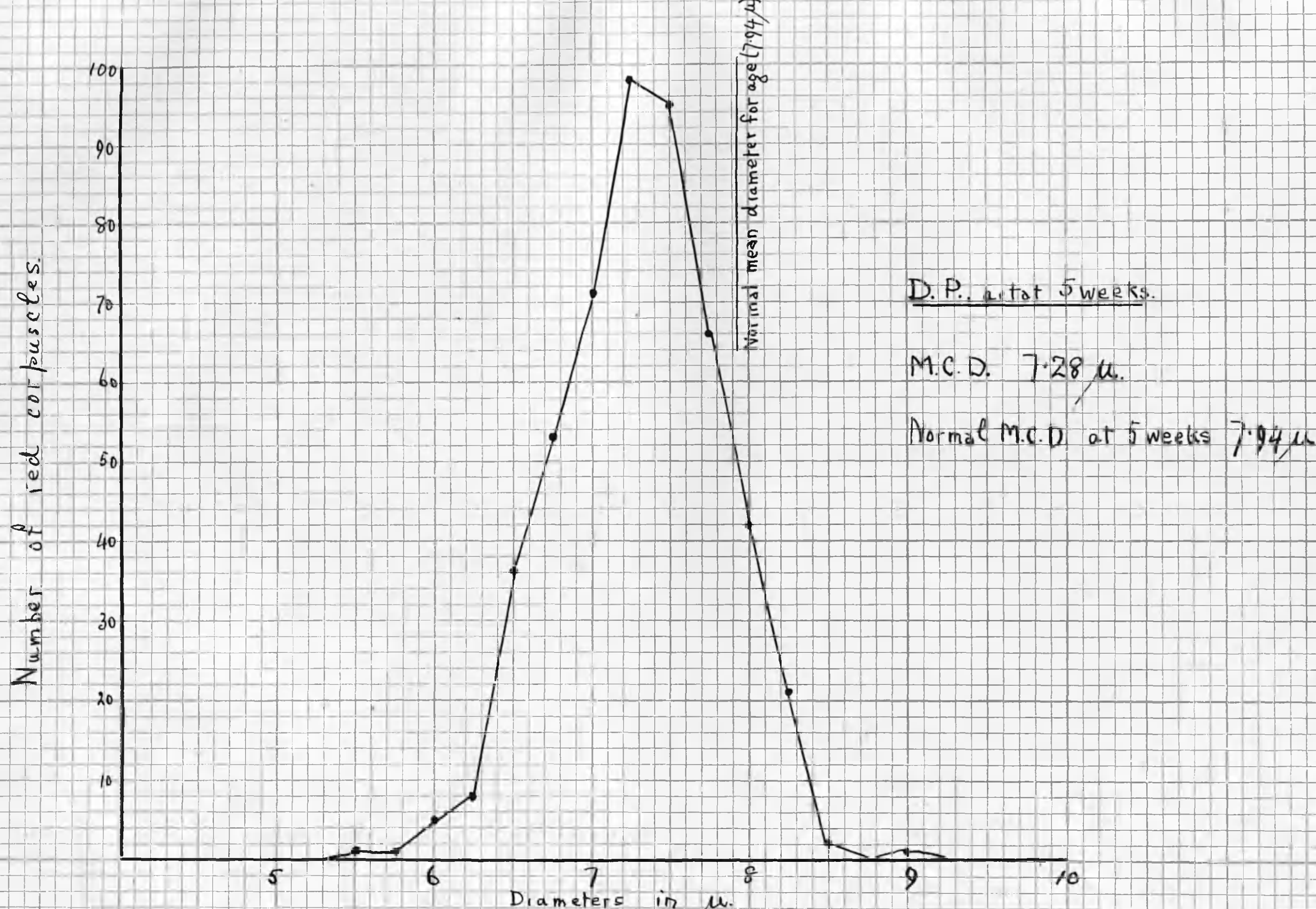


Fig. XXIV. Price-Jones Curve in a case of Congenital Hybertrophic Pyloric Stenosis.

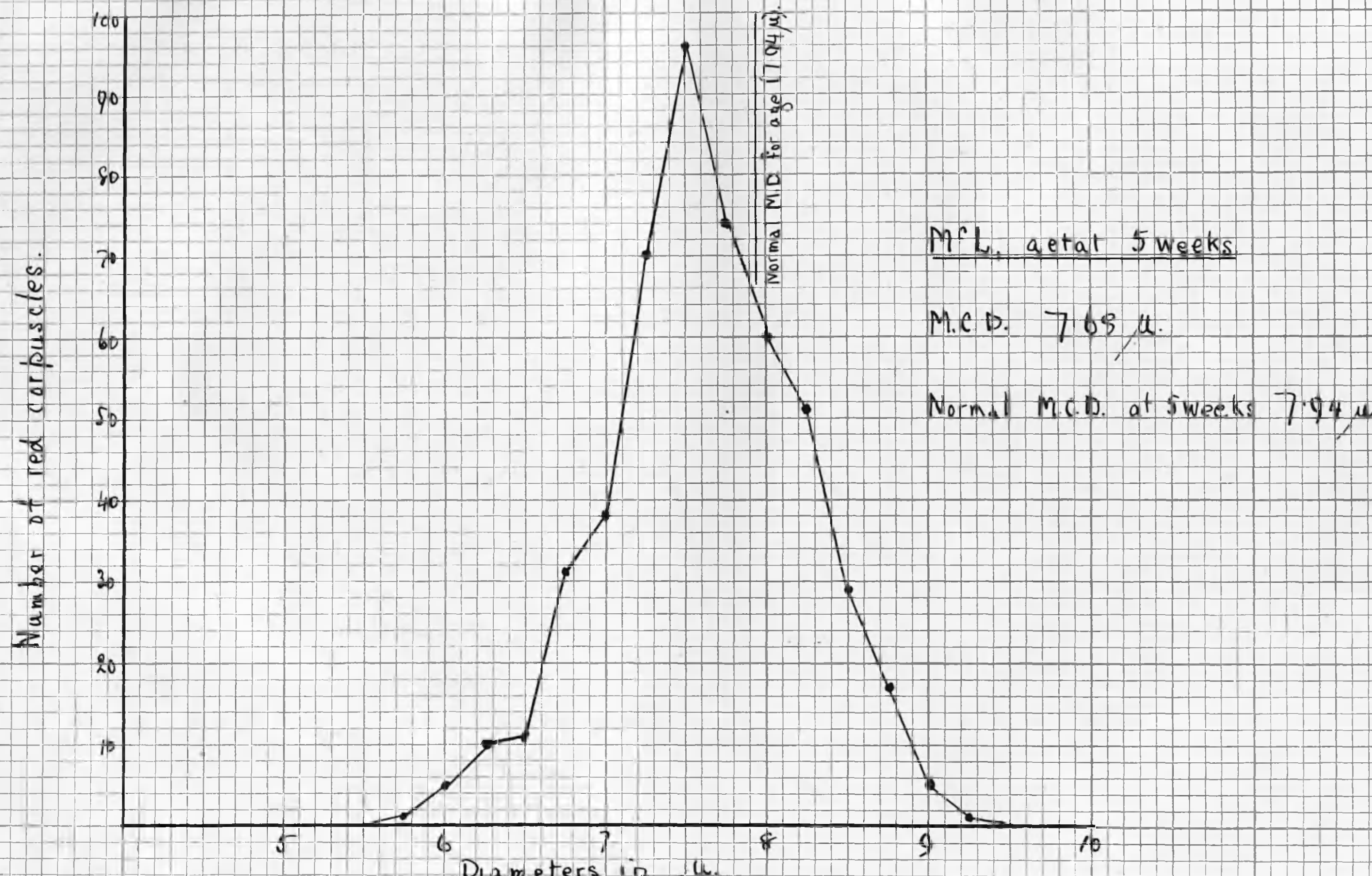


Fig. XXV. Price-Jones Curve in a case of Congenital Hypertrophic Pyloric Stenosis.

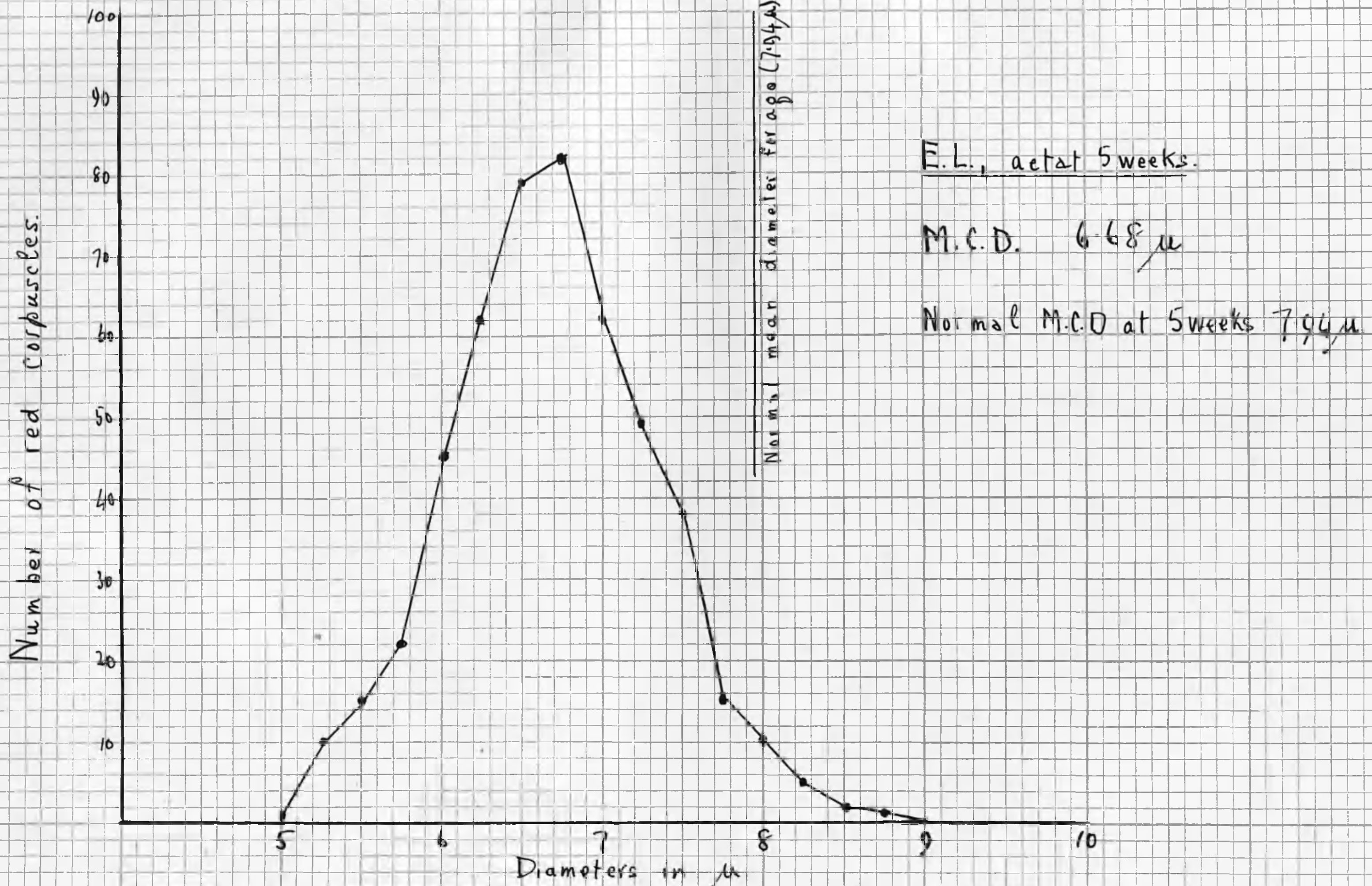


Fig. XXVI. Price-Jones Curve in a case of Congenital Hypertrophic Pyloric Stenosis.

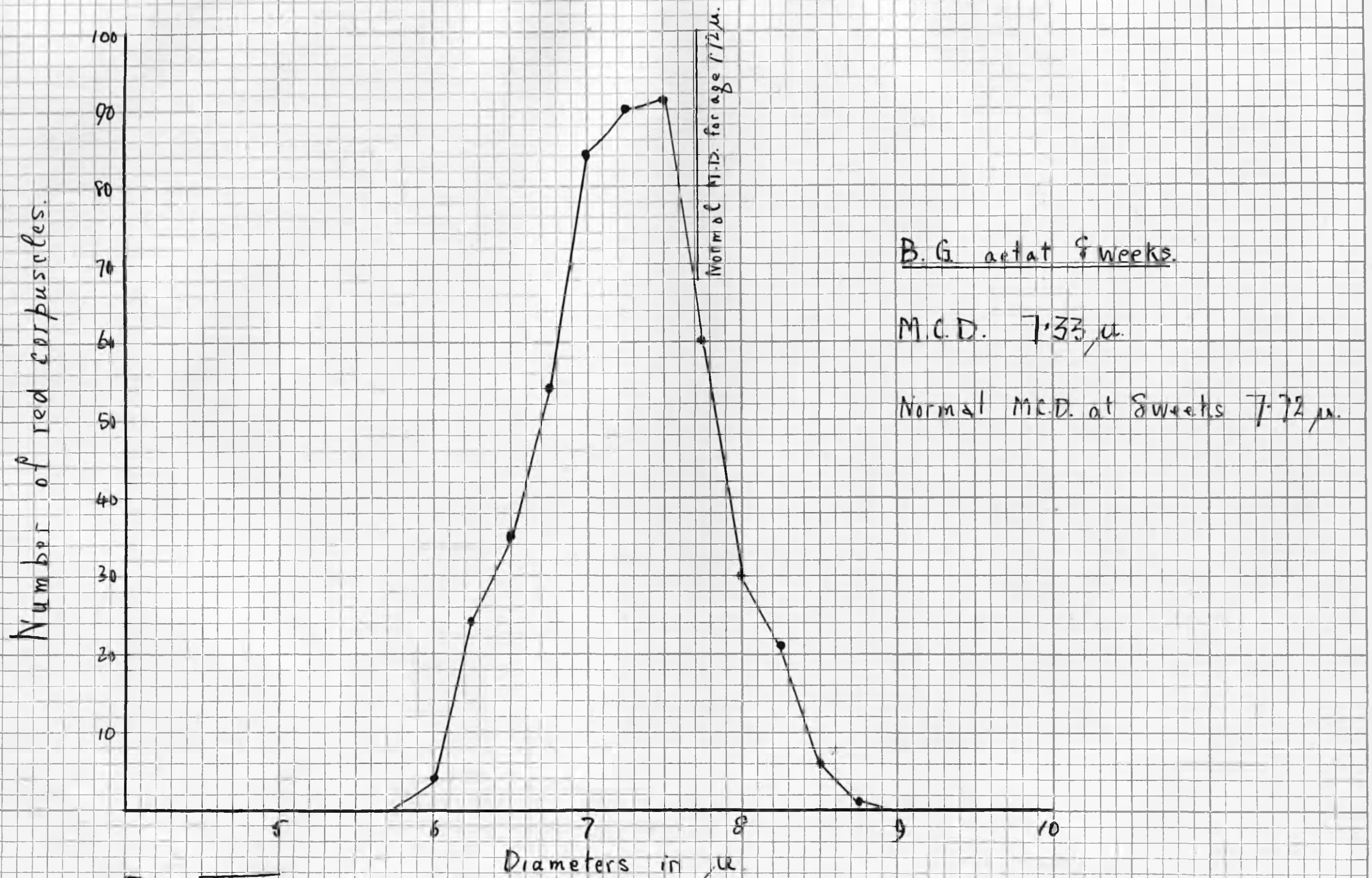


Fig. XXVII. Price-Jones Curve in a case of Congenital Hypertrophic Pyloric Stenosis.

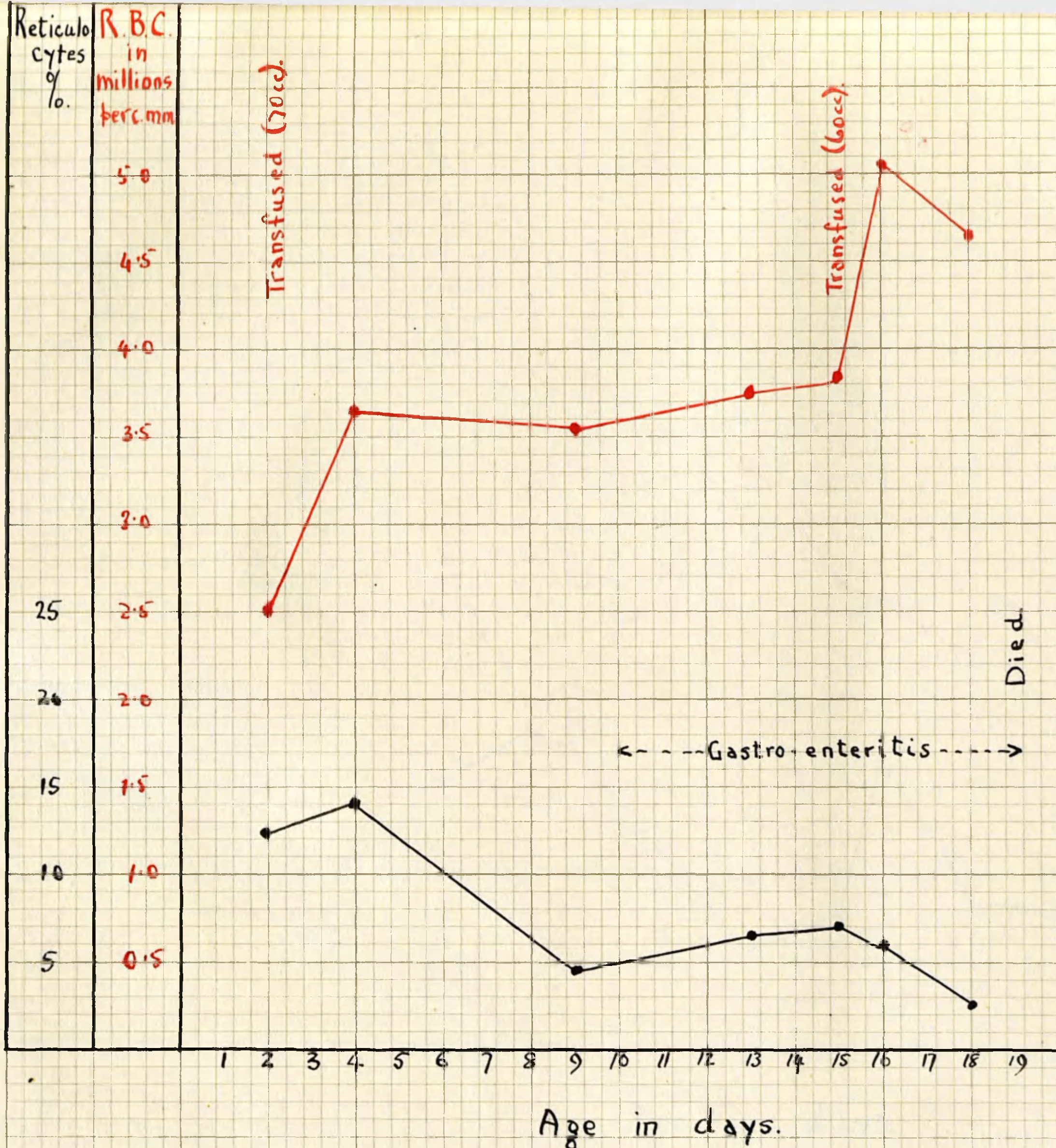


Chart I. Case No I. Icterus Gravis

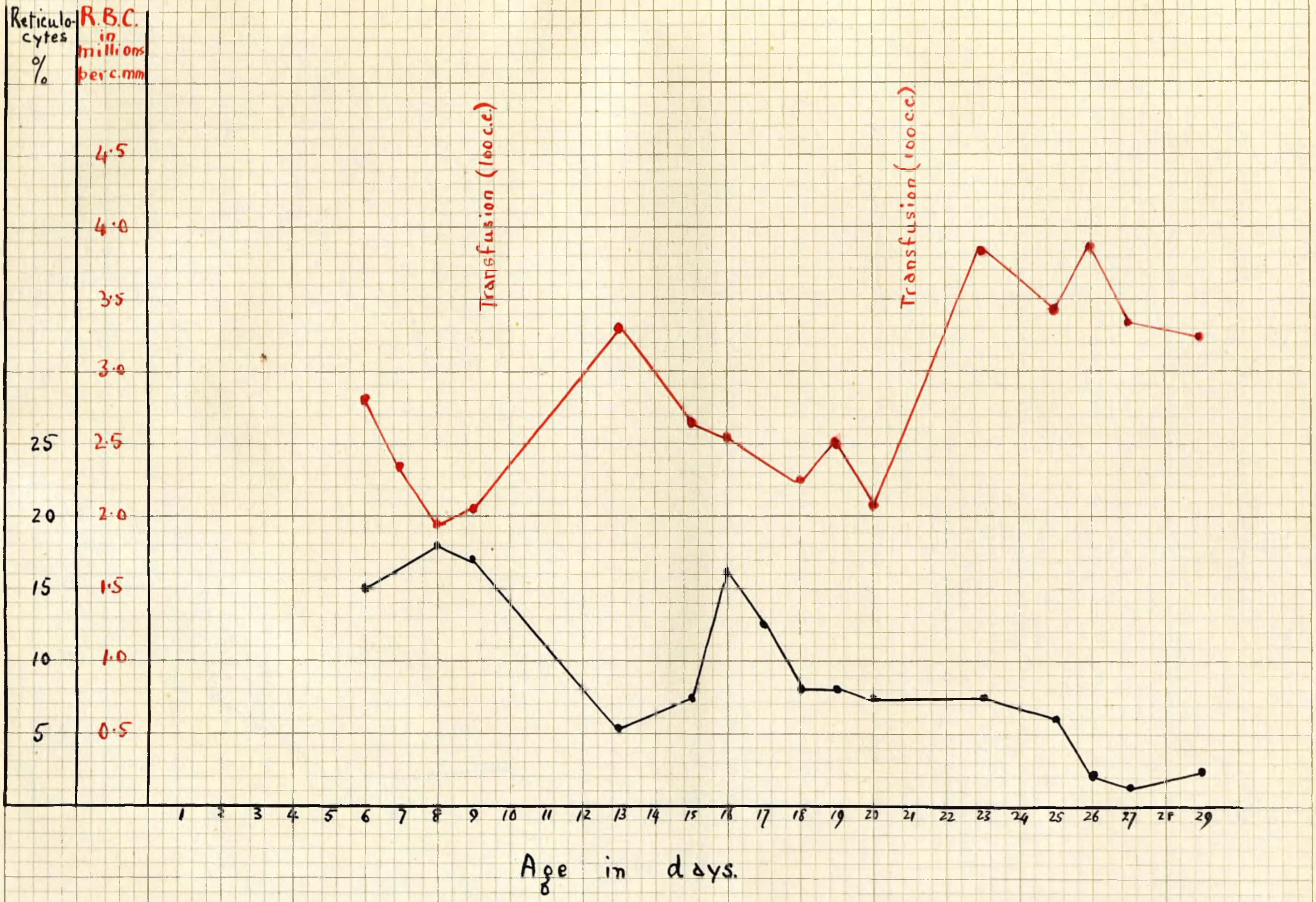


Chart II. Case 5. Icterus Gravis.

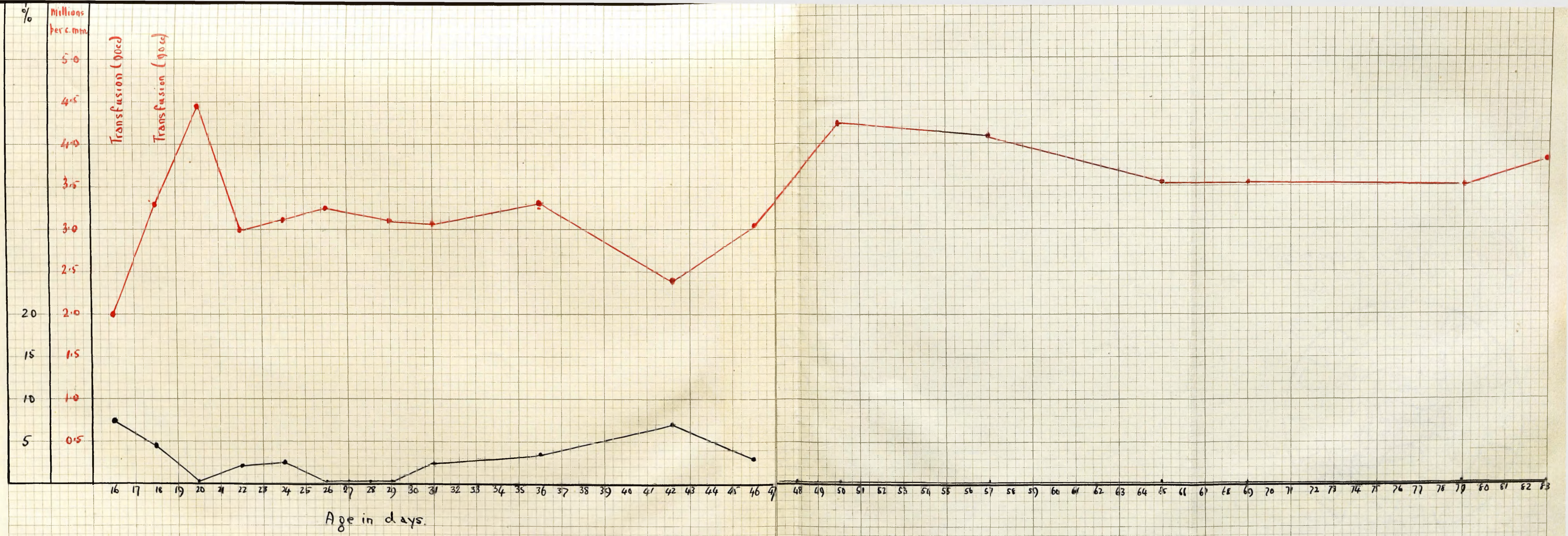


Chart III Case No. 6. Icterus Gravis.

Chart IV Case No 8. Icterus Gravis.

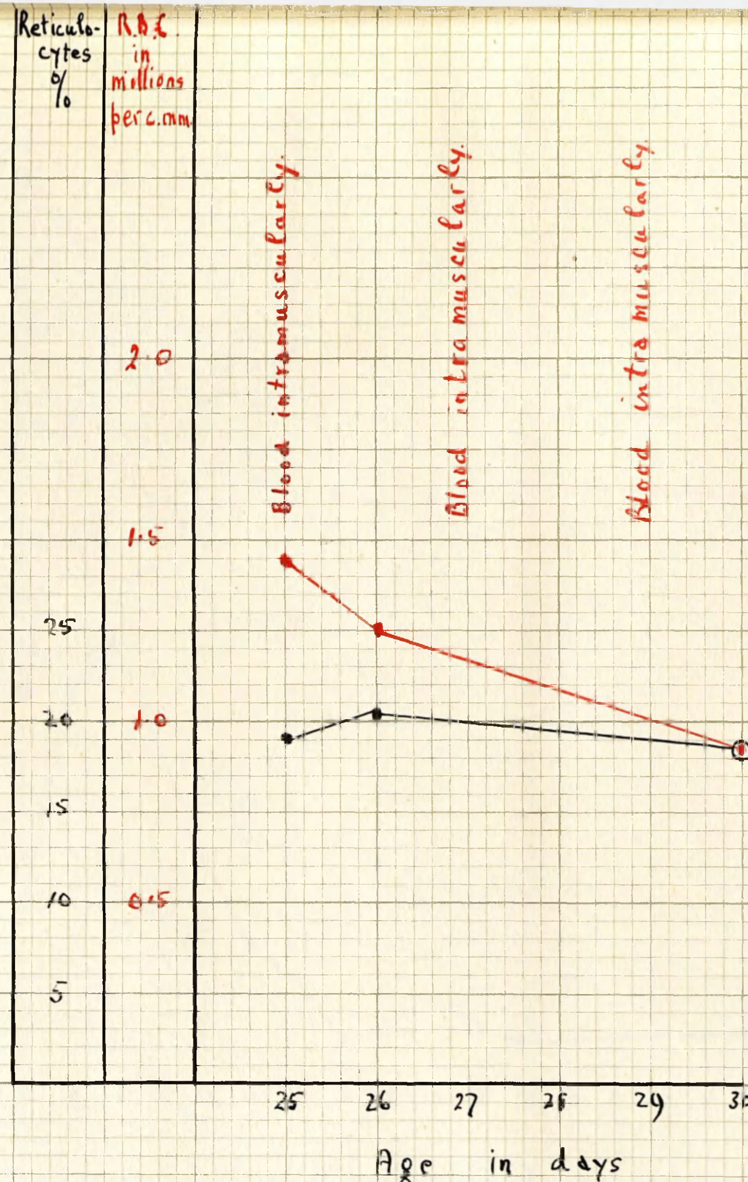


Chart V. Case No 9. Icterus Gravis

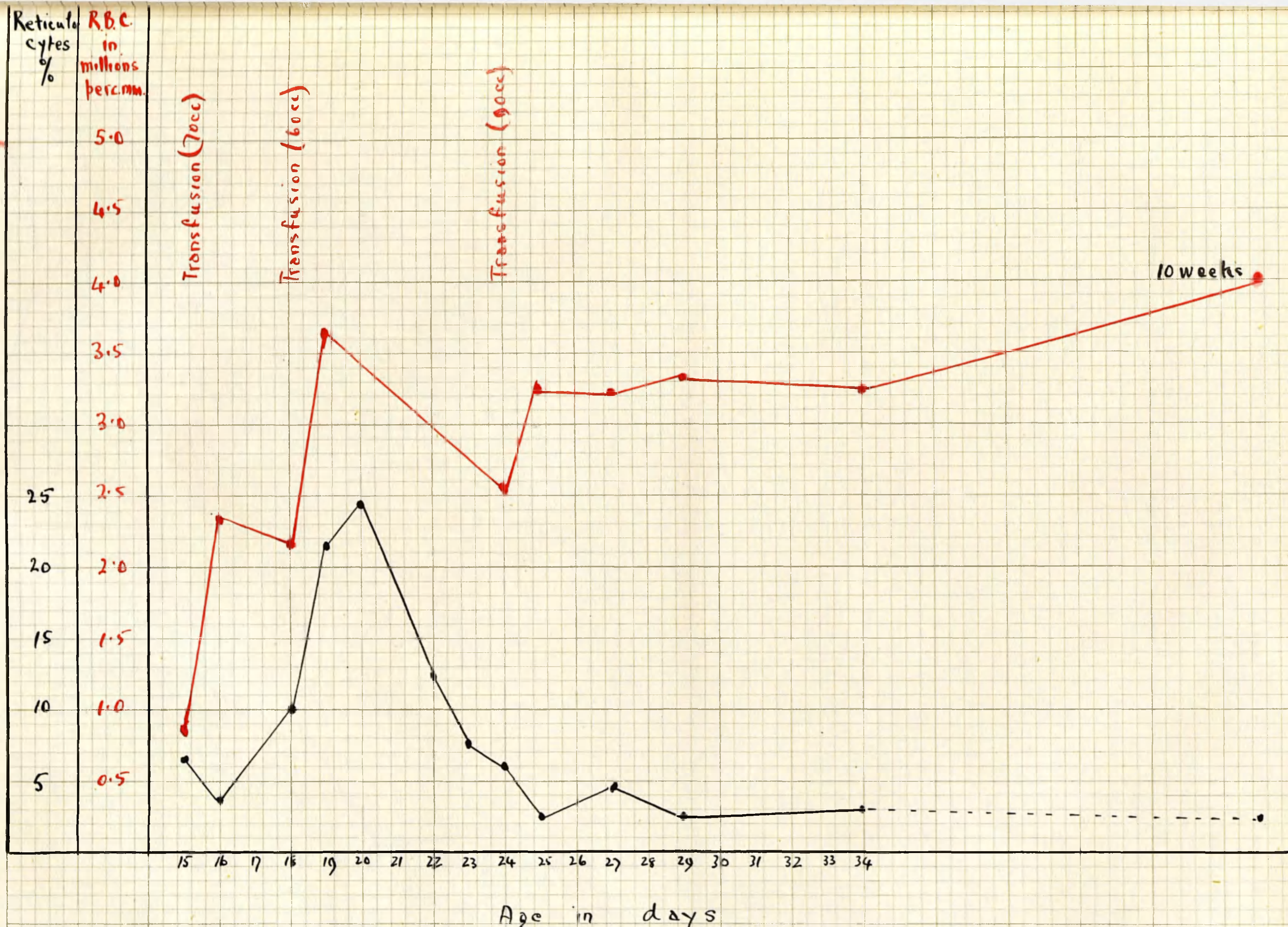


Chart VI. Case No 16. Icterus Gravis

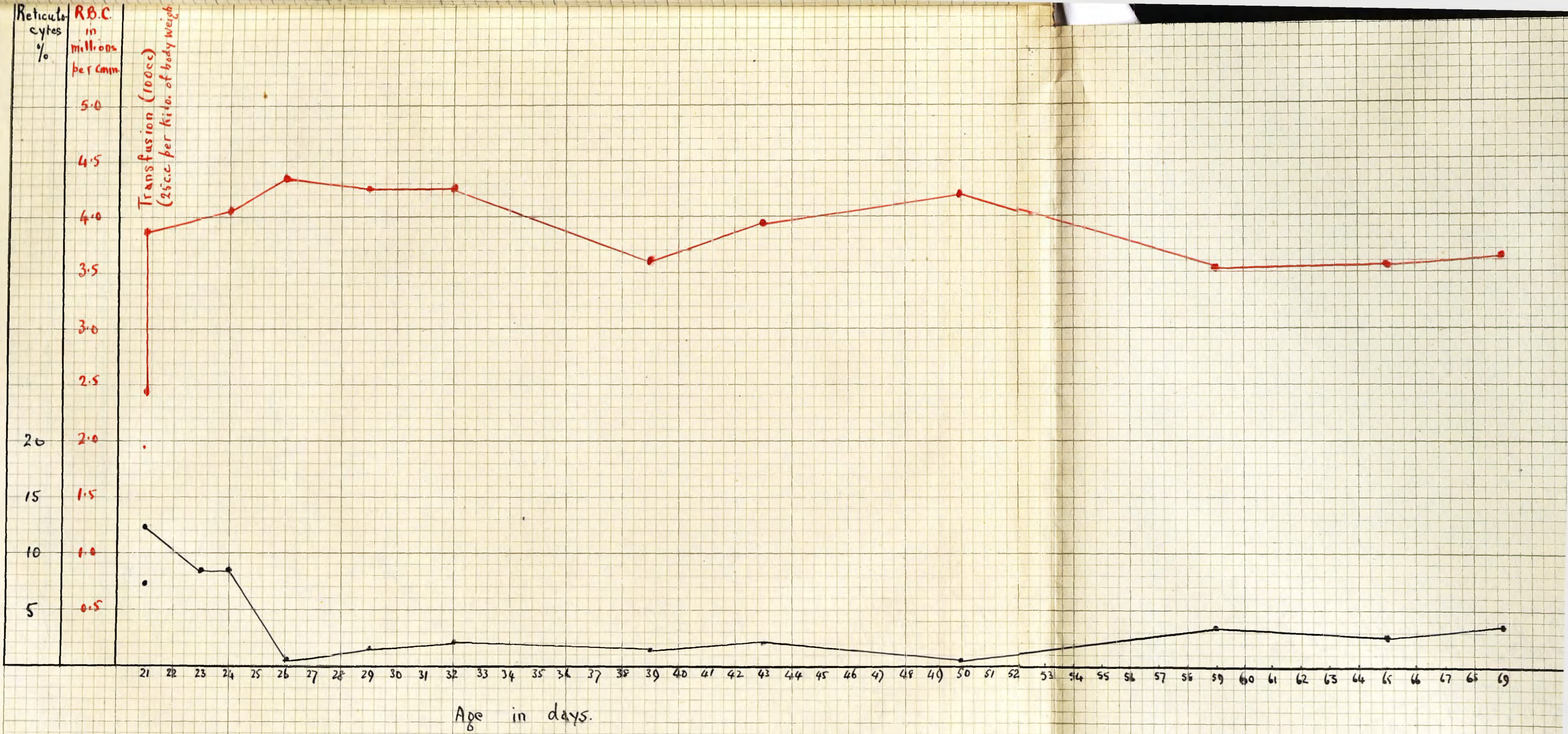


Chart VII. Case No 15. Icterus Gravis

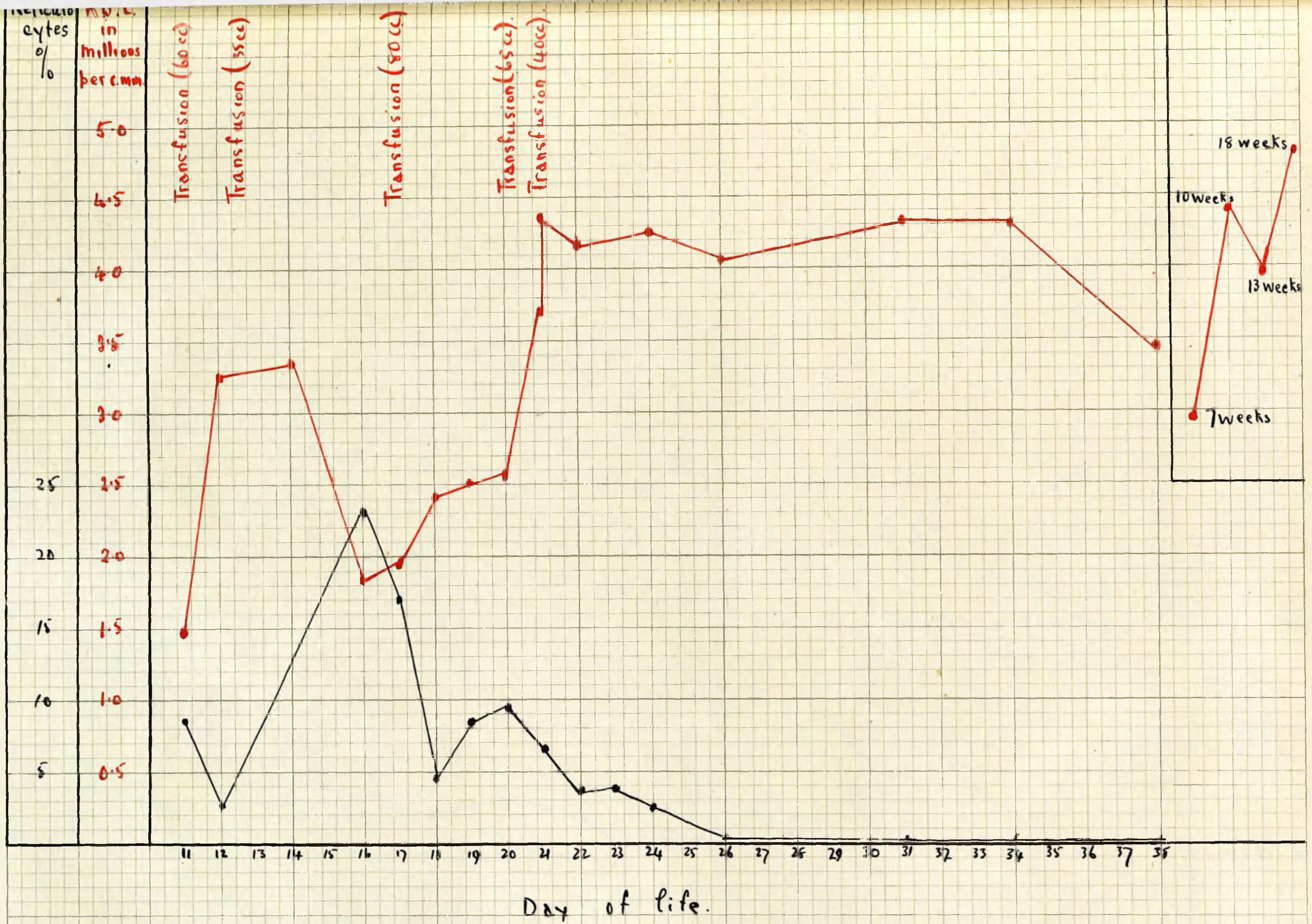


Chart VIII. Case No 17. Icterus Gravis.

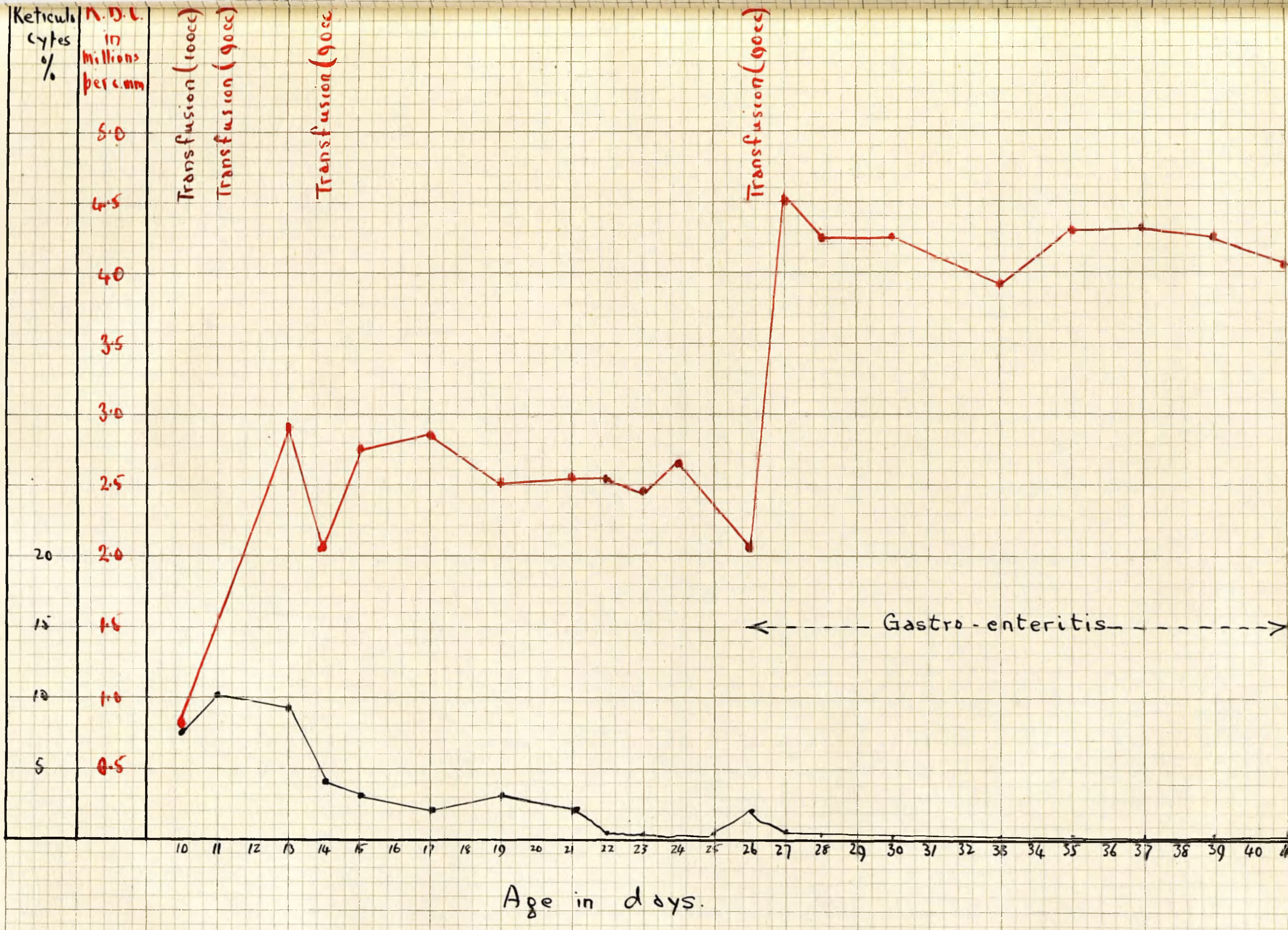


Chart IX. Case No. 20. Icterus Gravis.

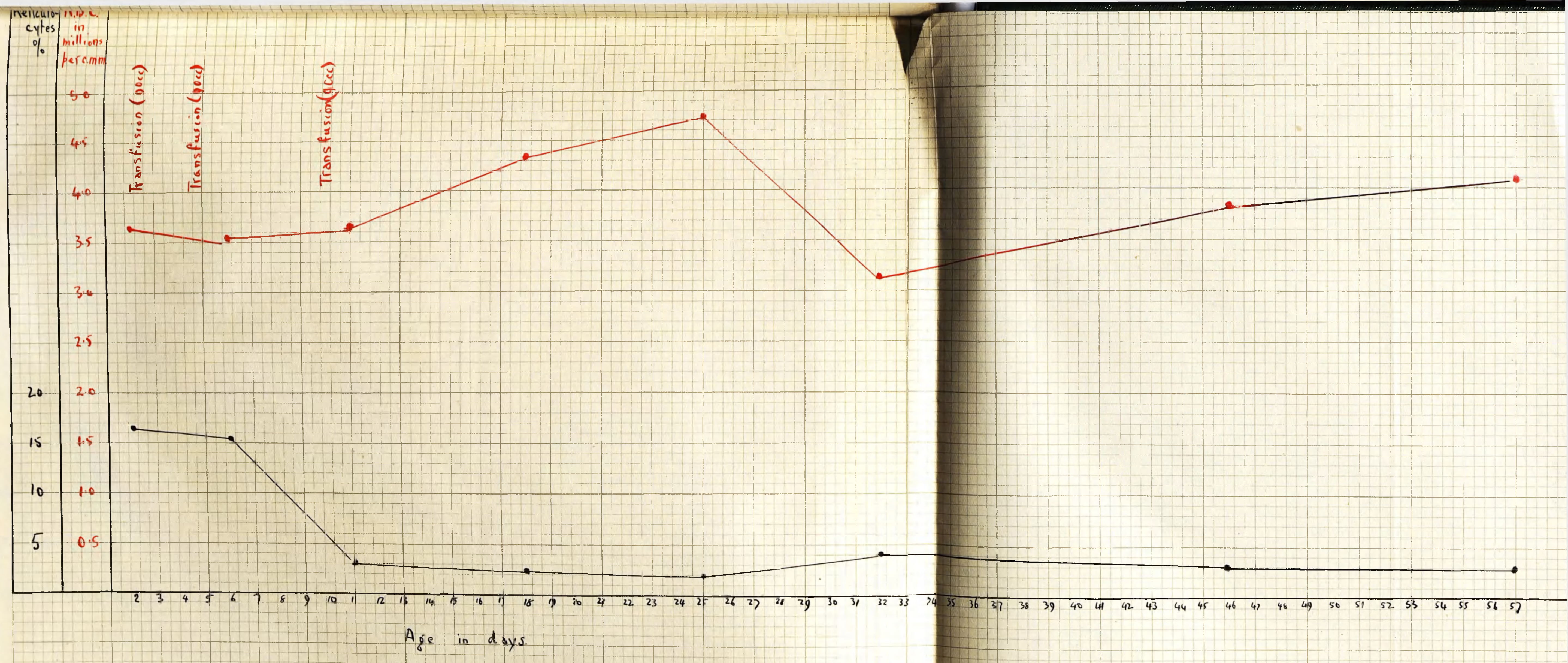


Chart X. Case 23. Icterus Gravis.

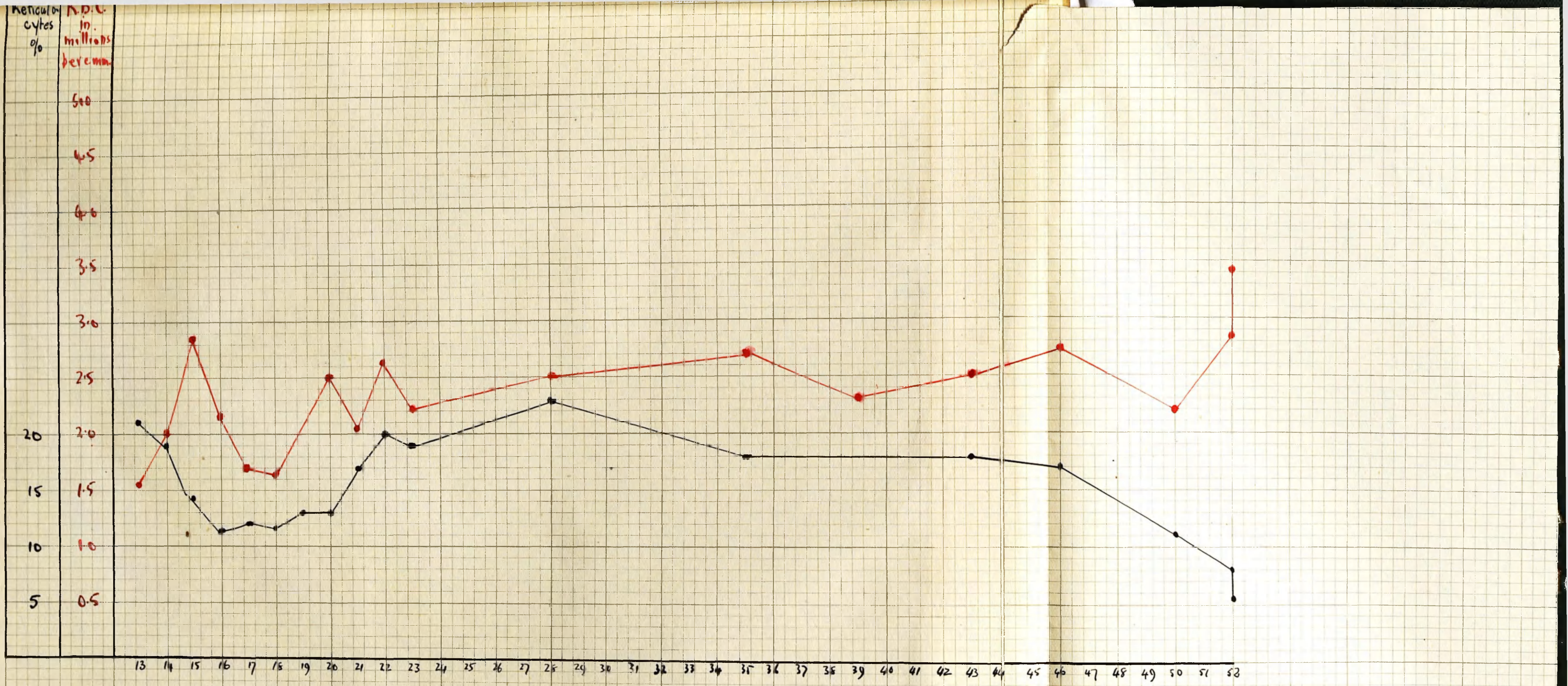


Chart XI. Case No. 24. Haemolytic anaemia without jaundice.

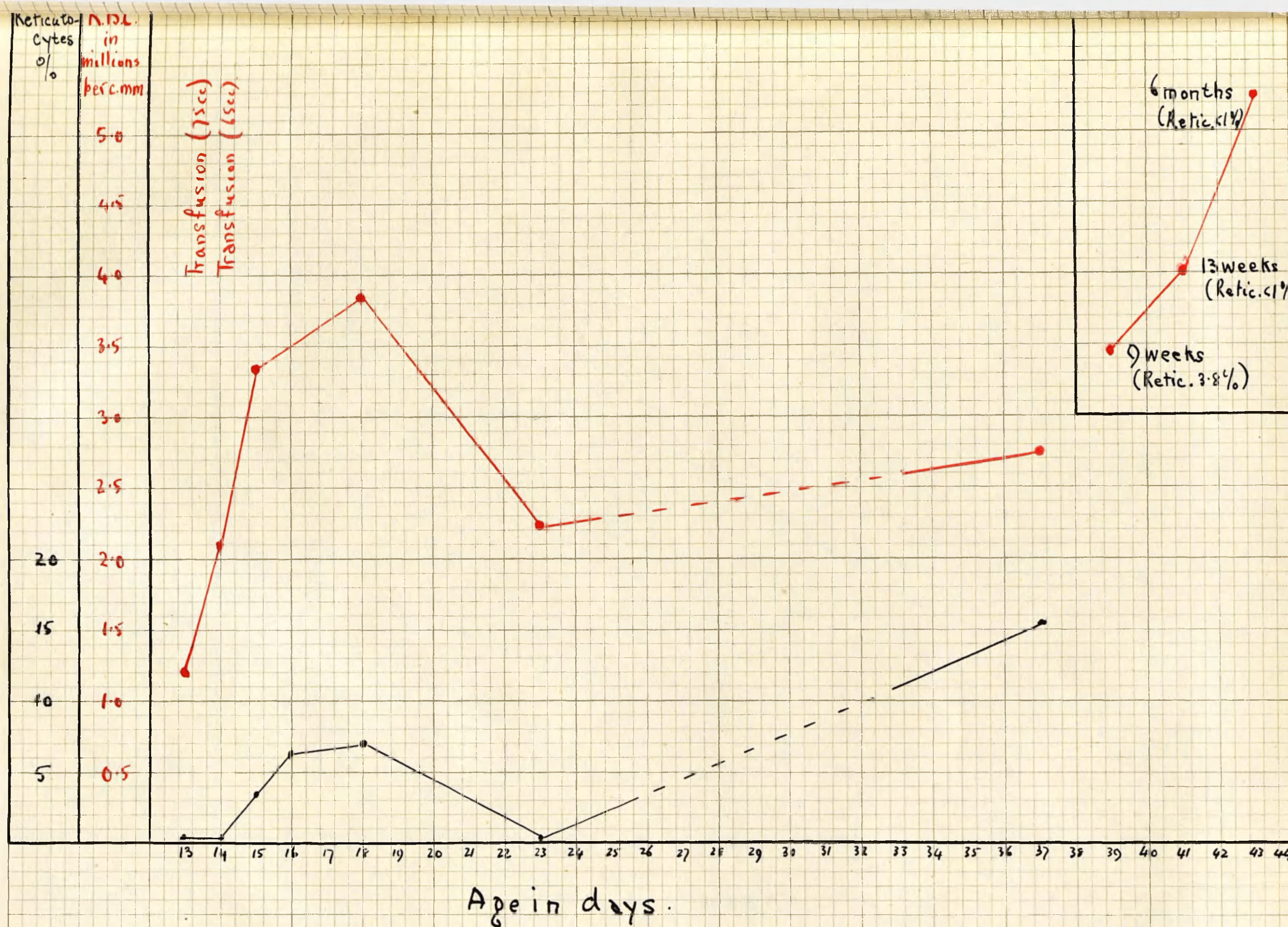


Chart XII. Case No. 28. Anaemia haemolytica neonatorum.

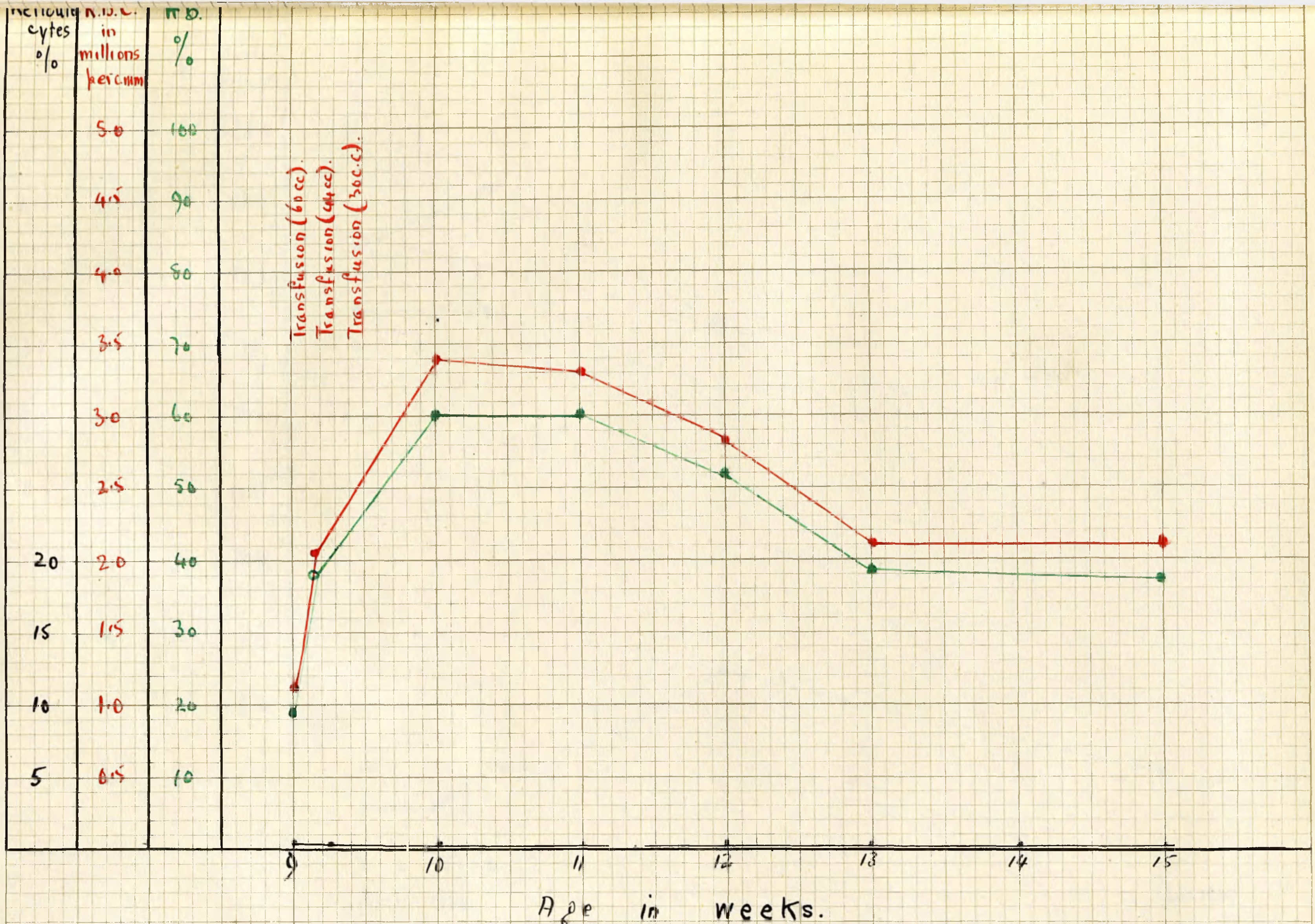


Chart XIII. Case No. 27. Haemolytic anaemia without icterus.

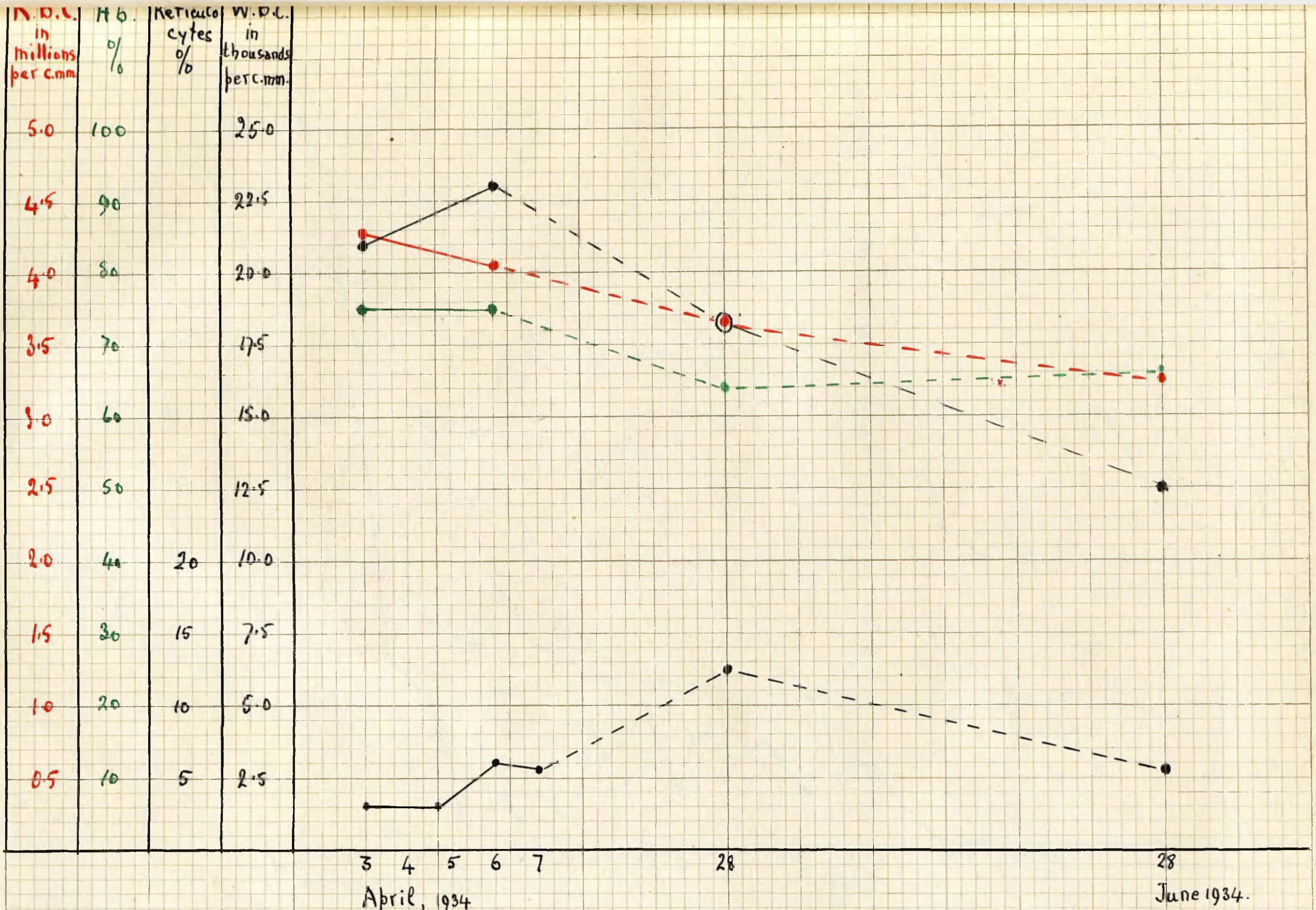


Chart XIV. Case No. 3. Jean L. Acholuric jaundice.

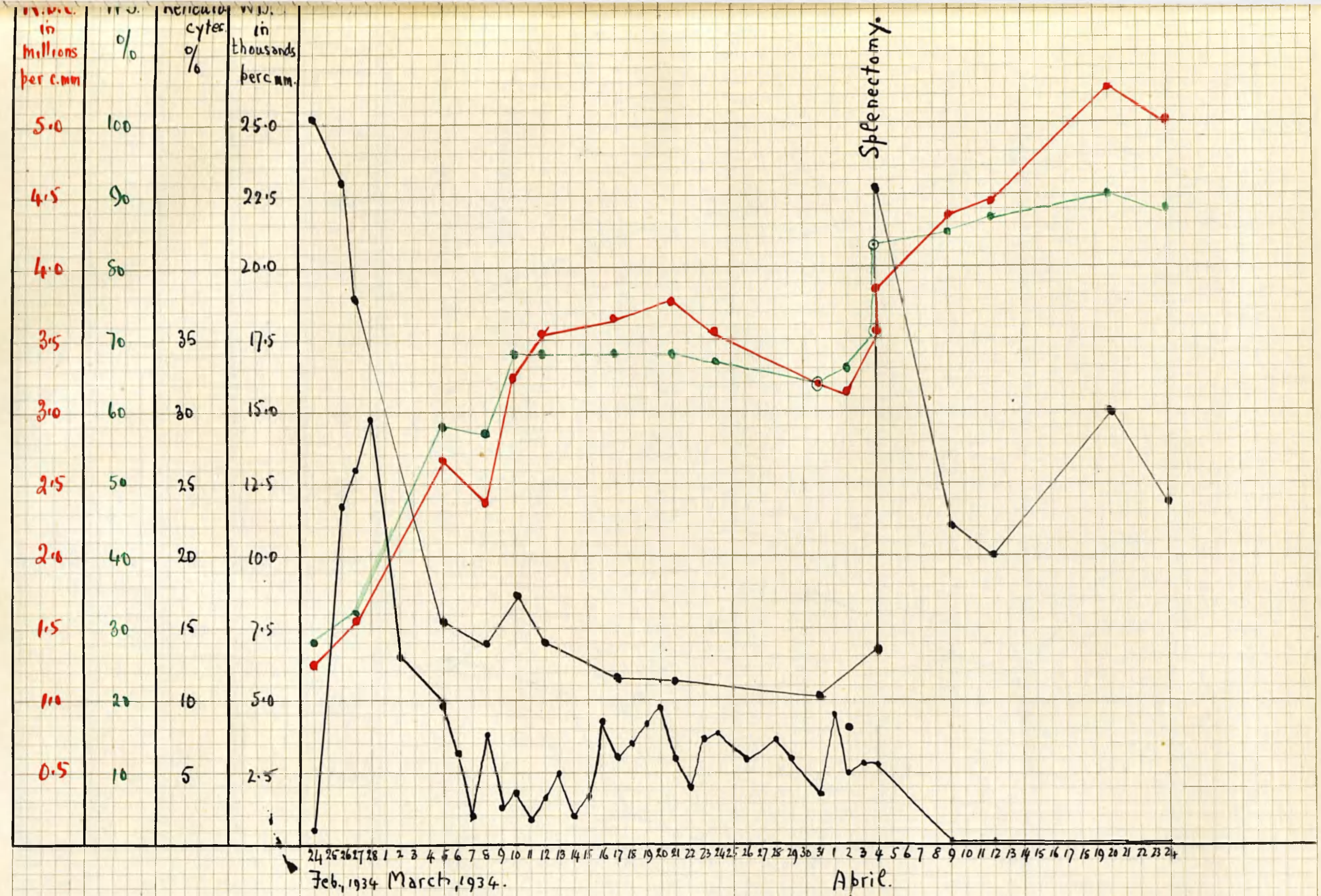


Chart XV. Case No. 4. Rena B. Acholuric jaundice.

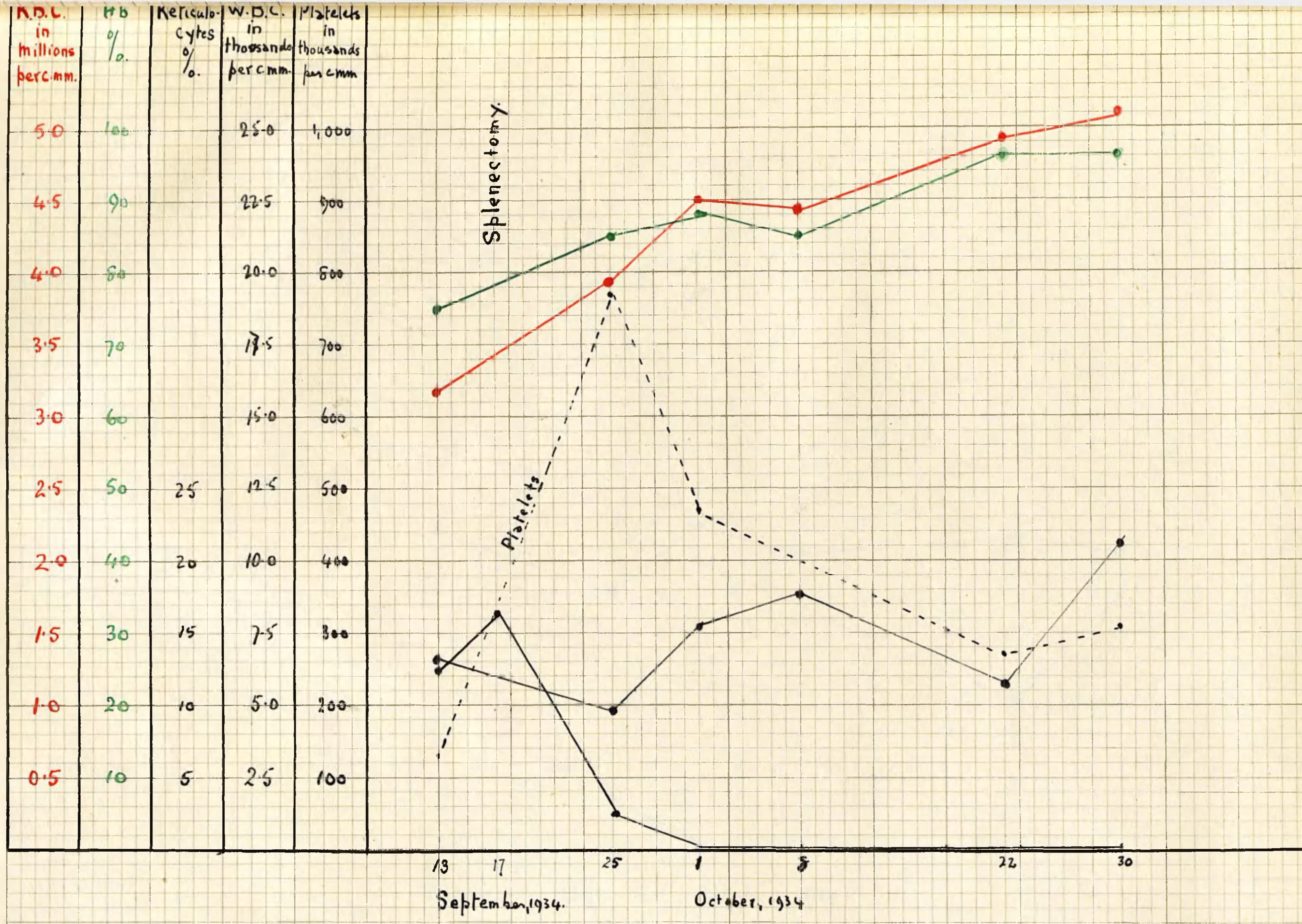


Chart XVI. Case No. 5 Jean H. Acholuric Jaundice.

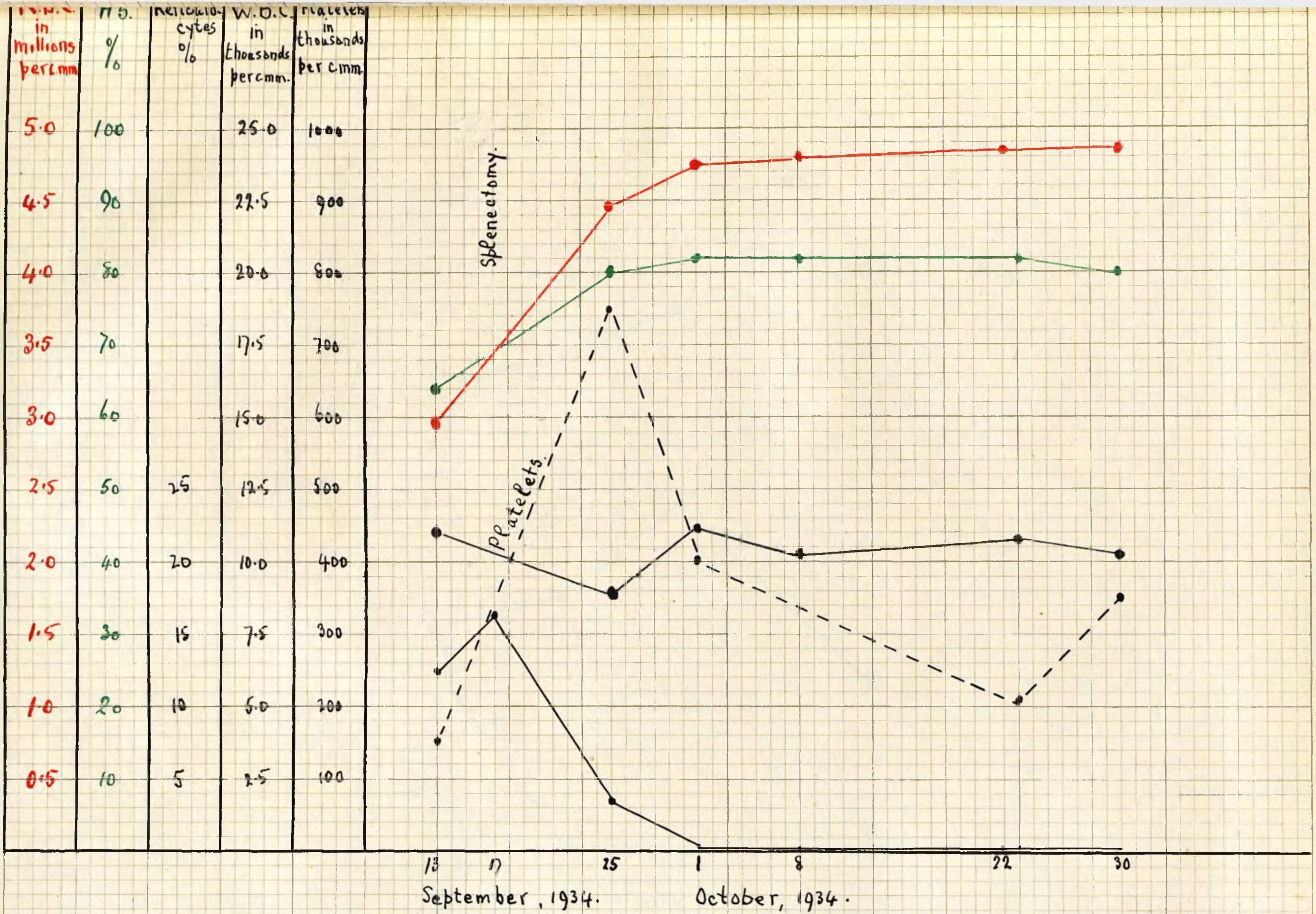


Chart XVII. Case No. 6. Nancy H. Acholuric jaundice.

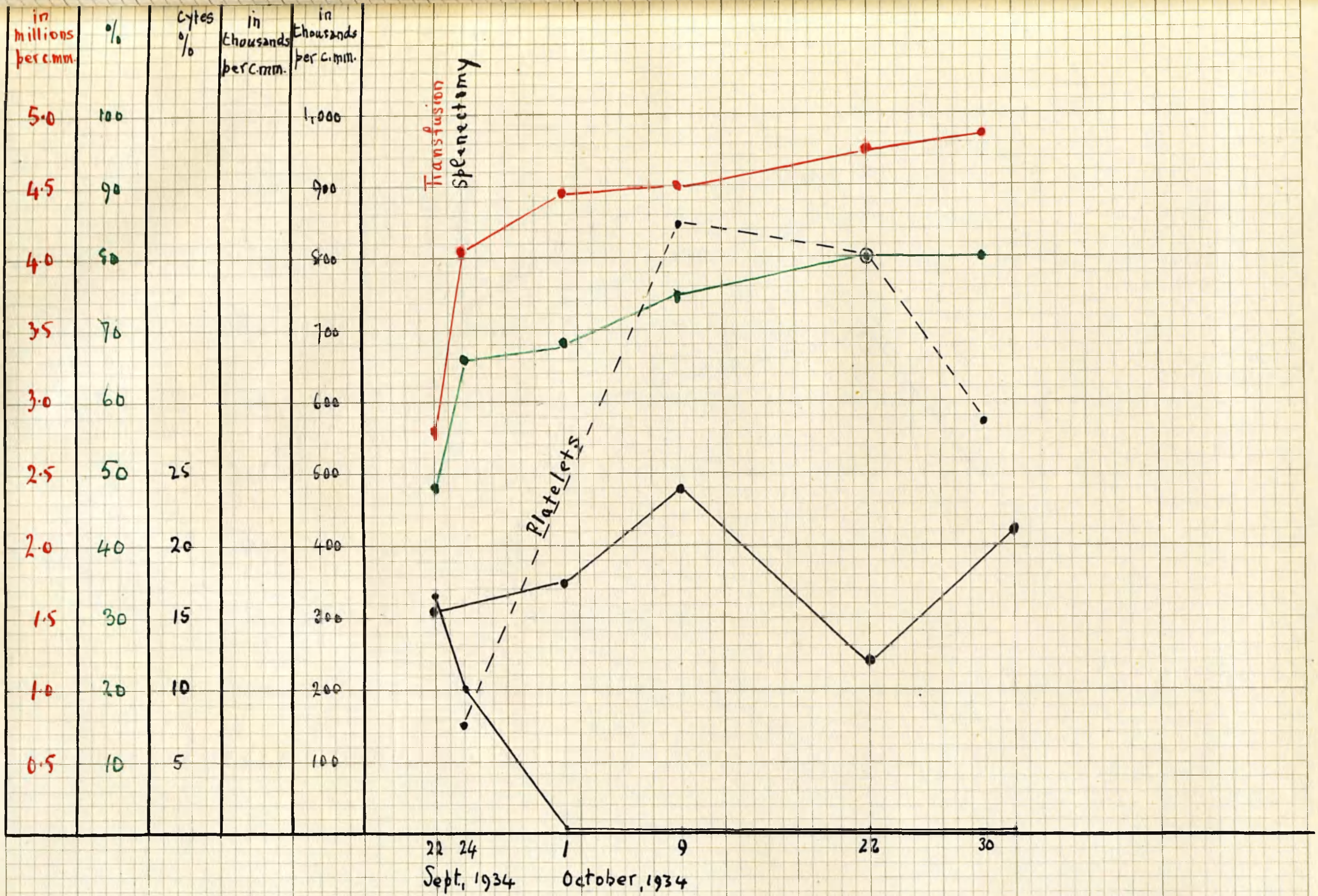


Chart XVIII. Case No. 7. John H. Acholuric jaundice.

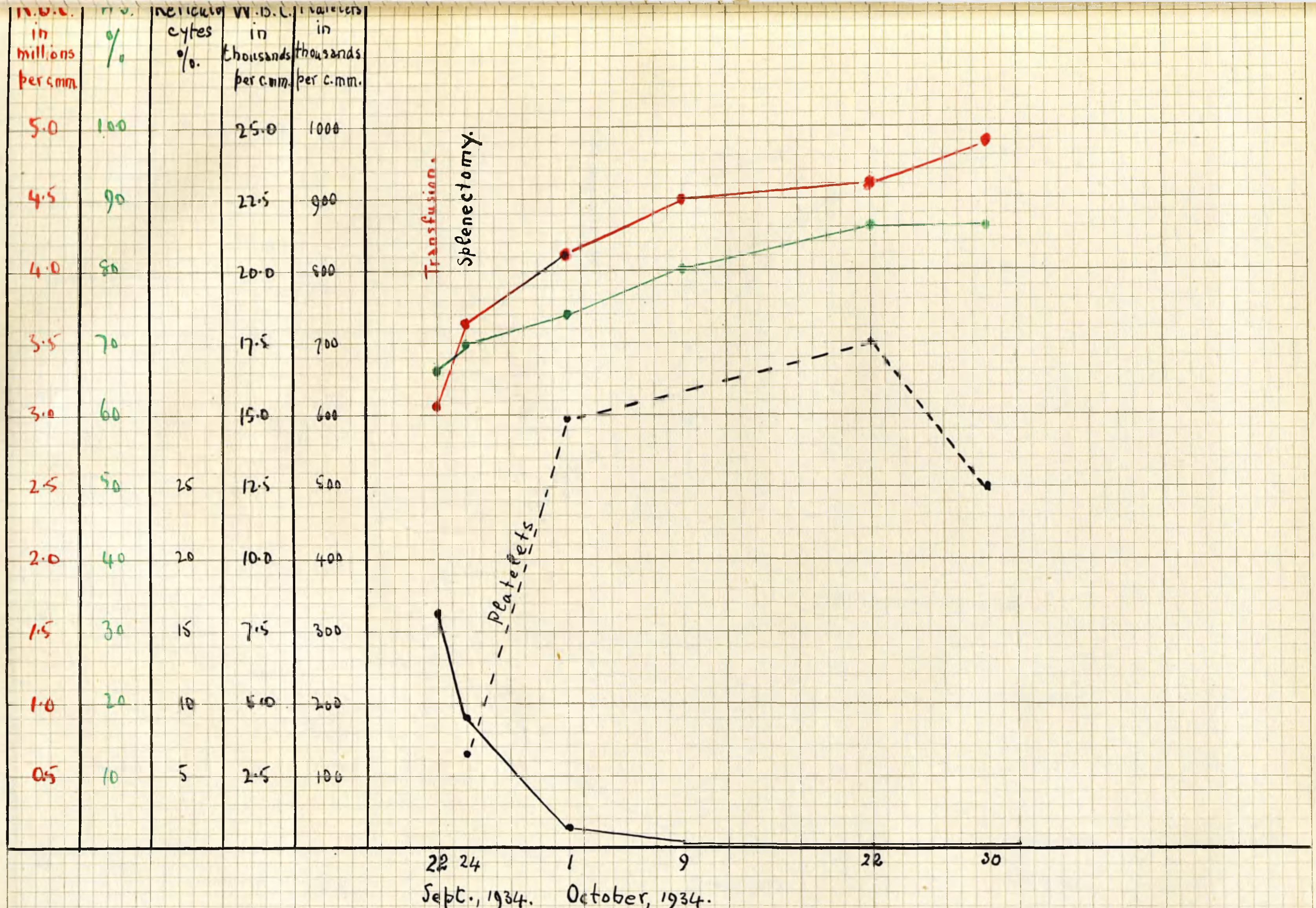


Chart XIX. Case No. 8. James H. Acholuric Jaundice.

Chart XX. Case No 9. Millicent M. Acholuric jaundice.

Plate I. Blood film: icterus gravis: x 350. Jenner and Giemsa.
Erythroblastaemia. A. Dividing erythroblast. B. Metamyelocyte.

Plate 2. Liver: icterus gravis: x 150. H. and E.
Slight disarrangement of cell columns. Brisk erythropoiesis in sinusoids.

Plate 3. Liver: icterus gravis: x 150. H. and E.
Cell columns slightly disarranged and tortuous. Congestion of sinusoids. Brisk erythropoiesis.

Plate 4. Liver: icterus gravis: x 75. H. and E.
Slight disarrangement of cell columns. Fatty infiltration. Erythropoiesis in sinusoids.

Plate 5. Liver: icterus gravis: x 65. H. and E.
Great disarrangement and degeneration of liver cells. A. excessive fibrous tissue round portal tract. B. Bile thrombus in duct. C. Small foci of erythropoiesis.

Plate 6. Liver: icterus gravis: x 110. H. and E.
Gross degeneration of liver cells. A. Foci of erythropoiesis.

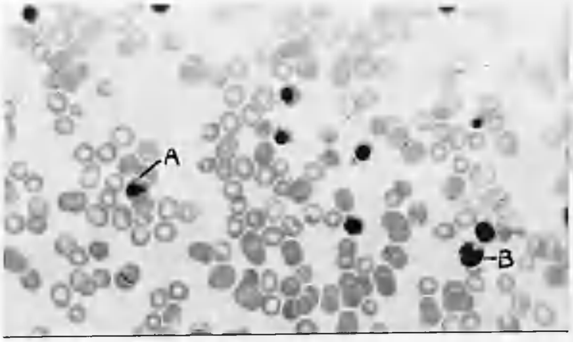


Plate 1.

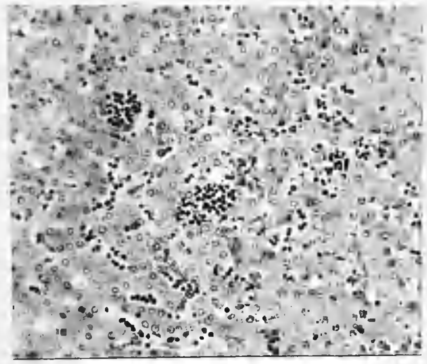


Plate 2.

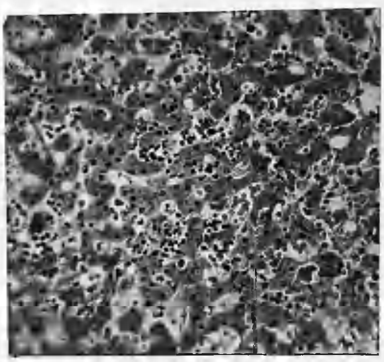


Plate 3.

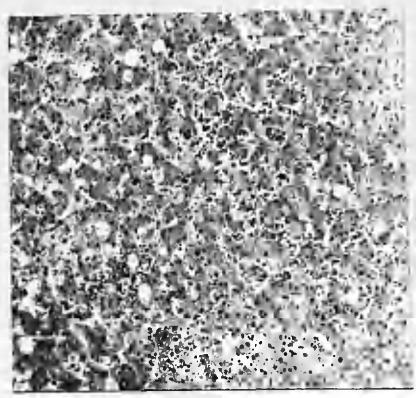


Plate 4.

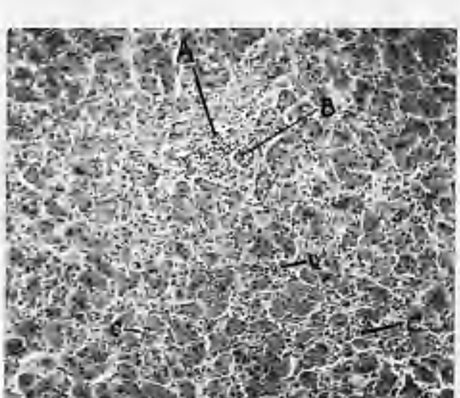


Plate 5.

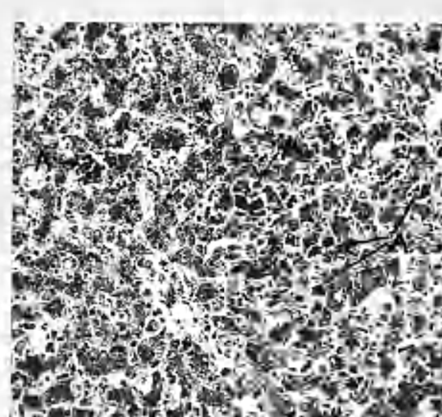


Plate 6.

<u>Plate 7</u>. Liver: icterus gravis: x 75. H. and E. Marked disarrangement and degeneration of liver cells. A. Large erythropoietic islet.

<u>Plate 8</u>. Liver: icterus gravis: x 130. H. and E. Gross degeneration of liver cells with formation of a syncitium, and grouping of nuclei.

<u>Plate 9</u>. Liver: icterus gravis: x 200. H. and E. Complete degeneration of liver cells. Grouping of nuclear remnants.

<u>Plate 10</u>. Liver: anaemia without oedema or jaundice: x 150. H. and E. Radial structure of lobule well-maintained. Liver cells healthy. Brisk erythropoiesis in sinusoids. Very numerous nucleated cells in circulating blood (A).

<u>Plate 11</u>. Liver: anaemia without oedema or jaundice: x 66. H. and E. Portal tract. Absence of thrombi in bile-ducts. Numerous nucleated cells in portal blood (A).

<u>Plate 12</u>. Liver: icterus gravis: x 870. Jenner and Giemsa. Megaloblasts in sinusoid. Pigmentation of liver cells.

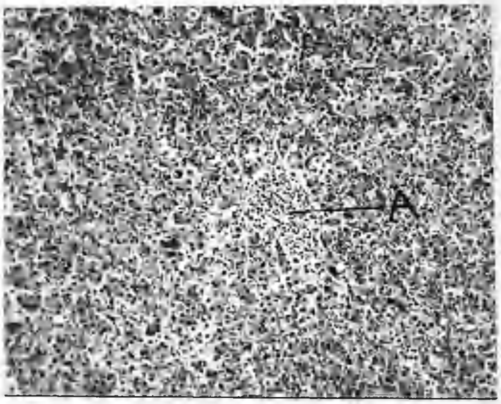


Plate 7.

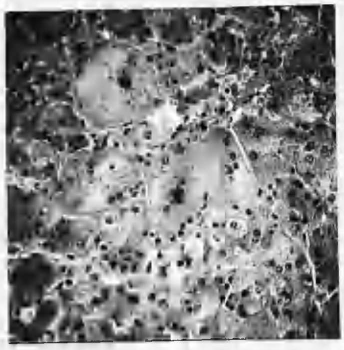


Plate 8.

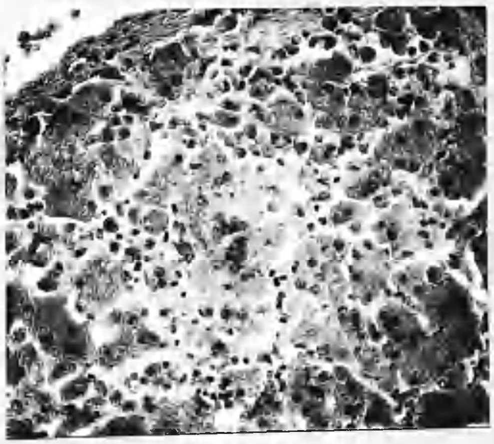


Plate 9.

Plate 10.

Plate 11.

Plate 12.

Plate 13.

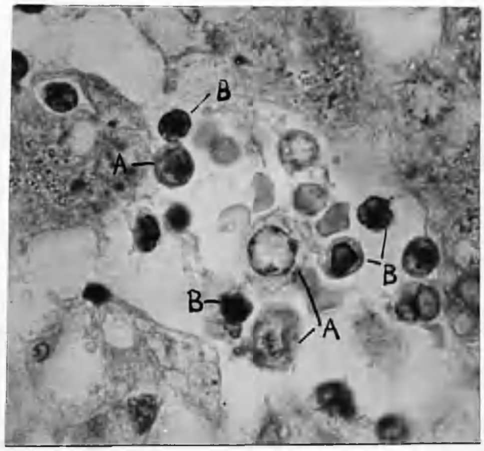


Plate 14.

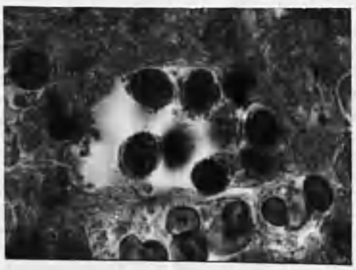


Plate 15.

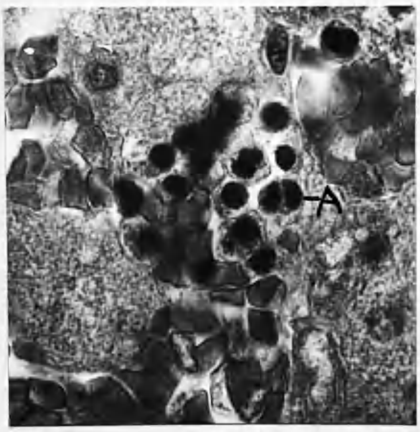


Plate 16.

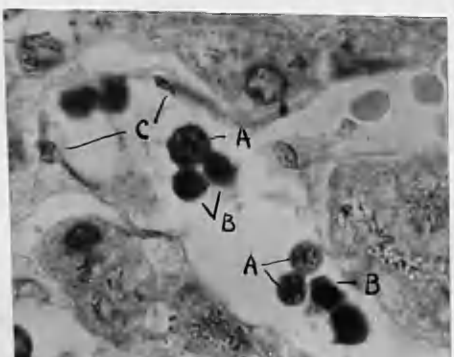


Plate 17.

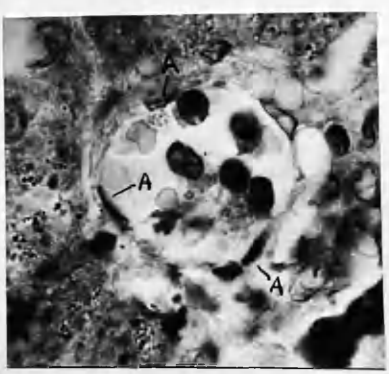


Plate 18.

<u>Plate 19</u>. Liver: icterus gravis: x 870. Jenner and Giemsa. Küpffer cells (A) surrounding an erythropoietic focus.

<u>Plate 20</u>. Liver: anaemia without oedema or jaundice: x 870. Küpffer cells (A) lying between erythroblasts and liver cells.

<u>Plate 21</u>. Liver: icterus gravis: x 200. H. and E. Leucopoiesis (A) near portal vessel (B).

<u>Plate 22</u>. Liver: icterus gravis: x 400. H. and E. Leucopoiesis. Higher magnification of part of plate 21.

<u>Plate 23</u>. Liver: icterus gravis: x 300. H. and E. Megakaryocyte in sinusoid.

<u>Plate 24</u>. Liver: icterus gravis: x 870. H. and E. A. Slightly swollen Küpffer cells. B. Phagocytic cells containing ingested material.

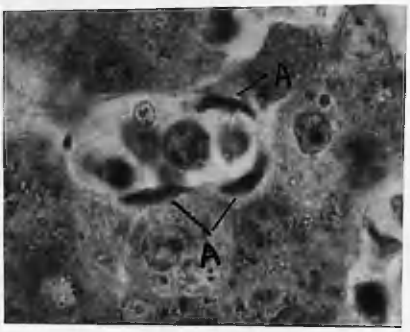


Plate 19.

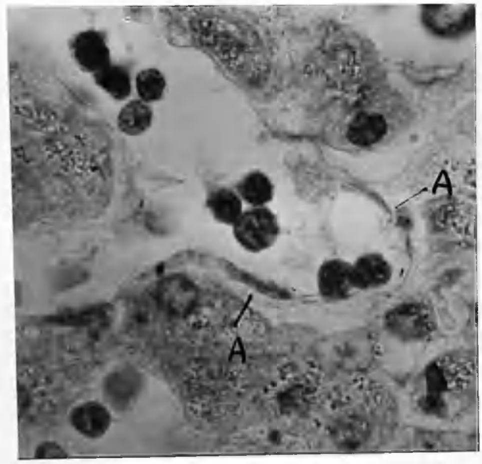


Plate 20.

Plate 21.

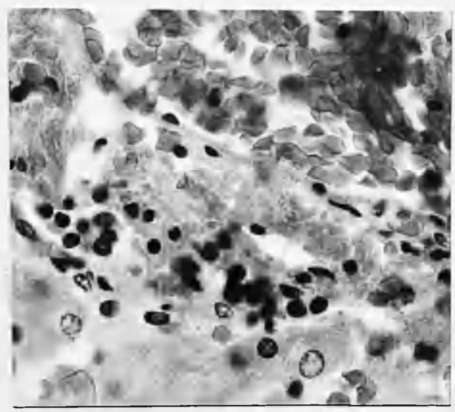


Plate 22.

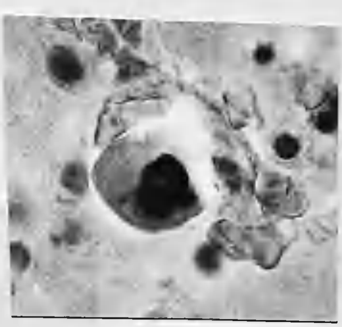


Plate 23.

Plate 24.

Plate 25. Liver: icterus gravis: x 750. H. and E.

A. Large phagocyte containing ingested cells.

Plate 26. Liver: icterus gravis: x 650. H. and E.

A. Degenerate and pigmented liver cells.

B. Erythropoiesis. C. Disintegrating phagocytes.

Plate 27. Liver: icterus gravis: x 50. Hydrochloric acid and pot. ferrocyanide. Iron in Küpffer cells, chiefly in portal and midlobular regions.

Plate 28. Liver: icterus gravis: x 110. Hydrochloric acid and potassium ferrocyanide. Haemosiderosis of Küpffer and liver cells.

Plate 29. Liver: icterus gravis: x 150. Hydrochloric acid and potassium ferrocyanide. Haemosiderosis of liver and Küpffer cells.

Plate 30. Liver: icterus gravis: x 650. Hydrochloric acid and pot. ferrocyanide. Haemosiderosis. Large masses (A) of granular haemosiderin in degenerating liver cells.

Plate 25.

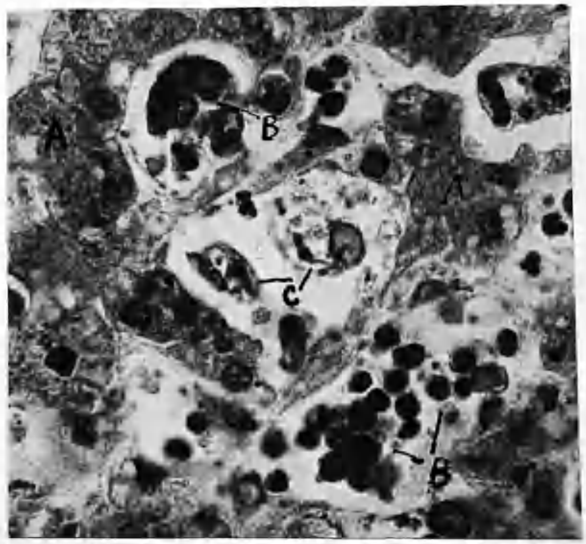
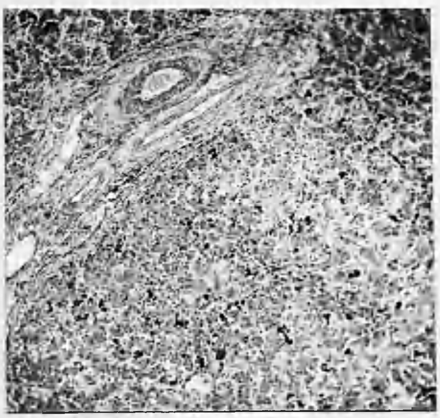


Plate 26.



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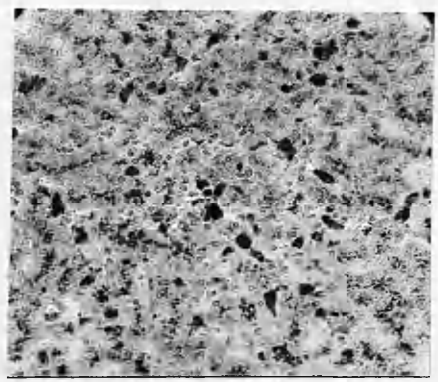

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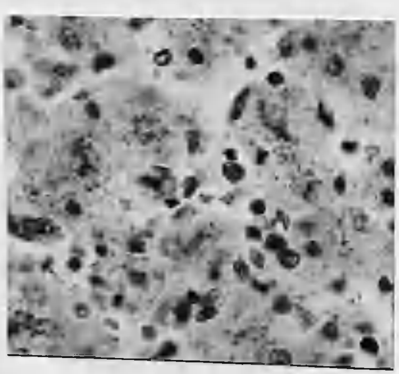


Plate 29.

Plate 30.

__Plate 31__. Liver: icterus gravis: x 650.

Hydrochloric acid and pot. ferrocyanide.

Haemosiderin (A) in degenerate liver cells.

__Plate 32__. Liver: icterus gravis: x 650.

Hydrochloric acid and pot. ferrocyanide.

A. Masses of haemosiderin in degenerate liver cells.

__Plate 33__. Liver: icterus gravis: x 400.

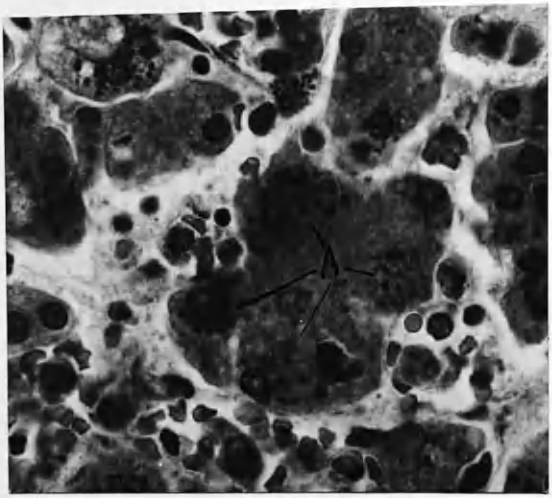
Hydrochloric acid and pot. ferrocyanide.

Haemosiderosis of degenerate liver cells. Case not transfused.

__Plate 34__. Liver: anaemia without oedema or jaundice: x 550.

Hydrochloric acid and pot. ferrocyanide.

Large amounts of haemosiderin in liver cells. Case not transfused.



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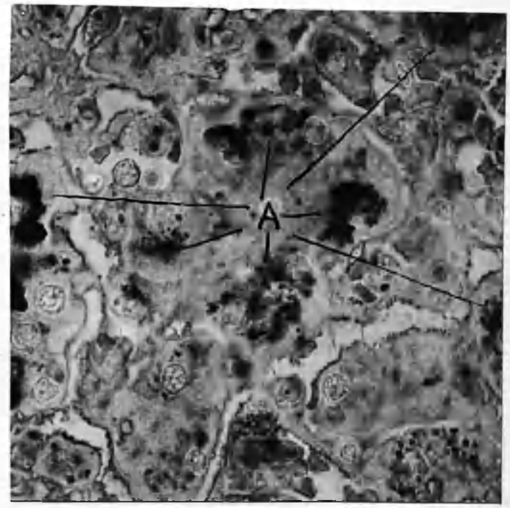


Plate 32.

Plate 33.

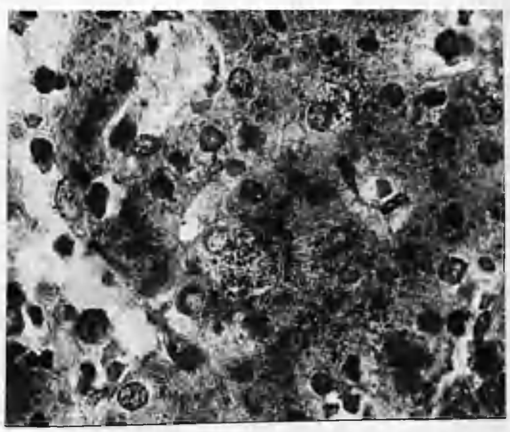


Plate 34.

Plate 35. Liver: icterus gravis: x 200. Gallego.
Normal staining of fibrous tissue.

Plate 36. Liver: icterus gravis: x 120. Gallego.
Normal amount of fibrous tissue round vessel and in lobule.

Plate 37. Liver: icterus gravis: x 200. Gallego.
Normal amount of fibrous tissue in areas of erythropoiesis and phagocytosis.

Plate 38. Liver: icterus gravis: x 120. Gallego.
Fine fibrosis in midlobular region.

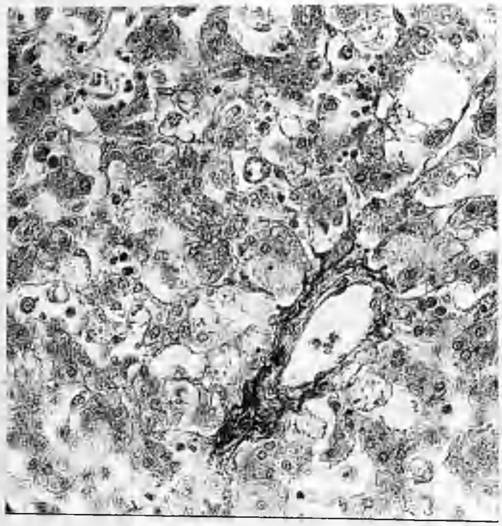


Plate 35.

Plate 36.

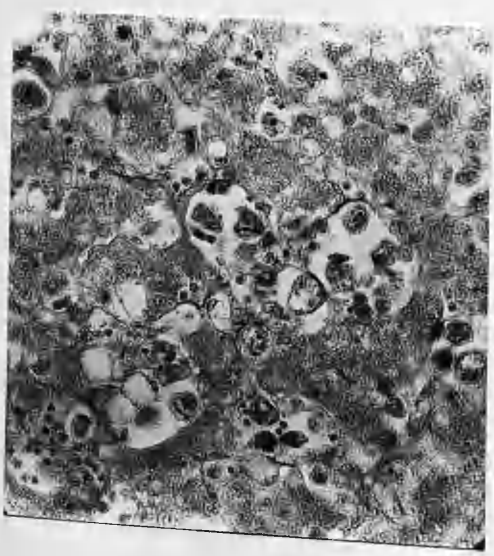


Plate 37.

Plate 38.

Plate 39. Liver: icterus gravis: x 120. Gallego.

A. Increase of fine fibrous tissue. Liver cells degenerate.

Plate 40. Liver: icterus gravis: x 200. Gallego.

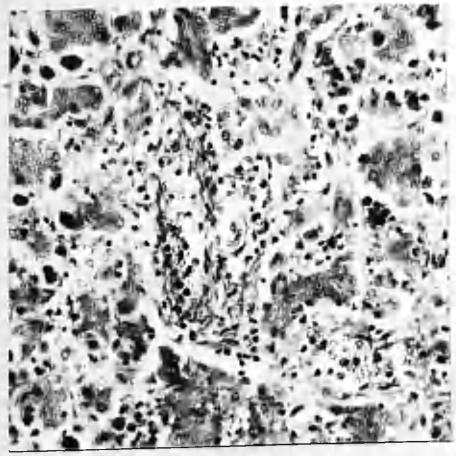
A. Increase of fine fibrous tissue in area where liver cells are degenerating.

Plate 41. Liver: icterus gravis: x 200. Gallego.

A. Increase of fine fibrous tissue between degenerating cell-groups.

Plate 42. Liver: icterus gravis: x 200. Gallego.

A. Very fine fibrosis in necrotic areas of liver.



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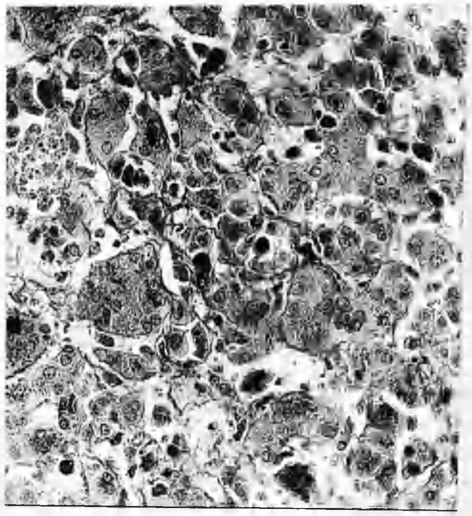

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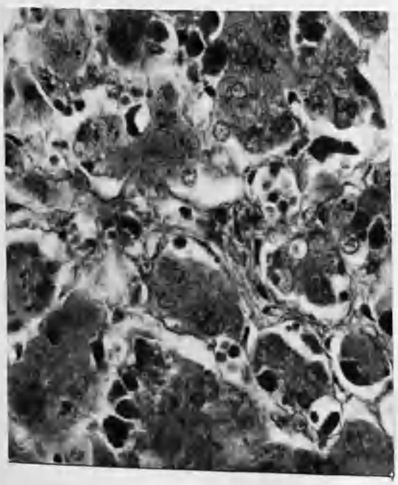


Plate 41.

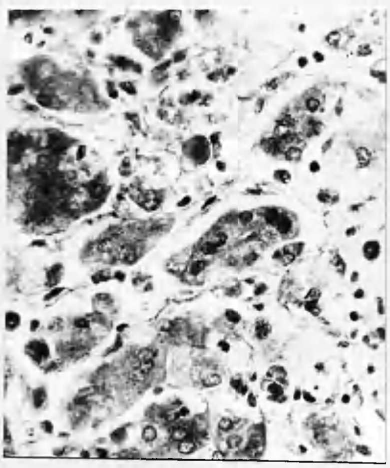


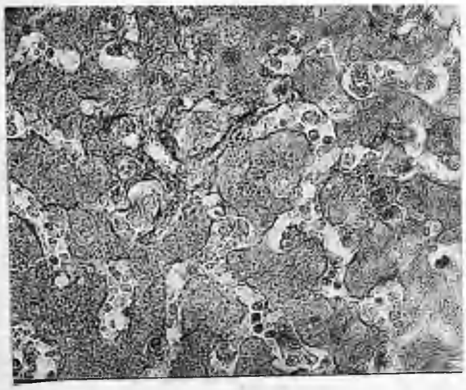
Plate 42.

Plate 43. Liver: icterus gravis: x 250. Gallego.
Thickening of reticulum.

Plate 44. Spleen: icterus gravis: x 45. H. and E.
A. Ill-defined Malpighian body; congestion of surrounding pulp.

Plate 45. Spleen: icterus gravis: x 200. H. and E.
A. Empty sinusoid; congestion of pulp.

Plate 46. Spleen: icterus gravis: x 870. Jenner and Giemsa.
Large intrasinusoidal erythropoietic focus.



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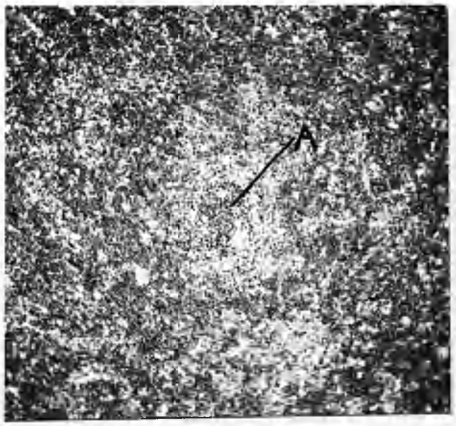

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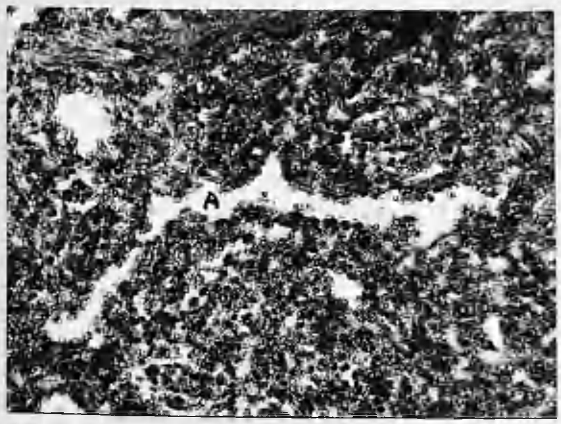


Plate 45.

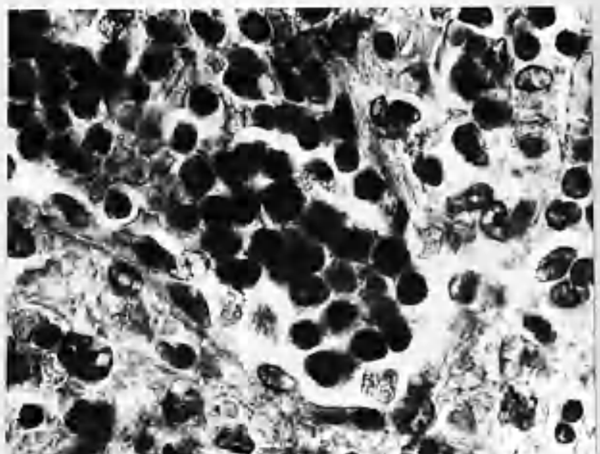


Plate 46.

Plate 47. Spleen: anaemia without oedema or jaundice: x 870. Jenner and Giemsa.
Small intrasinusoidal erythropoietic focus.
A. Megaloblasts. B. Erythroblasts.

Plate 48. Spleen: anaemia without oedema or jaundice: x 870. Jenner and Giemsa.
Megaloblastic erythropoietic islet.

Plate 49. Spleen: icterus gravis: x 870. Jenner and Giemsa.
Erythropoietic islet in sinusoid.
A. Erythroblasts. B. Normoblasts. C. Division of nucleus and extrusion of fragments.

Plate 50. Spleen: icterus gravis: x 450. H. and E.
A. Erythroblastic islet in sinusoid.
B. Unbroken cell-lining of sinusoid.
C. Empty sinusoid.

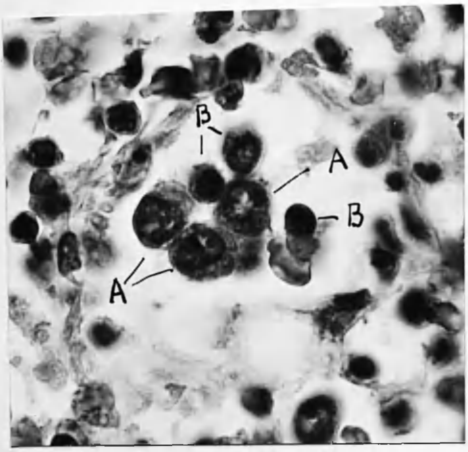


Plate 47.

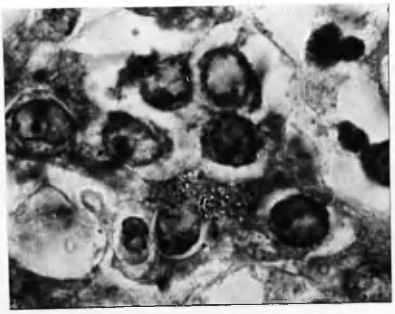
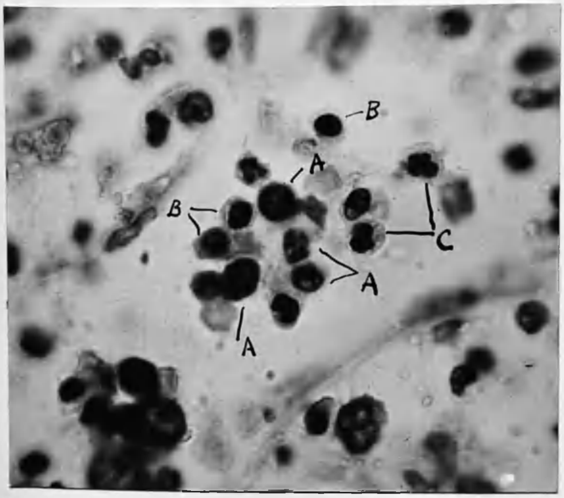


Plate 48.



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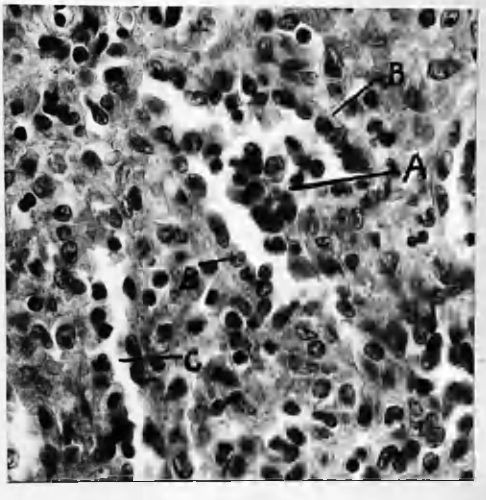


Plate 50.

Plate 51. Spleen: anaemia without oedema or jaundice: x 870. H. and E.
A. Phagocytes containing ingested cells, etc.

Plate 52. Spleen: icterus gravis: x 200. Hydrochloric acid and pot. ferrocyanide.
Haemosiderin in pulp phagocytes and in sinusoidal walls.

Plate 53. Spleen: icterus gravis: x 200. Hydrochloric acid and pot. ferrocyanide.
Iron in pulp phagocytes.

Plate 54. Spleen: anaemia without oedema or jaundice: x 75. Hydrochloric acid and pot. ferrocyanide.
Iron in pulp phagocytes. A. Small amounts in adventitia of vessel.

Plate 55. Spleen: icterus gravis: x 70. Hydrochloric acid and pot. ferrocyanide.
A. Malpighian body: iron free. Haemosiderosis of phagocytes in surrounding pulp.

Plate 56. Spleen: icterus gravis: x 70. Hydrochloric acid and pot. ferrocyanide.
Iron in fibrous tissue of capsule and trabeculae.

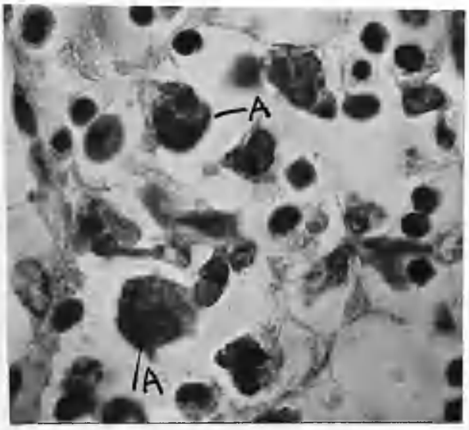
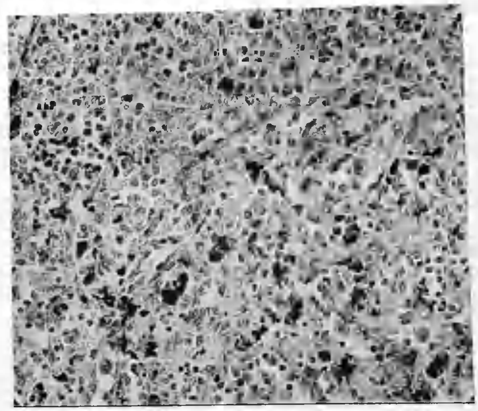


Plate 51.



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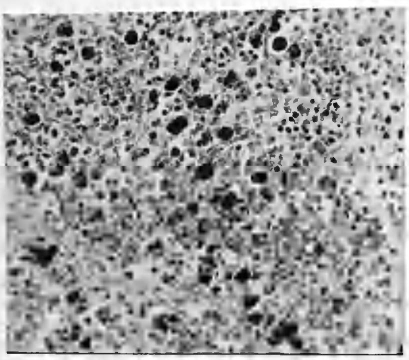

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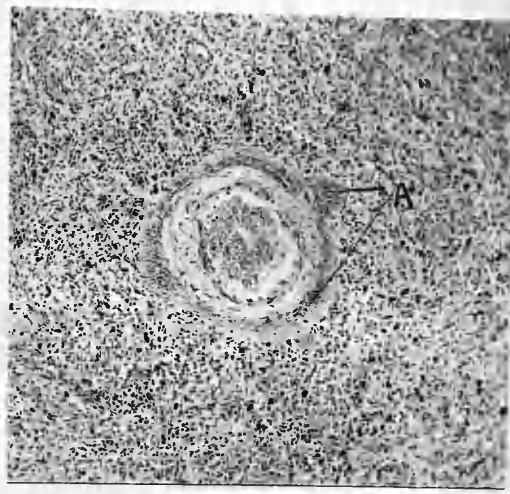

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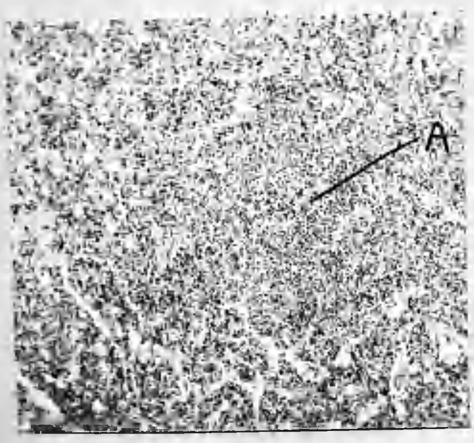

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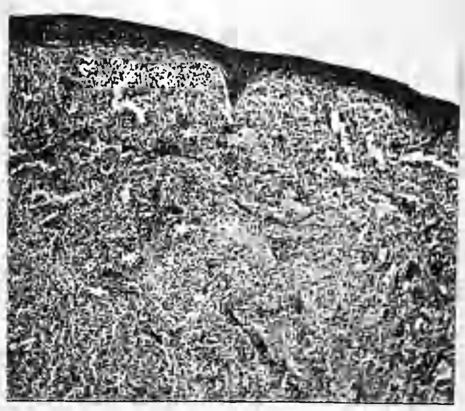


Plate 56.

__Plate 57__. Spleen: icterus gravis: x 200. Hydrochloric acid and pot. ferrocyanide.
Iron in reticulum.

__Plate 58__. Spleen: icterus gravis: x 300. Hydrochloric acid and pot. ferrocyanide.
Haemosiderosis of reticulum.

__Plate 59__. Spleen: icterus gravis: x 200. Gallego.
Normal delicate fibrous tissue of adventitious coat of blood-vessel. Normal fine reticulum of spleen.

__Plate 60__. Spleen: icterus gravis: x 250. Gallego.
Increase of fibrous tissue in adventitious coat of blood-vessel.

__Plate 61__. Spleen: icterus gravis: x 150. Gallego.
Thickening of splenic reticulum.

__Plate 62__. Spleen: icterus gravis: x 200. Gallego.
Thickening of reticulum and basement membranes of sinusoids.

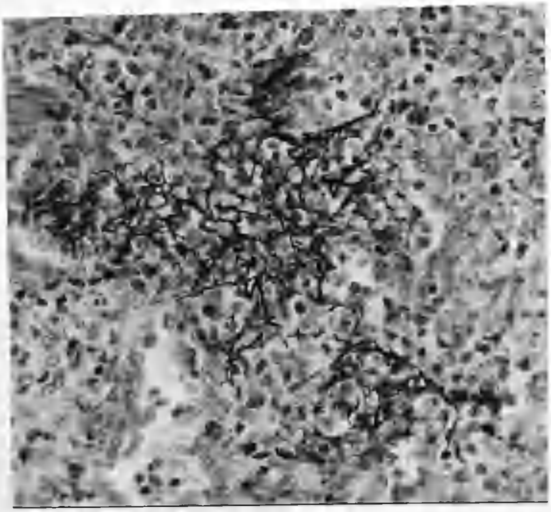


Plate 57.

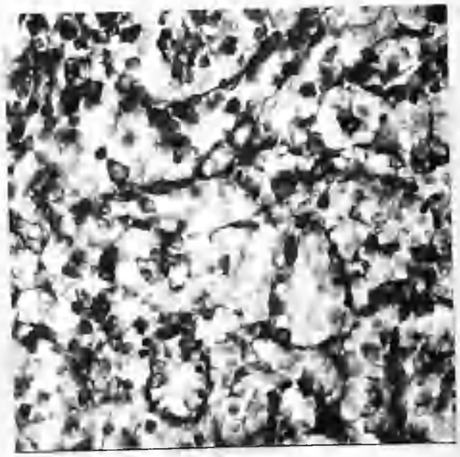


Plate 58.

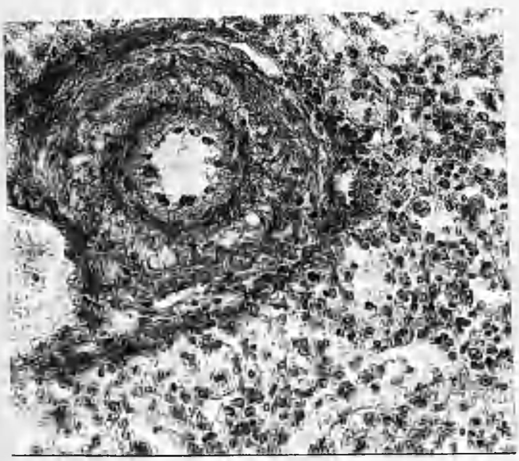
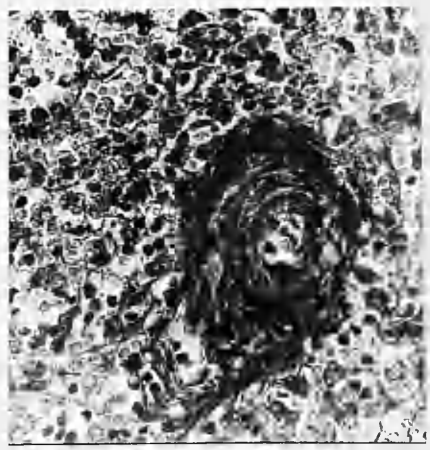


Plate 59.



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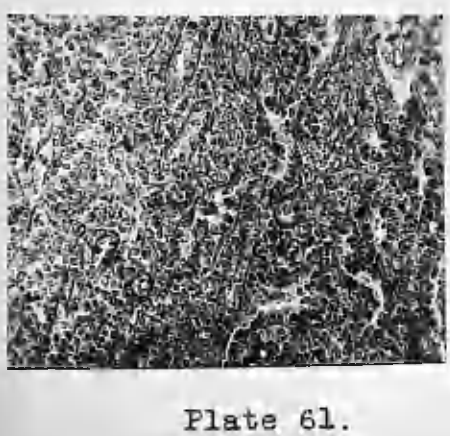


Plate 61.

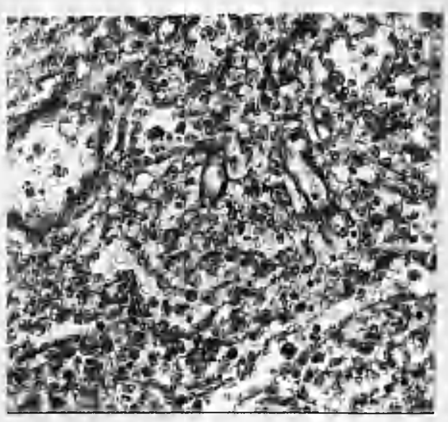


Plate 62.

Plate 63. Kidney: icterus gravis: x 200. Hydrochloric acid and pot. ferrocyanide.

A. Haemosiderin in cells of tubules.

Plate 64. Kidney: anaemia without oedema or jaundice: x 250. Hydrochloric acid and pot. ferrocyanide.

A. Haemosiderosis of tubules.

Plate 65. Bone-marrow: icterus gravis: x 400 Jenner and Giemsa.

A. Bony trabecula. Actively leucopoietic marrow. Chiefly myelocytes and metamyelocytes.

B. Eosinophile myelocyte.

Plate 66. Bone-marrow: icterus gravis: x 500 Jenner and Giemsa.

A. Erythropoietic focus. B. Megaloblasts.

Plate 67. Bone-marrow: anaemia without oedema or jaundice: x 150 Jenner and Giemsa.

A. Bony trabeculae. B. Fat cells. C. Hypoplastic and congested marrow.

Plate 68. Bone-marrow: icterus gravis: x 445 Jenner and Giemsa. Numerous erythroblasts with darkly staining nuclei.

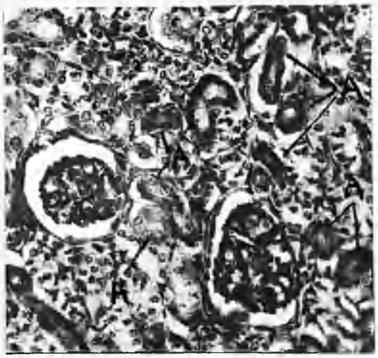


Plate 63.

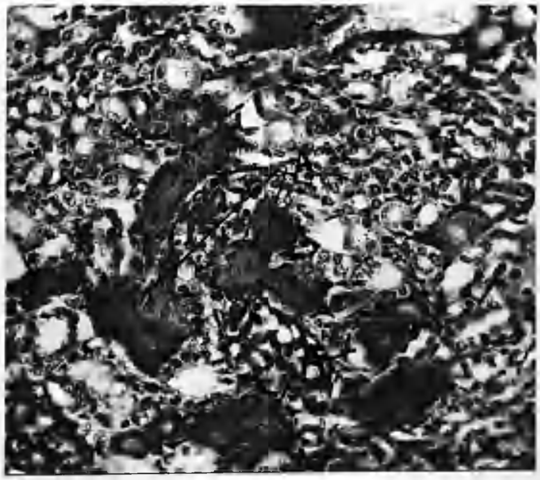
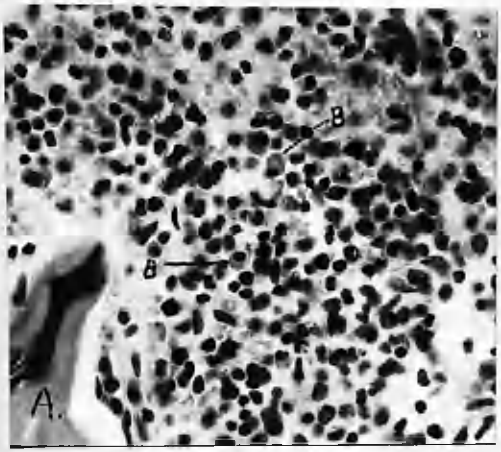


Plate 64.



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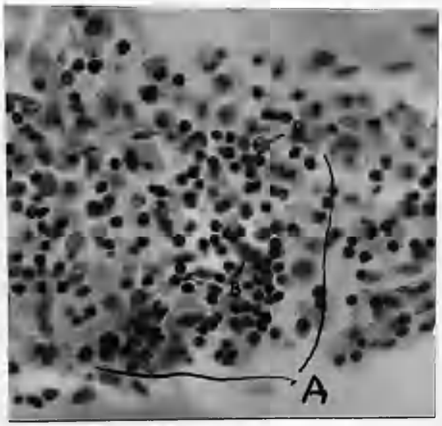


Plate 66.

Plate 67.

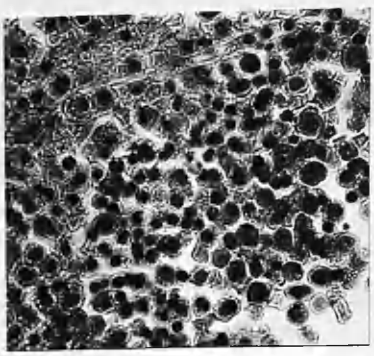


Plate 68.

Plate 69. Bone-marrow: icterus gravis: x 400 Jenner and Giemsa.

A. Erythropoietic islet. B. Capillary vessel.

Plate 70. Bone-marrow: icterus gravis: x 550 Jenner and Giemsa.

A. Intrasinusoidal erythroblastic islet.

B. Swollen and pigmented reticulo-endothelial cells.

Plate 71. Bone-marrow: icterus gravis: x 500 Jenner and Giemsa.

A. Intra-capillary erythroblasts.

Plate 72. Bone-marrow: icterus gravis: x 500 Jenner and Giemsa.

Darkly staining erythroblasts. A. Megaloblast.

Plate 73. Bone-marrow: icterus gravis: x 650. Leishman.

A. Normoblast. B. Myeloblast. C. Neutrophile premyelocyte. D. Eosinophile myelocyte.

Plate 74. Bone-marrow: icterus gravis: x 870. Leishman.

A. Early erythroblast. B. Late erythroblasts; two extruding parts of the nucleus. C. Myeloblast.

Plate 69.

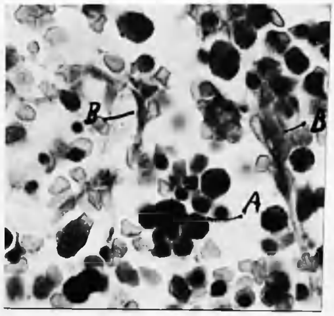
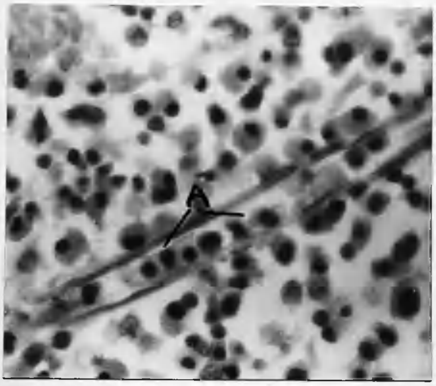


Plate 70.



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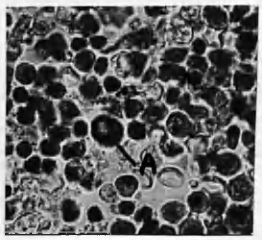

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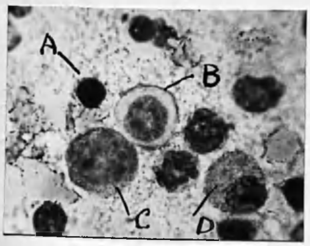

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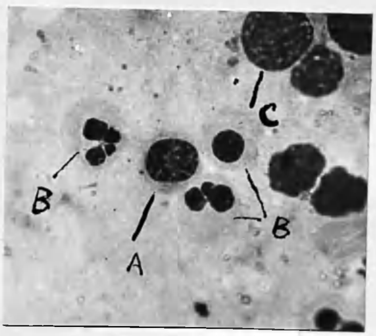


Plate 74.

Plate 75. Bone-marrow: icterus gravis: x 870. Leishman.
A. Early erythroblasts. B. Late erythroblasts; extrusion of nuclear fragments.

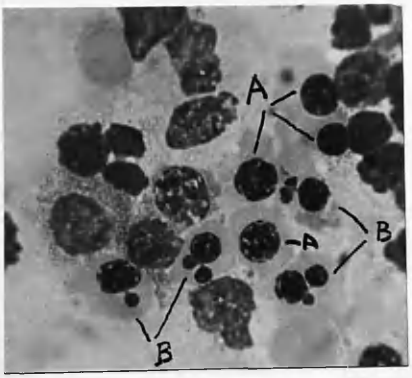
Plate 76. Bone-marrow: icterus gravis: x 870. Leishman.
A. Early erythroblast. B. Mitotic division of nucleus. C. Late erythroblasts. D. Myelocyte. E. Eosinophile myelocyte. F. Metamyelocyte.

Plate 77. Bone-marrow: icterus gravis: x 870. Leishman.
A. Early erythroblasts.

Plate 78. Bone-marrow: icterus gravis: x 650. Leishman.
A. Mitotic division of early erythroblast.
B. Normoblasts. C. Neutrophile myelocytes.
D. Neutrophile premyelocyte.

Plate 79. Bone-marrow: icterus gravis: x 650. Jenner and Giemsa.
Leucopoiesis. A. Myelocytes. B. Metamyelocytes.

Plate 80. Bone-marrow: icterus gravis: x 870. Leishman.
Neutrophile myelocytes.



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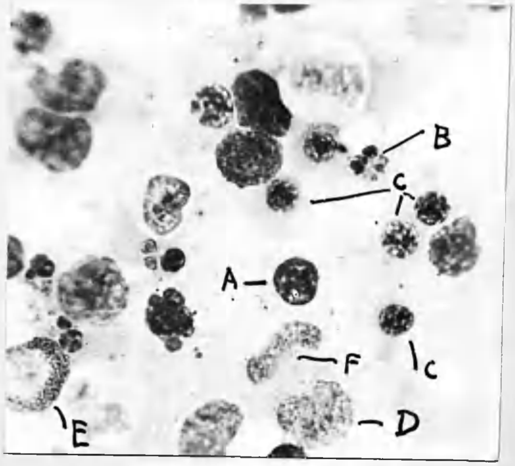

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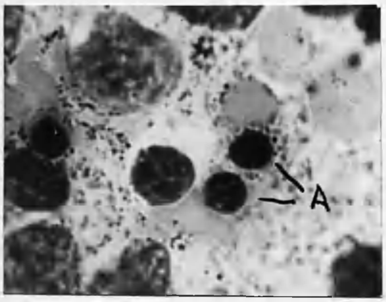
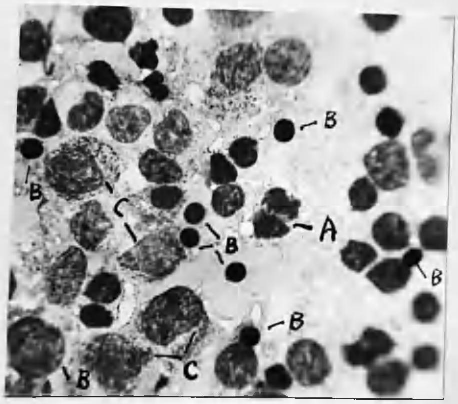


Plate 77.



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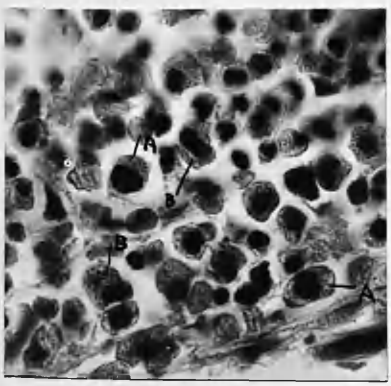

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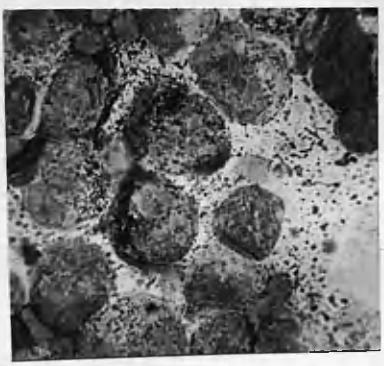

Plate 80.

Plate 81. Bone-marrow: icterus gravis: x 300. Jenner and Giemsa.

A. Megakaryocytes.

Plate 82. Bone-marrow: icterus gravis: x 550. Jenner and Giemsa.

A. Phagocytes containing ingested corpuscles, etc.

Plate 83. Bone-marrow: icterus gravis: x 550. Jenner and Giemsa.

A. Swollen and bile-stained reticulo-endothelial cells.

Plate 84. Bone-marrow: icterus gravis: x 550. Jenner and Giemsa.

A. Bile thrombus in capillary vessel.

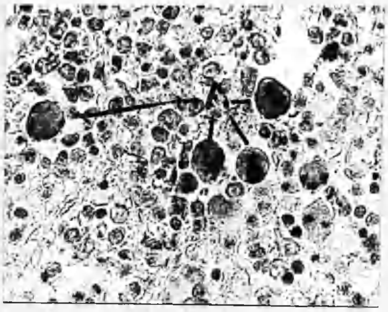

Plate 81.

Plate 82.

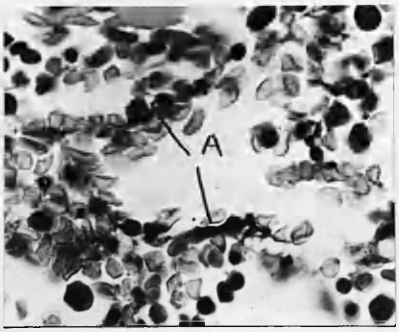

Plate 83.

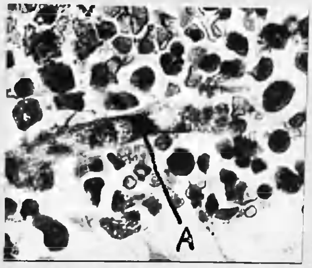

Plate 84.