Aminopropyl solid-phase columns can be used for phospholipid fractionation. A method has been developed to elute the acidic phospholipid phosphatidylglycerol (PG) separately, and it is applicable to both standard (phospholipid mixtures and pulmonary surfactants). The simplicity, rapidity, and high recovery make this method of isolating PG superior to other chromatographic procedures.

Several enzymatic procedures have been developed for determining the amount of surfactant phosphatidylglycerol (PG) in amniotic fluids. Some of these enzymatic findings disagreed with results obtained after thin-layer chromatography (TLC) (1, 2) and, especially when the amount of PG is approximately the same as that of lecithin (3), one has to doubt the enzymatic PG results. Usually, the amount of PG is determined by phospholipid-phosphorus assay, but then PG has to be isolated first. Several techniques are available, but TLC is time-consuming and "high-performance" liquid chromatography requires rather expensive equipment. The neutral and acidic phospholipids (including PG) can be separated by anion-exchange chromatography (4, 5), but these methods require large volumes of solvents. Relatively small volumes of solvents are, however, needed if solid-phase columns are used for ion-exchange chromatography. This note describes a "<10-minute" procedure for isolating PG by using solid-phase aminopropyl columns. The amount of PG can then be determined.

Materials and Methods

Two "100-mg" Aminopropyl Bond Elut columns (Analytichem Inc., Harbor City, CA 90710) were conditioned with hexane. One of these columns was loaded with mixtures of neutral lipids, the phospholipid standards (phosphatidylcholine, PC; phosphatidylinositol, PI; phosphatidylserine, PS; and PG, all from Sigma Chemical Co., St. Louis, MO) or with extracts of PG-rich but PI-poor sheep surfactant (6), PG-poor but PI-rich monkey surfactant (7), or mixtures of these two biological surfactants. The phospholipids were dissolved in 0.5 mL of dichloromethane and applied to the column under reduced pressure, to remove the solvent (eluate A). Next, the column was eluted with 1-mL portions of, respectively, solvents B1 (dichloromethane/2-propanol, 2:1 by vol) and B2 (acetic acid/diethyl ether, 2:98) and solvent C (two 1-mL portions of methanol), as described by Kaluzny et al. (8). The column, still containing the acidic phospholipids (PG, PI, PS) was attached above the second conditioned column. PG were eluted from the stacked columns with three 1-mL portions of solvent D (modified after Rouser et al. (4) and containing dichloromethane/methanol/ammonium hydroxide (28%)/ammonium acetate (10 mmol/L, in methanol), 28:7:1:1, by vol). PI and PS were then eluted with three 1-mL portions of solvent E (n-hexane/2-propanol/H2O/ammonium hydroxide (28%), 60:60:20:1, by vol).

The yield and recovery of the different phospholipids in each of the eluates was calculated after phospholipid-phosphorus determination (9), and "high-performance" TLC (HPTLC plates 60F254; Merck, Darmstadt, F.R.G.) was used to separate the phospholipids (10) and to control the purity of the different fractions.

Results and Discussion

Experiments were first performed with individual phospholipids. Eluates B1 and B2 contained the neutral lipids and the free fatty acids, as described by Kaluzny et al. (8). Eluate C contained the neutral phospholipid PC, whereas the acidic phospholipids PG and PI were retained. It was originally suggested by Kaluzny et al. (8) that methanol (solvent C) would elute all phospholipids. We cannot explain this difference, although we were not surprised to find that acidic phospholipids could only be eluted from the aminopropyl column after changing the ionic strength of the solvent. Within the range of 7–150 mmol, the loss of each phospholipid in eluate A and the two eluates B was less than 3%. The total analytical recovery, based on the phospholipid content of all eluates, ranged between 95 and 99%. Of the recovered phospholipids PC, PG, and PI, 95% are found, respectively, in eluates C, D, and E. The columns had to be stacked before solvent D was used, because otherwise approximately 25% of PI would be eluted with PG. There is less than 0.5% carryover of PC in eluates D and E, and the detection limit for PG and PI was 1 nmol. Overloading the columns (>250 nmol) resulted in decreased recovery and selectivity and increased the carryover of PC in eluate D.

We did experiments with the two biological surfactants (93–100 mmol of phospholipid) and their mixtures (stepwise changes of 20%), to simulate changes in the proportion of surfactant PG such as occur during fetal or neonatal lung maturation (11–13). Approximately 95% of the total phospholipid fraction was accounted for in eluates A–E, the same as found for the standards. After correction for recovery, 85–88% of both the sheep and monkey surfactant phospholipids was found in eluate C (the neutral phospholipids PC, sphingomyelin, phosphatidylethanolamine, and, if present, lysophosphatidylcholine), which agreed with the percentages found after TLC and determination of phospholipid phosphorus. Eluates D and E contained the 11–14% acidic surfactant phospholipids (PG, PI, and PS). Figure 1 shows the excellent relationship between the percentages of PG and PI of the mixed surfactants after sorbent extraction or TLC. The percentages of PI in eluate E are higher than those of PI after TLC because PS (approximately 1%) is also eluted with solvent E. These two phospholipids can be separated on SCX (benzenesulfonylpropyl) columns by using methanol and methanol–0.1 mol/L HCl (20/1, by vol) as solvents (unpublished results).

The described technique separates neutral and acidic phospholipids well and rapidly, more easily than by chromatography on diethylaminoethyl-cellulose (4, 5). More important, however, PG can be isolated from a pool of surfactant phospholipids within 5 to 10 min, and analytical recovery of
Fig. 1. Relationship between the determined percentages of PI and PG after sorbent extraction and their calculated percentages based on separation by "high-performance" thin-layer chromatography.

The percentages of PI and PG in the mixtures have been calculated from chromatographic results (in triplicate) for one sample of sheep surfactant (PI = 1.3 ±0.3%; PG = 11.8 ±0.4%) and one sample of monkey surfactant (PI = 8.4 ±0.7%; PG = 2.1 ±0.5%).

Phospholipids is high. Work is now in progress to evaluate the clinical use of the method.

We thank Becton Scientific BV, Rotterdam, The Netherlands, for supplying the Aminopropyl Bond Elut columns.

References