Analysis of Amniotic Fluid in the Management of the Rhesus Sensitised Pregnancy

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The pregnant rhesus negative woman, when sensitised by a rhesus positive foetus, carries in her blood antirhesus antibodies which are

readily detectable. A significant rise in titre of these antibodies during pregnancy will provide evidence that the baby is affected by haemolytic disease. Foreknowledge of this eventuality will alert one to the possible need for treatment of the baby, and in severe cases termination of the pregnancy before term could prevent stillbirth or lessen the severity of the haemolytic process. This evidence is by nature indirect, however, and in weighing the risks of prematurity against those of anaemia the need for a more direct assessment of the baby's condition is often acutely felt. A primipara sensitised in childhood by a rhesus positive blood transfusion or a multipara in whom serological studies in past or present pregnancies are lacking may be cited as examples posing difficulty, but the problem of whether to induce in a woman who has very high antibody titres and an obviously erythroblastotic infant before 35 weeks is perhaps the most troublesome decision to make.

Examination of amniotic fluid for its content of bilirubin provides such a direct method of assessment. This would seem surprising in view of the lack of correlation between bilirubin concentration of cord blood at birth and severity of the anaemia, yet Walker and Jennison (1962) report an accuracy of 91 per cent. in their predictions by this technique.

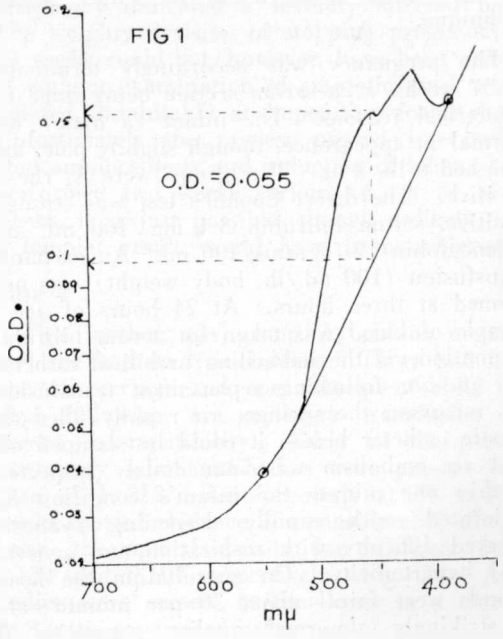
We record here our preliminary experiences with the analysis of amniotic fluid for bilirubin content in rhesus-sensitised pregnancies and illustrate the sort of information which can be obtained.

PROCEDURE

Amniotic fluid was obtained initially from rhesus-sensitised and normal women at the time of surgical rupture of membranes until the technique had been standardised, after which time samples were taken between the thirty-first and thirty-fifth week of gestation by transabdominal amniocentesis. The tap was performed by the attending obstetrician in each case and no difficulty or complications were experienced. The technique is well illustrated by Walker and Jennison (1962). Amniotic fluid was subjected to spectrophotometric examination as outlined below. Antibodies in the fluid were also sought and correlated with titres in maternal serum. This aspect is currently being studied further by one of us (G.W.D.H.).

THE MEASUREMENT OF BILIRUBIN IN AMNIOTIC FLUID

Because foetal destruction of haemoglobin is not the only factor which influences concentration of haem pigments in liquor amnii, it seems unlikely that pigment analysis will always reflect accurately the severity of foetal haemolytic disease. Nevertheless, estimation of bilirubin in liquor obtained by amniocentesis before the thirty-fifth week of pregnancy has proved most useful in practice. Bevis (1953) first observed that the degree of bile pigmentation of the amniotic fluid of foetuses affected by haemolytic disease had prognostic significance. Walker (1957) confirmed this and found that spectrophotometric curves of fresh specimens correctly predicted erythroblastosis foetalis in 95 per cent. of his cases. Naked-eye assessment of colour or use of the icteric index are dangerous over-simplifications, for haemoglobin in dilution imparts a jaundiced tinge which has no prognostic value. Direct spectrophotometric measurement of bilirubin, though applicable to serum, is unsuitable for liquor because of interference by haem pigments and opalescence due to vernix caseosa. For empirical purposes, however, an assessment of the bilirubin is possible from the spectral absorption curve of liquor, since bilirubin produces a peak or deviation at 450 mμ and since in unaffected babies absorption is approximately linear from 365-550mμ. Fig. 1 from Case 2 illustrates the type of curve obtained when the infant is affected. The optical density (O.D.) of bilirubin in liquor in normal and 101 rhesus-sensitised cases has been investigated by Liley



(1959), whose paper gives detailed instructions on the analysis of liquor and how to use the results obtained. If the O.D. at 450 mμ is plotted against duration of pregnancy in weeks, the point falls into one of three zones on his nomogram. From position of the plot and the slope of a line from this to any subsequent plot, a practical guide to the management of a case has been drawn up which it would be tedious to recapitulate here. We have found predictions based on Liley's nomogram reliable. The optical densities of liquor bilirubin in the illustrative cases considered here have been plotted out on a similar chart in Fig. 2.

ILLUSTRATIVE CASES

Case 1

The mother was blood group A rhesus negative and had inadvertently received a group A rhesus positive blood transfusion some years previously. The father was group AB rhesus positive. Her first pregnancy resulted in an infant severely affected by rhesus incompatibility, and three exchange transfusions were required. Amniocentesis was performed at 32 weeks and again at 34 weeks' gestation, the optical densities of bilirubin in liquor being respectively 0.075 and 0.057. Plotting these figures on the nomogram (Fig. 2), the curve fell in the mid-zone group, indicating a moderately affected baby, the indications being induction of labour at about 37 weeks. Weak anti-D antibodies were present in the liquor when tested by Löw's papain technique.

The pregnancy was accordingly terminated at 37 weeks, a caesarian section being done for obstetrical reasons. The infant (a male) was normal in appearance, though slightly pale, and weighed 6 lb. 3 oz. The groups were A_2 rhesus positive. The direct Coomb's test was strongly positive, serum bilirubin 3.4 mg./100 ml. and haemoglobin 12.7 grams/100 ml. An exchange transfusion (100 ml./lb. body weight) was performed at three hours. At 24 hours of age a sample of blood was taken for serum bilirubin estimation via the indwelling umbilical catheter, left *in situ* following replacement transfusion. On removing the syringe, air rapidly filled the plastic catheter before it could be clamped off, and air embolism was immediately suspected. Within one minute the infant's condition deteriorated, with rapidly deepening cyanosis, marked difficulty with respiration and generalised hypertonicity. On auscultation the heart sounds were faint—about 30 per minute—and a strikingly abnormal quality suggestive of pericardial friction was heard. The infant was turned slightly on to the left side and oxygen given via a funnel. At the same time, external cardiac massage was instituted. Within five minutes the child's condition improved and good colour rapidly returned. He remained limp and lethargic for some hours, but by the end of the day was quite normal and none the worse for this unusual and chastening experience. Subsequent progress was uneventful.

Comment.—Spectrophotometric analysis of liquor amnii on two occasions accurately predicted the severity of the disease in this case, and by terminating the pregnancy at 37 weeks the course was almost certainly influenced favourably, only one exchange transfusion being required.

The possibility of encountering a negative pressure in the umbilical vein and hence the risk of air embolism is not often stressed, but would appear to constitute a definite hazard to be guarded against.

Case 2

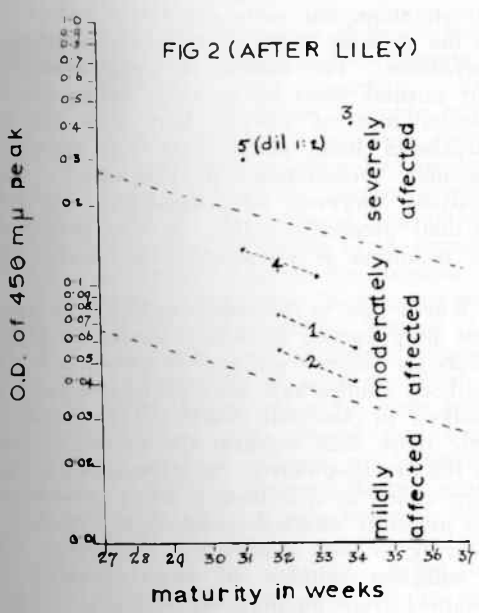
The mother was group A_1B rhesus negative and the father rhesus positive (genotype not known). There was one previous unaffected delivery. In the present pregnancy no antibodies were demonstrated at 22 weeks. At 26 weeks there were no complete agglutinins, but indirect Coomb's antibodies were present 1:2. Amniotic fluid analyses at 32 and 34 weeks gave optical densities for bilirubin of 0.055 and 0.041 respectively (Fig. 2). Weak antibodies were present in the fluid by Löw's papain technique.

These findings were suggestive of mild but definite haemolytic disease. Labour was induced at 38 weeks, at which time her antibodies were saline 1:4, Coomb's 1:2, trypsinised cells 1:4 and papainised cells 1:32. The infant was born normally weighing 7 lb. Cord blood showed direct Coomb's test positive +++, haemoglobin 18.5 grams/100 ml., serum bilirubin 4.0 mgm./100 ml. The blood group was B rhesus positive. An exchange transfusion was performed at three days (110 ml. per pound body weight) when the serum bilirubin had risen to 18.0 mgm./100 ml. Subsequent progress was uneventful.

Comment.—Here antibody results did not suggest an affected baby, yet analysis of the amniotic fluid accurately predicted the degree of haemolysis.

Case 3

This mother, group O rhesus negative, had had one miscarriage and then a normal delivery, both while overseas. During the latter pregnancy she is said to have had no antibodies. In the present gestation antibodies were first looked for at 33 weeks and found to be present in high titre (1:64 with papainised cells, 1:16 after Coomb's testing). Amniocentesis yielded deep yellow fluid, the optical density of the bilirubin peak reading 0.395 at 33 to 34 weeks' gestation. As will be seen from Fig. 2, these findings suggested impending foetal death. The liquor contained anti-D antibodies to a titre of 1:8 with papainised cells. Membranes were ruptured shortly afterwards (about 34 weeks' gestation), but as labour did not ensue a lower segment caesarian section was performed. The infant was hydropic, markedly pale and jaundiced, intubation being required to initiate breathing. Cord haemoglobin was only 2.7 grams/100 ml., serum bilirubin 4.2 mg./100 ml. and direct Coomb's test positive +. After allowing 30 ml. of blood to escape under high pressure from the umbilical cord, her condition improved somewhat so that a slow exchange transfusion was started using packed cells, 110 ml. being introduced and 160 ml. removed. After this procedure the infant's condition was a little better, but there was considerable indrawing of the chest and moist sounds were heard throughout both lung fields. A further exchange transfusion was performed 3½ hours after delivery, using fresh citrated whole blood, 450 ml. being removed and 380 ml. replaced. On completion there was a severe apnoeic attack, but the infant was resuscitated by manual inflation of the lungs and cardiac massage. By this exchange the bilirubin was reduced from 6 mg./100 ml. to 3.2 mg./100 ml. and the haemoglobin raised from 9.8 grams/100 ml. to 14.8 grams/100 ml. On the following day there was still very considerable respiratory distress, and in view of marked lower rib retraction, stay sutures were inserted on either side and attached to weights to help overcome the extreme negative intrathoracic pressure. At the same time a slow intravenous infusion of hypertonic glucose, bicarbonate and insulin was given in an attempt to combat acidosis and lower serum potassium. A further exchange transfusion was given 24 hours after delivery as the serum bilirubin had risen to 17.9 mg./100 ml. The situation remained about the same during the following evening, but on the next day there were increasingly severe apnoeic attacks and, despite other measures, the infant died that evening. Autopsy revealed the changes associated with hydrops foetalis. The heart was flabby and dilated and the lungs grossly oedematous. There was no evidence of hyaline membrane disease.



Comment.—Despite two apparently unsensitised gestations, antibody estimation in this pregnancy indicated a severely affected baby. The weighty decision of stopping pregnancy at 33 weeks could not, however, have been arrived at without examination of amniotic fluid, which pointed indubitably at impending foetal death. Unfortunately the process proved irreversible. Had amniocentesis and induction of labour been performed two weeks earlier, i.e., at 31 to 32 weeks, it is just possible, though unlikely, that a happier result would have been achieved.

Case 4

This mother was group A_2 rhesus negative and she had no live children. There had been one miscarriage and one subsequent child had died following an exchange transfusion for rhesus incompatibility. Early in the present pregnancy saline antibodies were present in a titre of 1:512. Amniocentesis at 31 and 33 weeks showed evidence of a considerably affected baby, the optical density for bilirubin being 0.134 and 0.107 respectively (Fig. 2). Antibodies were present in the liquor to a titre of 1:8 with papainised cells.

Surgical induction was performed at 35 weeks. The infant was well formed and weighed 6 lb. 11 oz. There was slight pallor, but no obvious jaundice. There was no enlargement of liver or spleen and no other abnormalities. Cord blood showed haemoglobin 11.1 grams/100 ml., direct Coomb's test +++ and serum bilirubin 5.0 mg./100 ml. The blood group was A rhesus positive. Clinical jaundice rapidly became apparent and an exchange transfusion was undertaken soon after birth (90 ml. per pound body weight). This lowered the serum bilirubin from 8.6 mg./100 ml. to 3.0 mg./100 ml. On the following day the serum bilirubin had risen to 16.2 mg./100 ml., so that another exchange transfusion was performed. On this occasion 150 ml. per pound were administered. The infant's condition was generally satisfactory, although there was slight cyanosis towards the end. A small top-up transfusion was necessary at six weeks, but progress was otherwise uneventful.

Comment.—Owing to the high antibody titre both before and during this pregnancy, examination of amniotic fluid proved of great value in confirming that the baby was affected and in categorising the degree of involvement. Induction at 35 weeks proved the optimal time to terminate the pregnancy and a good result was obtained.

Case 5

The mother had had a miscarriage followed by a child who required an exchange transfusion for mild rhesus incompatibility. Her blood groups were AB rhesus negative. At 31 weeks antibodies were found in the following titres: 1:32 saline, 1:64 with trypsinised cells, 1:128 with indirect Coomb's test, 1:512 with papainised cells. Amniocentesis at 31 weeks produced dark yellow fluid with an optical density for bilirubin of 0.295 after diluting 1:2 with distilled water (Fig. 2). A 1 in 16 titre of anti-D antibodies was obtained with papainised cells.

These findings indicated a very severely affected baby, and a caesarian section was performed immediately. On delivery there was a great deal of dark yellow amniotic fluid and the infant was born limp, markedly oedematous and pale. The abdomen was greatly distended by ascites and a huge liver. There was slight jaundice. The cord was cut and about 80 ml. of dilute blood was allowed to escape under great pressure from the arteries and veins. The infant was immediately intubated and the lungs manually inflated. There were then weak attempts at respiration. The umbilical vein was canalised and a further quantity of about 30 ml. of blood removed. A slow exchange transfusion, using partially packed fresh group O rhesus negative cells, was commenced. The infant was given continuous oxygen and manual inflation of the lungs was frequently required. After 100 ml. his condition improved considerably and breathing became well established. Digoxin and albumin 25 per cent. (3 ml.) were administered intravenously; 350 ml. of blood were introduced and 390 ml. removed with the hope that by so raising the haemoglobin the child might sufficiently improve to withstand a full exchange transfusion later. From this point on, however, there was gradual deterioration and he died nine hours after delivery with the picture of an intrapulmonary haemorrhage.

Comment.—Both antibody and amniotic fluid results indicated an extreme degree of sensitisation, and salvage of the infant was obviously impossible by present methods of treatment.

Discussion

Walker and Jennison, apart from the other workers cited here, have recently presented a well-argued case for the use of amniotic fluid study in rhesus-sensitised pregnancies. Although our small number of cases allows of no firm conclusions for or against this technique of investigation, our early experience adds weight to the growing mass of evidence indicating its usefulness. The results of liquor analysis in our normal cases or in cases falling into the bottom zone of Liley's chart have not been mentioned here, since there was no call to consider termination of pregnancy. These analyses, however, have reinforced our belief in their predictive value. It is surprising that the technique is not already more widely used.

Where one is provided with full details of past pregnancies, including serological studies, where the father's genotype is known and where antibody studies have been performed regularly, analysis of amniotic fluid will frequently do little more than confirm the prediction based on the overall picture. Such confirmation, however, culled as it is from a direct assessment of the presently affected infant, is nevertheless reassuring to both mother and obstetrician. Yet a sufficient number of surprise results are obtained from amniotic fluid analysis to make

the procedure worthwhile in all cases (Liley, 1961).

From our experience of local conditions the technique would appear to be of especial value in view of the frequency of partially or completely uninvestigated pregnancies. The problem is especially acute in such cases as our examples 3 and 5, where antibodies were sought for the first time early in the third trimester and found in high titre. The difficult decision to terminate pregnancy before 35 weeks in these cases could only have been taken with the confidence engendered by amniotic fluid examination.

Another situation especially applicable to local conditions is the rhesus negative pregnant woman living in a small centre. The decision as to whether she should be transferred for confinement to a town where facilities for special obstetric care and exchange transfusion are available may be made by the relatively simple and safe procedure of amniocentesis, the fluid being posted to the nearest laboratory for study. Such specimens do not deteriorate if sterile and protected from light (Liley, 1961). The initial tap should be performed at 31 weeks and another one two weeks later.

The strictures by Moncrieff (1959) regarding the safety of amniocentesis do not now seem valid in the light of the large number of taps performed without complication to mother or foetus (Bevis, 1953, 1956; Liley, 1961; Walker and Jennison, 1962).

Spectrophotometric curves of liquor have the disadvantage, for standard instruments, that they are rather time-consuming. Routine chemical methods for serum bilirubin fail principally because of the low bilirubin concentration in liquor and contamination by haemoglobin. The sensitive method for serum devised by Brucker (1959) has proved unsuitable for amniotic fluid because fading of the azopigment occurs, apparently due to the harmful action of antipyrine or bilirubin in aqueous solution containing diazonium salts. Watson (1962) has recently described a simple colorimetric method for bilirubin in amniotic fluid which might well come to replace the more laborious absorption curve. He found that exchange transfusion was not subsequently necessary for the infant when the liquor bilirubin concentration was under 0.8 μg./ml., but with bilirubin levels of 1.2 μg./ml. or more this form of treatment was always required. When the bilirubin concentration was over 2.0 μg. survival was not to be expected and a figure over 3.0 was probably lethal.

Reports on the detection of rhesus antibodies in amniotic fluid are scanty. Wild (1960) reported finding such antibodies, but not consistently unless the maternal antibody exceeded a titre of 1 in 28.

Using Löw's technique (Löw, 1955), antibody was found on each occasion in titres from 1 in 1 to 1 in 16. On no occasion were these antibodies detected by the anti-globulin technique. The strength of the antibody titre was proportional to that in the maternal serum, but this and the further decision as to whether the titre of antibodies in liquor amnii has prognostic significance must await the results of work still in progress.

CONCLUSION

Our early experience with the technique of amniocentesis and amniotic fluid study in rhesus-sensitised pregnancies indicates the safety and usefulness of the procedure.

Its routine use would offer special advantages in local conditions.

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