ADVANCES IN THE HANDLING OF HAEMOLYTIC DISEASE OF THE NEWBORN

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The Rhesus factor and its relation to haemolytic disease of the newborn was discovered in 1940.[1,2] In 1946 a method of simultaneous removal and replacement of blood was described as a means of treating the condition.[3] This method has withstood the test of time, and today exchange transfusion is still the only accepted method of dealing with the disease. Although the efficacy of exchange transfusion varies in different hands, with a reported mortality rate varying from 0·1% to as high as 15%, it is generally accepted to be an efficient and successful method of treatment.

However, to apply this treatment the infant must be born alive and viable. It is an unfortunate fact that once a woman has become sensitized to a blood-group factor, the foetal affliction usually becomes progressively more severe with subsequent pregnancies. The most important unresolved problem in haemolytic disease of the newborn is that of intra-uterine death which may occur as early as the 28th week of pregnancy. The approach to this problem has been to terminate the pregnancy prematurely, to reduce the exposure of the infant to the effect of the maternal antibodies, and induction of labour after the 36th week of pregnancy is now practised in most centres throughout the world. The indications for induction of labour are based on the maternal antibody titre and the history of a previously affected infant. In recent years even the latter requirement has been dropped, and a maternal antibody titre of 32, by the indirect antiglobulin technique, is considered adequate grounds for inducing labour after the 36th week of pregnancy. On serological data it is not possible in all cases to predict with certainty whether the foetus is affected, or how severely it is affected. Consequently some such prematurely terminated pregnancies produce mildly affected infants or, in the case of heterozygous husbands, completely unaffected Rh-negative infants.

The risk to the infant in pregnancies terminated after 36 weeks is negligible, and justifies the procedure even if the interference turns out to be unnecessary in a certain number of cases. However, the risks of prematurity increase alarmingly if the pregnancy is terminated before the 36th week of pregnancy and virtually preclude such a step because of the considerably increased danger to a possibly normal or mildly affected infant.

AMNIOTIC FLUID ANALYSIS

The determination of the amount of bilirubin in the amniotic fluid during the course of the pregnancy has proved to be a remarkably accurate indication of the severity of the red blood cell destruction in the foetus, and therefore of the probability of foetal death. Amniotic fluid analysis was pioneered by Bevis[4-7] and subsequently perfected by Walker,[8] Mackay[9] and Liley.[10] Liley's technique is widely used, and is based on the spectrophotometric measurement of the increased optical density of the amniotic fluid at 450 mμ. due to the presence of bilirubin. The technique is described in Liley's original article,[10] and in a subsequent publication[11] he indicated the possible sources of error associated with the technique.

Liley found a remarkably good correlation between the increase in the optical density of the amniotic fluid at 450 mμ. and the severity of the disease in the foetus as reflected by the outcome of the pregnancy. Based on his experience with 101 cases of Rhesus sensitization, he was able to predict 3 categories of severity—an upper zone, indicating severe red blood cell destruction with a high incidence of stillbirths; a lower zone, indicating an unaffected or mildly affected infant: and a middle zone, indicating a moderately affected foetus.

Early in 1964 the Natal Blood Transfusion Service commenced amniotic fluid analysis, and this report describes our experience with 46 cases of Rhesus immunization. Liley's technique[10] of analysing the amniotic fluid was used without modification, except for the use of a Beckman model B spectrophotometer instead of a Unicam SP 600 and SP 500 spectrophotometer.

Collection of Amniotic Fluid

Amniotic fluid was collected by introducing an appropriate needle, under local anaesthetic, through the maternal abdominal wall into the amniotic cavity. Five ml. of amniotic fluid was aspirated, transferred to a container, and sent to the laboratory immediately. The fluid was protected from light since bilirubin is photolabile.

Spectral Absorption Curve

The spectral absorption curve of the fluid was ascertained, as described by Liley,[10] by determining the optical density at 26 different wavelengths from 350 to 700 mμ., including a reading at 450 mμ. The results were plotted on a logarithmic scale against wavelength. If a significant amount of bilirubin is present, the graph will show a substantial peak at 450 mμ. The difference between the top of this peak and a straight line drawn from 350 mμ. to 550 mμ. represents the increase in optical density attributable to the presence of bilirubin. This deviation of the optical density from linearity at 450 mμ. when applied to Liley's criteria, will show whether the result falls in the upper, middle, or lower zone.

RESULTS

A total of 72 amniocenteses were performed in 46 patients. Since it is the object of this analysis to determine the validity of prognostication, based on amniotic fluid analysis by correlating the results with the severity of the disease in the foetus at birth, all cases in which it was not possible to assess the severity of the condition at birth were eliminated from the analysis. In addition, all cases in which amniotic fluid analysis was performed for the first time after the 36th week of pregnancy, were also eliminated. In the early stages of our investigation we were carried away by our enthusiasm, and a number of analyses were performed in patients presenting for the first time after the 36th week. We now realize that there is no indi-

cation for amniotic fluid analysis in the last 4 weeks of pregnancy.

Excluded from Analysis

Nineteen of the 46 cases were eliminated from the analysis for the following reasons:

1. Patient still pregnant—6 cases.
2. Amniocentesis performed for the first time after the 36th week of pregnancy—7 cases.
3. Foetus born at 24 weeks and too immature to allow for proper assessment of severity of disease—2 cases.
4. Premature labour with subsequent foetal death following transabdominal intra-uterine transfusion. The true foetal haemoglobin concentration could not be ascertained because of the presence of donor blood in the foetal circulation—1 case.
5. Amniotic fluid sample unsuitable for investigation owing to excessive turbidity or to the presence of an excessive amount of haemolysed blood—3 cases.

Included in Analysis

In the remaining 27 cases there were 9 that fell in the upper zone, 6 in the middle zone and 12 in the lower zone (Table I). For purposes of this evaluation a foetus with a PCV of 30% at birth (equivalent to a haemoglobin concentration of 10 G/100 ml.), was considered to have been at risk during its intra-uterine life. The results were correlated with the severity of the disease in the infants at birth with the following results (Table II):

1. Of the 9 upper-zone cases, 7 resulted in stillbirth, one in a severely affected hydropic infant that died shortly after birth, and one in a severely affected infant with a PCV less than 30% at birth. The infant survived following an exchange transfusion. However, labour had been prematurely induced at 36 weeks, and if the pregnancy had been allowed to go to term, it could have resulted in a stillbirth. The foetal mortality was 88%, and in the remaining 12% the foetus was considered to have been at risk during its intra-uterine life.
2. Of the 6 middle-zone cases, 1 infant was severely affected with a PCV less than 30% at birth, and 5 infants were not severely affected, with a PCV over 30% at birth. The foetal mortality was nil, and in 16% of the cases the foetus was considered to have been at risk during its intra-uterine life.
3. Of the 12 lower-zone cases, none was severely affected, 8 were mildly affected with a PCV over 30% at birth, and 4 were unaffected Rh-negative infants. The infant mortality rate was nil and none of the infants was at risk during its intra-uterine life.

These results are in keeping with Liley's and confirm the remarkably accurate prediction that analysis of the amniotic fluid permits, particularly in regard to those cases which are likely to result in intra-uterine death. This technique has revolutionized the handling of Rhesus-sensitized patients, and now offers hope, where little existed before, of salvaging the severely affected erythroblastotic infant that dies before the 36th week of pregnancy.

TABLE I. AMNIOTIC FLUID ANALYSIS IN 27 PREGNANT WOMEN SENSITIZED TO THE RHESUS FACTOR

Case	*Amniotic fluid analysis*		*Category*	*Outcome of pregnancy*	
	Stage	*Result†*		*Stage*	*Result*
1	31 weeks	1·64	Upper zone	32 weeks	Stillbirth
2	29 weeks	0·045	Lower zone	40 weeks	Unaffected
3	32 weeks	0·037	Lower zone	38 weeks*	Mildly affected, PCV not done
4	35½ weeks	0·180	Upper zone	36 weeks	PCV 27%
5	30 weeks	0·145	Middle zone	37 weeks	PCV 43%
6	31 weeks	0·520	Upper zone	33 weeks	Stillbirth
7	31 weeks	0·205	Upper zone	34 weeks	Stillbirth
8	32 weeks	0·032	Lower zone	40 weeks	PCV 45%
9	32½ weeks	0·015	Lower zone	40 weeks	Unaffected, Rh.-neg.
10	30 weeks	0·313	Upper zone	32 weeks	Stillbirth
11	30 weeks	0·111	Middle zone	37 weeks	PCV 29%
12	32½ weeks	0·080	Middle zone	33 weeks	PCV 38%
13	35½ weeks	0·028	Lower zone	40 weeks	PCV 38%
14	28 weeks	0·320	Upper zone	30 weeks	Stillbirth
15	30 weeks	1·816	Upper zone	33 weeks	Hydropic—died
16	27½ weeks	1·90	Upper zone	28 weeks	Stillbirth
17	36 weeks	0·016	Lower zone	38 weeks	PCV 54%
18	34 weeks	0·026	Lower zone	40 weeks*	PCV not done, mildly affected
19	32 weeks	0·035	Lower zone	40 weeks	Unaffected, Rh.-neg.
20	34 weeks	0·034	Lower zone	37 weeks	PCV 52%
21	34 weeks	0·014	Lower zone	38 weeks	PCV 52%
22	32 weeks	0·039	Lower zone	38 weeks	PCV 38%
23	28 weeks	0·068	Middle zone	36 weeks	PCV 65%
24	29 weeks	0·168	Middle zone	37 weeks	PCV 39%
25	28 weeks	0·084	Middle zone	36 weeks*	PCV not done
26	25 weeks	0·408	Upper zone	31 weeks	Stillbirth
27	24 weeks	0·037	Lower zone	40 weeks	Unaffected Rh.-neg.

†Deviation of optical density from linearity at 450 mμ
*Regarded as having birth PCV higher than 30% on subsequent clinical observation

DISCUSSION

The purpose of prenatal prediction of the severity of the disease in the foetus is to determine which of the following procedures should be followed:

1. Not to interfere with the pregnancy.
2. To terminate the pregnancy after the 36th week.
3. To terminate the pregnancy, by caesarean section if necessary, before the 36th week, provided that the foetus is deemed to be viable.
4. Intra-uterine transfusion[12,13] of compatible blood into the foetus if it is too premature to allow the pregnancy to be terminated. This is performed between the 28th and 34th weeks of pregnancy, approximately every 10 - 14 days until the foetus is sufficiently mature to be viable. Although this procedure is still in the experimental phase and fraught with technical difficulties, it has been done successfully* and undoubtedly offers a chance of survival to foetuses who would otherwise have died between the 28th and 36th week of pregnancy.

The relative inaccuracy of predictions based on serological data alone precludes any interference with a pregnancy earlier than the 36th week. Amniotic fluid analysis,

*See article 'Intra-uterine foetal blood transfusion' on p. 630 of this issue of the Journal.

TABLE II. CORRELATION OF AMNIOTIC FLUID ANALYSIS WITH SEVERITY OF DISEASE IN FOETUS

Amniotic fluid analysis result	*Number of cases*	*Stillbirth*	*Foetus hydropic*	*Birth PCV <30%*	*Birth PCV >30%*	*Foetus unaffected*	*Foetus at risk*	
							Total	*Per cent*
Upper zone	9	7	1	1	—	—	9/9	100
Middle zone	6	—	—	1	5	—	1/6	16
Lower zone	12	—	—	—	8	4	0/12	Nil

on the other hand, makes an accurate prediction of the severity of the disease possible, particularly in the more severely affected cases, thus allowing a more radical approach in those cases likely to result in stillbirth before the 36th week of pregnancy.

A PRACTICAL AND RATIONAL APPROACH TO THE HANDLING OF RHESUS-SENSITIZED CASES

The following routine procedure is advocated:

Routine serological analysis of maternal blood at 24 - 26 weeks is advised in all pregnancies.

If a maternal antibody is detected, and the titre suggests the possibility of a severely affected foetus, amniotic fluid analysis should be carried out at 26 - 27 weeks. In our experience a titre in excess of *16* in first affected infants, and titres in excess of *8* in subsequently affected infants, can result in foetal death before term, and therefore justify amniotic fluid analysis. The interpretation of maternal antibody titre varies with different laboratories, and every laboratory should establish its own criteria to select those cases requiring amniotic fluid analysis. In our experience about 80% of Rhesus-sensitized cases will require amniotic fluid analysis.

Interpretation of Amniotic Fluid Analysis (Table III)

(*a*) If the optical density of the deviation from linearity at 450 mμ. gives a result which falls in the lower zone, it indicates that the haemolytic process in the foetus is not severe, and the foetus is not at risk during its intra-uterine life. It is not necessary to undertake any further amniotic fluid analyses, or to perform further maternal antibody titrations. The pregnancy should be allowed to go to term. However, it should be realized that the infant may require an exchange transfusion at birth and appropriate arrangements should be made in readiness for this eventuality.

TABLE III. INTERPRETATION OF AMNIOTIC FLUID ANALYSIS

Weeks maturity	*Optical density of the deviation from linearity at 450 mμ.*		
	Lower zone	*Middle zone*	*Upper zone*
26	Below 0·070	Between 0·070 & 0·290	Above 0·290
27	Below 0·064	Between 0·064 & 0·270	Above 0·270
28	Below 0·059	Between 0·059 & 0·250	Above 0·250
29	Below 0·054	Between 0·054 & 0·230	Above 0·230
30	Below 0·050	Between 0·050 & 0·205	Above 0·205
31	Below 0·045	Between 0·045 & 0·185	Above 0·185
32	Below 0·041	Between 0·041 & 0·170	Above 0·170
33	Below 0·037	Between 0·037 & 0·155	Above 0·155
34	Below 0·034	Between 0·034 & 0·140	Above 0·140
35	Below 0·031	Between 0·031 & 0·130	Above 0·130
36	Below 0·029	Between 0·029 & 0·120	Above 0·120

(*b*) If the result falls in the upper zone, it indicates a high risk of intra-uterine death (in our experience as high as 80 - 90%), which may occur as early as the 28th week of pregnancy. If the foetus is considered to be viable, usually after the 34th week of pregnancy, immediate termination of pregnancy is advocated, by induction of labour or caesarean section if necessary. If the foetus is considered to be not viable, the only courses open are either to hope that the foetus will survive until it becomes viable or to undertake intra-uterine transfusion of compatible blood into the foetal peritoneal cavity every 10 - 14 days until it does become viable. Absorption of red blood cells from the peritoneal cavity is known to be surprisingly good;[14] if sufficient red blood cells compatible with the maternal antibody can be introduced into the foetal circulation this should ensure the survival of the foetus.

(*c*) If the result falls in the middle zone, it indicates that in a small proportion of cases (16% in our limited series) the foetus is at risk although unlikely to suffer intra-uterine death. In our experience the middle zone category rarely moves into the upper zone during the subsequent course of the pregnancy, which should be terminated by induction of labour at 36 weeks.

THE VALUE OF SEROLOGICAL INVESTIGATIONS

If facilities for amniotic fluid analysis are available, the only value of maternal antibody titrations is to determine which cases should be subjected to amniotic fluid analysis. If amniotic fluid analysis is undertaken there is no useful purpose served by further serial titrations of maternal antibody, or by determining the zygosity of the husband, since these results will in no way influence the course of action to be followed.

COMPLICATIONS OF AMNIOCENTESIS

Amniocentesis is not without danger to the foetus, and should not be undertaken without proper indications. In our experience the main risk is premature labour. If this occurs before the 34th week of pregnancy, it is likely to result in foetal death. Of a total of 72 amniocenteses performed in 46 patients, labour commenced within 3 days of the amniotomy in 2 cases. In one instance the amniocentesis was done at 24 weeks and the infant was stillborn. In the other the procedure was carried out at 32½ weeks. The infant was born alive with a PCV of 38% (case 12—Table I) but died during exchange transfusion. In a third case the foetal heart stopped approximately 72 hours after diagnostic amniocentesis was performed at 24 weeks. A macerated infant was delivered about 14 days later, and it was impossible to establish the cause of death. We presume that the foetal death was attributable to the procedure, probably as a result of placental haemorrhage. The 2 cases in which premature labour and/or foetal death occurred at 24 weeks have not been included in the series of 27 cases detailed in Table I, as the foetuses were too immature to allow for an adequate evaluation of the severity of the disease at birth.

Although it is desirable not to damage the placenta, there is no way of avoiding this in some instances. In 25% of our cases we obtained heavily bloodstained amniotic fluid, indicating placental transfixation. A fine-gauge needle should be used and the needle should be inserted and withdrawn along the same track to minimize placental trauma.

Four cases required several attempts before amniotic fluid was obtained, and in a further 2 cases no fluid was obtained despite multiple attempts. We ascribe this difficulty to the large oedematous placentae which usually accompany severe erythroblastosis. The one foetal death mentioned above occurred after multiple attempts to obtain amniotic fluid. We have come to the conclusion that if amniotic fluid is not obtained at the first or at most, the second attempt, the procedure should be abandoned. In such cases the desirability of making a further

attempt at a later date should be assessed in collaboration with the serologist. Clearly, if the indication for performing the amniocentesis in the first instance was borderline, no further attempts should be made and the pregnancy should be terminated by premature induction of labour at 36 - 37 weeks. On the other hand, if the patient has a very high antibody titre, with a history of one or more early stillbirths, further attempts are justified.

The danger to the mother is very small, being confined to the theoretical risk of sepsis, and to increased immunization due to foeto-maternal haemorrhage resulting from placental damage. One such case was encountered in our series.

SUMMARY

The handling and treatment of haemolytic disease of the newborn is briefly reviewed. The value of amniotic fluid analysis during pregnancy as a means of predicting the severity of the foetal red cell destruction is emphasized, and a rational approach to the handling of such cases is outlined.

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