Sedimentation of Lung-Derived Phospholipid during Low-Speed Centrifugation of Amniotic Fluid
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In most methods proposed for the assessment of fetal lung maturity, amniotic fluid is subjected to a preliminary low-speed centrifugation in an attempt to separate whole cells and cell debris from lung-derived surfactant phospholipid (lamellar body phospholipid). However, because lamellar body phospholipid is present in amniotic fluid in a membranous or particulate form, it is also partly sedimented by this procedure. The sedimentation of total phospholipid and lamellar body phospholipid by low-speed centrifugation has been quantitated for 49 samples of amniotic fluid from pregnancies of 30–41 weeks gestation. Isopycnic density-gradient centrifugation in a small air-driven ultracentrifuge was used to isolate lamellar body fractions from whole and centrifuged amniotic fluid. Centrifugation for 5 min at 1000 × g removed 0–70% of total phospholipid or lamellar body phospholipid, the mean values being 34 or 29%, respectively. There was an appreciable increase in lamellar body phospholipid relative to total phospholipid as a result of centrifugation in only 51% of the samples. In general, the effects of centrifugation were not related to gestational age of the fetus or the state of maturity of its lungs.

Respiratory distress syndrome occurs in infants born with immature lungs, which lack an adequate supply of surfactant. This phospholipid-rich substance is derived from lamellar bodies synthesized in the Type II cells of the alveolar epithelium (1). During pregnancy, surfactant is secreted into the potential air spaces of the fetal lung and passes into the amniotic fluid. A sudden increase in the concentration of surfactant or lamellar body phospholipid (LB-PL)3 in amniotic fluid signals that the lung has reached a stage of maturity compatible with normal function (1, 2).

Many methods have been devised for measuring the increase in amniotic fluid surfactant that accompanies fetal lung maturation. Some depend on the measurement of the phospholipid content or composition of amniotic fluid—for example, the widely used L/S ratio—others depend on measurement of physical properties of the lipids (3).

Theoretically, amniotic fluid phospholipids may be derived from many sources besides the lung (3). To remove whole cells and large particulate matter, which may contribute significant amounts of phospholipid, it is usually recommended that amniotic fluid be centrifuged at relatively low centrifugal force before proceeding with the assay. Many different centrifugal conditions have been recommended (3).

Soon after the L/S ratio procedure was introduced (4), it was suggested that surfactant may be present in human amniotic fluid in sedimentable form (5). However, a clear understanding of its characteristic morphology and sedimentation properties is recent. Surfactant phospholipid is present in the amniotic fluid in aggregates consisting of concentric whorls of membranes around dense cores, which seem to represent lamellar bodies in the process of unravelling (6). Although complete sedimentation of these lamellated structures requires centrifugation at 33 000 × g for 60 min or longer, some sedimentation occurs during centrifugation at only 80 × g for 5 min (7).

A new approach to the assessment of fetal lung maturity has recently been reported (2, 9, 10). The method involves determination of the phospholipid content of a lamellar body fraction isolated from amniotic fluid by isopycnic density centrifugation. Application of this method to amniotic fluid, before and after low-speed centrifugation, has made it possible to quantitate the loss of LB-PL during centrifugation and to assess whether the supernate is enriched with LB-PL relative to other phospholipids by this procedure.

Materials and Methods

Amniotic fluid was collected by transabdominal amniocentesis at 30–41 weeks of gestation, as part of the routine management of complicated pregnancies. Indications for amniocentesis included toxemia of pregnancy (13 cases), uncertain dates or suspected intrauterine growth retardation (13 cases), antepartum hemorrhage or threatened premature labor (seven cases), elective cesarean section (five cases), blood-group incompatibility (five cases) and maternal diabetes (four cases).

As soon as possible after collection, the amniotic fluid was centrifuged at 1000 × g for 5 min at room temperature. The supernatant fluid was decanted without disturbing the pellet and stored, together with a portion of uncentrifuged amniotic fluid, at −20°C to await further processing.

Lamellar body fractions were isolated from uncentrifuged and centrifuged amniotic fluid by layering the fluid over a Ficoll 70 solution and centrifuging at 115 000 × g for 15–20 min in a Beckman Airfuge (8). During this procedure, the lamellar body fraction accumulates as a band at the Ficoll/amniotic fluid interface. The total phospholipid and LB-PL content of the samples were determined as described previously (8). Freezing and thawing amniotic fluid once before use does not generally affect the determination of LB-PL (8).

For some samples, densitometer tracings used in determining the L/S ratio of centrifuged amniotic fluid were compared with densitometer tracings used in determining the L/S ratio of the corresponding lamellar body fractions. The L/S ratio procedure is described in detail elsewhere (10).

In a few cases, part of the pellet obtained after centrifuging amniotic fluid at 1000 × g for 5 min was prepared for electron microscopy as described previously (11).

Results

Comparison of data for amniotic fluid from different individuals showed that centrifugation at 1000 × g for 5 min resulted in a highly variable loss of total phospholipid and LB-PL into the pellet (Figures 1A and 1B). The mean loss of total phospholipid and LB-PL was 34% and 29%, respectively. Table 1 shows the effect of centrifugation on amniotic fluid phospholipid composition, with respect to the percentage of...

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3 Nonstandard abbreviations used: LB-PL, lamellar body phospholipid; L/S ratio, ratio of lecithin to sphingomyelin.

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LB-PL relative to total phospholipid. In some samples, the supernate became enriched in LB-PL relative to total phospholipid; in others the relative contribution of LB-PL was lessened (Table 1 and Figure 1C). Centrifugation resulted in an appreciable enrichment (enrichment factor >1.1) in only 51% of the samples.

When the results were expressed as in Figure 1, statistical analysis of the pooled data showed that there was no significant correlation between the effect of centrifugation and the gestational age of the fetus at the time of amniocentesis or the state of maturity of its lungs, as indicated by the LB-PL content of the amniotic fluid (9). However, when the results were expressed as in Table 1, it became apparent that samples already containing a high concentration of LB-PL tended to become enriched in LB-PL as a result of centrifugation. This group (group 3, Table 1) contained samples whose initial LB-PL concentration indicated that the fetal lungs were well beyond the critical stage of lung maturity.

Electron microscopy revealed the presence of aggregations of membranous material, frequently in concentric array, among the whole cells and debris sedimenting at 1000 × g (Figure 2). This material bears a striking resemblance to lamellar body fractions isolated from human amniotic fluid by isopycnic density-gradient centrifugation (8) or by differential centrifugation between 370 × g for 10 min and 9200 × g for 20 min (6).

Figure 3 shows densitometer tracings obtained during L/S ratio determinations of amniotic fluid and lamellar body fractions. The lamellar body fraction was rich in lecithin but contained little sphingomyelin, whether it was obtained from samples whose LB-PL content and L/S ratio indicated fetal lung immaturity or maturity (Figure 3). Clearly, the increase in the L/S ratio in amniotic fluid that is known to be associated with lung maturation is not due to a change in the L/S ratio of surfactant itself.

**Discussion**

In an investigation in which surfactant was isolated by using sodium bromide gradients and a large-scale preparative ultracentrifuge, it was shown that surfactant in lung washings of newborn infants contains little sphingomyelin, irrespective of the state of maturity of the infant's lungs (12). The data in Figure 3 are consistent with this finding, and thus provide support for earlier data (8) which suggested that the simple micro-method used for isolating LB-PL in the present investigation satisfactorily separates LB-PL from other phospholipids.

The data in Figure 3 also illustrate clearly that nonsurfactant phospholipid in amniotic fluid contributes to the L/S ratio and therefore plays a part in the assessment of fetal lung maturity by that method. The implications of this finding in relation to the effect of centrifugation on the L/S ratio have been discussed elsewhere (3, 7).

Evidently, centrifuging amniotic fluid under relatively gentle conditions (1000 × g, 5 min) causes a significant and highly variable loss of surfactant phospholipid (Table 1, Figures 1 and 2). Others have reported losses of total phospholipid (7, 13) or saturated lecithin (14) during low-speed centrifugation of amniotic fluid, consistent with the present findings. In many samples, centrifugation did not enrich the amniotic fluid with surfactant phospholipid relative to total phospholipid (Figure 1C), indicating that some samples of amniotic fluid contain a significant amount of nonsurfactant phospholipid that is not sedimentable under these conditions. Centrifugation conditions comparable to those used in the present investigation, or even harsher conditions, are being

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**Table 1. Effect of Centrifugation on the Phospholipid Composition of Amniotic Fluid**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of samples</th>
<th>Mean LB-PL mg/L (and SD)</th>
<th>Mean Uncentrifuged Centrifuged phospholipid, % (and SD)</th>
<th>Enrichment factor, B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>28 (9)</td>
<td>69 (17), 69 (17)</td>
<td>1.0 (0.3)</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>54 (9)</td>
<td>71 (10), 73 (12)</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>136 (50)</td>
<td>70 (16), 85 (18)</td>
<td>1.3 (0.4)</td>
</tr>
</tbody>
</table>

For definitions of groups and further details, see legend to Figure 1.

* A and B differ significantly, p < 0.05, paired t-test.
used routinely in many laboratories undertaking the assessment of fetal lung maturity (3).

Most methods for assessing fetal lung maturity depend on measuring, either directly or indirectly, the amount of surfactant phospholipid in amniotic fluid (3). Therefore, the variable removal of surfactant phospholipid during preliminary centrifugation represents a potential problem in any such method that includes this step in the procedure. Since Oulton (7) has shown that the minimal centrifugation conditions required to sediment whole cells (140 × g, 10 min) also cause the sedimentation of structures that appear to be of lamellar-body origin, the problem cannot be avoided by reducing the centrifugal force below 1000 × g, although this may help to minimize the problem. However, it should be noted that changing the centrifugation conditions may alter the "cut-off" value to be used for clinical interpretation of the results (3).

Assessment of fetal lung maturity by determining the LB-PL content of amniotic fluid (9) is not affected by this problem, because no preliminary centrifugation is required. In this method, separation of LB-PL from whole cells and cellular debris depends on the low buoyant density of surfactant, rather than on its sedimentation properties.

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References