HAEMOLYTIC DISEASE OF THE NEWBORN: CRITERIA OF SEVERITY

BY

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Haemolytic disease of the newborn can now be diagnosed with certainty at the moment of birth by means of the direct Coombs test. However, since this test seems to be positive in all cases of haemolytic disease, criteria are needed to assess the severity of individual cases. Such criteria are not only desirable for the purpose of deciding whether a case requires treatment but are essential for the orderly presentation of data. Of course it is possible to divide cases into the broad classes of hydrops foetalis, icterus gravis neonatorum, and congenital anaemia of the newborn, but close study of the disease reveals every possible gradation between these classes, and any division between them must be arbitrary. Because of this difficulty of grading it is impossible to tell whether different published series of affected infants include similar proportions of severe, moderate, and mild cases; and it is thus impossible to form precise conclusions on the value of different methods of therapy.

Wiener (1946) suggested that the form of anti-Rh in the mother's serum determines the clinical manifestations of haemolytic disease in her infant, but he has recently modified this opinion and now suggests that "blocking" antibodies play the dominant part and that their titre is roughly proportional to the severity of the disease (Wiener and Gordon, 1948); and Davidsohn and Stern (1948) have presented evidence that "blocking" antibodies are associated with more severe forms of the disease than are saline agglutinins. Nevertheless, present opinion has probably been fairly summed up by Diamond (1947): "A prognosis based on antibody tests . . . must be offered with reservations."

Doubtless the search for measurable criteria of severity has been discouraged by the general belief that, in some cases at least, the disease "comes on," or at least increases in severity, after birth. If this were true it would be extremely difficult to make comparisons between cases in view of the variable changes in haemoglobin and bilirubin values that occur after birth as normal phenomena. However, close study of the disease suggests that in fact the haemolytic process is maximal at birth.

This paper presents evidence that the haemoglobin value of the cord blood, determined by an accurate method, is a satisfactory criterion of severity and that knowledge of this value enables one, within limits, to give a reliable prognosis.

Clinical Material

Normal Infants.—In the course of this work cord blood was collected from 52 healthy full-term infants; in addition, venous samples were taken from many of these infants some hours after birth. In 12 of these latter cases the cord was deliberately clamped immediately after the birth of the

infant, and in a further 12 cases the cord was left untied for a full five minutes after birth. Finally, capillary samples of blood were taken on the first day of life from a further 33 healthy infants, including 22 from whom venous samples were taken simultaneously.

Infants with Haemolytic Disease.—Seventy-four affected infants were studied, although not all of the tests described below were made on every case. All these infants were born to D-negative mothers whose sera contained anti-D (with or without traces of anti-C or anti-E). In most cases the presence of antibody was first detected by routine tests during pregnancy. Sixty-nine of the infants were born alive, and all were demonstrated to be positive to the direct Coombs test; in a few cases in which the mother had not been tested during pregnancy, and in which the infant was seen by us only after it had developed signs suggestive of haemolytic disease, the diagnosis was first established by the finding of a positive direct Coombs reaction. The blood of five stillborn infants was not tested.

TABLE I

No. of Cases	Hb first Determined	Treatment		
		Nil	Simple Transfusion	Exchange Transfusion
50 {	30 Cord	10	1	19
	20 Few hours after birth	0	0	20
	17 2-5 days	2	13	2
	1 30 days	1	0	0
	6 Untested (5 stillborn, 1 moribund)	6	0	0
74		19	14	41

Fifty infants were seen and tested on the day of birth (see Table I), and the findings in this group form the data from which our main conclusions have been drawn. Ten of these infants were not treated—in three cases because they were moribund and in seven because they were considered to be too mildly affected; all of these seven recovered uneventfully. In one further mild case a simple transfusion of Rh-negative blood had to be given on the first day of life because the infant bled from its cord. The remaining 39 cases in this group were treated by exchange transfusion. Thus treatment was essentially uniform, and it seems justifiable to draw conclusions about the severity of the individual cases from the outcome.

In 18 further cases the infant was not tested until after the first day of life. The findings in this group have been used to supplement conclusions reached from the main 50 cases.

Of the 74 cases, 63 formed the material for a previous communication on treatment (Mollison and Cutbush, 1948).

The present paper is concerned with different aspects of the same group of cases and of a further group of cases seen more recently.

Methods

Blood Samples.—Whenever possible cord blood was examined. After tying the cord, blood was obtained with a dry syringe and needle by puncture of the umbilical vein at some point on the placental side of the ligature. When cord blood was not available a venous sample was obtained when the infant was first seen, usually within a few hours of birth. In almost all the cases in the present series, both in normal and in affected infants, this sample was taken by passing a fine plastic catheter up the umbilical vein; 1–2 ml. of blood was withdrawn and rejected before taking the sample for testing. A few venous samples taken on the first day and all those taken subsequently were obtained from a scalp vein, the external jugular vein, or the femoral vein; a scalp vein was used in the great majority of cases. Capillary samples were taken in duplicate from the heel after first warming it in water at approximately 40° C. for at least three minutes.

Determination of Haemoglobin.—Haemoglobin was measured as alkaline haematin in the photoelectric colorimeter; the method employed was essentially that of Wu (1922) as used by Clegg and King (1942). Into 4 ml. of N/10 HCl 20 c.mm. of blood was pipetted. After not less than 40 minutes 1 ml. of N/1 NaOH was added and mixed. (In cases of urgency, alkali was added at the end of 5–10 minutes after tests had shown that this did not lead to lower readings.) The samples were then read in optical cells of a thickness of 10 mm. against a water blank, using a green filter (Chance, O Gr I). During the early part of this work determinations were always made in duplicate, but after an analysis had revealed that the variance of replicate samples taken from venous blood was small only single determinations were made. Since the variance of replicate samples of capillary blood was rather greater, duplicate samples were estimated throughout.

The 20-c.mm., 4-ml., and 1-ml. pipettes used were calibrated and only those having errors within 0.3% of their stated capacity were employed.

The standard deviation of replicate samples was kindly calculated for us by Dr. Cheeseman (of the M.R.C. Group for Statistical Research) from 52 duplicate estimations on venous samples and 102 duplicate estimations on capillary samples. The venous samples had an S.D. of 0.26 from a general mean galvanometer reading of 24.4; this corresponds to an S.D. of 0.17 g. % of haemoglobin. The capillary samples had an S.D. of 0.46 from a general mean galvanometer reading of 25.2.

Haemoglobin Standardization.—The Gibson–Harrison permanent artificial standard (Gibson and Harrison, 1945), obtained from British Drug Houses, Ltd., was used throughout, the solution being boiled for four minutes and cooled before reading. In accordance with a personal communication from Dr. Q. H. Gibson the value of this standard was taken to be 16.1 g. % (in terms of iron) and not 16.0 g. %. As a further check the iron content of a sample of blood was determined by Dr. I. D. P. Wootton, using the "London modified" method described by King *et al.* (1948). This method is claimed to be accurate to within 0.3%. Eight replicate samples of the same blood were tested by our standard colorimetric method. The following results were obtained: Mean galvanometer reading of eight samples, 26.0; reading of Gibson–Harrison standard (boiled and cooled in the usual way and diluted to 2 parts in 5 with distilled water), 25.8. The blood sample is thus estimated to have had a haemoglobin value of 16.2 g. % (the measurements having been made at approxi-

mately 18° C.). Dr. Wootton's estimate of the haemoglobin concentration, equivalent to the iron content of this sample, was also 16.2 g. %.

Plasma Bilirubin Estimations.—The method of Haslewood and King (1937) adapted for the photoelectric colorimeter (King, 1947) was used, with the important modification (King and Wootton, 1948) of diluting the plasma with buffer if the readings were found or expected to exceed one and a half times the standard. This modification leads to considerably higher readings than those given by the original method and no doubt goes a long way towards meeting criticisms of the method (e.g., Gray and Whidborne, 1946). It is not yet known whether this modified precipitation method still gives lower results than the method of Malloy and Evelyn (1937). Determinations were always made upon venous samples; haemolysed plasma samples were discarded.

Serological Tests

(a) **Direct Coombs Test** (Coombs, Mourant, and Race, 1946).—After washing the erythrocytes three times in saline a 10% suspension (approx.) was prepared and one drop of this was mixed with one drop of anti-globulin serum on a flat opal tile. After half a minute the tile was slowly and gently rocked over a lighted box and examined with the naked eye for agglutination. If no definite agglutination developed within six minutes the test was considered negative. It was noted that different anti-globulin sera varied greatly in potency, and the reagent in use was tested from time to time as follows:

Normal Rh-positive cells were incubated with a serum known to contain only a trace of anti-Rh; the cells were then washed and tested against the anti-globulin serum. Even with potent reagents the reaction was never strong, and with moderate or weak anti-globulin sera there was no reaction at all. Through the generosity of colleagues we were fortunate in being able to maintain stocks of anti-globulin sera which would agglutinate such weakly sensitized cells.

The anti-globulin sera had been absorbed with human erythrocytes of different groups and were always diluted with saline to 1 in 20 or 1 in 50, depending upon their strength; they were nevertheless tested against washed unsensitized cells of different groups from time to time to see that they did not give non-specific agglutination. Occasionally when these tests were being read it was noted that a faint granularity developed after about 10 minutes' rocking on the slide, even though it was known that the cells were unsensitized. For this reason the convention was adopted of discounting any reactions developing after more than six minutes' rocking.

(b) **Indirect Coombs Test.**—Two volumes of serum were incubated at 37° C. with one volume of a 20% suspension of known O Rh-positive cells (usually CDe/CDe) for not less than 30 minutes. The cells were then washed and tested as above.

(c) **Titration in Saline and Albumin.**—The technique of Boorman *et al.* (1942) was used for saline titrations. For albumin titrations the sera were diluted in 30% bovine albumin and tested against cells suspended in 20% albumin (Diamond and Denton, 1945). Readings were made in conformity with the technique described in M.R.C. Memorandum No. 19 (1948).

(d) **Rh-testing.**—Mothers and infants were tested against a potent anti-D serum.

Measurement of Venous Pressure

In some of the earlier cases in this series it was noted that when the cord was cut a few hours after birth, for the purpose of carrying out an exchange transfusion, blood

gushed violently from the vein. In some of the later cases venous pressure was measured as follows:

With the infant supine on a flat surface, a plastic catheter was passed up the umbilical vein for a distance of approximately 2½ in. (6.25 cm.); a little blood was withdrawn and the catheter was then connected to a fine-bored saline manometer. The level of saline in the manometer was raised above the expected venous pressure and allowed to run in slowly until equilibrium was attained and the infant was quiet. The readings were referred to the level of the lower part of the front of the chest, using a wooden rule and spirit level.

Results: A. Haemoglobin Values

Normal Infants

(a) *Cord Blood*.—The results of 52 tests on normal infants are plotted in Chart 1; their mean value was 16.35 g.% and their

CHART 1.—Hb values in normal infants and in infants with haemolytic disease. I, cord blood; II, venous blood taken a few hours after birth. In II, series "A" are affected infants whose cord Hb is also plotted in I. Series B are infants whose cord Hb was not determined.

S.D. 1.34. The normal range throughout this paper has been expressed as "observed mean $\pm$ 2 S.D.," which gives a calculated range of 13.7–19 g.%, rather than the observed range of 14–19.4 g.%.

(b) *First Day; Venous Blood*.—The mean of 43 observations was 18.45 g.% (S.D.=2.04); these observations are also plotted in Chart 1. Again the calculated normal range (mean $\pm$ 2 S.D.) has been considered as the normal range in this paper.

(c) *First Day; Capillary Blood*.—The mean value of 33 observations* was 19.1 g.%, (S.D.=1.84).

(d) *Changes in Individual Cases*.—In Chart 2 the changes in four selected cases are illustrated. It will be noted that when the cord is tied late the haemoglobin value a few hours after

birth is considerably higher than that of cord blood, whereas when it is clamped immediately after birth there is little or no rise in the haemoglobin value. (For detailed observations upon this phenomenon, see De Marsh *et al.*, 1942; these illustrative normal cases are described because they have such a very important bearing on the changes observed in the affected infants.)

(e) Haemoglobin Values After

the First Day of Life.—Only a small group of normal infants was tested at intervals during the first 60 days of life. No infant had a haemoglobin value of less than 11 g.% during this period.

Affected Infants

(a) *Cord Blood*.—Thirty infants were tested; 14 had values within the normal range and 16 had values below it, ranging from 3.4 to 12.8 g.%. No deaths occurred among the infants with normal values, whereas there were 10 deaths among the 16 anaemic infants. Moreover, among the 10 infants with cord haemoglobin values below 8 g.% there were only two survivors (see Chart 1).

(b) *First Day; Venous Blood*.—Forty-three infants were tested; 14 had values within the normal range and 29 had values below it, varying from 3.7 to 14.3 g.%. These 43 cases included 23 whose cord-blood haemoglobin had been determined. As expected, the first-day values were on the average higher. At the same time the difference was a small one, and this was probably because in many instances the cord had deliberately been clamped as soon after birth as possible.

There were two cases of special interest: two infants whose cord-blood haemoglobin had been 12.3 and 12.8 g.% were found a few hours later to have values of 15 and 15.4 g.% respectively. Thus these two infants had values definitely below the normal range in cord blood, but a few hours after birth, doubtless owing to the fact that they recovered a fair amount of placental blood, they had values within the normal range; that is to say, haemoglobin values no lower than that of a normal infant recovering little or no placental blood.

Of the 20 infants whose cord blood was not tested but from whom a venous sample was taken a few hours after birth four died subsequently; the haemoglobin value of all these four infants was below 12 g.%.

(c) *First Day; Capillary Samples*.—In a few infants capillary samples were taken at the same time as venous samples; as in normal infants, the capillary samples gave distinctly higher values.

In Chart 3 are plotted the haemoglobin values observed in one affected and one normal infant. The affected infant is one of the two mentioned above whose cord haemoglobin value was 12.8 g.% and whose first-day venous haemoglobin value was 15.4 g.%; capillary samples taken at the same time as the "first-day venous" sample showed a further rise to 18 g.%. Thus it is clear that a haemoglobin value of 18 g.% in a capillary sample taken from an infant a few hours after birth does not exclude the possibility that a definite anaemia existed at the moment of birth.

The normal infant's cord haemoglobin value was 2.4 g.% higher than that of the affected infant, but because it recovered little placental blood its venous and capillary haemoglobin values a few hours later were no higher than those of the affected infant.

CHART 2.—Four normal infants selected to illustrate the effects of early and late tying of the cord.

CHART 3.—The Hb value of an infant may rise from 12.9 to 15.4 g.% within a few hours of birth, owing to the transfer of placental blood; on the other hand, the Hb value may change only from 15.2 to 15.8 g.% in an infant who receives a negligible amount of placental blood. The difference between the two infants is thus temporarily masked. Capillary samples usually give considerably higher values than venous samples and thus further mask anaemia.

*This group of observations is not strictly comparable with (b). Direct comparisons on the same infants have revealed distinctly greater differences between capillary and venous samples.

(d) *Haemoglobin Values After the First Day of Life.*—Seven infants with cord haemoglobin values between 15.1 and 18.7 g.% were left untreated; two other infants seen for the first time during the first three days of life were also judged to be very mildly affected (haemoglobin value within the normal range, mild icterus, weak positive direct Coombs reaction) and were not treated; one further infant was first seen on the 30th day of life and then found to have a moderately strong positive direct Coombs reaction. This infant was known to have shown no trace of jaundice in the neonatal period. The haemoglobin changes during the first two months of life in these ten mildly affected infants are plotted in Chart 4. In

CHART 4.—Hb changes in 10 very mild cases of haemolytic disease. Cord blood: range, 15.1–18.7 g.% (7 cases tested); 30-day venous sample: range 8.2–18 g.% (10 cases tested).

addition, an indication is given of the time for which the direct Coombs test remained positive. It is clear that an infant can have a positive direct Coombs reaction for one and even two months after birth without developing any serious degree of anaemia, and can have a positive reaction for two to three weeks without developing any subsequent anaemia at all.

In the cases treated by exchange transfusion the infant was usually provided with a concentration of some 4–5.5 million Rh-negative erythrocytes per c.mm., and this population of cells disappeared at the rate of only 1% a day (with a single exception). Only in two cases in which the infant grew unusually rapidly and in two cases in which the infant was provided with a concentration of less than 3.5 million Rh-negative cells per c.mm. did the haemoglobin concentration subsequently fall to such a level (7 g.%) that it was considered necessary to give a further transfusion. In the remaining infants the haemoglobin fell to 8.5–10 g.% at the end of 30–60 days and then slowly rose spontaneously.

B. Erythroblastemia

In 49 affected infants a blood film was examined on the first day of life. In 16 cases there were less than 10 nucleated red cells per 100 white cells; in 11 of these 16 the cord haemoglobin had been estimated and in only two was the value below 15 g.% (in these it was 12 and 12.9 g.%). In the remaining five infants, examined a few hours after birth, the haemoglobin varied from 11.6 to 16.9 g.%. All of these 16 infants recovered uneventfully, some without treatment.

In the remaining 33 cases there were at least 20 nucleated red blood cells per 100 white blood cells: in many instances the nucleated red cells far outnumbered the leucocytes and there were numerous primitive forms. Fifteen of these 33 infants died and the remaining 18 all required treatment.

C. Bilirubin Values

Normal Infants.—Cord blood. Twenty-one normal infants were found to have a mean value of 1.6 mg. per 100 ml. with a range of 0.8–2.6 mg.

Affected Infants.—(a) *Cord Blood.*—Thirty-two infants were tested; values ranged from 1 to 9.3 mg. per 100 ml. (see Chart 5).

Amongst 11 infants with cord bilirubin values over 4 mg. per 100 ml. there were six deaths. There were only two deaths amongst the 13 infants with bilirubin values between 3 and 4 mg. per 100 ml., and all of the eight remaining infants with values below 3 mg. recovered uneventfully. (b) *First Few Days of Life.*—In the 7 mildly affected infants (referred to above) whose cord haemoglobin lay between 15.1 and 18.7 g.% and who were left untreated, bilirubin values rose from 1–3.2 mg. per 100 ml. in the cord blood to peak values of 1.3–15.8 mg. between the second and fifth days of life.

It is difficult to make useful comparisons between this group of cases and those whose blood had a low haemoglobin value on the first day of life, because the latter infants were treated by exchange transfusion. However, it was striking that in this group the five infants who died at the age of 2–5 days all became profoundly jaundiced despite exchange transfusion, and as early as 24 hours after birth showed bilirubin values between 17 and 21 mg. per 100 ml.

In one infant (which did not develop kernicterus) exchange transfusion was not carried out until it was 12 hours old; during this period the plasma bilirubin rose from 8.1 to 19.3 mg. per 100 ml.

It is obvious that for purposes of comparison it is essential to take cord samples rather than samples a few hours after birth.

Correlation Between Haemoglobin and Plasma Bilirubin Values in Cord Blood

In Chart 6 the haemoglobin and plasma bilirubin values of the cord blood of 24 infants are plotted against one another. It will be noted that there is a correlation between low haemoglobin and high bilirubin values. As expected, the relationship does not appear to be linear. The haemoglobin value doubtless reflects the rate of blood destruction, so that, in general, higher bilirubin values are to be expected when the haemoglobin is low. Nevertheless the amount of bilirubin produced also depends upon the total amount of breaking-down haemoglobin, so that some falling off will be expected when the haemoglobin values are very low. From the Chart it appears that this is the case.

In Chart 6 the infants who died subsequently have been specially marked. It will be noted that there is some suggestion that the bilirubin value and haemoglobin value of a sample of cord blood, when taken together, give a better indication of the prognosis than either value considered separately. A slanting line could be drawn through the observations in such a way as almost completely to separate the living from the dying.

D. Serological Tests

Direct Coombs Test.

Tests were made on 69 Rh-positive infants born to mothers whose serum contained anti-Rh; all gave positive reactions, though a small number were weak. These weak reactions were shown by infants born to mothers.

CHART 5.—Cord bilirubin values in 32 affected infants compared with a group of 21 normals.

CHART 6.—Cord Hb concentration plotted against cord plasma bilirubin concentration in 24 affected infants.

whose sera contained low-titre albumin antibodies or saline antibodies of low or moderate titre. Further tests were made from time to time during the first three months of life. The results obtained in the untreated cases are shown in Chart 4, and it will be noted that even in mild cases the reaction remained positive for at least 10 days after birth and that in some cases it remained positive for as long as 60 days.

In infants treated by exchange transfusion with Rh-negative blood, Rh-positive cells often disappeared completely within a few days of transfusion or fell to a level of approximately 250,000 cells per c.mm. In these cases the direct Coombs test became negative and did not usually become positive again even when, at the end of 50 or 60 days, there were once more large numbers of Rh-positive cells in the circulation.

In infants treated by simple transfusion of Rh-negative blood there were usually more Rh-positive cells persisting in the circulation, and the direct Coombs reaction remained positive for long periods after transfusion. In one exceptional case the test was still positive 93 days after birth.

Tests for Free Antibody in Infant's Serum.—The sera of 41 infants were examined, either by the indirect Coombs test or by titration in albumin, or by both methods, for the presence of free Rh antibody; one or the other, or both, of the tests was positive in 35 cases.

In 18 cases sera were examined by both tests; in two cases where the infant's serum had a titre of only 2 in albumin the indirect Coombs reaction was negative, and in one case in which the serum gave a weak positive indirect Coombs reaction antibody could not be detected by the albumin test. In the remaining 15 cases the two tests were in agreement.

The amount of free antibody, determined by albumin titration, was not well correlated with the clinical manifestations. For example, amongst eight infants with a titre of 2 or less, three died, four were moderately severely affected, and one was very mildly affected and required no treatment. Among seven infants whose sera had an anti-Rh titre of 16 or more, four died, two were severely affected but recovered, and one was very mildly affected and required no treatment.

Persistence of free antibody does not by itself appear to be associated with tissue damage. In one infant given repeated simple transfusions of Rh-negative blood no Rh-positive cells could be detected between the 14th and the 30th days of life; at the end of this period the infant's plasma still contained Rh antibody to a titre of 32. By the 60th day there were once more many Rh-positive cells present, the direct Coombs reaction was positive again, and there was now only a trace of free antibody. The infant finally recovered completely.

Titre of Rh Antibody in Mother's Serum.—In 62 cases a sample of blood was taken from the mother within 24 hours of delivery. In every case the serum was titrated against CDe/CDe cells both in saline and in albumin. Seventeen of the 62 sera reacted equally well in saline and albumin (i.e., difference no greater than one tube). The remaining 45 sera gave higher titres in albumin; 26 of these gave no reaction at all in saline and nine reacted only in the first tube or the first and second tubes of the saline titration. The remaining 10 sera had titres of between 4 and 32 in saline, but in each case had an albumin titre that was at least four times greater (i.e., more than two tubes higher in the titration).

It is clear that any simple classification of these results must be arbitrary, but it is convenient to consider Rh antibodies in two groups—namely, (1) "predominantly saline agglutinins" (i.e., reacting equally well in saline and albumin): 17 cases; and (2) "predominantly albumin agglutinins" (i.e., titre in albumin two tubes or more higher than titre in saline): 45 cases.

It was noted that amongst the 17 cases in which the mother's serum contained predominantly saline agglutinins there were no stillbirths or deaths within the first 24 hours. However, there were three deaths at the age of 2–5 days, presumably from kernicterus. Amongst the 45 cases in which the mother's serum contained albumin antibodies (predominantly) there were four stillbirths, seven deaths within the first day of life, and four deaths at 2–5 days.

On these figures the difference in total mortality between the group of infants whose mothers' serum contained saline agglu-

tinins (mortality 18%) and the group in which the mothers' serum contained albumin agglutinins (mortality 33%) cannot be considered significant (S.E. of difference = 12.6%). Nevertheless the absence in the "saline group" of stillbirths and deaths within the first 24 hours of life is significant. The milder effects of saline agglutinins are further emphasized by Chart 7, in

CHART 7.—Titre of Rh antibody in mother's serum (at delivery) plotted against infant's first-day venous Hb value in 43 cases.

which the kind of antibody predominating in the mother's serum, and its titre, have been plotted against the infant's first-day haemoglobin value. It will be noted that in 10 out of the 12 cases in which the mother's serum contained saline agglutinins the infant's haemoglobin value was above 14 g.%.

With regard to sera containing albumin agglutinins predominantly, it appears that there is a correlation, though a poor one, between titre and degree of anaemia. There are few low haemoglobin values occurring with low maternal anti-Rh titres and few normal haemoglobin values occurring with high maternal anti-Rh titres. That this correlation is significant can be shown by dividing the cases into groups having, for example, an albumin titre of 128 or more and 64 or less respectively and showing that there is a significant difference in mortality. When the cases were analysed in this way and stillbirths (not appearing in Chart 7) were included, the results shown in Table II were obtained.

TABLE II.—Relation Between Titre of Albumin Agglutinins in Mother's Serum and Infant Mortality

Group	Total	Mother's Titre	Infants		Mortality
			Living	Dying	
I	28	1–64	22	6	21%
II	16	128 and over	5	11	69%

Observed difference between I and II = 48%. S.E. of difference 14%.

E. Venous Pressure

Venous pressure was measured in 14 infants; in infants without anaemia or with only slight anaemia it was found to vary between +3 and +5 cm. of saline. It is to be noted that this is the pressure below the diaphragm, in the proximal part of the umbilical vein, and represents the pressure above the front of the chest. In infants with severe or moderate anaemia the venous pressure was usually raised, values of +9 to +11 cm. usually being recorded (Table III). In these latter cases the cord often bled violently when cut, and this sign is probably a reliable indication of a raised venous pressure. In a few infants violent bleeding was noticed, although the pressure was not measured, and these cases have also been entered in Table III.

TABLE III.—*Venous Pressure in Cases of Haemolytic Disease*

Name	Haemoglobin		Venous Pressure* (cm. Saline)	Violent Bleeding When Cord Cut	Outcome
	Cord (G. %)	1st Day (G. %)			
Cu. . .	3.4	3.7	+9	+	Died 12 hours†
St. . .	5.3	5.0	+11	+	Died 12 hours†
Ho. . .	—	7.1	+10	?	Died 96 hours†
Wat. . .	5.7	5.7	?	+	Lived†
Ti. . .	—	7.3	?	+	Lived†
O'B. . .	7.4	—	+9	+	Died 13 hours
Th. . .	—	7.4	+7	0	Lived†
Ri. . .	—	8.4	+4	0	Lived†
Ve. . .	—	8.8	+5	0	Died 60 hours†
Bl. . .	9.4	10.9	?	+	Died 3 hours†
Du. . .	—	9.8	+3	0	Died 43 hours†
Pl. . .	—	11.9	?	+	Died 24 hours†
Bus. . .	12.2	13.7	+5	0	Lived†
Da. . .	12.3	15.0	+5	0	Died 72 hours†
Sa. . .	12.9	15.4	+4	0	Lived
He. . .	14.7	—	+5	0	Lived†
But. . .	15.4	15.1	+3	0	Lived
Ta. . .	15.4	16.9	+5	0	Lived†

Summary

No. Tested	Haemoglobin (G. %)		Raised V.P. or Bleeding
12	Cord	3.4-9.4	9
	1st day	3.7-11.9	
6	Cord	12.2-15.4	0
	1st day	13.7-16.9	

* Normal V.P. + 3 to + 5 cm. † Treated by exchange transfusion.

Deaths

Out of 74 cases there were five stillbirths, 10 deaths within the first 24 hours of life, and seven deaths between the second and fifth days of life. There were only two deaths later than this: one infant (Oa.), who was not seen before the third day of life and was treated by simple transfusion of Rh-negative blood, developed signs of kernicterus on the fourth day and eventually died, in a fit, at the age of 4 months; and one infant who recovered from haemolytic disease but developed congenital pyloric stenosis and died after operation. Of the remaining 50 survivors, only one has any signs suggestive of C.N.S. damage.

TABLE IV.—*Analysis of Fetal Cases*

Name	Haemoglobin		Erythro- blastaemia	Age at Death	Mother's Delivery Titre	
	Cord (G. %)	1st Day (G. %) (venous)			Sal.	Alb.
Ant. . .	—	—	Not examined	Stillbirth	—	+
Bu. . .	—	—	" "	" "	Nil	260,000
Tu. (2) . .	—	—	" "	" "	Nil	2,048
Gr. . .	—	—	" "	" "	16	128
Kie. . .	—	—	+++	" "	Nil	16
Yo. . .	—	—	Not examined	Few mins.	Nil	+
Ca. . .	5.1	—	+++	1 hr.	Nil	8
Tu. (1) . .	4.1	8.7	+++	9 hrs.	Unknown	—
O'N. . .	5.3	—	+++	3 hrs.	Nil	128
Cu. . .	3.4	3.7	+++	12 hrs.	8	128
St. . .	5.3	5.0	+++	12 hrs.	2	512
O'B. . .	7.4	—	+++	13 hrs.	Nil	256
Gi. . .	7.1	—	Not examined	11 hrs.	32	256
Ecc. . .	7.6	7.9	+++	60 hrs.	Nil	256
Ho. . .	—	7.1	+++	96 hrs.	Nil	256
Ve. . .	—	8.8	+++	60 hrs.	8	16
Bl. . .	9.4	10.9	++	3 hrs.	Nil	16
Du. . .	—	9.8	+++	43 hrs.	16	128
Pl. . .	—	11.9	+++	24 hrs.	4	16
Da. . .	12.3	15.0	++	73 hrs.	128	128
Hi. . .	—	14.7 (cap.)	Not examined	96 hrs.	32	64
Wal. . .	—	—	++	40 hrs.	Nil	16
Oa.* . .	—	9.1	+	4 mths.	Nil	256

* First examined on third day of life.
+++ = More than 70 nuc. r.b.c. per 100 w.b.c.
++ = 30-70 nuc. r.b.c. per 100 w.b.c.
+ = 10-30 nuc. r.b.c. per 100 w.b.c.

In Table IV the time of death is recorded together with the cord haemoglobin and the first-day venous haemoglobin (when known). It is evident that there is a correlation between low initial haemoglobin and death on the first day of life. Moreover, of the seven infants dying between the second and fifth day of life four had haemoglobin values well below the normal range a few hours after birth; one (Hi.) had a value just below the normal range and one (Da.) had a value just within the normal range; however, in this latter infant cord blood had

also been tested and found to be anaemic. In the seventh case the haemoglobin value was not determined before transfusion.

Discussion

It has already been mentioned that no infant with a cord haemoglobin value of over 14 g. % died, whereas of 10 infants with values under 8 g. % there were only two survivors. In the intermediate zone there were two deaths among six cases. The idea that the cord haemoglobin is a reliable index of severity receives further support from the observation that seven infants whose cord haemoglobin was within the normal range and who were left untreated pursued a benign course.

Data obtained from the infants first tested some hours after birth lend further support to the theory that initial anaemia is closely correlated with an unfavourable prognosis, since all four deaths in this group occurred in infants whose venous haemoglobin value a few hours after birth was below 12 g. %.

It has long been accepted that infants with hydrops foetalis have a severe anaemia at birth, and it might be suggested that our observations simply restate this fact. It is true that two of the infants whose cord blood was tested were frankly hydropic and others had varying mild degrees of oedema. However, these cases merged imperceptibly into the group who lived on beyond the first 24 hours only to die on the second, third, or fourth days of life; in most of these latter cases it was possible to establish by post-mortem examination that the infant had the changes of kernicterus.

It seems that two distinct mechanisms are operating, and it is to some extent coincidental that the degree of anaemia is correlated with both. It appears that very severe anaemia kills the infant by causing cardiac failure; doubtless the mechanism is similar in many respects to that of the hyperkinetic phase described by Howarth and Sharpey-Schafer (1947) in adults with severe anaemia. The process of birth presumably throws an additional strain on the infant's cardiovascular system and precipitates failure; doubtless also the transfer of placental blood, when it is allowed to occur, may add too great a load.

With regard to the deaths occurring two to five days after birth, two facts seem to be established. First, as Vaughan (1946) and Parsons (1947) have stressed, the deaths are not directly due to anaemia, since many of the infants have haemoglobin values within the normal range at the time of death; it is probable, as Hawksley and Lightwood (1934) first suggested, that the immediate cause of death in these cases is medullary failure consequent upon cerebral damage. On the other hand, these deaths are associated with a certain degree of anaemia at birth, as our observations make clear. The most likely explanation of this association is that the incidence of kernicterus is closely related to the severity of the haemolytic process.

If it is true that the severity of the haemolytic process not only determines the likelihood of the infant's dying from anaemia *in utero* or within 24 hours of birth but also largely determines the possibility of the infant developing kernicterus two to five days later, should it survive so long, other signs of rapid blood destruction besides anaemia should always be present in fatal cases of the disease.

The two most obvious signs to consider are erythroblastaemia and hyperbilirubinaemia. It has often been stated that erythroblastaemia is a variable sign in haemolytic disease, and in the present series of cases it was found in only 33 out of 49 infants examined on the first day of life. However, all of the 16 infants without erythroblastaemia were mildly affected, whereas among the 33 cases

showing erythroblastaemia there were none so mild as not to require treatment. Moreover, these 33 cases included 15 who died within the first five days of life, and in all of these erythroblastaemia was pronounced.

Raised bilirubin values must be interpreted more cautiously, since it is known that the newborn infant has a lowered capacity for excreting bilirubin, and this capacity varies widely from one infant to another; moreover, actual damage to the liver may occur in haemolytic disease (Hawksley and Lightwood, 1934). However, our observation of a moderate correlation between low haemoglobin and high bilirubin values suggests that in fact the hyperbilirubinaemia in infants with haemolytic disease is at least partly due to blood destruction.

The cord bilirubin value, taken alone, does not seem to be quite as satisfactory a criterion of severity as the cord haemoglobin, because only a narrow zone divides mild from severe cases. Not enough data are available to decide whether the haemoglobin and bilirubin values of cord blood, considered together, give a better estimate of severity than either value considered alone, but it is probable that they will prove to do so.

It appears that there is some connexion between the strength of the direct Coombs reaction and the severity of the haemolytic process, since in this series the weakest reactions were found in mildly affected infants. On the other hand, moderately strong reactions were not necessarily associated with signs of rapid blood destruction. One infant who never became jaundiced and developed only a very mild anaemia had a positive direct Coombs reaction for 60 days after birth.

The amount of free antibody in the infant's serum appears to be poorly correlated with severity, since some cases with only traces of free antibody were severely affected and others with moderate amounts of free antibody were mildly affected. At the same time it must be noted that the two infants with the largest amounts of free antibody were both severely affected.

It is evident that there is some correlation between the kind of antibody, and its titre, in the mother's serum and the severity of the disease in the infant, but that the correlation is low, so that exceptions are frequent. In this series the predominance of saline agglutinins over albumin agglutinins in the mother's serum was on the whole associated with milder forms of the disease.

When albumin agglutinins predominated in a serum their titre bore some relation to severity, for there were no very severe cases occurring with very low maternal titres, and deaths were significantly more frequent when the mother's titre was high. Similar conclusions have been reached by Davidsohn and Stern (1948).

Wiener (1946) suggested that the presence of saline agglutinins in the mother's serum caused a disease syndrome "erythroblastosis" associated with deep jaundice and kernicterus; this syndrome was said to come on after birth, since the agglutinins crossed the placenta only during labour and were then thought to cause agglutinative thrombi in the liver and brain; the presence of "blocking" antibodies was said to lead to a different syndrome—"congenital haemolytic disease," associated with anaemia and/or hydrops.

Wiener and Gordon (1948) now consider that saline agglutinins play only a minor part in haemolytic disease, though Wiener (1948) still believes that saline agglutinins may enter the infant's circulation "through placental defects or during labour."

A more convincing hypothesis is that saline agglutinins play no part in the aetiology of haemolytic disease and that sera which contain saline agglutinins can be regarded

as containing in addition a smaller amount of albumin agglutinins. This hypothesis would satisfactorily explain the milder effects of saline agglutinins and is also consistent with the evidence that the haemolytic process is already maximal at the moment of birth.

The idea that haemolytic disease "comes on" after birth has arisen from the fact that jaundice and pallor may be absent at birth but develop rapidly afterwards. However, it is well known that the plasma bilirubin rises rapidly after birth in *normal* infants, chiefly because of the poor excretory capacity of the liver in the newborn. Therefore a rapid rise in bilirubin after birth does not indicate that the rate of blood destruction is increasing. Our observations demonstrate that even in very mildly affected infants the plasma bilirubin is usually already raised at the moment of birth, and in severely affected infants is always far above the normal range.

A decline in haemoglobin value during the days following birth is also a phenomenon of all normal infants and has been shown to be due to a diminution in the production of erythrocytes after birth (Faxen, 1937; Shapiro and Bassen, 1941). It is very probable that the rapid fall in haemoglobin values during the first few days of life observed in many infants with haemolytic disease of the newborn is also due to a diminished production of erythrocytes rather than to any increase in the rate of destruction. In one severe case reported by Mollison (1943) the rate of destruction of Rh-positive erythrocytes in an infant with "icterus gravis" was measured on the fourth and seventh days of life and found to be distinctly slower on the second occasion. It is common knowledge that in infants with haemolytic disease the degree of erythroblastaemia diminishes rapidly after birth, and it is surprising that so much stress has been laid on blood destruction as a cause of the anaemia, to the complete exclusion of any consideration of changes in the amount of production.

It has been suggested that there is an inverse relationship between the degree of blood destruction and the development of kernicterus (Vaughan, 1946; Parsons, 1947). It seems to us that this belief has arisen as follows:

Infants whose cord haemoglobin value is below 8 g.% usually die within 24 hours of birth and therefore do not have time to develop kernicterus. Infants with cord haemoglobin values of, say, 10–12 g.% may acquire values of 12–15 g.% in venous samples taken a few hours after birth, and capillary samples may show values as high as 18 g.%. The infant's rapid production of cells does not fall off for two to three days after birth, and therefore an infant whose haemoglobin value was 12 g.% at the moment of birth may die on the third day of life with a haemoglobin value of, say, 16 g.%. The fallacy lies in regarding such a haemoglobin value as being necessarily normal. 16 g.% does lie within the normal range of haemoglobin values for the third day of life, but it is normal only for an infant receiving little or no placental blood. In an infant which has received a large amount of placental blood 16 g.% in a capillary sample taken on the third day of life is indicative of anaemia.

Summary

The haemoglobin value in the cord blood of an infant with haemolytic disease is well correlated with the severity of the disease. Infants whose cord blood contains less than 8 g.% are very likely to die within 24 hours of birth. These infants often show a raised venous pressure and probably die from cardiac failure. Infants with values over 14.5 g.% are very likely to recover without treatment. Deaths from kernicterus at two to five days occur in some of those infants who are anaemic at birth but not so anaemic as to die within 24 hours.

Haemoglobin values of blood samples taken after birth are much more difficult to interpret because of (a) the placental

transfer of blood, and (b) the large capillary/venous differences in the newborn.

The cord plasma bilirubin taken alone is a less valuable index of severity than the cord haemoglobin. However, taken in conjunction with the cord haemoglobin it is of definite value in assessing the severity.

In this series erythroblastæmia was present on the first day of life in all moderately severe cases and was a striking feature in all the fatal cases; in mild cases the number of nucleated red cells was usually within normal limits.

The strength of the direct Coombs test and the amount of free Rh antibody in the infant's circulation are of very limited value in assessing severity.

The form of Rh antibody predominating in the mother's serum and the antibody titre show some correlation with the severity of the haemolytic process in the infant. In practice exceptions are frequent, and antibody tests can be used only to forecast probabilities.

These findings emphasize the importance of determining the haemoglobin and bilirubin values of cord blood as a means of grading cases of haemolytic disease of the newborn. Not only is this of help in deciding whether treatment is needed, but it should make it possible to compare one treated series with another and to decide whether equal numbers of severe cases have been included.

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REFERENCES

- Boorman, K. E., Dodd, B. E., and Mollison, P. L. (1942). *British Medical Journal*, **2**, 535.
 Clegg, J. W., and King, E. J. (1942). *Ibid.*, **2**, 329.
 Coombs, R. R. A., Mourant, A. E., and Race, R. R. (1946). *Lancet*, **1**, 264.
 Davidsohn, I., and Stern, K. (1948). *Amer. J. clin. Path.*, **18**, 690.
 De Marsh, Q. B., Windle, W. F., and Alt, H. L. (1942). *Amer. J. Dis. Child.*, **63**, 1123.
 Diamond, L. K. (1947). *Proc. R. Soc. Med.*, **40**, 546.
 — and Denton, R. L. (1945). *J. Lab. clin. Med.*, **30**, 821.
 Faxen, N. (1937). *Acta paediatr., Stockh.*, **19**, Suppl. 1.
 Gibson, Q. H., and Harrison, D. C. (1945). *Biochem. J.*, **39**, 490.
 Gray, C. H., and Whidborne, Joanna (1946). *Ibid.*, **40**, 81.
 Haslewood, G. A. D., and King, E. J. (1937). *Ibid.*, **31**, 920.
 Hawksley, J. C., and Lightwood, R. (1934). *Quart. J. Med.*, n.s. **3**, 155.
 Howarth, S., and Sharpey-Schafer, E. P. (1947). *Lancet*, **1**, 18.
 King, E. J. (1947). *Microanalysis in Medical Biochemistry*, p. 149. Churchill, London.
 — Gilchrist, M., Wootton, I. D. P., O'Brien, J. R. P., Jope, H. M., Quelch, P. E., Peterson, J. M., Strangeways, D. H., and Ramsay, W. N. M. (1948). *Lancet*, **1**, 478.
 — and Wootton, I. D. P. (1948). Personal communication.
 Malloy, H. T., and Evelyn, K. A. (1937). *J. biol. Chem.*, **119**, 481.
 Medical Research Council (1948). Memorandum No. 19. H.M.S.O., London.
 Mollison, P. L. (1943). *Arch. Dis. Childh.*, **18**, 161.
 — and Cutbush, Marie (1948). *Lancet*, **2**, 522.
 Parsons, L. G. (1943). *Ibid.*, **1**, 534.
 Shapiro, L. M., and Bassen, F. A. (1941). *Amer. J. med. Sci.*, **202**, 341.
 Vaughan, V. C. (1946). *J. Pediatr.*, **29**, 462.
 Wiener, A. S. (1946). *N.Y. St. J. Med.*, **46**, 912.
 — (1948). *Ann. Allergy*, **6**, 293.
 — and Gordon, Eve B. (1948). *J. Lab. clin. Med.*, **33**, 181.
 Wu, H. (1922). *J. Biochem., Tokyo*, **2**, 173.

The British Council for Rehabilitation, which was founded four years ago, has issued the first number of a quarterly magazine (price 2s.) entitled *Rehabilitation*. It publishes a lecture by Sir Hugh Griffiths surveying the field and describing the aims and methods of rehabilitation. "Rehabilitation is treatment," he says, "but it is not the treatment of a disease or an injury but the treatment of the individual suffering from the disease or injury"; and Dr. Edward Dunlop, discussing rehabilitation in Canada, makes the point in another way—"Each disabled person has his own rehabilitation problems, varying in extent and character." The journal is intended for medical men and ancillary workers as well as managers of industry and patients. It is obtainable from the British Council for Rehabilitation, 32, Shaftesbury Avenue, London, W.1.

CHEMOPROPHYLAXIS OF EXPERIMENTAL FILARIASIS IN THE COTTON-RAT

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It is well known that the blood-sucking mite *Liponyssus bacoti* is a vector of *Litomosoides carinii*, a filarial parasite of the cotton-rat, and that the infection is used in this country and elsewhere for testing the filaricidal activity of drugs. The earlier investigations were made on wild cotton-rats which were found naturally infected in the field. As caution is necessary in the interpretation of the results of such experiments (Kershaw and Bertram, 1948), the more recent chemotherapeutic trials have been made on infections of known duration artificially induced in the laboratory.

This may be done by exposing rats to infection by placing them for a few days in an artificial rat-nest containing a colony of mites previously maintained on an infected rat. In our experience and that of other workers successful infection of a rat by this method cannot be predicted with any confidence. One of us (D. S. B.), in studies on the role of the mite as a vector, has found that it is much more efficient than was suggested by earlier work. Infection rates of over 80% have been obtained for infective forms in batches of mites, and, although a mite usually contains only a few worms, instances of about 20 or 30, or in one case even 67, infective worms in a single mite have been observed. This range of variation in the number of worms per mite in mites of the same batch tends to be wide and irregular. The selection of a suitable host for the provision of such highly infective mites will be dealt with in a later communication elsewhere. Using batches of these highly infective mites we have been able to eliminate some of the unknown or uncontrollable factors arising from the use of rat-nests by exposing rats to infection by releasing small numbers of the mites (about 20) on each rat for about 24 hours.

In a series of 41 untreated cotton-rats exposed to controlled infection in this way, 35 out of 37 of those rats from which gorged mites were recovered subsequently showed microfilariae in the peripheral blood, but four rats from which no gorged mites were recovered remained negative. Thus about 95% successful infection was obtained provided there was evidence of the mites having gorged on the rat. On the other hand, these results suggest that unless the mites feed freely on the rat infection does not occur.

We have shown elsewhere (Bertram, 1947) that the mites do not transmit all their worms at the first infective blood-meal. Consequently, any estimation of the number of worms transmitted to a rat necessitates dissections of a series of mites before, and of engorged mites after, the transmission meal. Since the number of worms per mite is so variable such estimates are likely to show