PRELIMINARY COMMUNICATIONS

Amniotic Fluid Lecithin: Sphingomyelin Ratio and Fetal Lung Development

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Summary

The normal range and trends of the ratio between lecithin and sphingomyelin in the amniotic fluid, estimated on thin-layer chromatograms, have been established for the last trimester. The accuracy of the ratio as an index of fetal lung development and of the risk of neonatal respiratory distress has been confirmed. The usual increase in the ratio towards term was not found in some pregnancies complicated by diabetes or severe rhesus incompatibility. Determination of the ratio may prove a valuable guide in the management of pregnancies with these complications.

Introduction

Respiratory distress due to the progressive atelectasis of hyaline membrane disease is a major cause of neonatal mortality. Typically it occurs in immature babies born after spontaneous premature labour or when early delivery is carried out for an obstetric complication such as rhesus incompatibility, but some mature infants of diabetic mothers are also affected.

Insufficient pulmonary surfactant, causing a high surface tension with reduced distensibility in the alveoli, has been implicated in the aetiology of this disorder (Avery and Mead, 1959; Pattle *et al.*, 1962; Adams *et al.*, 1965). It also seems likely that adequate surface activity in the alveolar lining facilitates re-expansion of collapsed neonatal lung after aspiration of milk or mucus. The main surface-active component is dipalmitoyl lecithin (Gluck *et al.*, 1970), and since fetal lung secretions reach the amniotic fluid (Scarpelli, 1967) its increased accumulation in the alveoli towards term is reflected in a sharply rising concentration of lecithin in the amniotic fluid from about the 33rd gestational week (Gluck *et al.*, 1971). As this terminal increase is not matched by a parallel increase in sphingomyelin Gluck *et al.* (1971) have suggested that the ratio between these two surface-active phospholipids in the amniotic fluid provides a useful index of fetal pulmonary maturation and of the likelihood of neonatal respiratory distress if delivery is not postponed. They also put forward a relatively simple method for determining this ratio from the areas of the lecithin and sphingomyelin spots on thin-layer chromatograms. Our preliminary clinical evaluation of the test is now presented.

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Methods

Amniotic fluid was usually obtained by amniocentesis, mostly from rhesus-immunized patients, many of whom had repeated tests. Other samples were obtained either at caesarean section or at amniotomy to induce labour or performed during labour.

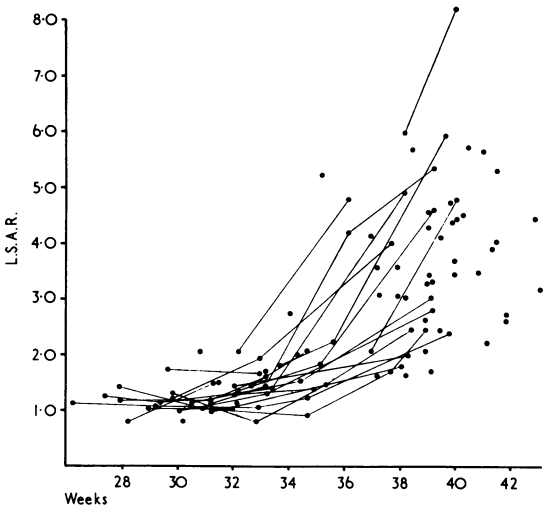
The biochemical procedure used follows that outlined by Gluck and his associates. The amniotic fluid phospholipids are first extracted in chloroform and methanol. After evaporation to dryness cold acetone is used to precipitate the surface-active phospholipids, which are redissolved in chloroform and then separated by thin-layer chromatography. The areas of the lecithin and sphingomyelin spots are measured by multiplying length by width, and the average ratio between them in duplicate aliquots of each amniotic fluid sample is reported as the "lecithin : sphingomyelin area ratio" (L.S.A.R.).

As a preliminary step the accuracy of this method was confirmed by showing a good statistical correlation between the L.S.A.R. and the ratio of measured concentrations of lecithin and sphingomyelin in 20 amniotic fluids ($r = 0{\cdot}9201$). The apparent unreliability of the method in the presence of meconium or chlorhexidine antiseptic cream was confirmed in vitro by determining the ratio before and after the addition of each of these substances. Amniotic fluids stained by meconium or mixed with chlorhexidine were therefore excluded.

Clinical respiratory distress was diagnosed in neonates in whom obvious difficulty in breathing developed at least 15 minutes after birth and was associated with rib recession and expiratory grunting. The diagnosis of hyaline membrane disease was based on the typical radiological appearances or necropsy findings or both.

Results

The L.S.A.R. is plotted in the Chart against gestational age in 105 amniotic fluid samples from 64 patients classified as normal, including 71 samples from 31 rhesus-immunized women who were subsequently delivered of unaffected normal infants after uncomplicated pregnancies. Normal limits for the L.S.A.R. during the third trimester were established from these results. Up to 32 weeks the normal range is 0·8 to 2·0; thereafter the



L.S.A.R. in 105 samples from 64 normal patients plotted against gestationa maturity in weeks. Serial tests in individual patients are joined.

L.S.A.R. rises, but much individual variation in the rate of increase results in a widening normal range, with values of at least 1·5 by 38 weeks and between 2·0 and 9·0 at term. The serial results also show that once the L.S.A.R. exceeds 2·0 a continued rise can be expected in normal pregnancies. The fetal sex had no obvious influence on these normal values.

In abnormal pregnancies (305 samples from 167 patients) our results to date suggest that the expected terminal rise in the L.S.A.R. may not occur when the fetus is severely affected by rhesus incompatibility (10 out of 14 cases with cord blood haemoglobin below 10·0 g/100 ml) and that it remains static or rises and then falls in some pregnancies complicated by maternal diabetes (3 out of 10 cases).

The L.S.A.R. was determined in 128 amniotic fluids obtained less than 48 hours before delivery in both complicated and normal pregnancies. As shown in the Table these results can be classified into three groups according to the risk of neonatal respiratory distress. In the high-risk group (L.S.A.R. < 1·5) clinical respiratory distress occurred in eight out of 10 infants, in four of whom hyaline membrane disease was present (one fatally affected). Four of the infants developing respiratory distress were born after at least 36 weeks' gestation; two were born during the 40th week, the mother of one being diabetic and the other mother showing abnormal glucose tolerance during the pregnancy. The L.S.A.R. was satisfactory (>2·0) in 102 samples, and none of the infants in this group had respiratory difficulties even though 11 were born at less than 36 weeks' gestation. In the intermediate group (L.S.A.R. 1·5-2·0) four of the 16 infants developed respiratory distress (hyaline membrane disease in one), and in one of these cases the L.S.A.R. was 1·9.

L.S.A.R. Less than 48 Hours before Delivery and Neonatal Respiratory Distress

L.S.A.R.	No. of Infants	Respiratory Distress	
		No.	%
<1·5	10	8	80
1·5-2·0	16	4	25
>2·0	102	0	0
Total ..	128	12	9

Discussion

These preliminary findings confirm the remarkable predictive accuracy of the L.S.A.R. in up-to-date samples of amniotic fluid—that is, obtained less than 48 hours before delivery. In particular, the almost invariable development of neonatal respiratory distress when such samples showed a ratio less than 1·5 supports the contention by Borer *et al.* (1971) that values below this critical level indicate dangerously immature fetal lungs. We are not, however, in agreement with their view that ratios above 1·8 define safely mature fetal lungs. Our experience suggests that only ratios above 2·0 can be regarded as safe from the point of view of neonatal pulmonary function, with values in the intermediate range of 1·5 to 2·0 indicating that there is some risk of serious respiratory troubles if the infant is born soon.

Besides confirming that an increase in the ratio between lecithin and sphingomyelin in the amniotic fluid is usual towards term our results show that the rate of increase varies from patient to patient, and they enable normal limits to be set for the L.S.A.R. during the last trimester. The range and trends of the L.S.A.R. in normal pregnancies will serve as a standard for comparison with the results of our continuing studies in abnormal pregnancies.

If confirmed by further experience the finding that the L.S.A.R. occasionally fails to rise or rises and then falls in patients with diabetes may lead to the antenatal detection of those infants (of diabetic mothers) with a particular risk of respiratory distress and hyaline membrane disease even when born near term. The level and trend of the L.S.A.R., determined by serial tests, may become a deciding factor in selecting the time for delivery of diabetic mothers.

The failure of the L.S.A.R. to rise in some pregnancies complicated by severe rhesus incompatibility may also be of practical importance, because much of the perinatal mortality of this disorder is due to the combined effects of respiratory distress and severe haemolysis in premature infants. We now take into account the result of an up-to-date L.S.A.R. test when expected severe haemolytic disease necessitates early intervention. A low L.S.A.R. in these circumstances at 33 or 34 weeks would lead us to prefer fetal blood transfusion to immediate delivery.

Sufficient data will soon be available to determine if lecithin and sphingomyelin levels in the amniotic fluid are affected by other obstetric complications. Acute complications such as placental abruption and fulminant pre-eclampsia may necessitate immediate delivery without regard to fetal maturity, but when chronic placental insufficiency is suspected an accurate prediction of fetal lung development would help in choosing the time for delivery. Up-to-date L.S.A.R. estimations would be of obvious value in timing elective caesarean sections and also induction of labour for assumed postmaturity, especially when the menstrual history is uncertain.

With these and other possible clinical applications in mind it is fortunate that measurement of the L.S.A.R. is both a reliable and a much simpler alternative to quantitative determination of lecithin in the amniotic fluid. The latter time-consuming procedure is unlikely to become available as a regular laboratory service, although it might be used as an additional test when immediate delivery is being considered but the L.S.A.R. is in the intermediate range (1·5 to 2·0).

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