Microviscosity of Amniotic Fluid Phospholipids, and Its Importance in Determining Fetal Lung Maturity

Thomas A. Blumenfeld,1 Howard S. Cheskin,1 and Meir Shnitzky2

Fluorescence polarization measurements of microviscosity (apparent viscosity within the hydrophobic center of lipid bilayers) of amniotic fluid correlate well with lecithin/sphingomyelin ratios determined by thin-layer chromatography. In addition to lecithin, phosphatidylglycerol and phosphatidylinositol are important for determining fetal lung maturity, but the lecithin/sphingomyelin ratio gives no information concerning these other phospholipids. The microviscosity of sphingomyelin significantly exceeded that of lecithin over the temperature range 25–37 °C; values for phosphatidylglycerol, phosphatidylinositol, and phosphatidylserine were lower. Phosphatidylglycerol, phosphatidylinositol, and phosphatidylserine, added individually, significantly decreased the microviscosity of dispersions with lecithin/sphingomyelin ratios corresponding either to fetal lung immaturity or maturity. Phosphatidylglycerol caused the greatest decrease in both.

Mixtures of the three phospholipids in the proportions found in term amniotic fluid decreased the microviscosity of fluids with either mature or immature lecithin/sphingomyelin ratios by 23–27%. When each was present in the proportion found in tracheal aspirate (twice that of term amniotic fluid), the decreases were 46–50%. This technique quickly and precisely indicates not only fetal lung maturity but also the presence of important phospholipids other than lecithin and sphingomyelin.

Additional Keyphrases: diagnostic aids • fetal status • phospholipids • respiratory distress syndrome

Microviscosity as measured by the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) is an excellent indicator of the relative amounts of lecithin and sphingomyelin in laboratory-prepared lipid dispersions, and when used for amniotic fluid it indicates fetal lung maturity equally as well as chromatographic measurement of the lecithin/sphingomyelin ratio (L/S) (1, 2). During gestation the pattern of change in amniotic fluid microviscosity parallels the expected development of the pulmonary surfactant system and accurately indicates its maturation (3), and thus, indirectly, that of the fetal lung.

In normal term pregnancies the phospholipid in greatest concentration in amniotic fluid is lecithin. Sphingomyelin, phosphatidylinositol, phosphatidylglycerol, phosphatidylserine, and phosphatidylethanolamine are also present in amniotic fluid, but at much lower concentrations (4). The relationships of the concentrations of these phospholipids are important indicators of fetal lung maturity. After 35 weeks of gestation the amniotic fluid L/S ratio is usually 2 or greater; if it is less than 2 at this time, the chance of an infant’s developing the respiratory distress syndrome is increased (5). The presence and relative quantities of the acidic phospholipids, phosphatidylglycerol and phosphatidylinositol, may also be an index to normal function of the newborn lung. Phosphatidylglycerol may markedly improve the function of lung surfactant in stabilizing alveoli; its absence may indicate fetal lung immaturity (4). Infants of diabetic mothers may have no phosphatidylglycerol present and may develop the respiratory distress syndrome even though their amniotic fluid L/S ratios indicate maturity (6).

This ratio, commonly used to determine fetal lung maturity, gives no information on phosphatidylinositol or phosphatidylglycerol. In contrast, microviscosity, as measured by fluorescence polarization, is affected by phospholipids other than lecithin and sphingomyelin (7, 8). In a previous study comparing fluorescence polarization and L/S ratios for amniotic fluid from 161 pregnancies, the test results disagreed for only eight specimens (1). In all eight the L/S ratio indicated lung immaturity and the fluorescence polarization value indicated maturity. This disagreement may have been caused by the presence of phospholipids that contributed to surfactant maturity and to a more mature value for microviscosity but did not influence the L/S ratio.

The purpose of the present study was to determine the effect of phosphatidylglycerol, phosphatidylinositol, and phosphatidylserine on the microviscosity of solutions having known L/S ratios.

Materials and Methods

Solutions containing dispersions of liposomes of single phospholipids and mixtures of phospholipids were prepared. Each solution contained a total phospholipid concentration of 0.75 g/liter in phosphate-buffered saline, pH 7.4. The dispersions contained lecithin (phosphatidylcholine), sphingomyelin, phosphatidylglycerol, phosphatidylinositol, and (or) phosphatidylserine.

Phospholipid dispersions were prepared from the following:

Lecithin: 1-α-phosphatidylcholine, dipalmatoyl, synthetic,
approximately 98% pure (P-0763; Sigma Chemical Co., St. Louis, MO) and L-α-phosphatidylcholine (type V-E, from egg yolk, cat. no. P-5763; Sigma Chemical Co.) in equal amounts by weight, a composition approximating that of lecithin in amniotic fluid.

L-α-Phosphatidyl-DL-glycerol (grade 1, from egg-yolk lecithin, approximately 98% pure, P-0514; Sigma Chemical Co.).

Sphingomyelin (from bovine brain, S-7004; Sigma Chemical Co.).

Phosphatidylinositol (from bovine brain, 3300 H; General Biochemicals, Chagrin Falls, OH).

L-α-Phosphatidyl-L-serine (from bovine brain, approximately 98% pure, P-6641; Sigma Chemical Co.).

Liposomal dispersions were prepared by the following procedure. One gram of each phospholipid was dissolved in (or diluted with) 1 L of chloroform/methanol (9/1, by vol). Phospholipid mixtures were produced by making mixtures of the appropriate phospholipid solutions (Table 1).

Each of the 18 solutions contained a total of 1.5 mg of phospholipid in 1.5 mL of chloroform/methanol. The solvent was evaporated under a stream of nitrogen, and 2 mL of phosphate-buffered saline (pH 7.4) was added to the residue. Each solution was sonicated (Sonifer Cell Disrupter, Model W350; Branson Sonic Power Co., Division of Branson Ultrasonics Corp., Danbury, CT) at 55 W until it was slightly cloudy (3–12 min). The solutions were then centrifuged (36 000 × g, 10 min, at 4 °C).

The fluorescence polarization (p value) of DPH in each dispersion was determined as follows:

Vigorously mix 100 μL of DPH (2 mmol/liter, in tetrahydrofuran) with 200 mL of the phosphate-buffered saline. Add 2 mL of this mixture to 0.5 mL of each liposomal dispersion and incubate at 37 °C for 30 min. Determine the fluorescence polarization (p) with a fluorescence polarimeter (Microcosimeter, Model MV-1; Elscint Inc., Hackensack, NJ). Place a thermometer in the cuvet; measure the temperature of the solutions after each p value is determined.

The fluorescence polarimeter provides monochromatic excitation at a wavelength of 365 nm and determines the fluorescence polarization of emitted light at wavelengths >418 nm.
The relationships of the microviscosities of sphingomyelin (S), phosphatidylglycerol (PG), phosphatidylinositol (PI), and phosphatidylserine (PS), to lecithin (L) at 25 and 37 °C were determined. In mixtures containing 53% of each phospholipid and lecithin, the microviscosity was increased by lecithin at both temperatures, with a greater effect at 37 °C. The temperature profiles of microviscosity for PI and PS were parallel straight lines over the temperature range studied, characteristic of phospholipids in the liquid-crystal state. The microviscosity of sphingomyelin was almost fourfold that of lecithin, whereas values for PG, PS, and PI were, respectively, 70, 57, and 53% lower than that of lecithin. At 37 °C the relations were the same, although to a lesser magnitude.

Mixtures of Phospholipids

The percentage composition, L/S ratio, and the microviscosity determined at 25 and 37 °C for each phospholipid dispersion are presented above in Table 1. The percentage differences in microviscosity of the single phospholipids and lecithin at 25 and 37 °C are illustrated in Figure 2. At 25 °C the microviscosity of sphingomyelin was almost fourfold that of lecithin, whereas values for PG, PS, and PI were, respectively, 70, 57, and 53% lower than that of lecithin. At 37 °C the relations were the same, although to a lesser magnitude.

Figure 3 shows the effect of adding PG, PI, PS, or lecithin, in amounts equal to 33% by weight of the total lipids, to L/S mixtures having ratios of 1 and 2. Addition of 33% lecithin to a mixture containing an L/S ratio of 1 resulted in a dispersion with an L/S ratio of 2. Addition of 25% lecithin to a mixture containing an L/S of 2 resulted in a dispersion with an L/S of 3. Addition of PG, PI, PS, or lecithin decreased the microviscosity of dispersions with L/S ratios of 1 and 2 at both 25 and 37 °C. The decreases were qualitatively predictable from the microviscosities of individual phospholipids. The decrease caused by additional lecithin (to form L/S ratios of 2 and 3) was less than that caused by the other phospholipids. At 37 °C, PI decreased the microviscosity of L/S mixtures more than did addition of an equal amount of lecithin, although at that temperature PI and lecithin have the same microviscosity. Apparently there is an interaction between phospholipids that tends to decrease the microviscosity of mixtures. The fact that linear averages of the microviscosities of individual phospholipids do not quantitatively predict microviscosities of the mixtures at either temperature is further evidence of the effect for this type of interaction (8).

Figure 4 shows the effects of the addition of 5.3 or 10.7% each of PG, PI, and PS. At 25 °C the microviscosity of dispersions with L/S ratios of 1 and 2 and containing 10.7% of these phospholipids by weight were decreased almost twice as much as those containing mixtures of 5.3% of each of these phospholipids. At 37 °C the microviscosity of a dispersion with an L/S ratio of 1 and containing 10.7% (by wt) each of PG, PI, and PS was decreased four times as much as the mixture containing 5.3% of each of these phospholipids. At this temperature the microviscosity of a dispersion with an L/S ratio of 2 and containing mixtures of 10.7% of each phospholipid was decreased three times as much as that of the mixture containing half as much of each of the three phospholipids.

Discussion

DPH fluorescence polarization in amniotic fluid and microviscosity determined from this measurement are excellently correlated with L/S ratio (1, 2), and the pattern of decrease in amniotic fluid microviscosity during gestation parallels the expected development of a fetal pulmonary surfactant system (3). Because of these relations, ease of performance, excellent precision, and low coefficient of variation, measurement of amniotic fluid fluorescence polarization is an alternative to L/S ratio measurement for estimating fetal lung maturity. Recent studies suggest that a fetal lung maturity profile should consist of a determination of PI and PG as well as the L/S ratio in amniotic fluid, because these compounds give important information not obtained from the L/S ratio alone (4). The microviscosity of amniotic fluid is affected not just by lecithin and sphingomyelin but by all phospholipids present. In this study we compared the microviscosity of laboratory-prepared liposomes of S, L, PG, PI, and PS, and determined the effect of added PG, PI, and PS, individually.
and in mixtures, on the microviscosity of liposomes with known L/S ratios.

The microviscosity of liposomes made from the various amniotic fluid phospholipids in this study differed considerably. Microviscosity of phospholipids is dependent on the polar head-group, the relative saturation and length of fatty acyl chains (8), and the temperature at which measurement is made. Temperature affects the microviscosity because it influences the physical state—liquid-crystal or gel—of phospholipids. At high temperatures the liposomes exist in the fluid liquid-crystal state with low microviscosity; at low temperatures the liposomes exist in the more rigid gel state and have a higher microviscosity (Figure 1).

At normal body temperature (37 °C), sphingomyelin is transitional between the liquid-crystal and gel states and has a higher microviscosity than L, PI, PG, or PS, all of which are presumably in the liquid-crystal state. At this temperature, PG has the lowest microviscosity of these phospholipids. Amniotic fluid microviscosity is routinely performed at 24–25 °C. At this temperature the rank order of the microviscosities of the phospholipids is the same as at 37 °C, but the magnitude of the differences is greatly accentuated at the lower temperature, making the test more sensitive in differentiating the phospholipid content of amniotic fluids.

The presence of PG improves the surfactant properties of lecithin (4), and thus is an important indicator of fetal lung maturity when present in amniotic fluid. The present study shows that it also has an important effect in decreasing microviscosity. PI and PS, other pulmonary phospholipids present in amniotic fluid, also have a lower microviscosity than lecithin (Figure 2) and decrease the microviscosity of lipid dispersions of lecithin and sphingomyelin similarly to, but not as much as, PG (Figure 3). Amniotic fluid phospholipid at term contains 5% PG, 5% PI, and 2% PS (4) and newborn tracheal aspirate phospholipid contains 11% PG, 5% PI, and 5% PS (10). When these three phospholipids each made up 5.3% of phospholipid mixtures containing L and S in ratios of 1 and 2, the microviscosity of the mixtures at 25 °C was about 25% less than that of the L and S mixtures alone. When the three phospholipids each composed 10.7% of the mixtures, the microviscosity was decreased twice as much, to approximately 50% (Figure 4). At 37 °C these decreases were 7–8% and 23–29%, respectively. These findings show (a) the microviscosity of lipid dispersions containing L/S ratios of 1 and 2 (corresponding to fetal lung immaturity and maturity in amniotic fluid) are decreased by adding physiologic quantities of PG, PI, and PS; and (b) increasing amounts of PG, PI, and PS decrease the microviscosity of phospholipid dispersion mixtures in approximately direct relation to their concentrations. These changes occur at normal physiologic temperature and the temperature at which the clinical microviscosity test is performed. These findings strongly suggest that amniotic fluid microviscosity is greatly influenced by phospholipids of physiologic importance other than L and S, and that amniotic fluid microviscosity is a more complete indicator of fetal lung maturity than is L/S ratio alone.

Amniotic fluid microviscosity begins to decrease at a mean gestational age of 30.4 weeks and continues to do so until term (3). This change coincides with increased concentrations of L, PG, and PI, while that of S remains relatively constant (4). Near term, the concentration of L is the highest of the amniotic fluid phospholipids and is the most important indicator of fetal lung maturity. The results of this study show that PG and PI complement L in decreasing microviscosity and that the decrease in amniotic fluid microviscosity during gestation reflects the chronology of the changes in the concentration of L, PG, and PI.

The measurement of amniotic fluid fluorescence polarization is precise (1, 3), correlates well with L/S ratio, and is technically easy to perform. A previous study of amniotic fluid fluorescence polarization determinations from 161 pregnancies (1) showed eight cases in which fluorescence polarization indicated fetal lung maturity earlier than the L/S ratio. These amniotic fluids may have contained phospholipids that were important indicators of fetal lung maturity and influenced the fluorescence polarization, but were not measured by the L/S ratio. The results of the present study support this hypothesis and suggest that in some cases amniotic fluid microviscosity determined by fluorescence polarization will more accurately indicate fetal lung maturity than does the L/S ratio.

References