MEAN LIQUOR BILIRUBIN VALUES IN RELATION TO SEVERITY OF HDN

![Graph showing mean liquor bilirubin values in relation to severity of HDN](image)

**FIG. 4.**—Mean liquor bilirubin values in relation to severity of haemolytic disease of the newborn.

for all grades of severity and even with normal pregnancy. Thereafter, however, the lines diverge and for normal or only mildly affected cases the bilirubin value progressively falls, while for stillbirth and very severe disease it progressively rises. Moderately affected infants fall in the intermediate zone.

At all stages of gestation one liquor bilirubin value unless extreme is misleading, but two consecutive values if falling give reassurance while high and rising suggest a bad prognosis. This strikes us as a relatively imprecise conclusion, but because one takes action on the basis of bilirubin level—and thereby possibly modifies the severity of the disease—more precise evaluation is not possible at present.

R. C. Whitfield and his colleagues have devised a novel method of evaluating changes in bilirubin level, joining two consecutive bilirubin values and projecting the line to strike their "action line" (Fig. 4). This scheme still needs careful evaluation but I doubt whether it is as accurate as has been suggested, for repeated bilirubin estimations in a patient would be expected to fall in a straight line, and in our experience this is not so. None the less, when its limitations have been defined, a scheme of this or similar nature may prove to be valuable.

**Conclusions**

Liquor examination for bilirubin is of considerable value in the management of pregnancy complicated by Rh isoimmunization. With appropriate precautions amniocentesis can be carried out safely and liquor evaluation can be standardized within a unit. The main sources of error are incorrect assessment of gestation, and contamination of the liquor with plasma or haemoglobin, but the latter are largely avoidable. The liquor bilirubin level is correlated with both cord haemoglobin (P<0.001) and the cord bilirubin (P<0.001) but there is such a large scatter at individual liquor bilirubin levels that the application of this technique in management is essentially empirical.

In some instances the bilirubin value gives a wrong forecast, usually by underestimating severity, and this is particularly true if only single values are considered. Forecasts are improved by considering the results of consecutive specimens, and we would regard this as essential before carrying out intrauterine transfusion or premature induction—both of which are dangerous procedures. We prefer a technique of estimating bilirubin that is not widely adopted but, using it based on a 0.5 cm. light path, we require that two consecutive liquor bilirubin ratios exceed 1:1 (O.D. 490-520 nm=\(>0.0415\)) and show a rise before we carry out intrauterine transfusion or will often induce labour at about 35 weeks if the ratio is more than 1:06 (O.D. 490-520 nm=\(>0.0255\)).

Unfortunately it is not possible to make any true evaluation of the merit or otherwise of such a policy, as this has not been tested by random trial. Nevertheless, when the father is heterozygous and there is a previous history of stillbirth, one can identify normal Rh negative pregnancies with 95% accuracy. Only a few years ago this would have been regarded as a considerable achievement.

**Intrauterine Transfusion**

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In 1963 A. W. Liley first suggested that intrauterine transfusion for fetuses with severe haemolytic disease would prolong life to a stage of gestation when conventional methods of treatment could be expected to give good results. The implication was that patients should be selected for this form of treatment only if intrauterine death before 35 weeks’ gestation was expected. The final selection of patients in both his and all subsequent series was based on the level of bilirubin in the amniotic fluid. Nevertheless, the features of all the different series have varied so much that it is impossible to make a valid comparison among them or, indeed, to make a reasoned evaluation of intrauterine transfusion.

**Technique**

In the original technique radio-opaque medium was injected into the liquor amnii at the time of diagnostic amniocentesis. This was ingested by the fetus, thereby outlining the fetal abdomen. The following day the patient was sedated and transferred to the radiology department, where under local anaesthesia a Touhy needle was inserted into the fetal abdomen. An epidural catheter was threaded through this into the peritoneal cavity and the needle withdrawn. Packed red cells were then injected to be absorbed via the lymphatics and to enter the circulation through the thoracic duct. Although placental localization is always desirable before diagnostic amniocentesis, it is essential before intrauterine transfusion. If it is to be performed early in pregnancy, transfemoral placental arteriography is usually indicated, but the ultrasonic method may be satisfactory.

Many techniques to facilitate correct siting of the catheter in the fetal abdomen have been advocated. To cut down radiation risks the use of an image intensifier is recommended, while the availability of videotape playback reduces the need for repeated x-ray examination. Our obstetric colleagues have preferred to omit injection of radio-opaque material into the liquor before transfusion as it can obscure the diagnostic pattern obtained when dye is subsequently injected into the peritoneal cavity to outline the fetal diaphragm and bowel. They prefer to take an anteroposterior and lateral x-
ray plate of the maternal abdomen using a wire mesh grid with an umbilical marker in situ, so that clinical examination can be related to the radiograph and the "target area" more easily identified.

Correct situating of the catheter is never simple, and minor modifications have been described such as the semi-stereotaxic technique, or passing an arteriography wire into the peritoneal cavity in preference to the radio-opaque dye. Before 26 weeks' gestation the technique may be extremely difficult, and in these cases an "impaling" technique or the introduction of the catheter under direct vision has been recommended.85-87

The injection of oily contrast media to outline the fetus has also been suggested, and some workers prefer to carry out transfusion with a spinal needle.86 Nevertheless, this facilitates the leakage of liquor into the space between the uterine wall and the amnion and may precipitate premature labour—a complication also associated with the use of self-retaining catheters.85 86

Blood
Most workers have been content to use group 0 Rh negative blood for transfusion, and to get maximal benefit and avoid the dangers of an excessive potassium level in the plasma the use of blood collected within 48 hours of transfusion is recommended. Concentrated cells with a packed cell volume of 80 to 90%, are usually employed. There is a risk that viable lymphocytes injected into the fetus during intrauterine life may survive and colonize the lymphoid tissue, with possible immunological implications.85 86 In 1965 the possibility of runt disease following intrauterine transfusion was postulated,86 89 and a suspected case described.88 The infant was a girl who had received multiple intrauterine transfusions with male donor blood and in whom 2% of the chromosomes were XY. Runt disease could be produced in piglets following intrauterine injection of viable lymphocytes intraperitoneally,89 but, in man, despite the theoretical possibility, only one other probable case has been described,89 associated with Yq chromosomal chimaerism.

Nevertheless, abnormalities in the immunoglobulin pattern of infants following intrauterine transfusion have been described,88 and we try and minimize the number of viable lymphocytes injected by using blood collected in acid-citrate dextrose rather than in heparin and by aspirating red cells from the bottom of the bottle. Even so, transfusion of some viable lymphocytes is unavoidable.

Volume and Frequency of Transfusion
This should be varied with fetal age, and we start by injecting 30 ml of packed cells before 25 weeks' gestation, 60 ml at 26 to 28 weeks, 80 ml at 28 to 30 weeks, and 100 ml after this time. More elaborate schemes have been described,88 and Liley87 calculated that the cord haemoglobin value was raised 1g./100 ml for every 4 to 7 g of haemoglobin injected intraperitoneally every 2 weeks. Before transfusion and his colleagues87 claimed to be able to predict the donor haemoglobin concentration in the fetus to within ±1.5 g./100 ml, but similar calculations for our material gave widely discrepant results. We attribute this to errors in estimating fetal size rather than in calculating the amount of blood absorbed and similar difficulties have been reported.88 The liquor bilirubin value is said to have little value in indicating the fetal condition following intrauterine transfusion, although the bilirubin/protein ratio is claimed to be reliable.88 Nevertheless, we do not agree that liquor bilirubin values never give useful information. In 72 of our cases with satisfactory estimates of liquor bilirubin this showed an overall rise in 47, and 26 resulted in stillbirth; of the 21 born alive, five died in the neonatal period. Of the 25 that did not show a rise, 19 resulted in livebirth, and only one of these infants died.

Further study may confirm that hormone assays87 or radiological or ultrasonic100 investigation will prove to be of value in monitoring progress. Most workers tend to repeat intrauterine transfusion within 10 to 14 days of the initial transfusion if this is carried out before 26 weeks and about every two to three weeks thereafter until delivery.

Dangers
Trauma to Fetus
Not unexpectedly, the use of a blind procedure often causes direct injury to the fetus. Reviewing 1,074 intrauterine transfusions, Queenan86 found an incidence of 10.5%, and at the Ross Conference86 the minimum risk was estimated at 6-5%, but many workers thought 15 to 20% a more realistic figure. Surprisingly the fetus often seems to survive such insults without permanent damage, though one infant in our series had an absent femoral pulse in one leg associated with a scar over the femoral triangle, but ultimately made a full recovery. Another has segmental paralysis and wasting of the abdominal wall, and a third evidence of peripheral nerve injury in the leg. Nevertheless, we have found that trauma may be so severe that intrauterine death follows, and this has also been found by others.88 104

Intrauterine death occurred within 48 hours of intrauterine transfusion in 25% of cases, and in many there was no evidence of serious trauma either at the time of operation or at necropsy. In some of the earlier cases possibly too large a volume of blood was injected too quickly, but in others the nature of injury was more subtle. We now prefer to inject blood at a very slow rate using a mechanical pump and take about one hour for the infusion of every 20 ml. We have also found it useful to monitor the fetal heart during transfusion by incorporating an electrode in the epidural catheter used for transfusion, this enables satisfactory E.C.G tracings to be obtained together with a simultaneous trace of the fetal heart rate.

Fig. 1 shows the development of bradycardia after the injection of as little as 20 ml of blood over a period of 45 minutes; spontaneous recovery occurred within about 10 minutes, though four similar episodes occurred during the transfusion.

Fig. 2 shows the heart rate of a fetus of 31 weeks' gestation who, after the injection of 15 ml of blood over a period of 45 minutes, developed appreciable bradycardia with prolonged QRS complexes and extrastoles.

This picture was reversed following injection of 0.2 mg of atropine, and the transfusion of 80 ml was completed without further change in heart rate or E.C.G. pattern. The infant survived. The prompt response to atropine and the fact that
Intrauterine Transfusion—Walker and Ellis

Results

In the different series the most noteworthy feature is the remarkable similarity (summarized in Table I) to the number of intrauterine transfusions per patient, the occurrence of stillbirth, and the prospect of ultimate survival. With the exception of the Canadian series, 35 to 40% survival has usually been achieved. Such results are disappointing and do not accord with the sweeping claims for improved survival attributed to the introduction of premature induction and later to intrauterine transfusion. Certainly we cannot show any impressive fall in perinatal mortality over the years, despite systematic liquor examination and intrauterine transfusion or premature induction, or both, in selected cases.

<table>
<thead>
<tr>
<th>Table I.—Results of Intrauterine Transfusion in Various Series</th>
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<tbody>
<tr>
<td>Centre</td>
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<td>U.S.A. Co-operative Study</td>
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<td>Hammersmith</td>
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On their face value the results may be misleading. Selection for and the timing of intrauterine transfusion are closely related to the ultimate results, and undoubtedly the best results would be achieved if cases were selected who did not need intrauterine transfusion and if this was carried out only a day or so before the patient was due to go into spontaneous labour. The disappointing results from early intrauterine transfusion have prompted Queenan to question whether this should ever be attempted before 25 weeks’ gestation.

Nevertheless, we believe that the poor results of early intrauterine transfusion are due not so much to technical difficulties but to the selection of the worst cases for early treatment. Thus we can relate the time of intrauterine transfusion directly to the previous obstetric history, and in Table II we have related the results of intrauterine transfusion to the previous obstetric history.

<p>| Table II.—Results of Intrauterine Transfusion in Relation to Previous History |
|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Previous History of H.D.N.</th>
<th>Total No. of Cases</th>
<th>Stillbirths (% of total cases)</th>
<th>Neonatal Deaths (% of livebirths)</th>
<th>Alive (% of total cases)</th>
</tr>
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<tbody>
<tr>
<td>&gt; 1 Stillbirth</td>
<td>11</td>
<td>73</td>
<td>Nil</td>
<td>27</td>
</tr>
<tr>
<td>1 Stillbirth</td>
<td>40</td>
<td>54</td>
<td>Nil</td>
<td>37</td>
</tr>
<tr>
<td>Livebirth with H.D.N.</td>
<td>34</td>
<td>44</td>
<td>41</td>
<td>35</td>
</tr>
<tr>
<td>None Previously Affected</td>
<td>13</td>
<td>38</td>
<td>12</td>
<td>54</td>
</tr>
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</table>

All Cases | 104 | 50 | 26 | 38 |

The survival rate overall was 40% but was only 30% if more than one stillbirth from haemolytic disease had occurred, compared with nearly 60% in first affected pregnancies. Even so, selection based on liquor bilirubin values had been made within the historical grouping, and therefore direct comparison with patients having a similar history but not treated by intrauterine transfusion is not possible.

Intrauterine transfusion was originally intended to benefit patients where early intrauterine death was expected. Hence it is disappointing to find that many cases selected for early
intrauterine transfusion already have hydrops at the time of the initial transfusion. Successful treatment of such fetuses has been reported, but the results generally are disappointing, and some regard the presence of fetal ascites as a contraindication to intrauterine transfusion. Nevertheless, if ascites is encountered probably nothing is lost by removing as much as possible and injecting donor blood. If after two weeks the fetus was still alive, we would certainly feel justified in repeating transfusion. Of our cases 30% had ascites removed on one or more occasions, and this was so for 58% of the stillbirths but for only 18% of the survivors. In one infant 110 ml of ascitic fluid was removed; when born two weeks later the infant had generalized oedema but no reaccumulation of ascites and no donor cells were present in the circulation. Thus, in the presence of ascites transfused cells are apparently less readily absorbed from the peritoneal cavity; absorption can occur, however, and has been shown using isotopically labelled red cells in man, and in dogs.

The absorption of erythrocytes following intrauterine transfusion is very variable and in our experience they have accounted for from 0 to 100% (average 68%) of the infant's blood at the time of delivery. Erythrocytes given intraperitoneally have been detected in the circulation within as little as two hours and they probably retain a normal oxygen dissociation curve in their new environment. Adhesions are sometimes detected—particularly between the liver and diaphragm in fetuses where donor blood had not been absorbed—and some workers have suggested that fibrinolytic agents injected into the peritoneal cavity may be valuable. Most reported series include one or two Rh-negative fetuses wrongly transfused, so that possibly normal fetuses may have been killed by intrauterine transfusion.

Results in Liveborn Infants

Even if the baby is born alive, severe haemolytic disease together with short gestation present serious problems. In our cases the mean cord haemoglobin value was 10 g./100 ml. compared with 10-6 g./100 ml. for 330 cases treated by premature induction, and 14.4 g./100 ml. for 2,626 cases of haemolytic disease allowed to go to spontaneous delivery. The cord bilirubin values were respectively 5-0, 4-6, and 3-2 mg./100 ml.

_Treatment of the infant_ was carried out in accordance with established practice, and all but two infants in our series met our cord blood criteria for early exchange transfusion. Though Wade and Ogden have reported a decreased need for repeated exchange transfusion in infants treated by intrauterine transfusion, this does not seem to be true for most published series, and for ours half the survivors have required repeated exchange transfusions, averaging 2-1 per survivor.

We have also noted an increased need for simple transfusion, and in one infant who had received five intrauterine transfusions and two exchange transfusions simple transfusion was also required on three occasions—the latest at 16 weeks of life. This phenomenon is unexplained, but we have noted very low reticulocyte counts in infants where nearly all the blood present at birth has been from the donor, even though in some the cord haemoglobin value was low. If all the circulating red cells are donor in origin, one would not expect reticulocytes to be present. Nevertheless, the surprising fact is that one can ever achieve a concentration of 100% donor cells in the presence of severe anaemia and that in some of these patients the finding appears little evidence of active erythropoiesis, though we have not made systematic bone marrow examinations.

"Obstructive Jaundice" is more common in infants treated by intrauterine transfusion and occurred in 30% of our cases, whereas the overall incidence in haemolytic disease is about 8%.

Hypoglycaemia is also common in severe haemolytic disease, but Lilley thinks that this is particularly associated with infants who have survived intrauterine transfusion, and estimates frequency at 80%.

Despite intrauterine transfusion some infants are hydropic at birth and their management presents serious difficulties; despite immediate exchange transfusion, positive pressure respiration, and correction of acidosis the results are disappointing. Though peritoneal dialysis may remove fluid the ultimate prognosis is not improved. This confirms Gordon's experience using intrauterine peritoneal dialysis.

_The neonatal death rate_ has been high in all series, and for our cases was seven times that of infants with haemolytic disease born following spontaneous delivery; this was not different from that in infants following premature induction. The main causes of neonatal death have been hydrops fetalis, pulmonary haemorrhage, kernicterus, and respiratory failure.

_Adequate follow-up studies_ are not yet possible because the oldest survivor is only a little over 6 years of age, but unless definite trauma has occurred during intrauterine transfusion the ultimate result does not seem to differ from that in other infants with similarly severe haemolytic disease. A high incidence of hernias has been recorded, and in our series 11 out of 39 surviving children had this complication, but most recovered without surgical treatment. Two infants also had signs of partial intestinal obstruction during the neonatal period and both resolved without surgery. This is also a recognized complication of intrauterine transfusion, particularly, but not necessarily, associated with the injection of blood or dye into the alimentary tract. Despite the frequent occurrences of direct injury, permanent sequelae are rarely seen but perhaps some infants do not survive to show it.

Intrauterine Exchange Transfusion

As exchange transfusion in liveborn infants with haemolytic disease is vastly superior to simple transfusion, not surprisingly this technique has been considered as an intrauterine procedure. This was first carried out in 1964. Various methods have been used, from that of Freda and Adamson, where a fetal leg was delivered within a diverticulum of amniotic sac and exchange transfusion carried out via the femoral vein, to that described by Asensio, in which the whole fetus was delivered by caesarean section and transfusion carried out through the internal jugular vein, after which the fetus was replaced in the uterus and premature labour prevented by the intravenous infusion of absolute alcohol in 5% glucose. The technique described by Seelen and his colleagues is much more elegant, a small incision being made in the uterus alongside the placental margin and a large placental vessel identified and used for exchange transfusion.

It seems to be generally agreed that the umbilical cord vein is not suitable for this technique. The technical procedure is evidently not so difficult as might be thought, but serious maternal infection has occurred, while fetal death and the onset of premature labour are common. Thus successes for this technique have usually been in infants delivered shortly after operation and where gestation was well advanced before the operation was attempted. One survivor required amputation of the leg.

We cannot improve on Asensio's conclusion "One year after the procedure and under the light of new experience obtained, we realized the need for immediate surgical intervention to save this fetus was more apparent than real. We were wrong, and most probably will now approach the problem with more caution."

Place of Intrauterine Transfusion

The concept of the technique of intraperitoneal transfusion of the fetus was so brilliant that it was promptly adopted in the
management of Rh immunization. This probably reflects the disappointing results achieved with other forms of treatment in the prevention of stillbirth. Its advent at the time when liquor examination was becoming established in the prediction of the severity of haemolytic disease meant that the liquor bilirubin value was adopted as the criterion for treatment. This was done before the accuracy of this estimation had been assessed—indeed, before the pattern of bilirubin in normal pregnancy had been properly studied. Thus the criteria for intrauterine transfusion were decided too hastily, and in applying many different techniques for estimating liquor bilirubin and in applying it to the management of Rh isoimmunization, which have been discussed in the previous article, testify to the problems involved.

Hence not surprisingly six years later, despite hundreds of communications on the subject, we still do not know the proper place of intrauterine transfusion in the management of Rh isoimmunization. If, as some authors suggest, it should not be carried out until, and with, Walker, W., of cases treated before 25 weeks' gestation, then it would appear to have lost the function for which it was originally devised. We would not accept this conclusion, because we do not believe that the evidence exists on which to make any valid judgement, but some help might be obtained by a retrospective study of cases treated in this way. We regret not having learnt from experience with exchange transfusion in liveborn infants the need for properly controlled trials in the evaluation of new techniques, especially those that are potentially dangerous.

We suggest that intrauterine transfusion may eventually have its chief role in preventing "latter intrauterine death" and doing away with the need for premature induction before 36 weeks' gestation. The combination of severe disease with short gestation presents serious problems in management and carries a high neonatal death rate and presumably an increased morbidity rate. Our evidence suggests that such risks diminish considerably beyond a gestational age of 36 to 37 weeks, but it may even be possible to avoid premature induction altogether in most cases of Rh isoimmunization.

It cannot be overstressed that selection of cases for intrauterine transfusion at any stage in pregnancy must be extremely stringent—far more so than it has been up to the present time. Moreover, any new schemes must be tested by properly controlled trials, if necessary on a collaborative basis, and if this is possible anywhere it must be possible in Britain.

The patients treated in the Newcastle series were under the obstetric care of A. Coxon, D. V. I. Fairweather, D. Tacchi, D. Millar, and E. Robertson.

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15. Walker, W., unpublished observation.
ANY QUESTIONS?

Coeliac Disease

Q.—Is there any evidence that the increased incidence of coeliac disease is related to earlier weaning and feeding with cereals?

A.—I know of no definite evidence either that the incidence of coeliac disease has increased (though it is probably recognized more often) or that its occurrence is related to the age of weaning.

The symptoms of coeliac disease are, of course, directly related to the introduction of the intrinsically toxic gluten in the diet. It is the relative result of sensitivity to the gluten fraction of wheat and rye proteins that follows. The question is whether the propensity of the small-bowel mucosa to react unfavorably to the presence of gluten might be induced by early introduction of gluten into the diet.

There are two main theories about the pathogenesis of gluten sensitivity. One suggests that patients with coeliac disease lack a peptidase enzyme necessary for proper digestion of gluten, so that a toxic residue accumulates in the gut. The other suggests an immunological mechanism with hypersensitivity to gluten. If the latter theory were correct the development of hypersensitivity might possibly be related to the time of first exposure to gluten.

An association between coeliac disease and cow's milk allergy has also been suggested. However, the current weight of opinion perhaps slightly favours the "milk allergy" theory, according to which it would seem more likely that the basic abnormality is usually present from birth and unchanged by diet in infancy—though the situation is far from simple, and there are certainly some instances of acquired gluten sensitivity.

In conclusion, the relative importance of genetic and environmental factors in the pathogenesis of coeliac disease is still uncertain. Environmental factors may play a part, and early exposure to gluten might be one of them, though there is no definite evidence that this is so. There are, incidentally, other arguments against the current practice of introducing cereals very early into the baby's diet. An excessive carbohydrate intake causes obesity in infancy, which may be the precursor of childhood and adult obesity.

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