has been sensitized and whose serum already contains Rh antibody. Hence it should be given to all unsensitized Rh negative women following delivery. The Rh group of the baby should be determined, as there is no need to treat a woman who has an Rh negative baby. The risk of sensitization is reduced if the baby is ABO incompatible, but the protection is far from absolute and Rh immunoglobulin should be given regardless of ABO blood group. The dose is 1 ml., and it is given intramuscularly within 72 hours of delivery.

An important point to remember is that protection is afforded only to the next Rh positive pregnancy. This pregnancy also carries the risk of transplacental passage of Rh positive fetal red cells at delivery, and hence ideally a further dose of Rh immunoglobulin should be given after each delivery of an Rh positive child.

Abortion also involves the risk of transplacental haemorrhage and sensitization can follow. Thus, provided adequate supplies of Rh immunoglobulin are available, all Rh negative women should be given injections of it. This is likely to become an important problem with the present-day rising incidence of abortion. One may easily visualize the tragedy of some unmarried girl being aborted and developing Rh antibody, and running into serious trouble when she eventually marries and wants to start a family.

*Failure Rate.*—Rh immunoglobulin is now known to be highly effective, though protection is not absolute and occasional failures do occur. There are several possible reasons for failure. Sensitization usually occurs at delivery, but occasionally the mother is sensitized owing to transplacental haemorrhage during pregnancy. Initially the antibody titre may be too low to detect, and the mother will appear to be unsensitized when the Rh immunoglobulin is given after delivery—whereas in fact she is already sensitized and Rh immunoglobulin cannot reverse the process at this stage. Another reason for failure is that the mother may have a particularly large transplacental haemorrhage which is not neutralized by the Rh immunoglobulin.

*Problems of Supply.*—Rh antibody (anti-D) is obtained either from Rh sensitized women or from Rh negative male volunteers who have been artificially stimulated with Rh positive blood. The supply of anti-D is therefore limited, but the supply position in Britain is steadily improving. In a situation where the supplies are limited it is customary to restrict treatment to unsensitized Rh negative primigravidae. When supplies become freely available, however, Rh immunoglobulin should be offered to all Rh negative women.

# Role of Liquor Examination

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That yellow liquor may be associated with severe haemolytic disease has been known since the turn of the century. Nevertheless, it was not until the 1950s that D. C. A. Bevis pointed out that this could be useful in management,[5 6] since when liquor examination has become a firmly established procedure. Its main purpose is to improve the selection of patients suitable for intrauterine transfusion or premature induction of labour.

## Amniocentesis

### Technique

In skilled hands amniocentesis is a relatively simple procedure carried out under local anaesthetic without premedication. Though it can be done at outpatient attendance, we have preferred to admit our patients overnight. A spinal needle is inserted into an area between the fetal limbs, or alternatively behind the fetal occiput; a few drops of liquor are allowed to escape from the needle, then a syringe is attached and 5 to 10 ml. withdrawn. Routine antibiotic cover is not needed.

Amniocentesis may also be used as part of the technique of intrauterine transfusion (when contrast medium is introduced to be swallowed by the fetus to outline the fetal alimentary tract); help in placental localization;[7] or confirm the diagnosis of hydrops fetalis.[8]

### Dangers

*Infection* following amniocentesis has been reported[9] but must be extremely rare and has not occurred in any of the 2,000 operations carried out in this centre. *Direct injury* to the fetus or umbilical cord has been reported,[10 11] but must also be exceedingly rare unless the fetus is moribund. *Premature labour* is not to be expected following the uncomplicated procedure.[9 10]

*Injury to the placenta* is a very common complication unless steps are specifically taken to avoid this. Placental localization is therefore recommended, especially if repeated amniocentesis is to be carried out, and is essential if intrauterine transfusion is contemplated. If a large fetal vessel is pierced there may be serious bleeding into the mother, retroplacentally or into the liquor,[12-14] and we have observed death of the fetus due to feto-maternal haemorrhage in a pregnancy where relatively mild haemolytic disease had been forecast.[15] Small feto-maternal bleeds are probably common;[16] moreover, undetected bleeds may occur, and rises in maternal antibody titre have been observed following amniocentesis.[17] On six or seven occasions we have observed pyrexia and rigors occurring a half to two hours after amniocentesis followed, in some cases, by an appreciable rise in antibody titre. We consider that such symptoms probably indicate large transplacental haemorrhage and carry a bad prognosis for the fetus. In the case of small feto-maternal bleeds, on the other hand, there is no evidence that the severity of haemolytic disease was greater than would have been expected had amniocentesis not been carried out. Similarly a study of subsequent pregnancies in these patients has shown no evidence of abnormal increase in severity of disease.[18]

Liquor samples containing gross amounts of blood are unsuitable for examination, for though red cells can be removed by centrifugation, persistent contamination by plasma will completely invalidate the results. Another source of error is that even small amounts of haemoglobin make quantitative measurement of bilirubin extremely difficult. If gross bleeding into the liquor occurs, results in specimens taken as much as two weeks later may not be valid.

### Indications

Because of the potential dangers, it is unreasonable to recommend repeated amniocentesis from early in pregnancy for all cases of Rh isoimmunization. We have not found selection for amniocentesis and timing based on the time of previous intrauterine death[19] or on the maternal

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antibody titre[20] to be of great value—except in the initially affected pregnancy in the latter case. We have therefore taken into account the previous history and in first affected pregnancies the antibody titre when deciding the need for and timing of liquor examination.[21] If a previous pregnancy has resulted in stillbirth or very severe disease, not only is there a high risk of stillbirth this time but many such intrauterine deaths will occur before 28 weeks' gestation. In such cases we would therefore carry out initial amniocentesis at about 20 weeks' gestation. In patients where previous moderate disease has occurred, intrauterine death, if it should occur, will do so after 35 weeks in two out of three cases, and in 80% for first affected pregnancies. In these cases we would carry out initial amniocentesis at about 30 weeks' gestation.

## Analysis of Liquor Amnii

Some workers consider the main pigment in amniotic fluid to be bilirubin,[22] but other haemoglobin breakdown products and haem precursors may also be present. If liquor is exposed to light, rapid photo-oxidation of bilirubin to a colourless compound occurs; therefore, samples immediately after collection should be placed in a brown sterile bottle and stored in a refrigerator until tested. Testing should usually be carried out within 12 hours, but delay up to 24 hours does not introduce significant error.

## Chemical Methods

Because bilirubin levels in liquor as low as 0·2 mg./100 ml. may be significant, standard methods of estimating plasma bilirubin are not satisfactory. Special biochemical techniques have therefore been devised but are often so elaborate that they are not of great practical value,[23–27] but the modified method of Jendrassik and Grof[28 29] is relatively simple and satisfactory.[30] The advantage claimed for biochemical methods is that they avoid errors due to interfering pigments[31–33] and sometimes allow a correct forecast in cases where that based on the spectrophotometric method is wrong.[34] Such discrepancies must be very rare and the latter method is generally preferred.

## Spectrophotometric Method

For spectrophotometry it is important that the liquor sample be clear and this can be achieved either by centrifugation or filtration. It is also important to take into account the length of the light path employed. Even using similar techniques and instruments, identical results are not achieved in different laboratories, so that finer points of discrimination need to be decided for each individual centre.

In Fig. 1 we have shown tracings on a linear scale for four samples of liquor obtained using an Optica Recording Spectrophotometer with a 0·5 cm. light path.

It will be seen that liquor containing predominantly bilirubin shows an absorption band at 450 nm., while that containing haemoglobin also has absorption bands at 410, 540, and 580 nm. The normal liquor shows a curvilinear trace with absorption in the lower visible range. If recorded on a logarithmic scale normal liquor gives a relatively but not perfectly straight line tracing.[35]

The quantity of bilirubin is estimated by measuring the bilirubin band, usually by Liley's technique,[36] in which optical density at different wavelengths is plotted on semilog paper and a straight baseline drawn in between 365 and 550 nm. The height of the bilirubin is then measured as optical density difference between the baseline and the liquor tracing at a wavelength of 450 nm. This is often unsatisfactory, as the many different baselines recommended show, [37–41] but the final differences in measurement are small. Similar calculations can be made for liquors traced on a linear scale, or the bilirubin may be measured in units[42] or classified in major grades.[39]

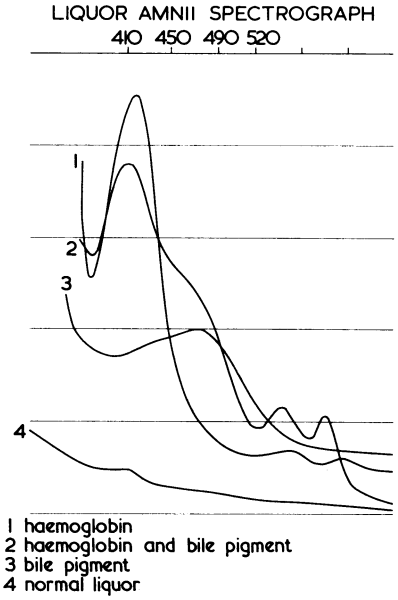


FIG. 1.—Liquor amnii spectrograph.

Whatever technique is employed, problems in measurement occur whenever an appreciable amount of haemoglobin is also present. The baseline can no longer be constructed to a rigid formula so that serious subjective errors are introduced. The best solution is to avoid haemoglobin contamination. In our early experience we detected significant amounts of haemoglobin in half the liquor samples, but during the past year haemoglobin was detected in only 28% of samples, being gross in only 2%. This decrease in contamination with haemoglobin we attribute to prior placental localization. Even so, haemoglobin contamination cannot always be avoided and to minimize errors from this cause, and to obtain a quantitative measure of the amount of bilirubin present, various methods other than the direct measurement of the 450 nm. peak have been advocated. Measurement of the difference between optical densities at various wavelengths has been recommended; 455 and 575 nm.,[43–44] 454 and 574 nm.,[45] 450 and 700 nm.,[46] 490 and 520 nm. [47] F. K. Bartsch has concluded that all the various methods give similar results and that none is significantly better than another.[48]

We prefer to measure the optical density difference between 490 and 520 nm., but because our apparatus gives a trace on a linear scale we have found it easier to express this as a transmittance ratio:

$$\frac{\text{transmittance 520nm.\%}}{\text{transmittance 490nm.\%}}$$

and the subsequent data given for our material are based on this measurement. Though there will be differences in detail from other methods of measurement, the general pattern and implications are the same.

## Liquor Bilirubin Levels

Even given that the method of measuring liquor bilirubin is satisfactory, difficulties in interpretation still arise because the normal values in pregnancy are not absolutely established. The bilirubin level falls during the latter weeks of pregnancy—this can be taken into account[36] when establishing guide lines for managing affected pregnancies (Fig. 2)—but these lines cannot be extrapolated to early pregnancy. Thus, numerous modifications for their interpretation have been introduced and some are illustrated in Fig. 2.[19 20 49–53] Undoubtedly some of the confusion arises because the liquor bilirubin level does not always indicate the severity of haemolytic disease in the fetus precisely. For example, in five pregnancies tracings of liquor taken at the same stage of gestation showed differing amounts of bilirubin; nevertheless, the severity of the haemolytic disease was practically identical in each case.[42]

Part explanation for these discrepancies may be that the bilirubin concentration depends on the volume of liquor, which may vary from patient to patient or at different times in the same patient. It is im-

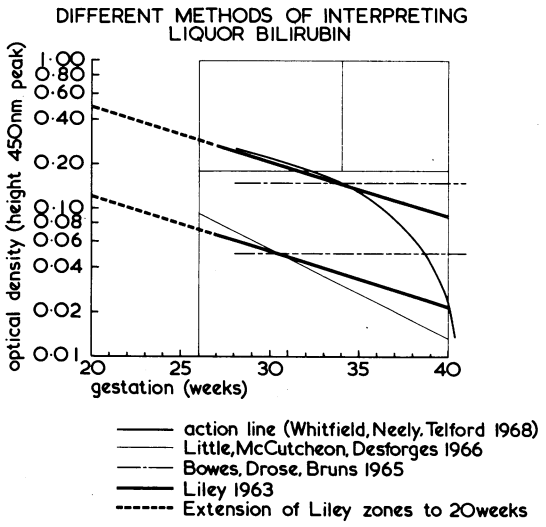


FIG. 2.—Different methods of interpreting liquor bilirubin.

practicable to measure the volume of liquor each time amniocentesis is performed, but, because the bilirubin value tends to parallel the protein level,[54] attempts have been made to allow for variation in liquor volume by calculating a bilirubin/protein ratio.[32 55-57] Another advantage claimed for the bilirubin/protein ratio is that it continues to give a valid indication of severity even after intrauterine transfusion, whereas the bilirubin level itself may be misleading. We were able to correlate both bilirubin and protein levels with severity of haemolytic disease,[58] but found that interrelation as a bilirubin/protein ratio was inferior to evaluation based on bilirubin alone. Possibly the liquor protein content influences the bilirubin level, as it does in the cerebrospinal fluid,[59] because in the very early weeks of pregnancy liquor protein values are extremely low and practically no bile pigment is present.[60 61] J. T. Queenan and his colleagues observed that the liquor protein level was maximal at about 20 weeks' gestation; they also found that the protein level correlated with the severity of haemolytic disease—and in particular that a rise towards the end of pregnancy was ominous, levels above 0·8 gm./100 ml. indicating the probability of hydrops fetalis.[62]

## Duration of Gestation

We have attempted to define the pattern of bilirubin in relation to gestation in normal pregnancies (Fig. 3).

This figure is based on 265 observations on 153 Rh negative pregnancies and 76 observations on 70 other normal pregnancies. Cases have only been included where an accurate assessment of gestation was possible and where there was no contamination with blood or haemoglobin. There is a rise in bilirubin to a maximum at about 18 to 20 weeks' gestation, which is maintained to about 26 weeks, whereas afterwards there is a continuous fall to near term. The two samples that

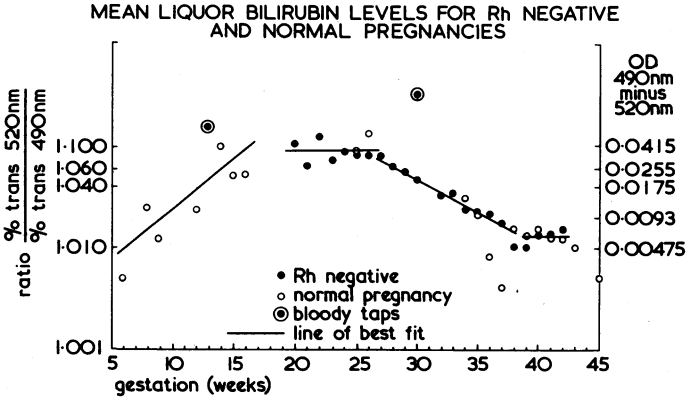


FIG. 3.—Mean liquor bilirubin levels for Rh negative and normal pregnancies.

were heavily contaminated with blood emphasize the magnitude of errors from this source.

The importance of having an accurate assessment of gestation when interpreting liquor values is evident, and other tests may be applied to the liquor sample to help establish this. Thus the osmolality of liquor near term is lower than that of maternal plasma,[63] while the creatinine concentration increases as pregnancy advances, until at term it may be three to four times as great as that in maternal plasma.[64] In the first trimester the major solute concentrations in the liquor resemble those found in maternal plasma; thereafter the osmolality falls progressively, as does the sodium concentration.[65] Conversely, the concentrations of urea and creatinine rise steadily after 30 weeks' gestation.[66-68] Histological examination of desquamated skin cells[69 70] may also give an indication of the stage of gestation, but T. Lind and his colleagues did not find this technique sufficiently precise for application in individual cases.[65]

## Severity of Haemolytic Disease

The liquor bilirubin value is superior to either the previous history or the maternal antibody titre in forecasting the severity of haemolytic disease in the fetus and, when known, dispenses with the need to take these further into account. For the sake of argument we have considered the value of a relatively high liquor bilirubin level (ratio 1·099) as a method of forecasting the probability, on the one hand, of stillbirth or very severe disease, or, on the other, of less severe disease or normal infants. The results are summarized in the Table.

*Liquor Bilirubin Level in Relation to Severe Haemolytic Disease of the Newborn at Different Stages of Gestation*

| Forecasted Diagnosis | Liquor Ratio Above or Below 1·009 | Percentage of Patients Affected at Different Stages of Gestation | | | |
|---|---|---|---|---|---|
| | | (20–26 Weeks) | (27–30 Weeks) | (31–33 Weeks) | (34–36 Weeks) |
| Rh negative or less severe disease | > | 32 | 7 | 15 | 4 |
| | < | 34 | 62 | 68 | 82 |
| Stillbirth or very severe disease | > | 34 | 22 | 12 | 7 |
| | < | Nil | 9 | 5 | 7 |
| Total no. of cases | | 35 | 45 | 224 | 664 |

Plotted in this way wrong forecasts by underestimating the severity appear in the bottom line, while wrong forecasts in overestimating severity are shown in the top line.

Firstly it will be observed that the proportion of high bilirubin values decreases as gestation advances, from 66% at 20 to 26 weeks to only 11% at 34 to 36 weeks. Secondly, the total errors in forecasting occurred at the respective gestational stages in 32, 16, 20, and 11% of cases. At the early stages of gestation errors were chiefly due to overforecasting severity, but at later stages of gestation to underforecasting severity; the converse is true with regard to forecasting less severe disease.

These findings confirm that gestation must be taken into account when evaluating the significance of the liquor-bilirubin level, but whether an accurate forecast can ever be made on one sample of liquor except in the extreme situation is questionable. There is no doubt that one can nearly always pick out Rh negative pregnancies but stillbirths do occur when the liquor is virtually clear.

In Fig. 4 the mean liquor bilirubin values at different stages of gestation are shown in relation to severity of disease in the fetus. Cases treated by intrauterine transfusion are not included. At 20 weeks' gestation high bilirubin levels are observed

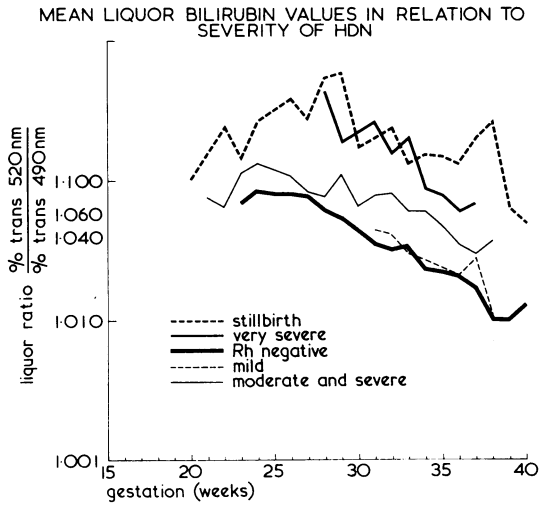


FIG. 4.—Mean liquor bilirubin values in relation to severity of haemolytic disease of the newborn.

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 can be misleading, but two consecutive values if falling give reassurance while if 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).[19] 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, though we 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.[71] 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.[77–76] Though 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*-

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