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Analysis of dipalmitoyl phosphatidylcholine in amniotic fluid by enzymatic hydrolysis and high-performance thin-layer chromatography reflectance spectrodensitometry

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Abstract

A novel test for the determination of dipalmitoyl phosphatidylcholine (DPPC) in amniotic fluid (AF) as free dipalmitoylglycerol (DPG), is described. Aliquots of amniotic fluid were hydrolyzed with *Bacilus cereus* phospholipase C, and the resulting diglycerides analyzed by $AgNO_3$-modified high-performance thin-layer chromatography (HPTLC)–reflectance spectrodensitometry. This HPTLC system provided resolution of DPG and palmitoylpalmitoleoylglycerol (POG) from other 1,2-diglycerides and cholesterol. The turn-around analysis time for triplicate aliquots of amniotic fluid was 40 min. Recoveries ranged between 90 and 98%. In summary, this method provides a quantitative, specific, highly reproducible, and fast turn-around means of analysis of DPPC in amniotic fluid.

1. Introduction

The level of phospholipids in amniotic fluid, and in particular, of dipalmitoyl phosphatidylcholine (DPPC), has been shown to correlate with the level of surfactant lining the alveoli and with fetal lung maturity (FLM). In 1971, a test for the assessment of FLM was developed by Gluck et al. [1]. This test measures lung surfactant-associated phospholipids in amniotic fluid by thin-layer chromatography (TLC). A lecithin/sphingomyelin (L/S) ratio of ≥ 2 in amniotic fluid is indicative of a mature lung. In 1976, Hallman et al. introduced the analysis of phosphatidylglycerol (PG) in amniotic fluid by one-dimensional TLC as an additional test for the assessment of FLM [2]. A few years later, Kulovich et al. introduced the analysis of PG in amniotic fluid by two-dimensional TLC [3]. Since first introduced by Gluck and Hallman, TLC analysis of surfactant-associated phospholipids in amniotic fluid has become the gold standard for the assessment of FLM [4].

Ogawa was one of the first investigators, along with Gluck, who attempted to measure surfactant lecithin in amniotic fluid and relate it to fetal lung maturity [5]. Others have determined foam stability [6], surface tension [7], the ratio of palmitic to stearic acid [8], surfactant apoprotein [9], or dipalmitoyl lecithin [10], in an effort to

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measure some specific index of fetal lung maturity. With the exception of the amniotic fluid-foam-stability test, these methods have either been difficult to use routinely or have been less reliable than both the L/S ratio and/or the analysis of PG, which thus remains the gold standard for the assessment of FLM.

However, none of the tests so far mentioned are suitable for the assessment of FLM in samples contaminated with either blood or meconium. In 1979 a new test was introduced by Torday et al. [11] based on the method described by Mason et al. for the isolation and analysis of disaturated phosphatidylcholine (DSPC) with osmium tetroxide [12]. According to this method, the unsaturated species of phosphatidylcholine are destroyed by treatment with osmium tetroxide and therefore, only the saturated counterparts, including DPPC, are measured. Torday et al. introduced the DSPC test as a highly specific method for the assessment of FLM in amniotic fluid samples contaminated with either blood or meconium. A further adaptation of this method was reported by Curbelo et al. where the concentration of DSPC in rhesus-monkey amniotic fluid was measured as a function of gestational age [13]. In their study a direct correlation between the concentration of DSPC in amniotic fluid and FLM was found.

DSPC, and in particular DPPC, constitutes the major lecithin component of lung surfactant accounting for almost 70% of the mature surfactant [14]. Unlike lecithin from lung surfactant, lecithins from other sources including blood or meconium, contain only trace amounts of DPPC [11]. Therefore, determination of DPPC in amniotic fluid would appear as the ideal approach to the monitoring of fetal lung surfactant in amniotic fluid and to the assessment of FLM. A method that closely approaches this ideal is that described by Torday et al. [11]. However, the main drawbacks of this method are that it requires relatively high volumes of amniotic fluid ($\geq$2 ml), it is time-consuming (3–4 h), it involves the use of highly toxic reagents, i.e. osmium tetroxide and carbon tetrachloride, and in addition to DPPC, it also measures other disaturated species of phosphatidylcholine.

In this report, a novel test for the analysis of DPPC in amniotic fluid, is introduced. To our knowledge, this is the first report of a method that directly measures the concentration of DPPC in amniotic fluid. This method can be applied to the monitoring of fetal lung surfactant in samples contaminated with blood and/or meconium and it provides a quantitative, specific, highly reproducible, and fast turn-around means of analysis of DPPC in amniotic fluid by enzymatic hydrolysis and micro-HPTLC–reflectance spectrodensitometry.

2. Experimental

2.1. Reagents

The lipid standards including dipalmitoyl phosphatidylcholine, palmitoylpalmitoleoyl phosphatidylcholine, dipalmitolyglycerol, palmitoylstearoylglycerol, dimyristoylglycerol, distearoylglycerol, palmitoylpalmitoleoylglycerol, palmitoyloleoylglycerol, all 1,2-diglycerides; free cholesterol, *Bacilus cereus* phospholipase C (Sigma Type XI, 1000 U/0.6 ml), and Kodak X-Omat film, were purchased from Sigma (St. Louis, MO, USA). L-3-Phosphatidylcholine, 1,2-di-[1-^{14}C]-palmitoyl (100–120 mCi/mmol) ([^{14}C]-DPPC) was obtained from Amersham Corporation (Arlington Heights, IL, USA). Dulbecco's phosphate-buffered saline (D-PBS)was obtained from Gibco-Life Technologies (Grand Island, NY, USA). Kimble disposable centrifuge tubes were obtained from Thomas Scientific. Precoated silica-gel HP-K high-performance microplates (5 × 5 cm, 250 μm thickness) were obtained from Whatman (Clifton, NJ, USA). Solvents were EM Science chromatographic grade. Inorganic salts were from J.T. Baker (Phillipsburg, NJ, USA) and of the highest purity available.

2.2. Hydrolysis of amniotic fluid phosphatidylcholines with Bacilus cereus phospholipase C

Amniotic fluid samples obtained by amniocentesis for cytogenetic analysis (15 to 18 weeks

gestational age) were spiked with dipalmitoyl phosphatidyl choline standard to obtain concentrations ranging between 0.2 and 30 μg/ml. Aliquots of 0.5 ml of amniotic fluid were then hydrolyzed with *Bacilus cereus* phospholipase C at concentrations that ranged between 20 and 300 U/ml, and incubated at 24°C and 37°C for up to 30 min. Aliquots of 25 μl were then removed at 5-min intervals for the various phospholipase C concentrations and temperatures selected and the resulting diacylglycerols analyzed as indicated below. Solutions of phospholipase C in D-PBS were stored at 4°C and were stable for up to 1 month.

2.3. Specificity of hydrolysis of phosphatidylcholines by Bacilus cereus phospholipase C

The effect of amniotic fluid hydrolysis with *Bacilus cereus* phospholipase C on the concentration of phospholipids other than phosphatidylcholine was also tested. Aliquots of 25 μL of amniotic fluid were added to 10-ml centrifuge glass conical tubes, and treated with either 25 μl of *Bacilus cereus* phospholipase C (160 U/ml) or 25 μl of D-PBS. The samples were then incubated at 37°C for 5 min, 0.3 ml of chloroform–methanol (C–M) (2:1, v/v) added, the emulsion separated at 600 *g* for 3 min, the lower phase aspirated and evaporated to dryness, and the lipid residue dissolved in 4-μl of C–M (1:1, v/v). Aliquots of 4-μl were applied to Whatman HP-K silica-gel plates, the plates predeveloped in C–M (1:1, v/v) to 1 cm, and developed in one dimension using chloroform–ethanol–triethylamine–water (30:34:30:8, v/v) as the mobile phase. Following development, the plates were dipped for 5 s in a 10% solution of $CuSO_4$ in 8% H_3PO_4 and placed on a CAMAG TLC plate heater III at 185°C for 8 min. The stained chromatograms were then scanned with a Shimadzu CS-9000 spectrodensitometer in the reflectance mode at 400 nm [15]. The values obtained for the various phospholipids present in the amniotic fluid samples including phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylcholine, and sphingomyelin were then compared to the untreated samples.

2.4. High-performance thin-layer chromatography

Aliquots of 25 μl of a *Bacilus cereus* phospholipase C solution (160 U/ml) in D-PBS were added to 25 μl aliquots of amniotic fluid, the reaction allowed to proceed to completion for 5 min at 37°C and the lipids extracted by liquid–liquid partitioning with 0.3 ml of C–M (2:1, v/v) (final C–M–AF ratio of 4:2:1, v/v). The resulting emulsion was separated in two phases by centrifugation at 600 *g* for 3 min, the lower phase aspirated, evaporated to dryness, and the lipid residue dissolved in 4 μl of C–M (1:1, v/v). Aliquots of 4 μl of the lipid extract were applied to $AgNO_3$-modified high-performance (HPTLC) Whatman 5 × 5 cm HP-K silica-gel microplates ($AgNO_3$-modified layers were prepared by dipping HP-K microplates in a saturated solution of $AgNO_3$ in methanol for 1 min and dried under a warm stream of air from a hair dryer for 10 s), predeveloped in C–M (1:1, v/v), the plates dried, and developed in one dimension using chloroform–acetone (95:5, v/v) as the mobile phase. Aliquots of 4 μl of dipalmitoyl glycerol standard at a concentration of 0.06 mg/ml, were applied to a separate lane for identification and quantification purposes. A blank consisting of 25 μl of phospholipase C and 25 μl of D-PBS and a control consisting of 25 μl of D-PBS and 25 μl of amniotic fluid, were also carried through the entire procedure. Following development, the plates were dipped for 5 s in a 10% solution of $CuSO_4$ in 8% H_3PO_4, allowed to drip in the vertical position for 30 s, and placed on a CAMAG TLC plate heater III at 185°C for 5 min. The stained chromatograms were then scanned with a Shimadzu CS-9000 spectrodensitometer in the reflectance mode at 400 nm.

2.5. Isolation of phosphatidylcholine from amniotic fluid

Aliquots of 0.1 ml of amniotic fluid obtained by amniocentesis at gestational ages ranging from 28 to 40 weeks were extracted with 0.6 ml

of C–M (2:1, v/v) as indicated previously. Aliquots of 50 μl of the C–M extract (1:1, v/v) were applied as a streak to Whatman HP-K silica-gel plates, the plates predeveloped in C–M (1:1, v/v) to 1 cm, and developed in one dimension using chloroform–ethanol–triethylamine–water (30:34:30:8, v/v) as the mobile phase. The band comigrating with phosphatidylcholine standard was scrapped, the silica gel extracted with 3 ml of C–M (2:1, v/v) and partitioned with 0.5 ml of distilled water. The corresponding emulsion was separated by centrifugation at 600 *g* for 5 min, the lower phase aspirated and evaporated to dryness. The purified phosphatidylcholine was then dissolved in 0.1 ml of D-PBS in a sonication bath, hydrolyzed with 0.1 ml of *Bacilus cereus* phospholipase C (160 U/ml) and the resulting diacylglycerols analyzed by HPTLC, as indicated previously.

2.6. Effect of blood and meconium contamination on the determination of DPPC

The effect of blood and/or meconium contamination on the determination of DPPC in amniotic fluid following hydrolysis of amniotic fluid with phospholipase C, was also evaluated. Blood and/or meconium, and/or urine (1 to 10% by volume) were added to 5 different amniotic fluid samples with DPPC concentrations ranging between 0.2 and 30 μg/ml and the concentration of DPPC determined as described previously. The concentration of DPPC as measured by free DPG was compared before and after addition of these contaminants.

2.7. Effect of freezing and thawing on the determination of DPPC

The effect of freezing and thawing on the concentration of DPPC in amniotic fluid obtained following hydrolysis of amniotic fluid with phospholipase C, was investigated. Aliquots of freshly collected amniotic fluid (within 60 min of amniocentesis) and of the same frozen specimens, were analyzed by the procedure described herein and the results compared. Frozen specimens were subjected at least to three freeze–thaw cycles.

2.8. Analysis of fatty acid methyl esters by gas chromatography

Aliquots of 0.1 ml of amniotic fluid, a solution of DPPC in D-PBS (10 μg/ml), and a solution of palmitoylpalmitoleoyl phosphatidylcholine (POPC) in D-PBS (10 μg/ml) were hydrolyzed separately with 0.1 ml of *Bacilus cereus* phospholipase C (160 U/ml) and the lipids extracted with 1.2 ml of C–M (2:1, v/v), as indicated previously. The corresponding lipid residues were dissolved in 100 μl of C–M (1:1, v/v), applied to $AgNO_3$-HPTLC Whatman 5 × 5 cm HP-K silica-gel microplates as a 4-cm streak, and developed in chloroform–acetone (95:5, v/v) to the top of the plate. Following development, the band comigrating with DPG and POG standards were scrapped from the plate, the silica gel extracted with 3 ml of C–M (2:1, v/v) and partitioned with 0.5 ml of distilled water. The corresponding emulsions were separated by centrifugation at 600 *g* for 5 min, the lower phases aspirated and evaporated to dryness. The lipid residues were hydrolyzed with 0.5 ml of 0.1 *M* sodium methoxide at 40°C for 1 h. After hydrolysis, the tubes were allowed to cool to room temperature, 1 ml of chloroform and 0.25 ml of distilled water added, the emulsion centrifuged at 600 *g* for 5 min and the lower phases aspirated and evaporated to dryness. The resulting fatty acid methyl esters were dissolved in a volume of *n*-hexane to give concentrations of 1 μg/μl. The fatty acid methyl esters were analyzed by GC on a WCOT capillary column (Supelco-Wax-10, 30 m × 0.53 mm I.D., 1.0 μm film thickness) with initial and final temperatures in the column oven of 150°C and 250°C, respectively, programmed to increase at a rate of 10°C/min. The analysis was performed on a Varian 3700 instrument equipped with flame-ionization detector operated at a temperature of 260°C. Fatty acid methyl ester peaks were identified by comparison of retention times of standard mixtures and quantified using a Hewlett-Packard 3392A inte-

grator, using methylheptadecanoate as the internal standard. The identity of DPG and POG as the components comigrating with DPG and POG standards was based on the presence of methylhexadecanoate (DPG) and methylhexadecenoate and methylhexadecanoate (POG) as the fatty acid methyl esters identified in the gas chromatogram.

2.9. HPTLC-autoradiographic analysis

Aliquots of 10 μl of [^{14}C]DPPC (30 μCi/ml) solutions in D-PBS were spiked into term amniotic fluid samples, hydrolyzed with *Bacilus cereus* phospholipase C and the resulting [^{14}C]-DPG extracted and redissolved in 4 μl of C–M (1:1, v/v). Aliquots of 4 μl of the hydrolyzed [^{14}C]DPPC extract and 0.3 μCi of [^{14}C]DPG were then applied to separate lanes of Whatman HP-K microplates and the diacylglycerides separated as indicated above. Following development, the plates were exposed to Kodak X-Omat film for 12 h at room temperature and the resulting bands scanned with a Shimadzu CS-9000 spectrodensitometer in the reflectance mode at 400 nm. Recoveries for DPPC were calculated by dividing the integrated area of [^{14}C]DPG in the lower phase by that of the [^{14}C]DPPC spiked, following enzymatic hydrolysis of [^{14}C]DPPC with *Bacilus cereus* phospholipase C.

2.10. Precision analysis

Aliquots of 25 μl of 20 different amniotic fluid samples with DPPC values ranging from 0.2 to 30 μg/ml were analyzed in triplicate for the DPPC concentration following the procedure herein described. The standard deviation corresponding to the three values obtained was utilized to calculate the intra-assay variation. Aliquots of 25 μl of a given amniotic fluid sample were also analyzed in triplicate for the DPPC concentration in 20 separate runs. The standard deviation corresponding to the 20 values of DPPC obtained was utilized to calculate the inter-assay variation.

3. Results and discussion

A lipid profile representative of a term amniotic fluid following enzymatic hydrolysis with *Bacilus cereus* phospholipase C is shown in Fig. 1. The major components released comigrated with dipalmitoylglycerol and palmitoyl-palmitoleoylglycerol (Fig. 1). These components were not detected in control amniotic fluid samples obtained from gestational ages ranging from 28 to 40 weeks in the absence of phospholipase C hydrolysis (data not shown). Enzymatic hydrolysis of dipalmitoyl phosphatidylcholine (DPPC) and palmitoyl-palmitoleoyl phosphatidylcholine (POPC) standards with phospholipase C resulted in bands that comigrated with dipalmitoylglycerol and palmitoyl-palmitoleoylglycerol, respectively, and with the components released following enzymatic hydrolysis of amniotic fluid. The identity of these components was further established by gas chromatographic analysis of the fatty acid methyl esters released following alkaline methanolysis. Gas chromatographic analysis of the fatty acid methyl esters released after alkaline metha-

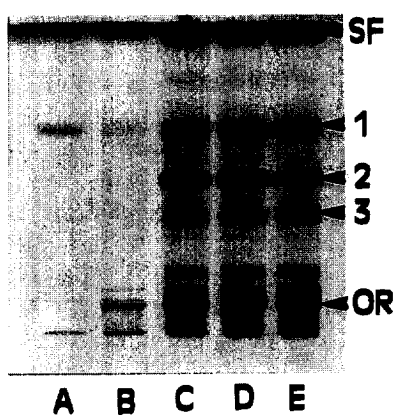

Fig. 1. Chromatogram of amniotic fluid lipids following enzymatic hydrolysis with phospholipase C. Lane A corresponds to 0.24 μg of dipalmitoylglycerol standard; lane B corresponds to phospholipase C blank; and lanes C, D, and E correspond to triplicate 25 μl aliquots of term amniotic fluid treated with phospholipase C (see Experimental). Bands: 1 = dipalmitoylglycerol; 2 = cholesterol; 3 = palmitoyl-palmitoleoylglycerol; SF = solvent front; OR = origin.

nolysis of the component comigrating with dipalmitoylglycerol resulted in a single peak that had the same retention time as methyl hexadecanoate(16:0). Similarly, gas chromatographic analysis of the fatty acid methyl esters released following alkaline methanolysis of the component comigrating with palmitoyl-palmitoleoylglycerol resulted in two peaks of equal intensity (1:1 peak ratio) that had the same retention times as methyl hexadecanoate(16:0) and methyl hexadecenoate(16:1), respectively. This data is consistent with the phospholipid composition of mature fetal lung surfactant obtained from amniotic fluid where the major components are the DPPC and POPC molecular species. In order to ascertain that phosphatidylcholine was the sole phospholipid source of the component comigrating with DPG following enzymatic hydrolysis with phospholipase C, phosphatidylcholine was isolated from amniotic fluids obtained by amniocentesis at gestational ages ranging from 28 to 40 weeks. The purified phosphatidylcholine was then hydrolyzed with phospholipase C and the resulting diacylglycerols analyzed by micro-HPTLC, as indicated previously. As shown in Fig. 2, DPG values obtained following phospholipase C hydrolysis of amniotic fluid and/or phosphatidylcholine isolated from the same amniotic fluid, increased with gestational age. The concentration of POG increased sharply with DPG concentrations above 1200 μg/100 ml (data not shown). The differences in the DPG values for both sets of samples were not statistically significant ($p > 0.5$). Therefore, analysis of DPG by HPTLC–reflectance spectrodensitometry following enzymatic hydrolysis of amniotic fluid with phospholipase C can be safely utilized to determine the concentration of DPPC in that amniotic fluid.

The rate of hydrolysis of DPPC was temperature, phospholipase C concentration, and time dependent. As shown in Figs. 3 and 4, optimal hydrolysis was obtained at a phospholipase C concentration of 160 U/ml at 37°C for 5 min. The rate of hydrolysis of POPC was also temperature, phospholipase C concentration, and time dependent (data not shown). Treatment of

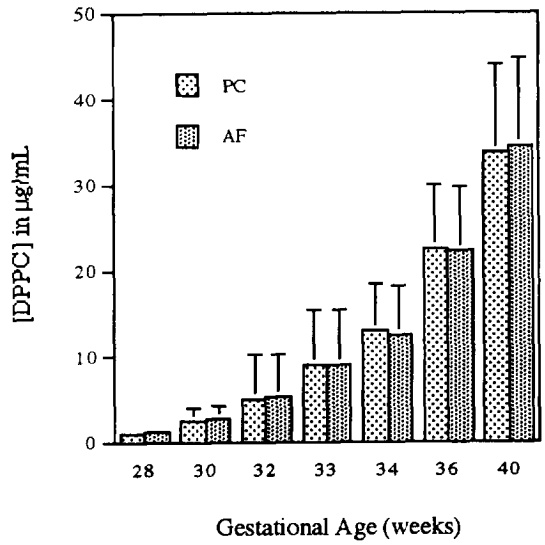

Fig. 2. Levels of DPPC in amniotic fluid as a function of gestational age. PC: phosphatidylcholine extracted from amniotic fluid was hydrolyzed with phospholipase C and the resulting DPG analyzed by HPTLC (see Experimental); AF: amniotic fluid was hydrolyzed with phospholipase C and the resulting DPG analyzed by micro-HPTLC (see Experimental). Each point represents the average of 5 separate experiments. Error bars are the standard deviations.

amniotic fluid with phospholipase C did not result in any significant hydrolysis of phospholipids other than phosphatidylcholine (data not shown). Addition of up to 10% of blood and/or meconium did not result in any significant change in the rate of hydrolysis ($p > 0.7$). The effect of blood and meconium contamination on the resolution of DPG and POG from other lipids is illustrated in Fig. 5. The effect of centrifugation on the concentration of DPPC and POPC, is also shown in Fig. 5. Lanes B and C and D and E of Fig. 5 correspond to a term amniotic fluid sample before and after centrifugation at 3000 g for 10 min. Centrifugation at this speed resulted in a net loss of 40 to 50% ($\pm 10.5\%$) of the DPPC present in the uncentrifuged samples. Increasing the centrifugation speed to 14 000 g resulted in a net DPPC loss of 90%. No significant differences were found in the values of DPPC obtained with the fresh samples compared to those obtained with the

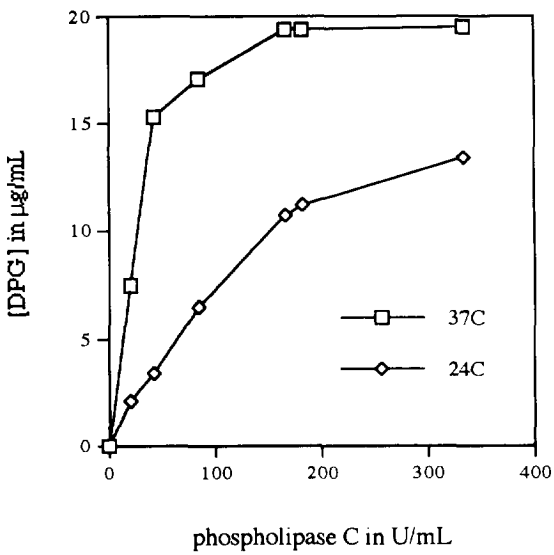

Fig. 3. Dependency of rate of hydrolysis of amniotic fluid with phospholipase C on enzyme concentration and temperature. Each point represents the average of separate experiments at the various phospholipase C concentration selected. Hydrolysis time was 5 min.

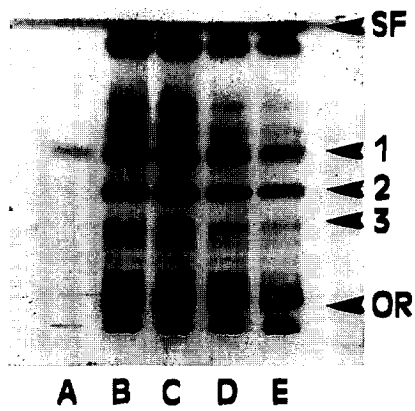

Fig. 5. Effect of centrifugation on the concentration of DPPC in amniotic fluid. Lane A corresponds to 0.24 μg of dipalmitoylglycerol standard; lanes B and C correspond to 25-μl aliquots of an uncentrifuged blood and meconium contaminated term amniotic fluid sample; lanes D and E correspond to the same amniotic fluid sample centrifuged at 1000 g for 10 min; Bands: 1 = dipalmitoylglycerol; 2 = cholesterol; and 3 = palmitoylpalmitoleoylglycerol; SF = solvent front; OR = origin.

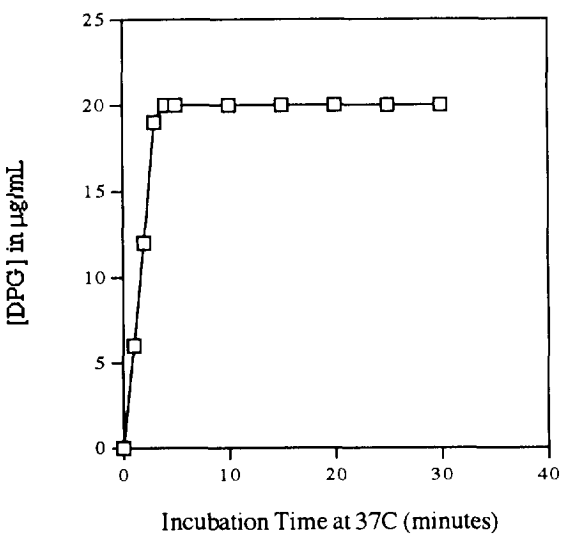

Fig. 4. Time dependency of amniotic fluid hydrolysis with phospholipase C. Each point represents the mean of 5 separate experiments at the time points selected.

frozen specimens subjected to three freeze–thaw cycles.

The efficiency of DPPC hydrolysis and extraction was determined by spiking amniotic fluid samples with known amounts of [^{14}C]DPPC and measuring the concentration of [^{14}C]DPG in the lower phase. The recoveries obtained ranged between 90% and 98% as determined by HPTLC-autoradiographic analysis.

The separation of DPG from other diacylglycerol species is shown in Fig. 6. DPG was resolved from dimyristoylglycerol, distearoylglycerol, myristolypalmitoylglycerol, palmitoylpalmitoleoylglycerol, palmitoyloleoylglycerol, and free cholesterol. Gas chromatographic analysis of the fatty acid methyl esters released following alkaline hydrolysis of phosphatidylcholine isolated from amniotic fluid indicated that methyl tetradecanoate(14:0), methyl hexadecanoate(16:0), methyl hexadecenoate(16:1), methyl octadecanoate(18:0), and methyl octadecenoate(18:1) comprised 2, 76, 15, 3 and 4% of the total fatty acid content, respectively. Based on this fatty acid profile, should other

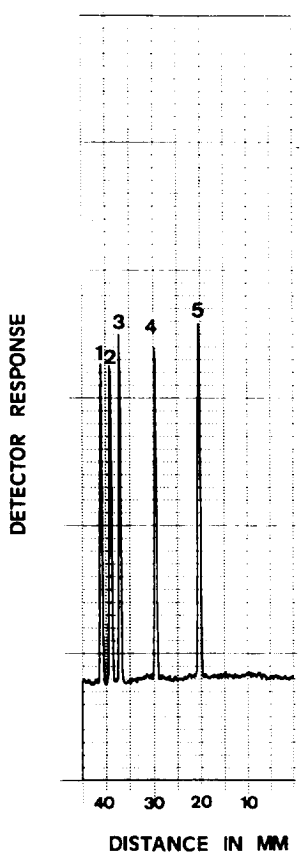

Fig. 6. Densitometric chromatogram of disaturated and monounsaturated diacylglycerols standards separated by HPTLC. Peaks: 1 = distearoylglycerol; 2 = dipalmitoylglycerol; 3 = myristolpalmitoylglycerol; 4 = dimyristoylglycerol; and 5 = palmitoylpalmitoleoyl glycerol. Aliquots of 0.1 μg of each standard were applied to separate lanes and resolved by micro-HPTLC (see Experimental).

1,2-disaturated diglyceride species different from that of the diglyceride standards used in this study comigrate with DPG, their contribution to the DPG value obtained will be ≤3%.

The lower limit of detection of DPPC in amniotic fluid as analyzed by the method described was 200 ng/ml with linear detector response extending to 30 μg/ml. The intra- and inter-assay variation for analysis of DPPC as measured by the standard deviation were 3 and 8%, respectively. The turn-around analysis time for triplicate aliquots of amniotic fluid was 40 min.

As compared to the method reported by Torday et al. [11] for the determination of disaturated phosphatidylcholine (DSPC) in amniotic fluid, analysis of DPPC by the method described here offers the following advantages: (1) it minimizes sample use: only 25 μl of amniotic fluid are required for analysis. Therefore, it will find wide applicability in those situations where sample availability is limited; (2) it provides a fast turn-around time, allowing analysis of triplicate aliquots of amniotic fluid in 40 min; (3) it minimizes the use of toxic reagents, i.e. osmium tetroxide and carbon tetrachloride; (4) it measures DPPC, the most abundant component of the mature lung surfactant; (5) it provides quantitative analysis of other key components of fetal lung surfactant, including POPC; and (6) it can also be applied to the analysis of frozen specimens of amniotic fluid. The main disadvantage of this method, as of any other method that provides quantitative analysis of a single component in a biological fluid, including the DSPC method, is that the value obtained is subjected to volume fluctuations. Therefore, in those situations where the volume of amniotic fluid is below the normal level (oligohydramnios) the concentration of DPPC in amniotic fluid will be artificially increased. And conversely, when the volume is higher than normal (polihydramnios), the concentration will be decreased.

In summary, analysis of DPG by micro-HPTLC–reflectance spectrodensitometry provides a quantitative, specific, highly reproducible, and fast turn-around means of analysis of lung surfactant-associated DPPC in amniotic fluid. Further studies are now in progress to determine the efficacy of this method in the assessment of fetal lung maturity.

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