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Note

The detection of phospholipids with acid fuchsin—uranyl nitrate reagent and its application for the estimation of phosphatidylcholine—sphingomyelin ratio (L : S) in amniotic fluid by thin-layer chromatography

Č. MICHALEC and J. REINIŠOVÁ

Laboratory of Protein Metabolism, Faculty of General Medicine, Charles University, Prague (Czechoslovakia)

The methods recently developed for predicting, before birth, infants potentially at risk from neonatal respiratory distress syndrome (RDS) undoubtedly represent a major advance in obstetric care. When positive results are obtained these methods enable the obstetrician to terminate a complicated pregnancy well before term, safe in the knowledge that the infant will not develop RDS; negative results warn that the fetal lungs are not mature and that delivery should be delayed even if the pregnancy may appear to be at term [1—3].

Amniotic fluid lecithin assay

The main component of surfactant is phosphatidylcholine (lecithin) and a method for measuring the amniotic fluid lecithin concentration by thin-layer chromatography (TLC) was developed [4]. Using this method, it was shown that an amniotic fluid lecithin level of about 3.5 mg/100 ml was critical: if it was above that level, RDS would not occur, but if it was below, RDS was almost inevitable.

The "shake" test

This is a quick and simple test for amniotic fluid surfactant based on the stability of bubbles formed by shaking amniotic fluid treated with ethanol. The test depends on the ability of the pulmonary surfactant in amniotic fluid to maintain a stable foam after shaking [5].

Amniotic fluid lecithin—sphingomyelin ratio (L : S index)

In 1971 Gluck et al. [6] developed a semiquantitative method for measuring the L : S ratio by TLC. The value of the L : S ratio depends on the fact that the concentration of lecithin in amniotic fluid is less than sphingomyelin until about 35—36 weeks gestation, at which time a surge in the lecithin concentration increases the L : S ratio, indicating fetal pulmonary maturation. The authors postulated that an L : S ratio of 2.0 or more indicates maturation of the fetal lungs with no risk of RDS, whereas infants with an L : S ratio of less than 2.0 have some clinical RDS, the lower the L : S ratio, the more severe the RDS.

Many modifications of the original method have been published in recent years [3, 7—13], and here we describe our present technique.

EXPERIMENTAL

A 2-ml sample of the centrifuged amniotic fluid was added dropwise to 2 ml of methanol, shaken and then 2 volumes of chloroform were added. After 2 min shaking and centrifugation the lower phase was removed and evaporated in a water bath at 60° under a stream of nitrogen. The dry residue was dissolved in chloroform (about 100 μl). A 20—30-μl volume of the chloroform solution was applied as a 1-cm line to the Silufol silica-gel sheets (4 × 10 cm; Sklárny Kavalier, Votice, Czechoslovakia). The foil was developed in chloroform—methanol—conc.NH_4OH (70:30:5) or chloroform—methanol—water (65:35:5). After a short drying at laboratory temperature the chromatograms were detected with acid fuchsin—uranyl nitrate reagent [14, 15]. Two-dimensional TLC was carried out with chloroform—methanol—conc.NH_4OH (70:30:5; 1st. dimension) and chloroform—acetone—methanol—acetic acid—water (5:2:1:1:0.5; 2nd. dimension). In some cases Vaskovsky's reagent [16] for phosphorus determination was used. After the detection washing of the chromatograms with ethanol is necessary. Two-dimensional chromatography was performed mainly for the verification of the results obtained by the one-dimensional technique. Densitometric scanning was performed with ERI 10 apparatus using filter No. 3 (VEB Zeiss, Jena, G.D.R.).

RESULTS AND DISCUSSION

Our present method was tested on 90 different amniotic fluids obtained by transabdominal amniocentesis (Figs. 1 and 2). The preparation of a calibration curve (phosphatidylcholine and sphingomyelin) is very useful for obtaining the more representative values of L : S ratio (Fig. 3). The simultaneous TLC of standard solutions of phosphatidylcholine and sphingomyelin is convenient.

Sometimes a double zone of sphingomyelin was observed (Fig. 1). In such cases the values of both fractions were summated and expressed as a total percentage of sphingomyelin.

The valuation of our modification was made by TLC of 14 samples of the same amniotic fluid. The mean value of the L : S ratio was 1.66 and the standard deviation ± 0.30. In the correlation with various clinical parameters it was observed that an L : S ratio of less than 2.0 indicates a risk of RDS.

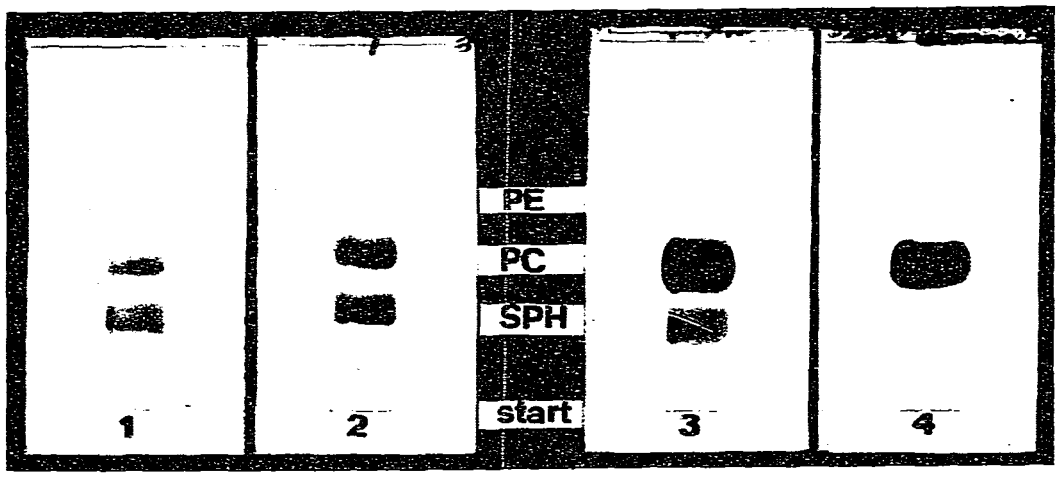

Fig. 1. One-dimensional chromatography of L:S ratio in amniotic fluid. Sorbent:Silufol sheets (4 × 10 cm). Solvent system:chloroform—methanol—conc. NH_4 OH (70:30:5). Detection:acid fuchsin—uranyl nitrate. SPH = sphingomyelin; PC = phosphatidylcholine; PE = phosphatidylethanolamine. L : S ratio: 1 = 0.9 (32 weeks of gestation); 2 = 1.3 (33 weeks); 3 = 2.7 (36 weeks); 4 = 8.5 (35 weeks).

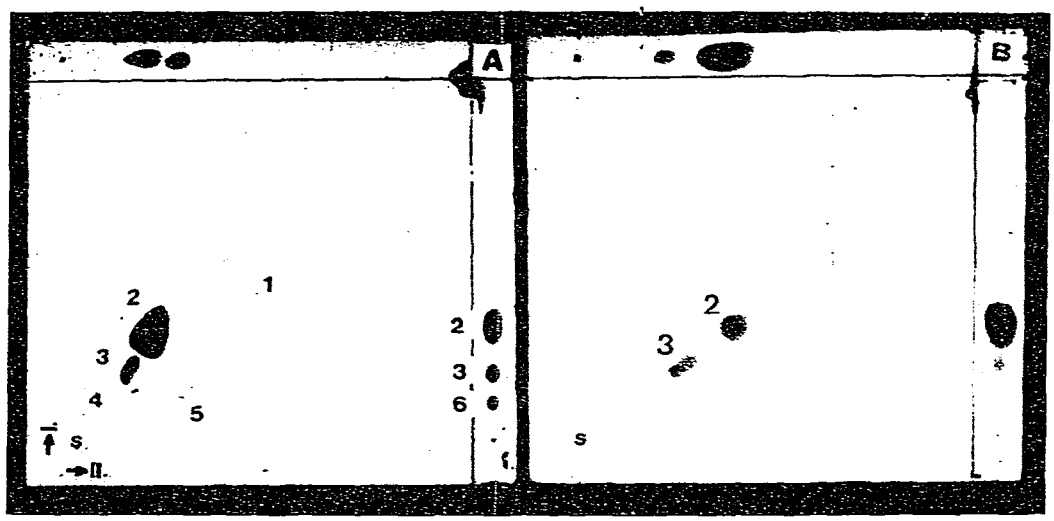

Fig. 2. Two-dimensional chromatography of phospholipids in amniotic fluid. Sorbent: Silufol sheets (10 × 10 cm). Solvent systems: 1st. dimension (I): chloroform—methanol—conc.NH_4OH (70:30:5); 2nd. dimension (II): chloroform—acetone—methanol—acetic acid—water (5:2:1:1:0.5). Detection: acid fuchsin—uranyl nitrate. A and B = two different samples of amniotic fluid. 1 = Phosphatidylethanolamine; 2 = phosphatidylcholine; 3 = sphingomyelin; 4 = phosphatidylinositol; 5 = phosphatidylserine; 6 = lysophosphatidyl—choline.

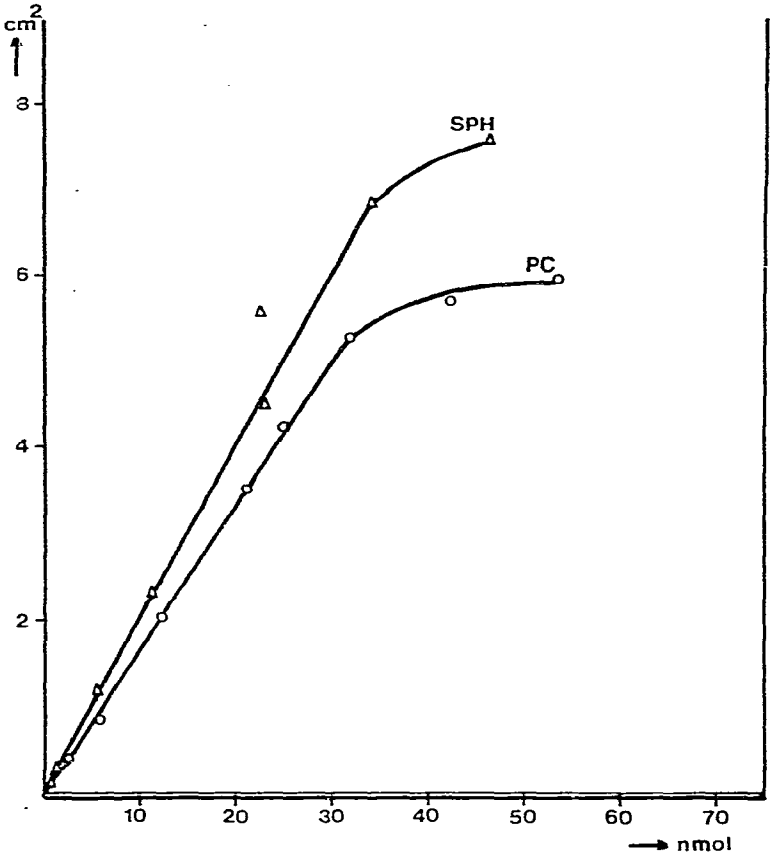

Fig. 3. Calibration curves of phosphatidylcholine and sphingomyelin after TLC on Silufol sheets and the detection with acid fuchsin—uranyl nitrate reagent. Densitometry: ERI 10 apparatus. PC = phosphatidylcholine; SPH = sphingomyelin.

The detection of phospholipids with acid fuchsin—uranyl nitrate reagent is very suitable for the semiquantitative evaluation of these substances in amniotic fluid. The application of the above-mentioned staining procedure is especially advantageous for detection of phospholipids carrying one negative charge (phosphate group) and one positive charge (choline or ethanolamine group) in their molecule [17].

In comparison with other TLC methods our modification has some advantages, especially in the use of a new staining procedure. According to our experiences we consider the present method as a simple and rapid determination of L : S ratio in routine laboratory practice.

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